Breast cancer tailored staging using molecular imaging
Teixeira, S.C.

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A warm thank you to Cees A. van der Mey who made this beautiful watercolour specially for the cover of this thesis.

Invitation

to the public defence of my PhD thesis entitled:

Breast cancer tailored staging using molecular imaging

On Thursday 14th of September 2017 at 10:00 in the Agnietenkapel
University of Amsterdam
Oudezijds Voorburgwal 229-231
Amsterdam

Reception/lunch afterwards at Dante Kitchen & Bar
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Breast cancer tailored staging using molecular imaging

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Breast cancer tailored staging using molecular imaging
PhD thesis, University of Amsterdam, the Netherlands
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Author: S. C. Teixeira
Cover: original watercolour by Mr. C. E. van der Mey representing the three major stages of breast cancer: low stage primary tumour (middle rose), presence of lymph node metastases (rose with palpable leaves on the left) and distant metastases (gray palpable rose on the right).
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BREAST CANCER TAILORED STAGING USING MOLECULAR IMAGING

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor

aan de Universiteit van Amsterdam

op gezag van de Rector Magnificus

prof. dr. ir. K.I.J. Maex

ten overstaan van een door het College voor Promoties ingestelde commissie,

in het openbaar te verdedigen in de Agnietenkapel op

donderdag 14 september 2017, te 10:00 uur

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Table of contents

Introduction .............................................................................................................................................. 9

Chapter 1: The role of PET/CT for nodal staging in primary stage II/III breast cancer patients 21

Part I – PET/CT: Improved baseline visualization

Chapter 2: Additional prone 18F-FDG PET/CT acquisition to improve the visualization of the primary tumour and regional lymph node metastases in stage II/III breast cancer ............... 43
Chapter 3: PET/CT with 18F-FDG predicts short-term outcome in stage II/III breast cancer patients upstaged to N2/3 nodal disease ................................................................. 59

Part II - PET/CT: Response monitoring

Chapter 4: Monitoring primary tumour response to neoadjuvant chemotherapy using MRI and 18F-FDG PET/CT in breast cancer subtypes ................................................................. 81
Chapter 5: Additional value of 18F-FDG PET/CT response evaluation in axillary nodes during neo-adjuvant chemotherapy for triple-negative and HER2-positive breast cancer ............ 101

Part III – MAMMI-PET: A new dedicated imaging modality

Chapter 6: Evaluation of a hanging-breast PET system for primary tumour visualization in patients with stage I-III breast cancer: comparison with standard PET/CT .......................... 125
Chapter 7: Dedicated breast PET (MAMMI-PET) in daily clinical practice: implications for radiation safety of nuclear medicine personnel ................................................................. 145
Chapter 8: A novel semi-robotized device for high-precision 18F-FDG-guided breast cancer biopsy ................................................................................................................................ 159
Introduction
Introduction

For women in the Netherlands the incidence of breast cancer is still the highest of all cancers, with 14,515 women presenting with primary breast cancer in 2015. Due to improved early detection, introduction of systemic treatment and increased breast cancer awareness, breast cancer mortality for female breast cancer patients in the Netherlands has dropped under the mortality for female lung cancer patients in 2006, and is still decreasing. In the US the highest incidence of all cancers in women is also still assigned to breast cancer.

Diagnostics and tumour classification

When a woman presents with a suspect lesion in the breast (primary lesion) the initial diagnostic work comprises of imaging the affected breast with mammography and screening the affected breast and both axillae with ultrasonography. Since the sensitivity of prone position MRI to detect primary lesions approximates 100% in some centres an additional MRI in prone position is performed to better delineate the primary tumour before start of systemic treatment (=baseline). Prone position MRI was introduced in 1985, using a dedicated coil, which enables three dimensional imaging of both breasts.

After initial imaging of the primary tumour, ultrasonography-guided fine-needle aspiration (FNA) and core biopsies are taken from suspect lesions in the breast and/or the axilla and proven malignant lesions are marked. These biopsy specimens are subsequently examined by the pathologist for confirmation of diagnosis and, in case of malignancy, for classification (histologic and immunologic) of the breast cancer subtype. Histologic classification of a tumour refers to the main groups of this tumour, lobular or ductal, and the immunologic classification refers to the presence of growth enhancing receptors as explained in the next paragraph. The combination of physical examination, imaging and image-guided biopsies reveals a diagnosis in more than 95% of the cases.

Classification of the tumour is used to guide additional systemic treatment (hormonal treatment, chemotherapy and/or and targeted therapy) tailored to the specific subtype. The presence of the oestrogen (ER) and progesterone (PR) receptors determine the sensitivity for hormonal treatment (ex. tamoxifen) and the overexpression of human epidermal Growth factor 2 determines the sensitivity for HER-2 targeted therapy (ex. trastuzumab).

Generally, three subtypes can be distinguished based on these receptors: Triple negative (both ER/PR and HER-2 receptors are negative), hormone-positive (ER/PR positive and Her-2 negative) and HER-2 positive subtype (HER-2 positive and ER/PR either positive or negative). In general,
patients with triple negative\textsuperscript{6} or HER-2 positive tumours\textsuperscript{7} show less favourable prognosis compared to patients with hormone receptor positive tumours.

**Staging and systemic treatment**

Breast cancer staging is defined using the TNM classification, valuable for subsequent treatment planning and prognostic stratification; T is related to the number and size of the primary tumour in the breast, N for location and number of lymph node metastases and M for the presence of distant metastases (for more detailed differentiation see Table 1).

Stage I patients have a low chance of developing regional (and distant) metastases compared to stage II and III breast cancer patients (see explanation under table 1). Stage II/III breast cancer patients consists of approximately 50% of all patients presenting with new onset breast cancer each year in the Netherlands\textsuperscript{8}. The treatment of a stage II/III breast cancer patient is always a multimodality treatment including systemic treatment and local regional treatment by a combination of surgery of the breast and axillary nodes and often radiotherapy\textsuperscript{9}. Stage IV breast cancer patients are further addressed in the paragraph “distant metastases”.

**Table 1:** TNM stages of primary breast cancer according to the Dutch guidelines, simplified.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0/Tis</td>
<td>No malignant tumour present in the breast</td>
</tr>
<tr>
<td>T1</td>
<td>Malignant tumour with a maximal diameter of 2 cm</td>
</tr>
<tr>
<td>T2</td>
<td>Malignant tumour with a diameter greater than 2 cm but of maximal 5 cm</td>
</tr>
<tr>
<td>T3</td>
<td>Malignant tumour with a diameter greater than 5 cm</td>
</tr>
<tr>
<td>T4</td>
<td>The malignant tumour has infiltrated the skin of the breast (including oedema)</td>
</tr>
<tr>
<td>N0</td>
<td>No axillary lymph node metastases present</td>
</tr>
<tr>
<td>N1</td>
<td>Minimal of 1 and maximal 3 axillary lymph node metastases present</td>
</tr>
<tr>
<td>N2</td>
<td>Minimal of 4 and maximal 9 nodes (N2a) or parasternal lymph node metastases</td>
</tr>
<tr>
<td>N3</td>
<td>Minimal of 10 axillary lymph node metastases or infraclavicular nodes (N3a) OR parasternal with the present of axillary lymph node metastases and/or supraclavicular lymph node metastases (N3c)</td>
</tr>
<tr>
<td>M0</td>
<td>No distant metastases present</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastases present</td>
</tr>
</tbody>
</table>

Tis= Tumour in situ, meaning the pre-stadium of breast cancer. The tumour has not yet ruptured anatomical boundaries. A T2-3N0M0 stage tumours or any T-stage tumour with a N1-2M0 is considered stage II and T3-4N0M0 tumours or any T-stage tumour with N2-3M0 is considered stage III.
Systemic treatment (chemotherapy, hormonal treatment and/or targeted therapy) can be given prior to surgery (preoperative systemic treatment or PST) or after surgery (adjuvant systemic treatment). One of the proven advantages of PST is the fact that the tumour volume may shrink converting the surgery plan of up to 40% of the patients from mastectomy to breast conserving therapy\textsuperscript{10}. Furthermore, initially tumour-positive (axillary) lymph nodes can be converted to ypN0 in 30-45% of the cases\textsuperscript{11,12}, possibly obviating the need for axillary lymph node dissection (ALND) or axillary radiotherapy (RT) in a subgroup of these patients. Depending on tumour subtype, a complete pathological response (pCR) rate of 40–50% can be achieved when applying PST\textsuperscript{13}, which is associated with a favourable prognosis, particularly in triple-negative tumours and HER-2 positive tumours\textsuperscript{14}. Another potential advantage of PST is that it allows for monitoring response to systemic therapy early on during treatment what might lead to replace or cease a specific and potentially toxic therapy regimen in case of treatment failure.

**Distant metastases**

Before the starting PST, with curative intent, primary breast cancer patients need to be screened for the presence of distant metastases as discovering distant metastases has significant consequence for prognosis and further treatment. Indeed, a breast cancer patient with distant metastases (TNM stage IV) is treated with so called “palliative treatment”, intended to prolong life and relief symptoms, instead of curing the disease. Recently, it has been described that a limited number of metastatic lesions or “oligometastatic lesions”\textsuperscript{15} may be treated locally with curative intent\textsuperscript{16–18}, there is, however, not yet a general protocol for this subgroup of patients. At the NKI the presence of a maximum of three distant metastases are considered being oligometastatic and when possible treated locally with radiotherapy (bone) or surgery (liver or lung).

Distant metastases screening in breast cancer patients is traditionally performed by ultrasound of the liver, a full-body bone-scan and a chest X-ray of the lungs\textsuperscript{8,19}. However, recent studies have shown that PET/CT identifies a higher number of distant metastases in breast cancer patients receiving PST (stage I/III), compared to conventional imaging\textsuperscript{3,19–25}. Subsequently, a new recommendation was added to the Dutch guidelines for breast cancer diagnostics and treatment (oncoline.nl) indicating that PET/CT is advised for distant metastasis screening in stage III and can be considered in stage II primary breast cancer patients\textsuperscript{9}. 
PET/CT

Positron emission tomography coupled with low-dose computed tomography (PET/CT) is an imaging modality using radiopharmaceuticals that are able to differentiate between pathologic (malignant or inflammatory) and physiologic (“normal”) activity in organs and tissues. These tracers are generally small molecules ligands, antibodies or drugs labelled with so-called “positron emitters” or radionuclides such as Fluor-18 ($^{18}$F). Fluorine-18 coupled with the glucose analog fluorodeoxyglucose ($^{18}$F-FDG or simply FDG) is the most commonly used tracer in oncology, but it has also diagnostic value in neurology and cardiology. The diagnostic value of FDG PET/CT in oncology is due to the increased glucose metabolism in malignant cells compared to physiologic uptake in non-malignant cells. Malignant cells show an increased uptake of glucose, and thus FDG, which can be visualised on a PET/CT image. For diagnostic purposes FDG uptake is commonly visually assessed but can additionally be quantified and expressed as “maximum standardized uptake value” (SUVmax). SUVmax is useful for comparing the lesions during PST, moreover, a positive correlation was found between an elevated SUVmax and subtypes with less favourable characteristics (triple negative, HER2 positive).

Due to its low sensibility for the detection of primary lesions <10 mm, standard supine PET/CT imaging has been described to be less adequate for the visualisation of primary breast cancer lesions. Recently a hanging-breast coil, similar to that of MRI, was introduced for prone position PET/CT imaging (figure 1). The implementation of prone position PET/CT acquisition resulted in less anatomical mismatch between the fused PET (FDG uptake) and CT images (anatomical structure) due to reduction of the breathing motion of the front thorax. Consequently, smaller tumour locations (= lymph node metastases) are correctly identified and several smaller studies have reported that prone position PET/CT may give a better estimation of tumour heterogeneity and the number of present lymph node metastases compared to supine PET/CT imaging.

MAMMI-PET and MammoCare

To overcome the limitation whole-body PET/CT has to detect lesions <10mm, a dedicated breast PET was developed: the MAMMI-PET (figure 3). This high-resolution full-ring system for dedicated hanging breast imaging without compression, was developed in the context of a EU-founded project to improve the detection of breast cancer.

The first MAMMI-PET prototype showed a high agreement in FDG uptake compared to PET/CT, even for tumours in the vicinity of the thoracic wall. Compared to the previous MAMMI-PET prototype the current version offers more stability and support for the patient and has a scanning
Introduction

range comparable with that of the clinically used positron emission mammography (PEM)\textsuperscript{42}. However, as the acquisition ring of this device is positioned close to the breast, with a diameter of approximately a tenth of the diameter of a full-body PET/CT scanner (figure 2), a less higher dose of FDG is needed for effectively scanning primary lesions\textsuperscript{39,40}. In addition to the fact that CT scanning is not performed, it overall results in a significant reduction of the radiation dose\textsuperscript{42}.

Since the MAMMI-PET system is not equipped with a CT scanner, attenuation correction is not available and quantification of FDG uptake expressed in SUV is not possible. Despite this limitation, image quality is good enough for visual evaluation with a better resolution compared to standard PET/CT devices\textsuperscript{44-46}. In this respect, MAMMI-PET is able to better visualise areas with different FDG uptake in larger primary breast tumours\textsuperscript{40}. These areas possibly indicate areas of different biological characteristics and thus tumour heterogeneity\textsuperscript{40}. For further investigating tumour heterogeneity it is required to use FDG-guided biopsies from different regions of interest and uptake. Currently a MAMMI-PET based biopsy device is being developed in the context of a project funded by the European Commission: The MammoCare (figure 4; http://www.mammocare.eu).

Rationale and thesis outline

The general aim of the research described in this thesis was to further investigate the additional clinical value of PET/CT to improve staging and response monitoring in female patients presenting with primary breast cancer. Secondly we aimed to assess the additional value of breast dedicated PET imaging and a dedicated FDG-guided biopsy system.

A more elaborate introduction on the use of PET/CT for breast cancer imaging is given in Chapter 1. In the first paragraph, a brief overview is given on the development of PET and PET/CT imaging in clinical oncology and the current role in breast cancer imaging, followed by a more detailed overview on the additional value of PET/CT imaging in stage II/III breast cancer, with special focus on the detection of (additional) lymph node metastases.

Part 1 of this thesis focuses on the additional value of prone position PET/CT for loco-regional staging. Chapter 2 addresses the additional value of PET/CT imaging in prone position for the visualization of the primary tumour and regional lymph node metastases compared to supine position PET/CT imaging. In Chapter 3 we analyse the effect on 3-year survival outcome of discovering additional lymph node metastases by prone position PET/CT.

In part 2 we explore the individual and additional value of FDG PET/CT for response monitoring of the primary tumour and axillary lymph node metastases during PST. Chapter 4 shows the comparison of response monitoring of the primary tumour during PST by MRI alone, PET/CT alone
and by a combination of both modalities. In Chapter 5 the FDG uptake of the primary tumour in the breast and axillary lymph node metastases were compared before and during PST to find the best prediction for a complete response in the final pathology per subgroup.

Part 3 presents the possibilities and consequence for daily clinical practice of introducing dedicated breast PET and PET-guided biopsy. Chapter 6 shows the comparison of MAMMI-PET with supine position PET/CT for visualization of histologically proven primary breast cancer lesions. In chapter 7 we assessed the expected additional radiation dose for the nuclear medicine technologists when MAMMI-PET would be implemented as an addition to the PET/CT protocol. In Chapter 8 the results of the first technical feasibility study of a MAMMI-PET inspired PET-guided biopsy device is presented: the MammoCare. Finally this thesis ends with a summary and future prospects.

Figure 1a: MRI coil for PET/CT imaging in prone position (chest down), the two openings are for the breasts. Figure 1b: a patient positioned on the coil before entering the PET/CT device.

Figure 2: PET/CT device with patient positioned in prone position. The diameter of the acquisition ring of the PET is approximately 85 cm.

Figure 3: The MAMMI-PET acquisition system. The acquisition ring is placed around the affected breast. The diameter of the acquisition ring is 17 centimetres.
Figure 4a: MammoCare system, with on the left the computerized console of the device and (on the right) the biopsy device. Patient is positioned in prone position.

Figure 4b: Top images show a coronal slice with a multifocal $^{18}$F-FDG avid tumour with two small regions of 10 and 12 mm (red arrows) and a sagittal slice showing one region of 31 mm. Below images show a coronal and a sagittal plane of the same heterogeneous tumour, with a total diameter of 94 mm (red dashed line).
References:


Chapter 1
The role of PET/CT for nodal staging in primary stage II/III breast cancer patients

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**Practice points**

- PET/CT upstages patients with stage II/III breast cancer as compared with conventional imaging.

- PET/CT has the highest specificity to differentiate between low N stage (N1) and high N-stage (N2/3) in stage II/III breast cancer patients.

- PET/CT imaging in prone position, with hanging breasts, seems more valuable than supine position PET/CT imaging for loco-regional staging in breast cancer.

- PET/CT imaging can be valuable for response monitoring during preoperative systemic treatment (PST) of axillary lymph node metastases and, in selected cases, also the primary breast cancer lesion.

**Summary**

Modalities that are conventionally used for regional and distant metastases staging, in clinical stage II/III breast cancer patients (ultrasonography, computed tomography [CT], MRI and bone scan), may underestimate the spread of the disease. The 18-fluoro-deoxyglucose whole body PET with CT ($^{18}$F-FDG PET/CT) is able to more accurately visualize the number of axillary lymph node metastases, the internal mammary chain, periclavicular lymph node metastases and distant disease than the previously mentioned modalities. Therefore, PET/CT scanning in stage II/III breast cancer results in a more individualized therapy plan. Additionally, $^{18}$F-FDG PET/CT scanning seems useful to monitor response of pre-operative systemic treatment in axillary lymph node metastases and for the primary tumour in ER+ and triple-negative breast cancer subtypes.

**Keyword**
breast neoplasms, FDG, radiotherapy, staging
Introduction

In The Netherlands, 15,759 women presented with primary breast cancer in the year 2013, of which 48% was stage II/III. At The Netherlands Cancer Institute the percentage of new stage II/III was similar, namely 54% for 482 new cases of breast cancer in the same year. Stage II/III breast cancer patients are patients who have a T2/T3 and/or at least N1 disease. The diagnostic work up for this group of patients consists traditionally of imaging for locoregional extent. The primary lesion is first visualized using radiological techniques as mammography and ultrasonography, and in some centres MRI is also used. Hereafter, guided by ultrasonography, fine-needle aspiration for cytology (FNAC) and core-needle biopsies are performed followed by pathologic examination for confirmation of diagnosis and histologic classification of the breast cancer subtype. The combination of these methods reveals a diagnosis in 90% of the cases.

Breast cancer treatment is a multimodality treatment including surgery, radiation treatment and systemic treatment (chemotherapy, endocrine therapy and targeted therapy). The sequence of treatment is changing during the last decade. Preoperative systemic treatment (PST), also called neo-adjuvant therapy (chemotherapy, endocrine therapy or targeted therapy), used to be given only to breast cancer patients with locally advanced breast cancer (LABC). Patients with LABC have stage T3/4 and/or N2/3 disease. PST may shrink the tumour volume converting the surgery plan from mastectomy to breast converting therapy, allowing up to 40% of patients to receive breast-conserving therapy. Although current consensus does not favor PST over adjuvant chemotherapy a few recent studies have reported that PST improves survival compared with postoperative systemic treatment, PST is also increasingly being used in clinical practice for earlier-stage breast cancer (T2/T3 and/or at least N1 or stage II/III). Furthermore, initially tumour-positive (axillary) lymph nodes can be converted to ypN0 in 30–45% of the cases, possibly obviating the need for axillary lymph node dissection (ALND) or axillary radiotherapy (RT) in this subgroup of patients. Depending on tumour subtype, a pathologic complete response (pCR) rate of 40–50% can be achieved. Early-response monitoring is therefore desired to adapt the chemotherapy regimen at an earlier stage to further improve remission rates.

In proven node-positive stage II or in stage III breast cancer it is advised to also screen for distant metastases when primary systemic treatment is given. Traditionally, distant metastases screening in breast cancer patients is performed by abdominal ultrasound (liver), bone-scan (bones) and/or chest x-ray (lungs). It has also been described that conventional imaging underestimates the overt lymph node spread and distant disease.

For regional and distant staging in stage II/III breast cancer, as well as for response monitoring, $^{18}$F-FDG PET/CT can be of additional value when compared with conventional imaging. This paper will explain to what extent PET/CT scanning may improve staging in stage II/III breast cancer.
cancer, with respect to regional lymph node metastasis and distant disease; and where PET/CT scanning could be of help for response monitoring, either of the primary tumour or for regional metastases. Furthermore, we will elaborate on technical adjustments of PET/CT scanning (particularly positioning in hanging breast position), whether this may have impact on diagnostic yield of PET/CT for nodal staging and have clinical consequences for both staging and response monitoring in stage II/III breast cancer.

PET/CT & its current role in breast cancer imaging

PET is an imaging modality that is able to differentiate between pathologic and physiologic activity in organs and tissues using biologically active compounds, substrates, ligands or drugs labeled with so-called ‘positron emitters’ or radionuclides such as Fluorine-18 (¹⁸F). Depending on the tracer used, the PET acquisitions give an overview of the glucose metabolism, amino acid transport, protein metabolism, receptor status, oxygen consumption or even cell division. PET is mostly used in oncology since ¹⁸-fluoro-deoxy-glucose (¹⁸F-FDG or FDG) was introduced as a tracer¹⁶. FDG is a glucose analogue labeled with radioactive fluorine (¹⁸F), a positron emitter with a half-life of 110 min. The positrons annihilate in tissue with electrons and subsequently two photons travel 180° in the opposite direction that are then captured by the PET scanner and reconstructed into an overview of its distribution. The increased uptake of glucose, and thus FDG, in malignancies¹⁷ is due to an increased expression of the glucose transporter molecules at the tumour cell surface compared with normal cells and related to an increased glucose metabolism¹⁶. The intensity of FDG uptake can be quantified and expressed as ‘maximum standardized uptake value’ (SUVmax), which can best be described as the radioactivity concentration per 2D or 3D region of interest (ROI).

The first experiences with PET as a standalone technique showed that it was difficult to localize the lesions without adequate anatomical information. Therefore it was inevitable to add a modality to provide anatomical information: the CT scan. After the first PET/CT prototype was introduced in 1998, the first PET/CT scanner became commercially available in 2001¹⁸. Initially, PET/CT was introduced for staging NSCLC and characterization of solitary pulmonary nodules, but the number of indications rapidly increased over time. Since its introduction, however, PET/CT has not been introduced as a modality for primary breast cancer imaging and lesion analysis because of the low sensitivity for small lesions. It was already described by Avril et al. that PET without CT misses 40% of the primary tumours with a diameter smaller than 1 cm¹⁹. After the addition of the CT scan, the sensitivity for detecting primary breast cancer lesions did not improve sufficiently (87–97%)²⁰–²² to replace MRI for T-staging with its sensitivity for the detection of the primary tumour approximating to 100%²⁰,²³.
Although not sufficiently explored, the FDG uptake of the primary lesion on PET/CT seems to correlate positively with parameters relating to higher tumour activity and more aggressive breast cancer lesions. There was a relation found between a higher SUVmax and a higher histological grade, negative oestrogen receptor or progesterone receptor status, or triple-negative subtype (p < 0.001)\textsuperscript{14,24,25}. Subsequently, as metastatic lesions are known to be generally more proliferative than the primary tumour, the first indication for PET/CT in breast cancer was for the assessment of distant metastases in high-risk patients. In several studies it was demonstrated that PET/CT shows a higher number of distant metastases when compared with conventional imaging, including abdominal ultrasound (liver), bone-scan (bones) and/or chest x-ray (lungs) in ‘at-risk’ patient groups (stage II/III and LABC)\textsuperscript{7,12–15}. One of the first major studies, including 60 patients before starting PST, demonstrated an overall sensitivity and specificity of PET/CT of 100 and 98%, respectively; compared with 60 and 83%, respectively, for conventional imaging\textsuperscript{12}. In 2012 Koolen et al. after including 154 patients with stage II/III breast cancer found that PET/CT found 42 additional distant lesions as compared with conventional imaging. Specifically, the bone scintigraphy and the thorax x-ray tests missed lesions that were detected on PET/CT. In this series PET/CT had a sensitivity and specificity of 100 and 96%, respectively \textsuperscript{14} for the detection of distant metastases. As a direct consequence of this article by Koolen et al. and other recent articles on the detection of distant metastases with PET/CT was a new recommendation in the Dutch guidelines for breast cancer diagnostics and treatment, stating that FDG-PET-CT can replace conventional staging methods for distant metastasis screening and is, therefore, advised for stage III and can be considered in stage II primary breast cancer\textsuperscript{8}.

Upstaging patients from M0 to M1, and subsequent stage IV, has a major impact on both prognosis and treatment, hence there are two important proposals regarding classifying patients as having distant metastases. First of all, distant metastases detected on PET/CT must be confirmed preferably with histopathology, or at least with additional imaging procedures, before considering changes to the treatment plan. Secondly, the number of distant metastases is highly important for treatment planning. It has been described that a limited number of metastatic lesions (maximum 3 or 5), so-called ‘oligometastatic lesions’\textsuperscript{26} can be treated with curative intent\textsuperscript{27–29}. At The Netherlands Cancer Institute the presence of a maximum of three metastatic lesions are considered as being ‘oligometastatic disease’ and the presence of more than three distant metastases are regarded as polymetastatic and are treated in a palliative setting. As PET/CT has been shown to find a higher number of metastatic lesions than conventional imaging it is therefore better able to differentiate between oligometastatic disease and metastases requiring palliative treatment.

A relatively new indication in which \textsuperscript{18}F-FDG PET/CT can also be of additional value in stage II/III breast cancer patient is for lymph node staging\textsuperscript{30}. Increased FDG uptake seen on the PET scan
corresponding with a lymph node fulfilling RECIST criteria\textsuperscript{31} on the CT and therefore fulfilling the criteria of being malignant, is easy to classify. However, slightly increased FDG uptake corresponding with a small lymph node can be difficult to classify and to properly localize. Indeed, a focus on PET without clear substrate on CT can be regarded as false positive in case of noise or uptake in brown fat tissue. However, true-positive uptake in a small lymph node can be missed when the SUVmax of the corresponding lymph node is leveled down on PET due to partial volume effect\textsuperscript{32}. A partial volume effect arises in volumetric images when more than one tissue type overlaps in one voxel (a voxel is the 3D correlate of a pixel\textsuperscript{33}) and, consequently, the SUVmax of the overlapping tissues is averaged in the image.

**Lymph node staging in stage II/III breast cancer: current practice**

A patient suspected to have breast cancer is advised to be screened for lymph node metastases in the axilla with ultrasonography, followed by ultrasonography-guided biopsy when a suspect lymph node is identified\textsuperscript{8}. Lymph nodes are considered suspect when a cortex of at least 2.3 mm thickness is observed or when the cortex is asymmetrical\textsuperscript{8}. In these cases FNAC and core biopsies of the node are advised according to the Dutch Guidelines\textsuperscript{8} and other guidelines. It has been demonstrated that ultrasonography can discriminate between suspect and non-suspect nodes with an accuracy of 67.9\%\textsuperscript{34}. In addition, using selective ultrasonography-guided needle biopsy of the axilla, 50\% of patients with axillary LN metastases can be identified prior to surgery with a false-negative rate of approximately 25\%\textsuperscript{35}. In literature, sensitivity between 50 and 80\% and specificity for ultrasonography-guided FNAC of approximately 100\%, have been reported\textsuperscript{36-38}.

When metastases are not detected with ultrasonography and FNAC is negative, a sentinel lymph node biopsy (SNB) can be performed. For SNB, preoperative lymphoscintigraphy is used to identify the lymph node(s) directly connected to the tumour (sentinel nodes) by using radiolabeled nanocolloids\textsuperscript{39}. In the operating room these radioactive lymph nodes can be detected with a probe and resected for pathological examination. In many hospitals the procedure is combined with the use of vital dyes to refine the sentinel lymph node identification by visualizing lymphatic vessels. Following the SNB, the tumour load in the resected lymph nodes can be assessed (by the pathologist); a differentiation can be made between no metastasis, isolated tumour cells (<0.2 mm), micrometastasis (0.2–2 mm) and macro metastases (>2 mm)\textsuperscript{40}. In recent years various experimental methods have been introduced as an alternative to the use of radiotracers and vital dyes. These techniques concern use of fluorescent agents\textsuperscript{41-43}, magnetic nanoparticles\textsuperscript{43} and hybrid tracers combining radioactivity and fluorescence in one signature\textsuperscript{44}. A promising new method to find the sentinel node is microbubble-guided ultrasonography\textsuperscript{45}.
SNB can be performed in clinically node-negative breast cancer patients before or after PST, with both advantages and disadvantages. The advantage of SNB after PST is a one surgical procedure together with excision of the primary tumour and the possibility to refrain from axillary treatment once a pCR is reached in the lymph nodes. The tumour load in the axilla after PST seems to be of more importance as a significant decrease in tumour volume implies less aggressive further treatment of the axilla. Disadvantages are the relatively low identification rates of approximately 85% and the higher FN rate of 11–15%. A similar approach, but with lymph node metastases present before PST, is the recently described Marking the Axillary lymph node with a Radioactive Iodine seed procedure (MARI). A lymph node containing metastasis is marked with an iodine-125-radiolabeled (125I) marker (or Iodine seed) before PST. The response can be analysed after PST by localizing and subsequent resection of the marked lymph node, with a false-negative rate of 7%. We prefer to apply this method for clinically node-positive patients as a significant decrease in tumour volume may result in a less aggressive further treatment of the axilla.

MRI has not yet been found to be sensitive enough for staging of the axilla in breast cancer patients. The type III washout curves of malignant axillary lymph node metastases show too much overlap with lymph nodes that do not contain metastases. Krammer et al. evaluated the capability of dynamic contrast enhanced MRI for the interpretation of axillary lymph nodes. In the study-group of 20 breast cancer patients, 30 lymph node metastases were evaluated. Of these 30 lymph node metastases 98% had a type III washout curve on MRI in comparison with 66% in the control group where 29 non-metastatic lymph nodes had been evaluated (p > 0.05).

To identify lymph node metastases, and mark them before the start of PST, ultrasonography guided biopsy is commonly used, but due to a low specificity for the identification of axillary lymph node metastases better techniques are required. In the subsequent paragraphs, the possible role of FDG-PET/CT for the detection of the lymph nodes harboring metastasis is discussed.

**Axillary lymph node staging in stage II/III breast cancer with PET/CT**

Only in stage II/III breast cancer is it advised to stage the axilla for lymph node metastasis with other modalities in addition to ultrasonography, in case of nonvisualization of metastases. In stage I breast cancer, the reported prevalence of lymph node metastases is between 5 and 28% and for carcinoma in situ (Tis) this prevalence is as low as 0.8%. Therefore, standard supine PET/CT is not routinely applied in these subgroups because of high costs and low yield. There are studies, however, that have included these low-risk breast cancer stages when screening for lymph node metastases. These studies showed a sensitivity for nodal staging with supine PET/CT between 20 and 77% and a specificity between 58 and 100%. More recently, Koolen
et al. showed a sensitivity and specificity of 73 and 100%, respectively, for the detection of axillary metastases in strictly T1 patients (n = 62) including PET/CT imaging in prone position\textsuperscript{59}. For prone position PET/CT imaging a stripped mock-up MRI coil (see Figure 1) can be used. Prone position PET/CT acquisition, as the patient is resting face down, also reduces the risk on mismatch between PET and CT images, due to the motion of breathing\textsuperscript{60} \[60\], which can be an explanation for the increase in sensitivity compared with supine PET/CT imaging.

Until recently, the sensitivity of supine PET/CT has also been described not to be generally better than conventional imaging for detecting axillary lymph node metastases in stage II/III breast cancer patients. PET/CT had a similar sensitivity to detect axillary lymph node metastases as ultrasonography, between 60 and 80%, both higher than the sensitivity of contrast-enhanced CT (33%)\textsuperscript{36–38,54,59}. After prone position PET/CT imaging had been introduced, the sensitivity increased from 70\textsuperscript{12} to 82\%\textsuperscript{10}. Koolen et al. (2012) included 311 breast cancer patients scheduled for PST and they all underwent PET/CT in prone position. The results were compared with pathology obtained by FNAC or SNB prior to PST. The sensitivity of PET/CT to detect axillary lymph node metastases was 82\% in this series (positive predictive value [PPV] 98\%). Of 28 patients with questionable axillary FDG uptake 82\% were histologically tumour positive\textsuperscript{10}.

In stage II/III breast cancer patients the negative predictive value of either prone or supine PET/CT for axillary lymph node staging is below 80\% (53–79\%)\textsuperscript{12,21,55,56,59,61}. Therefore, when PET/CT does not show any uptake in the axilla, the axilla cannot be considered to be free of metastases. For this reason, post-PST SNB is advised. Post-PST SNB has the best-possible sensitivity, can be performed during excision of the primary tumour and takes into account the response to PST. In contrast to the described negative predictive value, PET/CT is described to have a specificity approximating to 100\% for the detection of axillary lymph node metastases\textsuperscript{10,62,63}. This implies that lesions found to be highly suspicious on PET/CT have to be confirmed by histological examination. Currently, at The Netherlands Cancer Institute, prone position PET/CT acquisition is performed for regional staging in addition to the ultrasonography (with biopsy) of the axilla in patients scheduled for PST.

As described before, the presence of axillary lymph node metastases at time of diagnosis is of particular importance for the prescription of PST. However, as patients with stage N2/3 will receive additional treatment to the axilla and extra-axillary regions with RT, it is necessary to have to knowledge of all lymph node basins that are at risk at initial stage. PET/CT covers all regional lymph node basins, including the mediastinum, internal mammary chain (IMN) and the neck. In the next paragraph, the value of PET/CT in assessing the lymph node status of other basins then the axilla is described.

At The Netherlands Cancer Institute, we have adapted the following policy to manage the axilla in patients who will undergo PST: we perform screening with ultrasonography of the regional
Figure 1: Patient with breast cancer positioned on a stripped MRI coil to enable prone position PET/computed tomography imaging with breasts hanging free.

Figure 2: Fused PET/computed tomography images in coronal view of three different patients with proven breast cancer and lymphogenous metastases. Image (A) shows FDG active lymph nodes smaller than 1 cm in the axilla and in one supraclavicular lymph node. Images (B) and (C) show the possibility that PET/CT gives to count the suspect lymph nodes to decide whether or not to add radiotherapy to the treatment. On image (B) three suspect lymph nodes can be discriminated and on image (C) four (or five) suspect lymph nodes can be discriminated.
lymph node basins, followed by FNAC when a suspect lymph node is located. We perform a whole-body PET/CT in all patients for regional lymph node screening and for distant metastases screening. When the PET/CT images show clear evidence for lymph node metastases we do a targeted ultrasonography with FNAC to prove the existence of malignancy in the lymph node. In case of proven lymph node metastases, either detected by ultrasonography or PET/CT, a MARI procedure\textsuperscript{48,49} is performed. When neither ultrasonography nor PET/CT discovered lymph node metastases, we perform a post-PST SNB\textsuperscript{46,47,64,65}.

**Upstaging N-stage by PET/CT & its effect on the therapy plan**

As a direct consequence of whole body imaging with PET/CT in supine position, all regional lymph node basins where breast cancer lesions metastasize can be assessed properly by this technique\textsuperscript{63}. Adequate staging is required as it may result in a change of the RT plan\textsuperscript{9–11}. Irradiation of regional nodes is currently advised in patients at high risk for locoregional recurrence. The patients classified as high-risk have ≥4 tumour-positive axillary nodes, involvement of nodes in the IMN or periclavicular area, T4 tumours, or T3N1 tumours\textsuperscript{66}. Although there is no universal agreement on the treatment of intramammary chain nodes, they are considered to be extra-axillary lymph node metastases.

The role of PET/CT for visualizing extra-axillary lymph node metastases has not yet been studied extensively. In a study by Aukema et al., 60 patients were scheduled to receive PST and were additionally staged with prone position PET/CT. Conventional imaging in this series consisted of ultrasonography and FNAC of the ipsilateral axilla, infra- and supraclavicular region and, if indicated, based on physical examination or MRI, also of additional regions. In this study, PET/CT visualized extra-axillary lymph node metastases in 17 patients (28%) compared with seven by detected by ultrasonography-guided FNAC. Consequently the RT plan was changed in seven (12%) patients due to upstaging with PET/CT\textsuperscript{11}. In 2012, Berndsford et al. (n = 103) described that PET/CT found new extra-axillary malignancy in 15 patients (15%), which lead to an upgrade of initial staging in 14% and ultimately a modification of planned treatment in 8% of the patients\textsuperscript{9}. In their study, similar inclusion criteria were applied, but PET/CT was performed in supine position. In the same year, Koolen et al. included 311 patients scheduled to receive PST who also underwent PET/CT in prone position. In this series PET/CT found 58 new suspect N3-nodes (26 IMNs, 32 periclavicular nodes) that were not detected by other techniques changing regional RT planning in 16% of the patients\textsuperscript{10}.

Considering the upstaging by PET/CT as compared with conventional imaging, PET/CT so far appears to be the most adequate method to visualize all present lymph node metastases in stage
PET/CT for nodal staging in primary stage II/III breast cancer patients before start of PST (see Figure 2), and therefore place the patient in the adequate nodal stage. More studies are required to confirm this role of PET/CT, but initial results are highly promising.

Response monitoring

MRI is still preferred as technique of choice for response monitoring of systemic treatment of the primary tumour in breast cancer patients. Since PET/CT has a sensitivity of 87–97% for the detection of primary tumours it cannot compete with the sensitivity of MRI that approximates the 100%, especially in tumours that are less proliferative or aggressive. By contrast, MRI is not the modality of choice for the identification of axillary lymph node metastasis; due to the limited scanning range it has a PPV of 69–75%. Furthermore, MRI is also found to be inaccurate for the response monitoring in ER+ breast cancer subtypes. The question can be put forward, whether subgroups of patients can be identified in which PET/CT can replace MRI for therapy monitoring.

The number of studies on the value of PET/CT alone for response monitoring of axillary lymph nodes is limited. In a study by Koolen et al. where the early response to PST in the axilla was assessed in 80 stage II/III breast cancer patients, PET/CT performed in prone position showed to have a specificity of 95% and a PPV of 86% to accurately identify histologic pCR. This is in line with a study by Rousseau et al. in 2011 who included 52 stage II/III breast cancer patients and found a specificity and PPV of 75 and 95%, respectively, while performing PET/CT in supine position.

The value of response monitoring of the primary lesion with PET/CT depends on the subtype of the breast tumour. It has been described that primary lesions with the HER2+ subtype do not show a correlation between its decrease in FDG uptake on PET/CT and a favourable response after final pathology. In another study by Koolen et al., who assessed the response to PST in 98 patients with stage II/III breast cancer, it was shown that the area under the ROC curve was 0.35 (0.12–0.64) for HER2+ compared with 0.90 (0.76–1.00) for ER-positive/HER2-negative and 0.96 (0.86–1.00) for triple-negative tumours. The HER2+ subtype tumours show an increase in SUVmax during PST where MRI shows a good response. PET/CT can therefore not be used yet for the response monitoring in this subgroup, which has been reported to account for 10–15% of newly diagnosed breast cancer. Consequently, when the histology of the primary lesion is known, one could make a choice between MRI and PET/CT based on the subtype. For this purpose it should be taken into account that PET/CT might have an added value when performed...
in prone position as the high-resolution acquisition of the thorax can be made within 10 min\textsuperscript{60}, while covering all regional lymph node basins of interest.

Whether PET/CT can be of added value to the value of the MRI concerning response monitoring is still under investigation. A first illustration of how response monitoring during PST by either MRI or PET/CT depends on breast cancer subtypes, was recently presented. Pengel et al. included 93 patients scheduled to receive PST while response monitoring of the primary lesion was performed with PET/CT and MRI. They found an AUC for the relative reduction in SUVmax on PET/CT to be 0.78 (95\% CI: 0.68–0.88), and for the relative reduction in tumour diameter at late enhancement on MRI to be 0.79 (95\% CI: 0.70–0.89). When combining the information from PET/CT and MRI while taking into account the breast cancer subtype, the AUC was 0.90 (95\% CI:0.83–0.96)\textsuperscript{73}. There is not enough evidence available yet on the performance of PET/CT compared with MRI for response monitoring during PST taking into account the breast cancer subtypes. More studies including larger patient numbers are required to establish a recommendation.

**Conclusion & future perspective**

In this overview, we describe that PET/CT, after being preferred for identification of distant dissemination in lungs, bones and liver in comparison to conventional imaging, also has a better overall sensitivity for lymph node staging in stage II/III breast cancer patients compared with conventional imaging. Furthermore, it can be successfully used for response monitoring during PST to predict pCR of lymph node metastases and, in triple-negative and ER+/HER2- breast cancer subtypes, also for the primary tumour. For both axillary staging and response monitoring there seems to be an advantage in introducing prone position PET/CT imaging.

The data presented in this review indicate that PET/CT improves the detection of regional lymph node metastases before start of PST, resulting in an upstaging and consequently adjustment of the treatment plan. However, as discussed earlier, the actual treatment of the axilla continues to be debated. In early breast cancer and where there is limited risk of nodal involvement, ALND does not improve survival and can therefore be omitted, as was presented by the ACOSOG Z11 trial\textsuperscript{74} or treated with RT (AMAROS)\textsuperscript{75}. Without applying ALND in these cases morbidity can thus be significantly reduced without compromising either regional control or survival. Such an ‘axillary sparing’ strategy could also be employed in the higher risk patients, such as those with larger tumours and proven axillary macro-metastases; they could be candidates for PST, if they respond well to systemic treatment.

We have explored venues over which this goal can be reached: when the PET/CT (and ultrasonography) shows no lymph node metastases, the patient starts with PST and we perform
PET/CT for nodal staging in primary stage II/III breast cancer

a post-PST SNB. When the PET/CT does find axillary lymph node metastases we perform a MARI procedure\textsuperscript{48,49} by marking the most prominent lymph node with an Iodine seed. If at final pathology the SNB is positive or the MARI lymph node shows incomplete response we opt for ALND or RT of the axilla depending on the staging by PET/CT before start of PST. It can therefore be concluded that PET/CT is preferred in stage II/III breast cancer for regional and distant staging. For response monitoring PET/CT can be of value for the regional lymph nodes and for the primary tumour in ER+/HER2- and triple-negative subtypes. For the response monitoring of the primary tumour in HER2+ breast cancer subtype, PET/CT is not preferred due to the inadequate correlation with the achievement of pCR.

As a future perspective we would like to present the current development of more specific PET/CT tracers that, linked to very specific agents, are able to target a certain type of tumour. In case of a HER2+ breast cancer lesion these tracer target the HER2 receptor. Some examples for the visualization of HER2+ breast cancer are trastuzumab (Herceptin\textsuperscript{®} [Roche Nederland BV, Woerden, The Netherlands]) and Bevacizumab (Avastin\textsuperscript{®} [Roche Nederland BV, Woerden, The Netherlands]). These two antibodies can be linked to various radionuclides\textsuperscript{76} and have been described to successfully visualize HER2+ breast cancer lesions\textsuperscript{76–78}. HER2+ lesions can also be visualized using anti-HER2 affibodies. These are three-helical scaffold proteins based on a modified B-domain of a staphylococcal protein A, and can be linked to Gallium (68Ga)\textsuperscript{79,80}. These possible PET/CT tracers are not yet used as standard in clinical practice but show potential for the visualization of specific tumour types.

Together with the promising results in recent literature mentioned in this review, and these new developments of possible tracers, PET/CT can continue to compete in the specialized field of diagnostic tools used in stage II/III breast cancer imaging before and during PST.

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Part I
PET/CT: Improved baseline visualization
Chapter 2
Additional prone $^{18}$F-FDG PET/CT acquisition to improve the visualization of the primary tumour and regional lymph node metastases in stage II/III breast cancer.

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Abstract

Purpose: To prospectively compare prone and supine acquired $^{18}$F-FDG PET/CT for visualization of primary tumours and regional lymph nodes in stage II/III breast cancer patients.

Materials and Methods: One hundred ninety-eight patients were included consecutively from August 2010 to April 2012. One hour after administration of 180-240 MBq $^{18}$F-FDG PET/CT, PET/CT images of the thorax were firstly acquired in prone position. Subsequently, a standard PET/CT in supine position from skull base to thighs was made. Both sets of images were tested in a univariate and a multivariate analysis for the number of lesions per breast or lymph node (LN) region and anatomical mismatch between PET and CT images.

Results: Images in prone position showed less compression of breast tissue, more primary tumour (PT) multifocality ($P < 0.001$) and more avid axillary LNs ($P < 0.001$) compared with supine position. Anatomical mismatch of the axillary LN metastases was found more often on supine PET/CT images compared with prone ($P = 0.004$). Prone images showed a smaller PT functional volume compared with supine position ($P < 0.001$).

Conclusions: Prone position PET/CT improved the visualization of PT multifocality and the number of detected axillary lymph nodes. Therefore, it is a valuable addition to standard supine PET/CT in the protocol for locoregional assessment in stage II/III breast cancer patients.

Key Words: PET/CT, breast neoplasms, $^{18}$F-FDG, staging, prone imaging
Introduction

In locally advanced breast cancer, it has recently been reported that preoperative systemic treatment (PST), also known as neoadjuvant chemotherapy\(^1\) improves survival compared with postoperative systemic treatment\(^2\). Therefore, PST is increasingly being used in clinical practice for stage II/III.

Before the planning of PST, the classification of the tumour type is similarly important to the baseline staging of the disease, locally and at distance. Suspect primary breast cancer lesions are traditionally evaluated on imaging modalities to detect anatomical disruption (mammography and ultrasound) or causing changes in blood perfusion (magnetic resonance imaging [MRI])\(^3\). The MRI is the golden standard for the planning of the operative treatment, as it has a sensitivity of 93\% for detecting multicentric disease\(^4\) and is therefore important to rightfully convert the surgery plan from breast conserving surgery to mastectomy if more than one cancer lesions are present in the breast\(^5\). The staging of locoregional LNs is achieved using ultrasound guided biopsy or, in case of a negative ultrasound or biopsy, the sentinel node procedure\(^3,6\).

For years, abdominal ultrasonography, chest radiography, and a bone scan were used for distant metastases screening. Recently, \(^{18}\)F-FDG PET/CT in supine position was introduced as a possible complementing diagnostic tool for this purpose\(^7-9\) as PET/CT has an excellent sensitivity (100\%) and a high specificity (96\%–98\%) to find distant metastases compared with conventional modalities\(^10,11\). In our hospital, \(^{18}\)F-FDG PET/CT in supine position is used as a staging procedure for distant metastases and to supplement locoregional imaging of the primary tumour (PT) and LNs for accurate curative treatment planning (PST, operation and radiotherapy). However, standard \(^{18}\)F-FDG PET/CT may result in less optimal PT visualization because of gravity-related tissue compression and breathing artifacts\(^12\). Partially, because of these issues, MRI is currently performed in prone position for locoregional detection and response monitoring of breast cancer\(^13\).

To enable direct comparison with MRI before and during PST, prone positioning with hanging breasts was introduced for PET/CT using a mock-up coil similar to MRI (Fig. 1). Koolen et al showed that this prone position PET/CT was able to identify up to 95\% of all tumours in patients scheduled to receive PST\(^14\) and most T1 breast cancer lesions, even when smaller than 10 mm\(^15\). Furthermore, combining prone and standard supine \(^{18}\)F-FDG PET/CT, locoregional LN metastases were detected with a sensitivity of 82\% and a specificity of 92\%, changing the radiotherapy indication in a substantial proportion of patients based on detection of $\geq 4$ FDG-avid axillary LN or detection of occult N3 nodes\(^16-18\).

We have previously shown the feasibility of PET/CT in prone position as an additional tool to standard supine PET/CT\(^12\). In line with these previous findings, a more recent study by Abramson
et al\textsuperscript{19} showed in 24 patients that prone position PET/CT found statistically significant more axillary LN metastases than standard supine position PET/CT. The objective of the present study was the comparison between standard \(^{18}\)F-FDG PET/CT in supine position and PET/CT in prone position for visualization of FDG-avid primary tumours and regional lymph nodes in stage II/III breast cancer patients.

**Materials and methods**

**Patient Inclusion**

From August 2010 to April 2012, all stage II/III breast cancer planned to receive PST were asked to undergo prone and supine position \(^{18}\)F-FDG PET/CT acquisitions after giving informed consent. We analysed all \(^{18}\)F-FDG PET/CT baseline acquisitions.

**PET/CT**

As a preparation for the PET/CT acquisitions, patients were required to have fasted during 6 hours before \(^{18}\)F-FDG administration, and blood glucose levels were required to be lower than 10 mmol/l. To reduce uptake of \(^{18}\)F-FDG by brown fat, 10 mg diazepam was administered orally\textsuperscript{20}. Patients received a single dose of 180 to 240 MBq (4.9–6.5 mCi) of \(^{18}\)F-FDG intravenously, depending on their body mass index.

After a resting period of 60±10 minutes, PET/CT studies were acquired using a whole body PET/CT scanner (Gemini TF, Philips, Cleveland, OH). First, a PET/CT of the thorax was performed in prone position using a mock-up coil for hanging breast imaging (Fig. 1)\textsuperscript{12}. The acquisition of the PET (3.00 minutes per bed position and 2x2x2 mm voxel reconstruction) was complemented with a low-dose CT acquisition without contrast for attenuation correction and anatomical localization.

Subsequently, a whole body PET/CT scan was performed in supine position (1.30 minutes per bed position) with a low-dose CT scan (5 mm CT slices) from the base of the skull to the upper half of both femora. From July 2011, the standard 5 mm CT slice reconstruction of the supine position scans was complemented with a 2-mm CT slice reconstruction, and for all the patients included after this adjustment, we used the new CT reconstruction to compare with the 2 mm prone position PET/CT scans. The administered activity, time of administration, and patient’s body weight were used for additional calculation of maximum standardized uptake values (SUVmax).

**Image Reading**

All images acquired in prone or supine position were analysed with both CT and PET images, separated and fused, using orthogonal multiplanar reconstruction. The number of separate
FDG-avid lesions in the breast and regional lymph node stations were visually assessed after anatomical localization. The $^{18}$F-FDG uptake was measured qualitatively in the primary breast lesion(s) and locoregional nodes using a 4-degree scoring system$^{17}$: (0) similar to surrounding breast tissue or lymphatic tissue, (1) slightly more than surrounding structures, (2) moderately intense, and (3) very intense. Furthermore, we looked at anatomical mismatch, graded as present when there was less than 50% overlap of the suspect lesion between the PET and the CT image. Additionally, the most FDG-avid PT1 and second most FDG-avid breast lesion (PT2), and the most FDG-avid locoregional lymph node in the axilla (LNA) and/or outside the axilla (LNO) were analysed semiquantitatively using the SUVmax derived from a 3D growing region, separating the tumour from the surrounding nonpathologic tissue. The lower threshold to calculate the standardized functional volume using the iso50-method (VOI50)$^{21}$ was 50% of the SUVmax of the particular lesion. For

The multivariate analysis, the same variables were used as for the univariate analysis, only adding scanning position (=prone or supine).

**Statistical Analysis**

Both univariate and multivariate analyses were performed using R (version 3.0.2.).

**Univariate Analyses**

For the univariate analyses, we used the distance from PT to pectoral muscle, tumour volume, breast volume, and age as testing variables for the visibility of the PT, as well as age as testing variable or the visualization of LN metastases. We compared the prone with the supine imaging scans using the McNemar’s exact test for binominal variables (e.g., anatomical mismatch), the signed rank test for continuous variables (e.g., SUVmax), and the Stuart-Maxwell test for multinomial variables (e.g., number of LNs). A $P < 0.05$ was found to be significant for the univariate analysis.

**Multivariate Analysis**

The scanning position (prone vs supine) and the SmmCT were added as a testing variable for the anatomical mismatch. Logistic regression was used for binominal variables (e.g., anatomical mismatch). Continuous variables (e.g., SUVmax) were tested using a linear regression and multinomial variables (e.g., number of LN) were tested using a Poisson regression. We looked at the visualization (for LNs) or visualization score (PTs), number, SUVmax, and anatomical mismatch of PTs and LNs, which results in 6 separate analyses. A $P < 0.008$ was found to be significant after using the Bonferroni stratification for the number of multivariate tests ($0.05/6 = 0.008$).
Results

A total of 198 patients (mean age, 50 years; range, 26–82) were included between August 2010 and April 2012. Consequently, both prone and supine images of 201 breasts with at least one pathologically proven primary breast cancer lesion were evaluated, including 100 images of which the supine PET images were fused with the 5 mm supine CT slices and 101 of which the supine PET images were fused with the additional 2 mm CT slice reconstruction. Table 1 shows the baseline characteristics of the patients.

Univariate Analysis

Prone position PET/CT images showed more often a higher number of PT (P < 0.001) and a higher SUVmax of the tumour (P < 0.001) and LNs (P < 0.001), more multifocality as illustrated in Figure 2 (58 vs 40, P < 0.001), and a higher number of axillary LNs (IQR, 2–5.5 vs 1–4, P < 0.001) compared with the supine position images. There was no difference in the amount of extra-axillary LNs visible on PET/CT (P = 0.153). Of all FDG-avid PTs on the prone position scan, 97.5% (n = 195) was classifiable as highly suspicious (visual score 2) comparing with 91.5% (n = 184) of the PT visible on the supine position scans (P < 0.001). The VOI50 measured of PT1, axillary LNs, and extra-axillary LNs was significantly greater (P < 0.001) on the supine position scans. In the 2 mm CT supine versus 2 mm prone group, the anatomical mismatch for the primary
tumour, axillary, and nonaxillary lymph nodes, there was no significant difference (P = 1). In the 5 mm supine versus 2 mm prone group, the mismatch of the primary tumour was not significantly different (P = 1), but the anatomical mismatch for the axillary lymph node metastases (P = 0.052) and the nonaxillary lymph nodes (P = 0.045) were almost significant.

**Multivariate Analysis**

Prone position positively influenced the visualization of multifocality (P < 0.001), as shown in Table 2. The SUVmax of PT1 was not influenced by the scanning position (P = 0.02) including the different scanning protocol and reconstruction for both scanning position but by a greater tumour volume (P = 0.001) and increased breast volume (P < 0.001). As shown in Table 3, prone position PET/CT imaging visualized a higher total number of LNs (P < 0.001) and of axillary LNs (P < 0.001), but not of extra axillary LNs separately (P = 0.37). Prone or supine position did not significantly influence the degree of 18F-FDG uptake (visualization score). The standard 5 mm CT slice reconstruction of the standard supine PET/CT was the only factor causing an increase in anatomical mismatch between PET and CT for axillary LNs (P = 0.004), as demonstrated in Figures 3 and 4.

**Table 1: Description of patient population**

<table>
<thead>
<tr>
<th>Baseline characteristics of 198 patients (201 scans)</th>
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<td>Age in years</td>
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<tr>
<td>Unknown 1 (0%)</td>
<td></td>
</tr>
<tr>
<td>cN</td>
<td></td>
</tr>
<tr>
<td>0 59 (29%)</td>
<td></td>
</tr>
<tr>
<td>1 93 (46%)</td>
<td></td>
</tr>
<tr>
<td>2 10 (5%)</td>
<td></td>
</tr>
<tr>
<td>3 38 (19%)</td>
<td></td>
</tr>
<tr>
<td>Unknown 1 (0%)</td>
<td></td>
</tr>
<tr>
<td>Sentinel Node</td>
<td></td>
</tr>
<tr>
<td>No 121 (60%)</td>
<td></td>
</tr>
<tr>
<td>Yes, before PET/CT 52 (26%)</td>
<td></td>
</tr>
<tr>
<td>Yes, after PET/CT 28 (14%)</td>
<td></td>
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</tbody>
</table>

n=number of breast and lymph node region analysed, 3 patients had bilateral tumours. Data are presented as number (percentage), unless otherwise specified. cT incitates tumour stage; cN, lymph node stage.
Table 2: Multivariate analyses

<table>
<thead>
<tr>
<th></th>
<th>Tumour focality P</th>
<th>Score (0-3) P</th>
<th>SUVmax P</th>
<th>AM P</th>
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<tbody>
<tr>
<td>PT1:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine vs prone</td>
<td>&lt;0.001</td>
<td>0.024</td>
<td>0.02</td>
<td>0.441</td>
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<tr>
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<td>0.672</td>
<td>0.897</td>
<td>0.235</td>
<td>0.113</td>
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<tr>
<td>PT1 volume</td>
<td>0.002</td>
<td>0.391</td>
<td>0.001</td>
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</tr>
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<td>Breast volume</td>
<td>0.156</td>
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</tr>
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<td>0.445</td>
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<td>0.025</td>
<td>0.586</td>
</tr>
<tr>
<td>PT2:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine vs prone</td>
<td>X</td>
<td>0.324</td>
<td>0.022</td>
<td>0.021</td>
</tr>
<tr>
<td>Distance to pectoral muscle</td>
<td>X</td>
<td>0.127</td>
<td>0.526</td>
<td>0.185</td>
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<tr>
<td>PT1 volume</td>
<td>X</td>
<td>0.067</td>
<td>0.357</td>
<td>0.138</td>
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<tr>
<td>Breast volume</td>
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<td>0.012</td>
<td>0.661</td>
</tr>
<tr>
<td>Age</td>
<td>X</td>
<td>0.071</td>
<td>0.79</td>
<td>0.164</td>
</tr>
</tbody>
</table>

Most avid primary tumour (PT1), second most avid primary tumour (PT2), anatomical mismatch between PET and CT image (AM)

Table 3: Multivariate analyses.

<table>
<thead>
<tr>
<th></th>
<th>No. LN P</th>
<th>Anatomical Mismatch P</th>
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<td>LNA:</td>
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<td>Age</td>
<td>0.37</td>
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<tr>
<td>5mmCT</td>
<td>X</td>
<td>0.004</td>
</tr>
<tr>
<td>LNO:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine vs prone</td>
<td>0.366</td>
<td>0.406</td>
</tr>
<tr>
<td>Age</td>
<td>0.004</td>
<td>0.514</td>
</tr>
<tr>
<td>5mmCT</td>
<td>X</td>
<td>0.047</td>
</tr>
<tr>
<td>All LN:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine vs prone</td>
<td>&lt;0.001</td>
<td>X</td>
</tr>
<tr>
<td>Age</td>
<td>0.069</td>
<td>X</td>
</tr>
<tr>
<td>5mmCT</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Most avid axillary lymph node (LNA), most avid nonaxillary lymph node (LNO), lymph nodes (LN). X=not applicable. We only included the 5mm CT reconstruction (5mmCT) as a testing variable for the anatomical mismatch between PET and CT.

Discussion

Prone position PET/CT images were superior to supine position images for the visualization and discrimination of FDG avid primary breast lesions and locoregional lymph nodes in patients with...
Figure 2: Sagittal fused PET/CT images of the breast area taken from the same patient. On image (A), acquired in prone position, 2 lesions are distinguishable as separate primary tumours, whereas on image (B), taken in supine position, only 1 lesion can be pointed as primary tumour.

Figure 3: Transversal CT and fused PET/CT images of the thorax taken from the same patient. Images (A) and (B) are made in prone position showing the $^{18}$F-FDG uptake in the axillary lymph node. Images (C) and (D) are acquired in supine position showing $^{18}$F-FDG uptake lateral from the lymph node, not covering the area on the CT.
stage II/III breast cancer. This finding is important because for optimal therapy planning and prognostic information, it is important to implement the most effective diagnostic methods in daily practice for the visualization of multicentricity and multifocality, locoregional (lymph node) metastases, and distance metastases.

Both the univariate and multivariate analysis showed that the PET/CT in prone position detected the primary tumour and multifocality more often. In a previous publication prone position, PET/CT was found to detect between 83% and 100% of the 3 main types of breast cancer with a specificity of 98%14. Currently, MRI is considered as gold standard for both local staging of the primary tumour at baseline and response monitoring during PST as it has, to date, the best accuracy for detecting multifocality or multicentricity11. Recently, Pengel et al reported that when both the MRI and PET/CT are acquired in prone position, comparable value are found for monitoring response of the primary tumour during PST22.

Prone position images also showed a significant higher number of axillary LN metastases and a higher number of axillary and non-axillary LN metastases combined, but not of extra-axillary LN metastases separately. Possibly, the number of patients included with extra-axillary LNs (n=39) was not sufficiently high to show a difference in this analysis. Unfortunately, we did not have the possibility to confirm all findings with final pathology as all patients underwent PST. However, a recent study by Koolen et al evaluated PET/CT in prone position, showing a sensitivity of 82% and a specificity of 92% for the detection of axillary LNs before PST16, and a study of Cooper et al showed a mean sensitivity and specificity for gadolinium enhanced MRI of respectively 88% and 73%23. Taking into account the high specificity of PET/CT, this device might have a possible role for preoperative assessment of locoregional LN metastasis.

In our multivariate analysis, anatomical mismatch between the PET and the CT of the axillary LNs was less often found on the prone position images when comparing with supine images with standard 5 mm CT slice reconstruction. Curiously, the univariate analyses did not show this particular result. In a clinical setting where the prone position acquisitions cannot be added to the standard supine position PET/CT, it can be considered to reconstruct the CT images with 2 mm slices to possibly diminish the disadvantage of anatomical mismatch for axillary lymph nodes.

Most PTs showed a larger functional volume on supine positioning images (Figs. 5 and 6). To exclude the possible overprojection of multiple (multicentric) lesions on the supine position scan, a signed rank test in the group of patients with only one tumour in the breast was applied, but the differences remained (P=0.228 and P=0.1). These findings are discordant with those of Moon et al24 who reported a significantly greater maximum tumour diameter (1.3±0.6 cm vs 1.6±0.6 cm, P < 0.001) on prone position.

The lower SUV values in supine images seen in the univariate analyses could explain the outliers in volume assessment for the supine images: with a lower SUVmax the iso50 threshold, as applied
in the present study, tends to include more normal surrounding breast or lymph node tissue in the volume measurement. Another possible explanation is that glandular tissue is compressed in supine position, which may result in higher SUV values in normal tissues surrounding the tumour and thus a larger iso50 volume. Based on these considerations, we cannot recommend the iso50 volume as a method for tumour volume evaluation; especially in case of low SUVmax values, it does not provide meaningful tumour definitions. After the multivariate analyses, the difference in scanning position (the difference in voxel size and reconstruction) did not outweigh the influence of the variables distance of tumour until the pectoral muscle, tumour volume, breast volume, or age.

**Figure 4:** Anatomical mismatch 2. Transversal CT and fused PET/CT images of the thorax taken from the same patient. *Image (A)* was taken from the prone position scan, the arrow points at an FDG-avid parasternal LN, with neatly fused PET and CT. *Image (B)* was taken from the supine position scan, the top arrow pointing at the PET signal and the bottom arrow pointing at the CT image of the LN.

In conclusion, PET/CT acquired in prone position for hanging breast imaging seems to improve visualization of both the PTs and the axillary LN metastases. Without any additional FDG administration and with an acquisition time of only 10 to 15 minutes, extra prone PET/CT could be incorporated to the standard PET/CT protocol in addition to supine scans for a more accurate assessment of locoregional involvement. As a future perspective, PET/CT in prone position may also be considered to supplement prone MRI for baseline locoregional staging and monitoring of therapy response in stage II/III breast cancer patients as it adds additional information about the glucose metabolism and, thus, about the behaviour of malignant lesions in the breast and regional lymph nodes during PST.
Figure 5: The iso50 volume of the primary tumour of prone position compared supine PET/CT with 2 mm CT reconstruction for patients with only 1 tumour inside the breast. The line drawn separates the area in which the supine position scan (IsoVol TB, y-axis) shows a primary tumour with a greater volume from the area where the prone position scan (IsoVol HB, x-axis) shows a primary tumour with a greater volume.

Figure 6: The iso50 volume of the primary tumour of prone position compared supine PET/CT with 5 mm CT reconstruction for patients with only 1 tumour inside the breast. The line drawn separates the area in which the supine position scan (IsoVol TB, y-axis) shows a primary tumour with a greater volume from the area where the prone position scan (IsoVol HB, x-axis) shows a primary tumour with a greater volume.
References


Chapter 3
PET/CT with $^{18}$F-FDG predicts short-term outcome in stage II/III breast cancer patients upstaged to N2/3 nodal disease.

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Renato A Valdés Olmos, Department of Nuclear Medicine, NKI-AvL
Abstract

**Introduction:** $^{18}$F-FDG PET/CT has high positive predictive value for the detection of avid lymph node metastases in breast cancer patients. We analysed the effect of upstaging lymph nodes by PET/CT on short-term outcome in stage II/III breast cancer patients.

**Patients and methods:** A total of 278 stage II/III primary breast cancer patients (mean age 48.9 years, range 19e75 years) were re-staged with $^{18}$F-FDG PET/CT before start of pre-operative systemic treatment (PST). Patients were divided in three groups based on risk for local recurrence: a low e (T2N0), intermediate e (T0-2N1 and T3N0) and a high-risk group (T0-3N2e3, T3N1 and T4). Within these groups we looked at local recurrence-free survival (LRFS), recurrence-free survival (RFS) and overall survival (OS) within the first 3 years of follow-up.

**Results:** With a median follow-up (FU) of 50 months the LRFS, DDFS and OS were 88%, 89% and 92% respectively for the whole group. PET/CT upstaged 43 patients from the low- and intermediate risk group to the high-risk group, based on detection of ≥ 4 avid axillary nodes or occult N2/3-disease. Patients upstaged with PET/CT had more events for all three analyses compared to the original risk groups, which resulted in a significantly worse RFS (69.8%; p= 0.03) a nearly significantly worse LRFS (p=0.052) and no effect in OS (p=0.433).

**Discussion:** Additional PET/CT staging allows breast cancer patients to be treated according to the true stage, still stage II/III breast cancer patients upstaged to N2/3 by PET/CT have worse short-term outcome, despite adjustment of treatment, than patients staged high-risk with conventional imaging.

**Keywords:** PET/CT; Radiotherapy; Breast cancer; Survival; Upstaging
PET/CT with 18F-FDG predicts short-term outcome in stage II/III breast cancer patients

Introduction

The treatment of breast cancer patients is multidisciplinary, often combining surgery with systemic treatment and/or radiotherapy. Preoperative systemic treatment (PST), also called neo-adjuvant therapy, is becoming the preferred treatment for stage II/III breast cancer patients. Giving systemic treatment pre-operatively, and consequently reaching a pathologic complete response (pCR) is known to be predictive for better survival outcome in ER positive/Her2 negative (ER+), HER2 positive/ER negative and triple negative (TN) breast cancer subtypes. Before start of PST it is relevant to discover overt distant metastases for which abdominal ultrasound (liver), bonescan (bones) and/or chest X-ray (lungs) are traditionally used. Several studies report that Positron emission tomography coupled with low-dose computed tomography (PET/CT) using 18-fluorodeoxy-glucose (18F-FDG) outperforms conventional imaging. PET/CT is specifically helpful to improve staging of regional lymph nodes.

Ultrasound of the axilla, including targeted fine-needle aspiration (FNAC) or core biopsies of suspicious lymph nodes, has a sensitivity to detect axillary lymph node metastases of between 50% and 80%. Sentinel lymph node biopsy (SNB) before PST is able to reveal the N-stage with 96.9% accuracy but is becoming less preferred as it implies an additional surgical procedure for the diagnostic evaluation of a restricted region (axilla). PET/CT has also been has been reported to be more adequate for lymph node staging than conventional imaging in stage II/III breast cancer patients, as it is able to visualise more additional 18F-FDG avid extra-axillary lymph node metastases.

Patients with a higher tumour load in the axilla (>3 nodes) or with presence of non-axillary lymph node metastases (N 2/3) usually receive an axillary lymph node dissection (ALND) followed by complementary regional radiotherapy to the axilla. Giving additional regional radiotherapy in these high-risk patients has been reported to reduce the rate of breast cancer recurrence and with longer follow-up, also overall survival.

In 2013 Koolen et al. found that PET/CT upstaged 42 (23%) stage II/III breast cancer patients to the radiotherapy requiring (high-risk) group. Following recent literature on the high predictive value of PET/CT when 18F-FDG avid lymph nodes are detected we adapted the radiotherapy fields after PST in our institute for primary breast cancer patients scheduled for PST based on newly found N+ on PET/CT.

Our expectation is that by upstaging breast cancer patients into the high-risk group, and consequently adding radiotherapy to the treatment plan, this may positively influence survival. Outcome was evaluated using recurrence free survival (RFS), local recurrence free survival (LFS); and overall survival (OS).
Patients and methods

Patient inclusion
From September 2007 to October 2011 we included 311 consecutive breast cancer patients with a primary breast cancer lesion of at least 3 cm or at least 1 lymph node metastasis, who were candidates for PST. Thirty-three patients had proven distant metastases and were therefore excluded, leaving 278 patients for this analysis. This study was approved by the local ethics committee and in accordance with the Helsinki Declaration of 1975, as revised in 1983, and all patients gave written informed consent. The patient population for this analyses was previously reported by Koolen et al.14

Lymph node imaging
Conventional imaging for lymph node staging consisted of ultrasound of the axilla and ipsilateral periclavicular areas. When a lymph node was found to be suspect; having a cortex larger than 2.3 mm was present and/or an asymmetrical and irregular cortex, or displaced hilar fat,15 a FNAC was performed. When ultrasound was negative or FNAC inconclusive a SNB followed before start of PST.

All patients were subsequently staged with a whole body PET/CT scanner (Gemini TF, Philips, Cleveland, USA). As standard preparation for 18F-FDG PET/CT the patients were required to have fasted during 6 h and blood glucose levels were supposed to be lower than 10 mmol/l before 18F-FDG administration. To reduce uptake of 18F-FDG by brown fat 10 mg diazepam was administered orally.16 Patients received a total dose of 180-240 MBq of F18-FDG intravenously, depending on their body mass index, and were then asked to rest for 60 ± 10 min.

A PET/CT scan of the thorax was performed in prone position (3.00 min per bed position and 2 x 2 x 2 mm voxels reconstruction) with hanging breasts using a mock-up MRI coil.14

Subsequently, a whole body PET/CT scan was performed in supine position from skull base to the upper half of both femora (1.30 min per bed position). Each PET acquisition was followed by a low-dose CT acquisition (40 mA, 2 mm slices) without contrast for attenuation correction and anatomical localisation. Subsequently, images were evaluated using orthogonal multiplanar reconstruction and simultaneous display of PET, CT and fused PET/CT. Lymph nodes were defined as being tumour positive if proven by US with FNAC or identified as 18F- FDG avid lymph nodes on PET/CT imaging.

Treatment
PST was given as previously reported.14 Briefly, HER2- negative patients received six cycles of cyclophosphamide and doxorubicin in a dose-dense schedule (every 2 weeks) and HER2-positive
patients received a trastuzumab-based regimen, consisting of paclitaxel, trastuzumab, and carboplatin. PST was followed by resection of the primary tumour by breast conserving surgery (followed by radiotherapy) or mastectomy.

Patients who had tumour-positive axillary nodes before PST underwent ALND. All patients undergoing BCT received postoperative breast irradiation. Patients with more than 3 axillary lymph node metastases (cN2 (4+)) and patients with stages cN2-3 or ypN2-3 received additional radiotherapy to the regional LN area. The internal mammary chain (IMC) was irradiated in case of a PET positive node or pathologically proven tumour-positive nodes (at cytology or SLNB). After ablative surgery, irradiation of chest wall was based on the following institutional guidelines: all cT3-4, ypT3-4, cN2-3, cN2 (4+), and ypN1-3 tumours. In patients with ypN1 the periclavicular area was added to the radiation field. See Figure 1.

**Risk-groups**
The group was divided in three subgroups according to the pre-operative risk estimation of local and regional recurrence: low-risk (T2N0), intermediate-risk (T0-2N1 and T3N0) and high-risk (T0-3N2-3, T3N1, T4). 14 Forty-three patients who were classified with conventional imaging as being
low-risk, intermediate risk and appeared to have 4 FDG-avid nodes in the axilla or extra-axillary lymph node metastases ($\geq$N2a), were therefore newly classified as high-risk (=upstaged), and subsequently scheduled to receive adjuvant radiotherapy.\textsuperscript{14} The patients that had been classified as high-risk patients by conventional imaging and appeared to have additional extra-axillary lymph node metastases in other regions were also scheduled to receive additional radiation fields.

**Statistical analyses**

Following the expected positive effect of early treatment with radiotherapy in the upstaged group the main emphasis in analyses of recurrence was on the difference in locoregional recurrence and overall survival of the three risk groups and the upstaged group: patients with low-risk after PET/CT, patients with Intermediate risk after PET/CT, patients with high-risk before PET/CT and patients classified into high-risk after PET/CT (the upstaged group). The follow-up was defined from the date PET/CT acquisition was made until death or last follow-up. In these four groups we looked at recurrence free survival (RFS), local recurrence free survival (LRFS) and overall survival (OS) of the first 3 years. For the definition of RFS the development of local recurrence, distant metastasis or death was defined as an event. For the definition of LRFS local recurrence and death were defined as an event. For OS only death was defined as an event. We used the Kaplan-Meier method to describe survival in the four groups. To differentiate between the events in each subgroup we drew Venn-diagrams.

Log-rank tests were used to compare the survival between the three risk groups and the upstaged group. Additionally a comparison was made between the patients that were classified as high-risk group before PET/CT and the upstaged group. All analyses were performed using R (version 3.0.2.).
Results

The baseline characteristics of the included 278 female patients are presented in Table 1. In Figures 2-4 the Kaplan-Meier curves are depicted for RFS, LRFS and OS. Before PET/CT, conventional imaging had resulted in the following classification: 47 low-risk patients, 144 intermediate-risk patients and 87 high-risk patients. PET/CT imaging resulted in the upstaging of 5 low-risk patients and 38 intermediate-risk patients.

Recurrence free survival (Figure 2)
The 3-year RFS for the whole group was 87% (95% C.I. 83-91). The patients who were not upstaged following PET/CT showed 2 (4.9%) events in 41 patients in the low-risk, 18 (16.8%) events in 107 patients in the intermediate-risk and 14 (16.1%) events in 87 high-risk patients. The upstaged patients had 13 (30.2%) events in 43 patients. The RFS of the four groups was significantly different ($X^2=9.07$ on 3df; $p=0.028$) but the RFS of the group that remained in the high-risk group was not significant different from the upstaged group ($X^2=2.85$ on 2 df; $p=0.091$).

Local recurrence free survival (Figure 3)
The 3-year LRFS for the whole group was 88% (95% C.I. 85-92). The patients who were not upstaged following PET/CT showed 2 (4.9%) events in 41 patients in the low-risk, 14 (13.1%) events in 107 patients in the intermediate-risk and 10 (11.5%) events in 87 high-risk patients. The upstaged patients had 11 (25.6%) events in 43 patients. There seems to be a trend as the LRFS in the groups that remained after PET/CT was clearly different in the image and was almost significantly different from the upstaged group ($X^2=7.72$; $p=0.052$). Focusing on the difference in LRFS between the group that remains in the high-risk group and the upstaged group we find $X^2=3.51$; $p=0.061$.

When looking at the separate patients in both groups, 6 patients had a recurrence in the upstaged group, of which 5 had only a regional recurrence (of which two died) and 1 patient had both a local and a regional recurrence. In the group of patients staged as high-risk with conventional imaging 5 patients had a recurrence of which 3 had a regional recurrence, 1 patient had a local recurrence and one patient had both a local and a regional recurrence. None of these patients survived the first three years of follow-up.

Overall survival (Figure 4)
The three-year overall survival was 92% (95% C.I. 89-95) in the whole group, 95% (95% C.I. 89-100) for the patients who remained in the low-risk group, 92% (86-97) for the patients...
Table 1: Baseline characteristics of 278 female patients n (%)  

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<th>Age in years</th>
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<td>2 (1%)</td>
<td>1</td>
<td>22 (8%)</td>
</tr>
<tr>
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<td>0</td>
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<td>1</td>
<td>187 (67%)</td>
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<td>T2N0</td>
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<td>20</td>
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<tr>
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<td>T4</td>
<td>20</td>
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<td>1</td>
<td>18 (7%)</td>
<td>2</td>
<td>144 (52%)</td>
</tr>
</tbody>
</table>

cT=tumour stage, cN=lymph nodes stage, CI=conventional imaging, N=number, %=percentage. Data is present as number (percentage, unless otherwise specified.
Figure 2: The Kaplan-Meier curves for the recurrence free survival (RFS) after PET/CT. Low-low is the group that remained in the low-risk group after staging with PET/CT, intermediate-intermediate remained in the intermediate-risk group and high-high remained in the high-risk group. L/I-high is the group of patients upstaged by PET/CT.

Figure 3: The Kaplan-Meier curves for the local recurrence free survival (LRFS) after PET/CT. Low-low is the group that remained in the low-risk group after staging with PET/CT, intermediate-intermediate remained in the intermediate-risk group and high-high remained in the high-risk group. L/I-high is the group of patients upstaged by PET/CT.
who remained in the intermediate-risk group and 94% (95% C.I. 88-99) for the patients who remained in the high-risk group. The three-year survival was 85% (95% C.I. 75-97) for the group upstaged by PET/CT.

Patients that remained in the same groups after PET showed 2 (4.9%) events in 41 patients in the low-risk, 11 (12.3%) events in 107 patients in the intermediate-risk and 9 (10.3%) events in 87 high-risk patients. The upstaged patients had 7 (16.3%) events in 43 patients. The OS in the four groups was not significantly different ($X^2$=2.74; $p=0.433$). In this analysis we see even less difference between the OS of the group that remained in the high-risk group and the upstaged group ($X^2$=0.9; $p=0.406$).

**Venn diagrams**

In Figures 5-8 the Venn diagrams are depicted explaining the events found in the analyses for RFS, LRFS and OS.
**Figure 5:** Venn diagram of patients remaining in the low-risk group (low-low): N=41. **Image a:** Local recurrence free survival (LRFS), two patients died of which 1 had a local recurrence (and a distant metastasis). No patient with a local recurrence was still alive. **Image b:** Distant disease free survival (DDFS), two patients died of which one had distant metastases (and a local recurrence). One patient died without distant disease but no patients with distant metastases were still alive.

**Figure 6:** Venn diagram of patients remaining in the intermediate-risk group (intermediate-intermediate) N=107. **Image a:** Local recurrence free survival (LRFS), 11 patients died of which 2 had a local recurrence (and distant metastases). Three patients had a local recurrence and were still alive. And 9 patients died without having a local recurrence. **Image b:** Distant disease free survival (DDFS) eleven patients died of which 7 had distant metastases (two of which had also a local recurrence). Four patients died without distant disease and four patients with distant metastases were still alive.

**Figure 7:** Venn diagram of patients remaining in the high-risk group (high-high) N=87. **Image a:** Local recurrence free survival (LRFS), 9 patients died of which 5 had a local recurrence (of which 4 also had distant metastases). One patient had a local recurrence and was still alive. Four patients died without a local recurrence (but with distant metastases). **Image b:** Distant disease free survival (DDFS) 9 patients died of which eight had distant metastases. One patient died without distant metastases (but with local recurrence) and five patients with distant metastases were still alive.
The adjustment of the radiotherapy fields following upstaging of patients with stage II/III breast cancer with 18F-FDG PET/CT into the high-risk group did not lead to less regional recurrences compared to high-risk patients classified with conventional imaging, when looking at short-term outcome. We did find that patients upstaged by PET/CT into the high-risk group showed a worse short-term RFS than patients who remained in the original risk groups after PET/CT staging. This effect was also seen for LRFS and for OS, although not significantly. We expect to find a more pronounced effect with a longer follow-up time. The worse RFS in the upstaged group could be related to the biology of the primary tumour. When analysing the four groups we found that, compared to the intermediate (21.5%) and the high-risk group (25.3%), the upstaged group (37.2%) had a higher percentage of TN breast cancer patients. In Table 2 we added an extensive comparison between the patients staged as high-risk by conventional imaging and the patients upstaged by PET/CT. It is remarkable that the upstaged group consisted of patients with only T2 tumours (p<0.0001) and had a higher number of stage N2 and N3, although not significantly. When comparing the histology the upstaged group consisted of more patients with ductal carcinoma (98% vs 76%; p=0.0033). On the other hand the conventional high stage group had included more patients with unfavourable T-stages and thus more often patients who underwent mastectomies (64% vs 44%, p=0.043). Looking at pCR after neo-adjuvant chemotherapy, the patients in the upstaged group showed less often pCR in both breast and axilla (16% vs 24%), although the pCR comparison was not significantly different between both groups. Overall it
seems that the upstaged group is a more homogenous group including patients with tumours more prone to develop lymphogenous metastases.

And, although we did not find that the groups treated with radiotherapy had a similar local recurrence rate as the intermediate risk group, which would be explained by a positive effect of treatment with radiotherapy, it can be expected that without adequate treatment the local control in these patients would be worse. The 43 upstaged patients described in more detail illustrate our expectation. Of the low and intermediate patients that have been upstaged by PET/CT 9% became ypN0 after pre-operative systemic treatment, in these patients the indication for additional radiotherapy would have been missed. In 6% patients became ypN1, meaning that without staging with PET/CT the additional fields would have been missed (Figure 9). A visual illustration on how PET/CT was able to upstage a low-risk patient is shown in Figure 10.

The clinical impact of current findings is still not established yet. Following favourable results of recent larger trials the St. Gallen’s consensus experts recommend that patients with at least 4 axillary lymph node metastases should undergo radiotherapy of the inter-mammary chain and peri-clavicular areas. Uncertainty persists about how patients with 1-3 axillary lymph node metastases should be treated. Following the results presented by Poortmans et al. and Whelan et al. it has been recently recommended that regional node irradiation should be considered for patients with 1 to 3 axillary lymph node metastases in case of young of age with unfavourable tumour characteristics (large diameter, extensive lymphovascular invasion, high histologic grade etc.). This might also be the group where ALND might be avoided and avoiding the possible consequential morbidity. In our patient group this would imply that part of our intermediate risk-group patient should receive radiotherapy to lymph node stations and be treated as our defined high-risk patients.

However, at the NKI, our radiation oncologists agreed to irradiate in the high-risk patients according to the definition mentioned. Nevertheless, still much discussion persists regarding the application of regional radiotherapy, considering the higher occurrence of contra-lateral breast cancer in patients who receive IMC and/or whole breast irradiation, and the high costs of all-field radiotherapy.

The PET/CT could be helpful in differentiating between presence of N1 and N2/3 disease as it is the most accurate to discover clinically unsuspected stage N3 lymph node metastases, and its high positive predictive value for detecting lymph node metastases reaches the 100%. Despite that in previous articles radiotherapy was given either to all patients undergoing radiotherapy of the axilla, or after confirmation by SNB, we have selected patients for all-field radiotherapy by taking advantage of the high predictive value of PET/CT for the detection of lymph node metastases. According to the Dutch Breast Cancer Guideline (http://www.oncoline.nl/breastcancer) at the Netherlands Cancer Institute all breast cancer patients scheduled for pre-
operative systemic treatment undergo 18F-FDG PET/CT to exclude metastases at distance. If PET/CT shows FDG-avid lymph nodes in the axilla, targeted ultrasound-guided biopsy is taken and an 125-iodine-seed is implanted in the lymph node for surgical identification and histopathological examination after chemotherapy. When no abnormal lymph nodes are shown a post-NAC sentinel lymph node procedure is performed. Although recently several studies have reported that post-NAC SNB is feasible larger recently published studies, the SENTINA25 and the ACOSOG Z107126 trials, emphasize that when SNB is done after NAC the detection rate is lower 80.1% vs 99.1%) and the false negative rate is higher (14.2-24.3%) than when done before NAC.

The most important shortcoming of our study is the short follow-up time of three years as this amount of time is probably too short for definite conclusions. Nevertheless we found that recurrence free survival is significantly less favourable in patients upstaged by PET/CT. These patients need additional treatment of the lymph node basins at risk, preferably with radiotherapy. We feel the need to communicate our findings to contribute to the discussion on who to irradiate in all fields after PST.

In conclusion we found that stage II/III breast cancer patients upstaged with PET/CT into the radiotherapy needing high-risk group showed a worse recurrence free survival, despite adjustment of treatment, than patients placed in the high-risk group following conventional imaging. These patients need to be identified upfront so adequate additional radiotherapy can be planned.

Conflict of interest statement
None to declare.
Table 2: Comparison between both groups of high-risk patients

<table>
<thead>
<tr>
<th>C.I. high-risk</th>
<th>Upstaged patients</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>87</td>
<td>43</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>50</td>
<td>47</td>
</tr>
<tr>
<td>Range</td>
<td>43-58</td>
<td>38-56</td>
</tr>
<tr>
<td>Follow-up</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (95% CI)</td>
<td>48.95 (44.1-53.4)</td>
<td>56.38 (44.7-67.2)</td>
</tr>
<tr>
<td>3 years completed</td>
<td>74</td>
<td>38</td>
</tr>
<tr>
<td>Stage before chemotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>1</td>
<td>2 (2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>2</td>
<td>14 (16%)</td>
<td>43 (100%)</td>
</tr>
<tr>
<td>3</td>
<td>50 (57%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>4</td>
<td>20 (23%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>cN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4 (5%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>1</td>
<td>43 (49%)</td>
<td>16 (37%)</td>
</tr>
<tr>
<td>2</td>
<td>5 (6%)</td>
<td>3 (7%)</td>
</tr>
<tr>
<td>3</td>
<td>35 (40%)</td>
<td>24 (56%)</td>
</tr>
<tr>
<td>Subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER+/Her2-</td>
<td>39 (45%)</td>
<td>17 (40%)</td>
</tr>
<tr>
<td>Her2+</td>
<td>26 (30%)</td>
<td>10 (23%)</td>
</tr>
<tr>
<td>TN</td>
<td>22 (25%)</td>
<td>16 (36%)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adeno</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Ductal</td>
<td>66 (76%)</td>
<td>42 (98%)</td>
</tr>
<tr>
<td>Ductolobular</td>
<td>1 (1%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Lobular</td>
<td>16 (18%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Metaplastic</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Papillar</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4 (5%)</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>2</td>
<td>48 (55%)</td>
<td>25 (58%)</td>
</tr>
<tr>
<td>3</td>
<td>26 (30%)</td>
<td>13 (30%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>9 (10%)</td>
<td>3 (7%)</td>
</tr>
<tr>
<td>Breast surgery*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mastectomy</td>
<td>56 (64%)</td>
<td>19 (44%)</td>
</tr>
<tr>
<td>Breast conservation</td>
<td>30 (34%)</td>
<td>24 (56%)</td>
</tr>
<tr>
<td>Axillary surgery</td>
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<td></td>
</tr>
<tr>
<td>SNB only</td>
<td>6 (7%)</td>
<td>6 (14%)</td>
</tr>
<tr>
<td>ALND only</td>
<td>75 (86%)</td>
<td>35 (81%)</td>
</tr>
<tr>
<td>Both</td>
<td>5 (6%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>None*</td>
<td>1 (1%)</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>
### Table 2: Comparison between both groups of high-risk patients (continued)

<table>
<thead>
<tr>
<th>C.I. high-risk</th>
<th>Upstaged patients</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pCR rate</strong></td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>No pCR in axilla and breast</td>
<td>40 (46%)</td>
<td>18 (42%)</td>
</tr>
<tr>
<td>pCR in breast</td>
<td>7 (8%)</td>
<td>6 (14%)</td>
</tr>
<tr>
<td>pCR in axilla</td>
<td>12 (14%)</td>
<td>5 (12%)</td>
</tr>
<tr>
<td>pCR in breast and axilla</td>
<td>21 (24%)</td>
<td>7 (16%)</td>
</tr>
<tr>
<td><strong>ypTc</strong></td>
<td></td>
<td>0.49</td>
</tr>
<tr>
<td>0</td>
<td>30 (34%)</td>
<td>16 (37%)</td>
</tr>
<tr>
<td>1</td>
<td>25 (29%)</td>
<td>19 (44%)</td>
</tr>
<tr>
<td>2</td>
<td>21 (24%)</td>
<td>6 (14%)</td>
</tr>
<tr>
<td>3</td>
<td>7 (8%)</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>4</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>yN</strong></td>
<td></td>
<td>0.31</td>
</tr>
<tr>
<td>0\textsuperscript{a}</td>
<td>35 (40%)</td>
<td>12 (28%)</td>
</tr>
<tr>
<td>1\textsuperscript{b}</td>
<td>15 (17%)</td>
<td>9 (21%)</td>
</tr>
<tr>
<td>2</td>
<td>14 (16%)</td>
<td>9 (21%)</td>
</tr>
<tr>
<td>3</td>
<td>17 (20%)</td>
<td>7 (16%)</td>
</tr>
<tr>
<td><strong>Local radiotherapy</strong></td>
<td></td>
<td>0.11</td>
</tr>
<tr>
<td>Breast</td>
<td>33 (38%)</td>
<td>24 (56%)</td>
</tr>
<tr>
<td>Thoracic wall</td>
<td>49 (56%)</td>
<td>16 (37%)</td>
</tr>
<tr>
<td>None</td>
<td>5 (6%)</td>
<td>5 (7%)</td>
</tr>
<tr>
<td><strong>Regional radiotherapy</strong></td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>No radiotherapy</td>
<td>16 (18%)</td>
<td>6 (14%)</td>
</tr>
<tr>
<td>Parasternal only</td>
<td>2 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Periclavicular only</td>
<td>20 (23%)</td>
<td>7 (16%)</td>
</tr>
<tr>
<td>Parasternal and periclavicular</td>
<td>6 (7%)</td>
<td>9 (21%)</td>
</tr>
<tr>
<td>Only axilla</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Axilla and parasternal</td>
<td>3 (3%)</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Axilla and periclavicular</td>
<td>24 (28%)</td>
<td>9 (21%)</td>
</tr>
<tr>
<td>Axilla, parasternal and periclavicular</td>
<td>15 (17%)</td>
<td>9 (21%)</td>
</tr>
</tbody>
</table>

Both groups meaning patients classified as high stage according to conventional imaging (C.I.) and patients upstaged by PET/CT.

\textsuperscript{a} One patient had an occult primary tumour.

\textsuperscript{b} One patient without axillary surgery

\textsuperscript{c} One breast tumour was occult and 2 multifocal tumours in high-risk group were not included.

\textsuperscript{d} Four patients in the high-risk group and 6 in the upstaged group had N0, but had only undergone SN and were not included.

\textsuperscript{e} Two patients had a N1 but had only undergone SN and were not included.
Impact on LRR and indication for RT

Patients moved to high risk (n = 278)

- **47** Low risk  
  (T2 N0)  
  1 patients (5%) ≥4 axillary nodes  
  5 patients (11%) occult N3

- **113** Intermediate  
  (T2 N1)  
  24 patients (21%) ≥4 axillary nodes (N2 4+)  
  22 patients (19%) occult N3

- **87** High risk  
  (cT3-4 N2-3)  
  0 No changes in indication for RT  
  15 patients (17%) RT fields adapted

(M1 patients excluded)

**Figure 9:** Details of clinical relevance of PET/CT staging for the group of upstaged patients.

**Figure 10:** Coronal PET/CT image showing FDG-avid lymph node metastases in the left axilla and in de left infra- and supraclavicular area.
References


19. Overgaard M, Nielsen HM, Overgaard J. Is the benefit of postmastectomy irradiation limited to patients with four or more positive nodes, as recommended in international consensus reports? A subgroup analysis of the DBCG 82 b&c randomized trials. Radiother Oncol 2007;82(3):247–53.


Part II
PET/CT:
Response monitoring
Chapter 4
Monitoring primary tumour response to neoadjuvant chemotherapy using MRI and $^{18}$F-FDG PET/CT in breast cancer subtypes

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Kenneth G Gilhuijs, Department of Radiology/Image Sciences Institute, UMCU
Abstract

Introduction
To explore guidelines on the use of MRI and PET/CT monitoring primary tumour response to neoadjuvant chemotherapy (NAC), taking breast cancer subtype into account.

Subjects and Methods
In this prospective cohort study, 188 women were included with stages II and III breast cancer. MRI and 18F-FDG-PET/CT were acquired before and during NAC. Baseline pathology was assessed from tumour biopsy. Tumours were stratified into HER2-positive, ER-positive/HER2-negative (ER-positive), and ER-negative/PR-negative/HER2-negative (triple-negative) subtypes, and treated according to subtype. Primary endpoint was pathological complete response (pCRmic) defined as no or only small numbers of scattered invasive tumour cells. We evaluated imaging scenarios using MRI only, PET/CT only, and combinations.

Results
pCRmic was found in 35/46 (76.1%) of HER2-positive, 11/87 (12.6%) of ER-positive, and 31/55 (56.4%) of triple-negative tumours. For HER2-positive tumours, MRI yielded the strongest predictor (AUC: 0.735; sensitivity 36.2%), outperforming PET/CT (AUC: 0.543; p=0.04), and with comparable results to combined imaging (AUC: 0.708; p=0.213). In ER-positive tumours, the combination of MRI and PET/CT was slightly superior (AUC: 0.818; sensitivity 55.8%) over MRI alone (AUC: 0.742; p=0.117) and PET/CT alone (AUC: 0.791). However, even though relatively large numbers of ER-positive tumour patients were included, no significant differences were yet found. For triple-negative tumours, MRI (AUC: 0.855; sensitivity 45.4%), PET/CT (AUC: 0.844; p=0.220) and combined imaging (AUC: 0.868; p=0.213) yielded comparable results.

Discussion
For HER2-positive tumours, MRI shows significant advantage over PET/CT. For triple-negative tumours, comparable results were seen for MRI, PET/CT and combined imaging. For ER-positive tumours, combining MRI with PET/CT may result in optimal response monitoring, although not yet significantly.

Key words: Breast cancer; neoadjuvant chemotherapy; response monitoring; MRI; PET/CT.
Introduction

Neoadjuvant chemotherapy (NAC) for breast cancer has the potential benefit to reduce tumour size; enabling conversion from breast ablation towards breast-conserving surgery \(^1\text{-}^3\) as well as reduction in axillary lymph node dissections \(^4\text{-}^6\). In addition, the response to chemotherapy can be monitored; which enables switching to alternative non-cross resistant chemotherapy or ceasing treatment after insufficient response. Thus, patients may either benefit from a more appropriate NAC regimen or they will be protected from undergoing further ineffective toxic treatment \(^7\).

Monitoring treatment response during NAC is typically performed using ultrasound or dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI). The latter has the potential to discriminate between viable tumour cells and NAC-induced fibrotic tissue and has shown to be a strong predictor for tumour response \(^8\text{-}^10\). Although MRI has several advantages over conventional imaging techniques, the predictive value of MRI is not perfect and it strongly depends on the molecular subtype and morphologic appearances of tumours \(^11\). MRI performs well in human epidermal growth factor receptor 2 (HER2)-positive tumours, and in oestrogen receptor (ER)-negative/progesterone receptor (PR)-negative/HER2-negative (triple-negative) tumours, but it is less accurate in ER-positive tumours \(^12\).

Hence, other imaging techniques are under investigation to monitor tumour response \(^13\). Currently, positron emission tomography using fluorodeoxyglucose, integrated with computed tomography \((^{18}\text{F-FDG PET/CT})\), is used for preoperative staging in patients scheduled for NAC \(^14\). More recently it has been investigated to monitor response of breast cancer to NAC \(^15\text{-}^16\). The results for PET/CT also showed dependence on breast cancer subtype, indicating good performance in ER-positive and triple negative tumours, but relatively poor performance in HER2-positive tumours \(^17\).

MRI visualizes changes in morphology and vascularization of tumours whereas PET/CT visualizes changes in the glucose metabolism of tumours. Therefore, a complementary value of these techniques has been hypothesized. This complementary value for response monitoring is important knowing both imaging techniques vary accuracy depending on breast cancer subtype. Recently, an explorative study showed a potential complementary value of MRI and PET/CT. However, this study had an insufficient number of patients to determine how MRI and PET/CT could be combined in the daily clinical workflow to benefit optimally from their complementary value \(^18\).

The aim of the present study is to explore guidelines on the use of MRI and PET/CT in the clinical workflow to monitor response of the primary tumour to NAC, taking breast cancer subtype into consideration.
Material and methods

Patient cohort
Patients were included between September 2008 and June 2013 in this prospective cohort study. Eligibility criteria included primary invasive breast cancer of at least 3 cm and/or at least one tumour-positive axillary lymph node. This study was approved by the institutional review board and written informed consent was obtained from all patients. Of this current study, 93 patients were reported earlier by Pengel et al. 18.

Pathology prior to NAC
Core-needle biopsies of the primary tumour were taken prior to NAC. Tissue was routinely processed and stained using hematoxylin and eosin. Histopathology was assessed by an experienced breast pathologist (J.W.). Tumour type was recorded as invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC) or any ‘other’ tumour type. The oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status were determined according to the Dutch guidelines (www.oncoline.nl). For ER and PR, immunohistochemistry was used. A 10% threshold was used to discriminate between negative (<10% staining) or positive (≥10% staining) hormone receptor status. Immunohistochemistry for the HER2 was scored as 0, 1+, 2+ or 3+ to differentiate between negative (<2+) and positive (>2+) HER2 receptor status. At score 2+, in-situ hybridization was used to differentiate between a negative and positive status. Tumours were stratified into ER-positive and HER2-negative subtype (ER-positive), HER2-positive subtype (HER2-positive) and ER-negative/PR-negative/HER2-negative (triple-negative) subtype.

NAC
The NAC regiment differed per subtype 18. In short, HER2-positive tumours were treated in three cycles of eight weeks with paclitaxel, carboplatin and trastuzumab (day 1, 8, 15, 22, 29 and 36)19. ER-positive and triple-negative tumours were treated with three courses of ddAC (doxorubicin and cyclophosphamide on day 1, every 14 days, with PEG-filgrastim on day 2). Following these three courses, tumours were reported as ‘favourable’ or ‘unfavourable’ responders based on previously reported MRI response criteria by Loo et al.8. In the context of a larger study, a ‘favourable response’ was followed by three more courses of ddAC whereas an ‘unfavourable response’ was followed by three courses of docetaxel and capecitabine, which criteria were reported earlier by Rigter et al. 20.
Response imaging

MRI and PET/CT were performed at the start of chemotherapy (baseline imaging) and during chemotherapy (interim imaging). Specified as after the first cycle of eight weeks (in HER2-positive tumours) or after three courses of chemotherapy (in ER-positive and triple-negative tumours)\(^{21}\).

MRI

MRI was performed using a 3.0-T scanner (Achieva, Philips, Best, The Netherlands) with dedicated bilateral seven-element SENSE breast coil. Patients were scanned in prone orientation. Six consecutive coronal 3-D THRIVE SENSE T1-weighted sequences were acquired (1.1 x 1.1 x 1.1 mm\(^3\) voxels; 90s acquisition time; TR/TE 4.4/2.3 ms, flip angle 10\(^\circ\), FOV 360mm); One unenhanced series and five series following the intravenous injection (power injector; 3 mL/s) of gadolinium-containing contrast (Dotarem 0.5 mmol/ml; Guerbet; Aulnay-sous-Bois, France) which was followed by 30 mL of saline.

MR imaging was assessed by radiologists with breast MR experience using a protocol as previously described\(^{8,22}\). In short, a custom-build viewing station was used which enabled simultaneous viewing of two series reformatted and linked in three orthogonal directions. Subtraction images for initial enhancement (90s after contrast agent injection), late enhancement (450s after contrast agent injection), maximum intensity projections, and color-coded visualization of contrast curves were available. The latter visualized enhancement into persisting, plateau or a wash-out curve in accordance with the definitions used by Kuhl et al.\(^{23}\). The largest tumour diameter was assessed at initial (LD initial) and at late (LD late) enhancement. The largest diameter spanned the total lesion-bearing region including seemingly normal tissue in between and in any of the three orthogonal directions. Relative changes on MRI (MRI \(\Delta\)) between interim and baseline imaging were calculated separately for LD initial and LD late.

PET/CT

Imaging with PET/CT was performed after a six-hour fasting period at blood glucose levels of <10 mmol/l. Ten milligrams of diazepam were administered orally to prevent brown adipose tissue activation\(^{24}\). Depending on body mass index an intravenous dose of 180 or 240 MBq FDG was administered. After a resting period of 60 ± 10 min, PET/CT (Gemini TF; Philips, Cleveland, Ohio) was performed with the patient in prone orientation using a stripped mock-up MRI coil. The CT scan (10 mAs, 2mm slices) preceded the PET scan (3 min per bed position; 2 x 2 x 2 mm\(^3\) voxels). An additional standard supine whole-body PET/CT scan for distant staging was performed at baseline imaging prior to NAC. A panel of experienced readers evaluated the images in an orthogonal multiplanar reconstruction; which simultaneously display PET, CT, and fused PET/CT imaging. FDG uptake was measured using maximum standardized uptake values (SUV-max) in
a 3D region of interest containing the primary tumour (SUV-max tumour) and, when present, in the lymph node (SUV-max lymph node) showing the strongest uptake\textsuperscript{17}. Relative changes on PET/CT (PET/CT $\Delta$) between interim and baseline imaging were calculated separately for the SUV-max tumour and the SUV-max lymph node.

Pathology after NAC

In this study, according to the definition of Sataloff\textsuperscript{25}, pathological complete response (pCRmic) after completion of NAC, was defined as either complete absence of tumour cells or presence of only a small number of scattered invasive cells in the breast resection specimen (ypTmic). Pathological non-complete response (non-pCRmic) was defined as any remaining viable residual disease in the breast due to partial tumour response, stable or progressive disease.

Analyses

Baseline characteristics

Analyses were performed using SPSS (version 20.0; Chicago, Illinois). Associations were assessed between pCRmic and patient age, tumour histology, tumour subtype, MRI curve-type prior to NAC, MRI LD initial, MRI LD late, SUV-max tumour, SUV-max lymph node, as well as the change of these latter four characteristics during NAC. Two-sided Pearson’s chi squared, Fisher’s exact, and Mann-Whitney U tests were used for this purpose.

Imaging scenarios

At the interim-imaging stage, post-hoc analysis was performed to systematically evaluate and compare six different imaging scenarios for response monitoring per subtype: MRI only, PET/CT only, MRI and PET/CT at baseline with MRI only or PET/CT only at interim imaging, MRI followed by PET/CT, or MRI followed by PET/CT only under certain conditions (Figure 1). For every imaging scenario, the patient, tumour and scenario-specific imaging characteristics were entered into multivariate analyses (binary logistic regression with backward feature selection, p-to-remove: 0.10). Receiver operating characteristics (ROC) curves were acquired and areas under the curve (AUC) were assessed. Subsequently, patients were stratified according to breast cancer subtype. The AUC of the different scenarios were compared using the DeLong test\textsuperscript{26}. For this purpose, the scenario to monitor response using MRI only was used as a reference. ROC-curves were fitted using bi-exponential fitting\textsuperscript{27}, and an operating point at 90% specificity was selected to assess the accompanying sensitivity. In other words, the probability of correctly predicting a non-pCRmic was determined under the condition that the probability to correctly predict a pCRmic is at least 90%.
Results

Baseline patient and pathology characteristics

A total of 188 patients were included (mean age 47 years, range 25 – 73 years), baseline characteristics are shown in Table 1. According to ypTmic, which was used as pCRmic in this current study, overall 77/188 of patients (41%) achieved a pCRmic and a non-pCRmic was seen in 111/188 of patients (59%). Patients with pCRmic were significantly (p<0.001) younger (mean age: 44 years) than patients with non-pCRmic (mean age: 50 years).

Considering subgroups, for the ER-positive subgroup a pCRmic was seen in 11/87 patients (12.6%). Conversely, pCRmic was seen in 35/46 patients (76.1%) with HER2-positive tumours and in 31/55 patients (56.4%) with triple-negative tumours. No residual disease in the breast and axilla (ypT0/is ypN0) was seen in 26/46 (56.2%) of HER2-positive tumours, 4/87 (4.6%) of ER-positive tumours, and 22/54 (40%) of triple-negative tumours.

Baseline imaging

On baseline MRI, the mean tumour size was 47 mm (LD initial) and 39 mm (LD late) (Table 2). No significant differences in size were observed between tumours showing pCRmic versus non-pCRmic. On baseline PET/CT, a significant difference was found between SUV-max in the tumour and response at pathology; tumours resulting in pCRmic had higher SUV-max (10.3) compared to those not leading to pCRmic (8.2) (p=0.029). In addition, baseline SUV-max in the lymph nodes was higher in tumours resulting in pCRmic (5.7) than in those resulting in non-pCRmic (4.5), although this was not significant in the overall patient group (p=0.056).

<table>
<thead>
<tr>
<th>Patient and tumor characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scenario 1</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Baseline imaging</td>
</tr>
<tr>
<td>Interim imaging</td>
</tr>
</tbody>
</table>

Figure 1: The six potential imaging scenarios investigated to monitor response of tumours during neoadjuvant chemotherapy.
Table 1: Baseline patient and tumour characteristics prior to neoadjuvant chemotherapy (NAC).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>pCRmic</th>
<th>Non-pCRmic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (%)</td>
<td>188</td>
<td>77 (41%)</td>
<td>111 (59%)</td>
<td></td>
</tr>
<tr>
<td>Age (years), mean (sd)</td>
<td>47 (11)</td>
<td>44 (11)</td>
<td>50 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tumour stage prior to NAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>20</td>
<td>11</td>
<td>9</td>
<td>0.391</td>
</tr>
<tr>
<td>T2</td>
<td>116</td>
<td>47</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>43</td>
<td>17</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>9</td>
<td>2</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Nodal stage prior to NAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>40</td>
<td>15</td>
<td>25</td>
<td>0.862</td>
</tr>
<tr>
<td>N1</td>
<td>105</td>
<td>43</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>12</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>31</td>
<td>14</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDC</td>
<td>167</td>
<td>72</td>
<td>95</td>
<td>0.222</td>
</tr>
<tr>
<td>ILC</td>
<td>18</td>
<td>4</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Clinical Subtype, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-positive/HER2-negative (%)</td>
<td>87</td>
<td>11 (12.6%)</td>
<td>76 (87.4%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HER2-positive (%)</td>
<td>46</td>
<td>35 (76.1%)</td>
<td>11 (23.9%)</td>
<td></td>
</tr>
<tr>
<td>Triple negative (%)</td>
<td>55</td>
<td>31 (56.4%)</td>
<td>24 (43.6%)</td>
<td></td>
</tr>
</tbody>
</table>

Patient and tumour characteristics of all 188 patients versus pathological complete response (pCRmic) after NAC. sd: standard deviation. IDC = Invasive ductal carcinoma. ILC = Invasive lobular carcinoma. ER = Oestrogen receptor. HER2 = Human epidermal growth factor receptor 2.

Table 2: Imaging characteristics prior to neoadjuvant chemotherapy.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>pCRmic</th>
<th>Non-pCRmic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI baseline; curve type</td>
<td></td>
<td></td>
<td></td>
<td>0.212</td>
</tr>
<tr>
<td>Persisting</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Plateau</td>
<td>81</td>
<td>30</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Wash-out</td>
<td>107</td>
<td>47</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>MRI baseline; tumour size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD initial (mm); mean (sd)</td>
<td>47 (24)</td>
<td>46 (23)</td>
<td>47 (25)</td>
<td>0.691</td>
</tr>
<tr>
<td>LD late (mm); mean (sd)</td>
<td>39 (21)</td>
<td>38 (18)</td>
<td>40 (22)</td>
<td>0.608</td>
</tr>
<tr>
<td>PET/CT baseline; SUV-max</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUV-max tumour; mean (sd)</td>
<td>9.1 (6.0)</td>
<td>10.3 (7.2)</td>
<td>8.2 (4.7)</td>
<td>0.029</td>
</tr>
<tr>
<td>SUV-max lymph node; mean (sd)</td>
<td>5.0 (5.1)</td>
<td>5.7 (5.3)</td>
<td>4.5 (4.8)</td>
<td>0.056</td>
</tr>
</tbody>
</table>

Imaging characteristics at MRI and PET/CT plotted versus the pathological complete response (pCRmic) and non-pCRmic of tumours to neoadjuvant chemotherapy. LD initial = Largest tumour diameter on initial enhancement. LD late = Largest tumour diameter on late enhancement. SUV-max = Maximum standardized uptake value.
Monitoring primary tumour response to NAC using MRI and/or PET/CT

Figure 2: MRI and PET/CT imaging of different breast cancer subtypes. The top row shows MR subtraction images with color-coded visualization of contrast curves (persisting/green; plateau/blue; wash-out/red), the middle row shows maximum intensity projection of MR subtraction imaging, and the bottom row shows standardized uptake values on PET/CT imaging. For each example, imaging prior (left) and during (right) neoadjuvant chemotherapy is shown. Image (A) A 48-year-old women with an ER-positive invasive ductal carcinoma (IDC) showing a moderate response on MRI and PET/CT imaging, with a non-pathologic complete response (non-pCRmic) on final pathology. Image (B) A 52-year-old woman with a HER2-positive IDC showing a good response on MRI but a moderate response on PET/CT imaging, with a pCRmic on final pathology. Image (C) A 28-year-old woman with a triple-negative IDC showing a good response on MRI and PET/CT, with a pCRmic at final pathology.

Table 3: Imaging characteristics during NAC.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>pCRmic</th>
<th>Non-pCRmic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI Δ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD initial Δ (%); median (sd)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-42 (34)</td>
<td>-66 (33)</td>
<td>-26 (24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LD late Δ (%); median (sd)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-58 (38)</td>
<td>-82 (28)</td>
<td>-42 (35)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PET/CT Δ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUV-max tumour Δ (%); median (sd)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-53 (27)</td>
<td>-67 (17)</td>
<td>-43 (28)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SUV-max lymph node Δ (%); median (sd)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-65 (32)</td>
<td>-74 (31)</td>
<td>-57 (31)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Relative change (Δ) of largest tumour diameter on initial (LD initial) and late (LD late) enhancement on MRI ([LD interim – LD baseline / LD baseline] x 100%) and relative change of the maximum standardized uptake value (SUV-max) on PET/CT ([SUV-max interim – SUV-max baseline / SUV-max baseline x 100%] plotted versus pathological complete response (pCRmic) after NAC.
**Interim imaging**

During NAC, the relative change in size of tumours on MRI that reached pCRmic after NAC was significantly larger than the change in those that did not reach pCRmic (p<0.001) (Table 3). This was observed at initial enhancement (-66% change versus -26% change) as well as at late enhancement (-82% versus -42%) (Table 3). On PET/CT, the relative change in SUV-max of tumours resulting in pCRmic after NAC versus those resulting in non-pCRmic was significantly larger (-67% versus -43%; p<0.001). A comparable observation was made for changes in SUV-max in the lymph nodes (-74% versus -57%; p=0.001). Examples of MRI and PET/CT imaging are shown in Figure 2.

**Scenarios**

An overview of the optimal model per scenario is given in table 4. At interim imaging, the models resulting from scenarios 1 and 2 are identical, suggesting that baseline information from PET/CT does not add value to response monitoring without interim PET/CT. Comparable observations were found for scenarios 5 and 6: without interim MRI, baseline MRI does not add complementary information.

The AUC and confidence intervals of the models are shown in table 5. At interim imaging, in the overall group, MRI appears to yield the strongest predictor of tumour response to NAC. When considering MRI as the reference, no other scenario yielded obviously superior performance.

For HER2-positive tumours, MRI was also the strongest predictor, performing significantly better than PET/CT. For this subtype, PET/CT was not found to have additional value. With scenario 1 (MRI only), at an operating point of 90% specificity, a sensitivity of 36.2% was achieved (Figure 3).

For ER-positive tumours, a favourable performance was seen from adding PET/CT to MRI, although no significant difference was seen to the MRI only scenario. Monitoring using PET/CT only also yielded favourable performance over that using MRI only. With scenario 4 (MRI combined with PET/CT in incomplete responders), at the 90% operating point, a sensitivity of 55.8% was achieved.

For triple-negative tumours only very small differences were seen between the different scenarios. With scenario 1 (MRI only), at a 90% specificity, a sensitivity of 45.5% was achieved.
### Table 4: Characteristics remaining in the scenario models.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Characteristics</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scenario 1</td>
<td>Age</td>
<td>0.961</td>
<td>0.925 – 0.998</td>
</tr>
<tr>
<td>B: MRI</td>
<td>Clinical subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I: MRI</td>
<td>Triple-negative</td>
<td>reference</td>
<td></td>
</tr>
<tr>
<td>ER-positive</td>
<td>0.150</td>
<td>0.056 – 0.402</td>
<td></td>
</tr>
<tr>
<td>HER2-positive</td>
<td>1.147</td>
<td>0.401 – 3.282</td>
<td></td>
</tr>
<tr>
<td>LD initial Δ</td>
<td>0.171</td>
<td>0.030 – 0.975</td>
<td></td>
</tr>
<tr>
<td>LD late Δ</td>
<td>0.126</td>
<td>0.027 – 0.580</td>
<td></td>
</tr>
<tr>
<td>Scenario 2</td>
<td>Age</td>
<td>0.961</td>
<td>0.925 – 0.998</td>
</tr>
<tr>
<td>B: MRI &amp; PET/CT</td>
<td>Clinical subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I: MRI</td>
<td>Triple-negative</td>
<td>reference</td>
<td></td>
</tr>
<tr>
<td>ER-positive</td>
<td>0.150</td>
<td>0.056 – 0.402</td>
<td></td>
</tr>
<tr>
<td>HER2-positive</td>
<td>1.147</td>
<td>0.401 – 3.282</td>
<td></td>
</tr>
<tr>
<td>LD initial Δ</td>
<td>0.171</td>
<td>0.030 – 0.975</td>
<td></td>
</tr>
<tr>
<td>LD late Δ</td>
<td>0.126</td>
<td>0.027 – 0.580</td>
<td></td>
</tr>
<tr>
<td>Scenario 3</td>
<td>Clinical subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B: MRI &amp; PET/CT</td>
<td>Triple-negative</td>
<td>reference</td>
<td></td>
</tr>
<tr>
<td>I: MRI &amp; PET/CT in all patients</td>
<td>ER-positive</td>
<td>0.200</td>
<td>0.063 – 0.633</td>
</tr>
<tr>
<td>HER2-positive</td>
<td>2.208</td>
<td>0.607 – 8.028</td>
<td></td>
</tr>
<tr>
<td>SUV-max tumour Δ</td>
<td>0.032</td>
<td>0.003 – 0.359</td>
<td></td>
</tr>
<tr>
<td>LD late MRI</td>
<td>0.100</td>
<td>0.023 – 0.434</td>
<td></td>
</tr>
<tr>
<td>Scenario 4</td>
<td>Clinical subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B: MRI &amp; PET/CT</td>
<td>Triple-negative</td>
<td>reference</td>
<td></td>
</tr>
<tr>
<td>I: MRI &amp; PET/CT in patients with incomplete response on MRI</td>
<td>ER-positive</td>
<td>0.235</td>
<td>0.069 – 0.803</td>
</tr>
<tr>
<td>HER2-positive</td>
<td>3.277</td>
<td>0.689 – 15.592</td>
<td></td>
</tr>
<tr>
<td>LD late Δ</td>
<td>0.155</td>
<td>0.030 – 0.801</td>
<td></td>
</tr>
<tr>
<td>SUV-max tumour Δ</td>
<td>0.017</td>
<td>0.001 – 0.324</td>
<td></td>
</tr>
<tr>
<td>Scenario 5</td>
<td>Clinical subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B: MRI &amp; PET/CT</td>
<td>Triple-negative</td>
<td>reference</td>
<td></td>
</tr>
<tr>
<td>I: PET/CT</td>
<td>ER-positive</td>
<td>0.256</td>
<td>0.089 – 0.740</td>
</tr>
<tr>
<td>HER2-positive</td>
<td>4.902</td>
<td>1.484 – 16.195</td>
<td></td>
</tr>
<tr>
<td>SUV-max tumour Δ</td>
<td>0.017</td>
<td>0.002 – 0.157</td>
<td></td>
</tr>
<tr>
<td>Scenario 6</td>
<td>Clinical subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B: PET/CT</td>
<td>Triple-negative</td>
<td>reference</td>
<td></td>
</tr>
<tr>
<td>ER-positive</td>
<td>0.256</td>
<td>0.089 – 0.740</td>
<td></td>
</tr>
<tr>
<td>HER2-positive</td>
<td>4.902</td>
<td>1.484 – 16.195</td>
<td></td>
</tr>
<tr>
<td>SUV-max tumour Δ</td>
<td>0.017</td>
<td>0.002 – 0.157</td>
<td></td>
</tr>
</tbody>
</table>

Characteristics remaining in scenario 1 to 6, with corresponding odds ratios (OR) and 95% confidence intervals (CI). B = Baseline imaging. I = Interim imaging. LD initial = Largest tumour diameter on initial enhancement. LD late = Largest tumour diameter on late enhancement. SUV-max = Maximum standardized uptake value. Δ = Relative change.
Table 5: Area under the curve (AUC) and 95% confidence interval (95% CI) of all scenario models.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>AUC (95% CI)</th>
<th>Overall p-value</th>
<th>HER2-positive p-value</th>
<th>ER-positive p-value</th>
<th>Triple-negative p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scenario 1</td>
<td>0.894 (0.847)</td>
<td>0.735 (0.534)</td>
<td>0.742 (0.571)</td>
<td>0.855 (0.758)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Versus</td>
<td>0.894 (0.847)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scenario 2</td>
<td>0.890 (0.843)</td>
<td>0.688 (0.508)</td>
<td>0.795 (0.674)</td>
<td>0.864 (0.768)</td>
<td>0.250</td>
<td>0.250</td>
</tr>
<tr>
<td>Scenario 3</td>
<td>0.892 (0.846)</td>
<td>0.708 (0.513)</td>
<td>0.818 (0.704)</td>
<td>0.868 (0.775)</td>
<td>0.250</td>
<td>0.250</td>
</tr>
<tr>
<td>Scenario 4</td>
<td>0.868 (0.816)</td>
<td>0.543 (0.362)</td>
<td>0.791 (0.668)</td>
<td>0.844 (0.737)</td>
<td>0.250</td>
<td>0.250</td>
</tr>
<tr>
<td>Scenario 5</td>
<td>0.868 (0.816)</td>
<td>0.543 (0.362)</td>
<td>0.791 (0.668)</td>
<td>0.844 (0.737)</td>
<td>0.250</td>
<td>0.250</td>
</tr>
</tbody>
</table>

The AUC of the interim scenarios were compared using scenario 1 (MRI only) as a reference. *Significant difference compared to scenario 1.

Figure 3: Fitted receiver operating characteristics (ROC) curves of the optimal imaging scenario for HER2-positive, ER-positive and Triple-negative tumours. Image A) ROC-curve of scenario 1 (MRI only) in HER2-positive tumours. Image B) ROC-curve of scenario 4 (MRI combined with PET/CT in incomplete responders) in ER-positive tumours. Image C) ROC-curve of scenario 1 (MRI only) in triple-negative tumours.
Discussion

The aim of this study was to explore guidelines in monitoring tumour response to NAC, taking breast cancer subtype into account and using different imaging scenarios: MRI only, PET/CT only, or a combination thereof. To pursue this aim, MRI and PET/CT were performed both prior to NAC as well as during NAC. Post-hoc analyses were performed to assess and compare the efficacy of scenarios. By systematically considering all combinations at different therapeutic windows in the clinical workflow, we found that the optimal imaging scenario depends considerably on breast cancer subtype.

For HER2-positive tumours, monitoring of tumour response to NAC was most accurately accomplished using MRI only. Approximately one third of the patients (36.2%) who did not achieve pCRmic could be identified at the cost of incorrectly assuming residual disease in 10% of the patients. PET/CT performed significantly less accurate (p=0.04), while combination of these techniques did not show obvious improvement.

For triple-negative tumours, monitoring of response was also most accurately accomplished using MRI only. Approximately half the number of patients (45%) who did not achieve pCRmic could be identified, at the cost of incorrectly assuming residual disease in 10% of patients. For these tumours, small difference was seen between the performance of PET/CT and MRI. This suggests that PET/CT is an appropriate alternative to MRI for patients with triple-negative tumours with contraindications for MRI.

For ER-positive tumours, PET/CT showed slightly favourable performance compared to MRI, and results suggest that response monitoring of ER-positive tumours may be optimized by combining MRI with PET/CT. Using this latter scenario, half the number of patients without pCRmic could be identified while residual disease was incorrectly assumed in 10% of the patients. However, even though relatively large numbers of ER-positive tumour patients were included, no significant differences were yet found.

It is widely recognized that the different breast cancer subtypes prompt different treatments, variant responses to treatment, are linked to different prognosis, and as seen in this current study different subtypes are also linked to different optimal imaging scenarios.

In prior studies, the strictest definition of pCRmic (i.e., no residual invasive disease in the breast or axilla: ypT0 ypN0) was found to be associated with increased disease-free and overall survival in subgroups of patients, mostly so in HER2-positive and triple-negative tumours. However, in this study we chose pCRmic: complete absence of tumour cells or presence of only a small number of scattered invasive cells in the resection specimen (ypTmic) as the endpoint of this study was not survival, but rather assessment of sensitivity and specificity of imaging for response monitoring. Second, the association between ypT0 ypN0 and survival has not been shown for luminal A
tumours, which comprise the largest subgroup of breast cancers, among which the ER-positive tumours. Thirdly, as comparable numbers of ypT0 ypN0 were found compared to the results of other studies: 5-10% of ER-positive tumours, 20-30% of triple-negative tumours, and 30-65% of HER2-positive tumours (treated with a combination of NAC and trastuzumab)\textsuperscript{28-31}, there was insufficient power to use ypT0 ypN0 as endpoint in ER-positive tumours in this study. Using ypTmic, tumour response was found in 11/87 ER-positive tumours (12.6%), providing sufficient power to assess the sensitivity of MRI, PET/CT and combination thereof. In future studies, other study endpoints could be considered, such as the possibility for breast conserving surgery following NAC, as improvement of surgical options is still one of the major reasons to consider NAC\textsuperscript{32}.

Future studies could also consider the inclusion of diffusion-weighted MR imaging (DW-MRI), as promising results have been shown in the use of DWI to monitor early tumour response of breast cancers to NAC\textsuperscript{33}. For PET/CT imaging, the SUV-max of tumours and lymph nodes were evaluated because these are most commonly assessed in clinical practice. However, future study could consider other imaging characteristics such as the total lesion glycolysis\textsuperscript{34}. Also, the use of $^{18}$F-fluoroestradiol or $^{89}$ZR-trastuzumab could be considered for PET/CT response monitoring in certain breast cancer subtypes\textsuperscript{35,36}. In addition, future studies could focus on automated techniques to extract complementary information from MRI and PET/CT to monitor breast cancer response\textsuperscript{37}.

**Conclusion**

For imaging response of breast cancer to neoadjuvant chemotherapy, MRI was found optimal to monitor response for HER2-positive and triple-negative tumours. For HER2-positive tumours, MRI has an advantage over PET/CT imaging as well as over combined techniques. However, for triple-negative tumours, PET/CT is an appropriate alternative in patients with contraindications for MRI. For ER-positive tumours, PET/CT shows favourable performance over MRI, and combining PET/CT with MRI could provide optimal response monitoring. However, even though relatively large numbers of ER-positive tumour patients were included, significant differences could not yet be shown.

**Acknowledgments**

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References


Chapter 5
Additional value of $^{18}$F-FDG PET/CT response evaluation in axillary nodes during neo-adjuvant therapy for triple-negative and HER2-positive breast cancer

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Abstract

Purpose
$^{18}$F-FDG PET/CT can monitor metabolic activity in early breast cancer during neo-adjuvant systemic therapy (NST), but it is unknown if the metabolic breast and axillary response differ. We evaluated the correlation between metabolic breast and axillary response at various time points during NST. Furthermore, we analysed if the combined metabolic response improves pathologic complete response (pCR) prediction compared to using the metabolic breast response alone.

Methods
$^{18}$F-FDG PET/CT was performed at baseline (PET1), 2-3 weeks (PET2), and 6-8 weeks (PET3) of NST in patients with triple-negative (TN) and HER2-positive node-positive breast cancer. SUVmax and ΔSUVmax were determined separately for breast and axilla. Spearman’s correlation coefficients (r) between both localisations were calculated. The accuracy of pCR total (ypT0/is,ypN0) prediction using the metabolic response in breast, axilla or both was examined using logistic regression analysis.

Results
Hundred-five patients were included: 45 TN and 60 HER2-positive tumours. The metabolic response in breast and axilla correlated moderately in TN tumours (r=0.57) using ΔSUVmax between PET1-PET3 and poorly in HER2-positive tumours (r=0.49) using SUVmax at PET2. In TN tumours, metabolic breast response predicted pCR well without improvement after adding axillary response (c-index 0.82 versus 0.85, p=0.63). In HER2-positive tumours, metabolic breast response predicted pCR poorly with improvement after adding axillary response (c-index 0.64 versus 0.72, p=0.06).

Conclusions
$^{18}$F-FDG PET/CT response during NST differs between breast and axilla. In TN tumours, pCR total prediction can be made independent of metabolic axillary response. In HER2-positive tumours, axillary response may improve pCR total prediction. These findings may help guide PET/CT-response-based changes during NST. Trial registration: NTR NTR1797. Registered 29 May 2009, retrospectively registered.

Key words: breast cancer, $^{18}$F-FDG PET/CT, neo-adjuvant treatment, early response monitoring
Background

Neo-adjuvant systemic treatment (NST) is increasingly used in early breast cancer to allow downstaging of the primary tumour to facilitate breast-conserving surgery. Initially tumour-positive lymph nodes may convert into tumour-negative lymph nodes during NST, which permits less aggressive treatment of the axilla as well. In vivo response monitoring and adapting ineffective therapy regimens may become important additional assets of a neo-adjuvant approach.

Magnetic resonance imaging (MRI) is increasingly used as standard of care for response evaluation in the breast during NST in the Netherlands. Functional imaging with radiolabelled fluor-18-deoxyglucose (18F-FDG) positron emission tomography combined with computed tomography (PET/CT) can visualise the glucose metabolism in the primary tumour and affected lymph nodes. Furthermore, detection of changes in tumour glucose metabolism in response to treatment enables early response monitoring. Optimal long-term outcome is seen after pathologic complete response in breast and axilla (pCR total) but the sensitivity to NST may differ between both sites. Nevertheless, most previous neoadjuvant PET/CT studies focussed on the metabolic response of the breast alone. Substantially fewer studies evaluated the early metabolic response of the axilla, the combined response in breast and axilla or the agreement between both.

Therefore, the aim of our study, performed in HER2-positive and triple-negative (TN) breast cancer patients, was twofold. First, we assessed the correlation between the metabolic response in breast and axilla. Second, we evaluated the additional value of incorporating the metabolic axillary response over the breast response alone in predicting pCR total.

Methods

We performed a prospective single-centre study with sequential PET/CT scanning before and during NST in women with primary stage II-III HER2-positive or TN breast cancer. Patients were included from September 2008 until June 2014. The institutional review board approved the study protocol and all included patients provided written informed consent. Only patients with a visible primary tumour and affected lymph nodes at baseline PET/CT were included in this analysis. Forty-five of these patients were included in a previous report.

Pathological evaluation

At baseline, core biopsies were obtained from the primary tumour for pathologic diagnosis and oestrogen receptor, progesterone receptor, and HER2-status, according to Dutch national
guidelines (www.oncoline.nl). A marker was placed at the primary tumour site to guide surgery and pathologic evaluation. Breast conserving or ablative surgery was performed based on tumour characteristics, and patient’s preference. Baseline nodal status was assessed by physical, ultrasound, and PET/CT examination with by fine needle aspiration of suspicious lymph nodes. Biopsies of the primary tumour and fine needle aspiration of the lymph nodes were aimed to be obtained prior to baseline PET/CT. Patients with clinical node-negative disease underwent a sentinel node procedure (SNP) either before or after NST. In case of node-positive disease at baseline a level I-II axillary lymph node dissection was performed or the initially positive marked lymph node(s) was removed guided by marking the dominant axillary node(s) with radioactive iodine seeds (MARI-procedure).2 PCR was assessed by experienced breast pathologists, and was defined as no residual invasive tumour cells irrespective of in-situ lesions.6 PCR breast, pCR axilla, and their combination (pCR total) were determined.

**Treatment**

Patients with TN tumours received three cycles dose-dense doxorubicin/cyclophosphamide (AC) followed by MRI-evaluation. Patients with an unfavourable MRI response switched to three cycles capecitabine/docetaxel [CD] or three cycles carboplatin/paclitaxel [CP]22. Patients with a favourable response were randomized between three additional cycles of AC or CD/CP. Patients with homologous recombination deficient (HRD) tumours were randomized between three cycles CD/CP or an additional AC-cycle followed by intensified alkylating chemotherapy consisting of cyclophosphamide/thiотeпа/carboplatin (CTC). Patients with HER2-positive tumours received 24 cycles weekly paclitaxel/trastuzumab/carboplatin (PTC) with trastuzumab only in weeks 7, 8, 15, 16, 23, and 24.23 In case of an unfavourable MRI response after eight weeks of NST patients switched to four cycles 5-fluorouracil/epirubicin/cyclophosphamide/trastuzumab (FEC-T).

**PET/CT procedures**

A PET/CT was performed at baseline (PET1), after two to three weeks of treatment (PET2), and after six to eight weeks (PET3). Patients were instructed to fast for six hours prior to the scan and blood glucose levels were required to be <10mmol/L. Based on the patient’s body mass index 180-240MBq 18F-FDG was administered intravenously and 10mg diazepam was given orally to reduce 18F-FDG-uptake by brown fat. Following a resting period of 60 ± 10 minutes, in accordance with EANM procedure guidelines, a PET-scan (3.00 min per bed position and image reconstruction to 2x2x2mm voxels) of the thorax was performed according to the hanging breast protocol, using a whole-body scanner (Gemini TF; Philips, Cleveland, OH)24. A low-dose CT-scan (2mm slices) without intravenous contrast preceded the PET acquisition for anatomical localisation. In order to be able to make a valid comparison between scans within an individual and between
individuals the same imaging system and protocol including the target time interval between $^{18}$F-FDG injection and PET acquisition were used throughout the study. At baseline a standard supine whole-body PET/CT was performed as well as part of disease staging.

**Supplementary figure 1:** CONSORT diagram
**Image reading**

The acquired PET/CT images were evaluated by a panel of experienced reviewers (BK, MvR, ST), supervised by two nuclear medicine specialists (RVO, WV). All baseline scans were qualitatively assessed for sufficient $^{18}$F-FDG-uptake of the primary tumour and lymph node metastases, defined as the ability to visually distinguish known tumour locations from adjacent non-malignant tissue (i.e. pathological versus physiological uptake, respectively) with an estimated ratio of $>2.0$, to allow subsequent quantitative response evaluation. Quantitative $^{18}$F-FDG-uptake of the primary tumour and the most active level I-II axillary lymph node was measured as the maximum standardised uptake value (SUVmax) within a 3D region of interest (ROI). Level III lymph nodes were not included, as these are not routinely resected during axillary clearance. If the automated ROI generation was unreliable due to a low tumour-to-background ratio, the ROI was manually drawn. In case of a complete metabolic response on the subsequent scans the baseline ROI localisation was used for calculation of the SUVmax.

**Statistical Analyses**

All analyses were performed separately for TN and HER2-positive tumours. Descriptive statistics were used to outline patient, tumour, and treatment characteristics. For response analyses the most active axillary lymph node was included. The absolute SUVmax values at the different time points and the relative percentage changes in SUVmax (hereafter referred to as SUVmax and $\Delta$SUVmax respectively) were determined in breast and axilla, and their association was calculated using Spearman’s correlation coefficient ($r$). The association of the various PET/CT parameters at different time points with pCR was tested using logistic regression analyses and presented as the c-index (equivalent of the area under the curve [AUC] in ROC analyses). Correlation and c-index results were interpreted according to previously described classifications $^{25,26}$. The change in c-index when adding axillary response to a model including breast response alone was tested for significance based on the algorithm proposed by DeLong et al.$^{27}$.

Data were analysed using SPSS version 22.0 (SPSS Inc. Chicago, USA) and STATA (version 13; StataCorp, College Station, TX, USA). $P$-value of $<0.05$ was considered statistically significant. No adjustment for multiple testing was made.
### Table 1: Baseline and treatment characteristics according to subtype

<table>
<thead>
<tr>
<th></th>
<th>TN (n=45)</th>
<th>HER2+ (n=60)</th>
<th>All (n=105)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>50 (36-55)</td>
<td>45 (37-52)</td>
<td>47 (37-54)</td>
</tr>
<tr>
<td>Tumour size on MRI (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>31 (22-45)</td>
<td>38 (22-60)</td>
<td>33 (22-50)</td>
</tr>
<tr>
<td>Disease stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>19 (42%)</td>
<td>26 (43%)</td>
<td>45 (43%)</td>
</tr>
<tr>
<td>III</td>
<td>26 (58%)</td>
<td>34 (57%)</td>
<td>60 (57%)</td>
</tr>
<tr>
<td>Baseline axillary staging method</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive, pre-SNP&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Positive, FNA</td>
<td>44 (98%)</td>
<td>60 (100%)</td>
<td>104 (99%)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>13 (29%)</td>
<td>25 (42%)</td>
<td>38 (36%)</td>
</tr>
<tr>
<td>3</td>
<td>16 (36%)</td>
<td>14 (23%)</td>
<td>30 (29%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>16 (36%)</td>
<td>21 (35%)</td>
<td>37 (35%)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ductal</td>
<td>43 (96%)</td>
<td>55 (92%)</td>
<td>98 (93%)</td>
</tr>
<tr>
<td>Lobular</td>
<td>0 (0%)</td>
<td>4 (7%)</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>HR-status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER- and PR-</td>
<td>45 (100%)</td>
<td>29 (48%)</td>
<td>74 (71%)</td>
</tr>
<tr>
<td>ER+ and/or PR+</td>
<td>0 (0%)</td>
<td>31 (52%)</td>
<td>31 (30%)</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45 (100%)</td>
<td>0 (0%)</td>
<td>45 (43%)</td>
</tr>
<tr>
<td>PTC&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0 (0%)</td>
<td>60 (100%)</td>
<td>60 (57%)</td>
</tr>
<tr>
<td>PET assessment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PET1 performed</td>
<td>45 (100%)</td>
<td>60 (100%)</td>
<td>105 (100%)</td>
</tr>
<tr>
<td>PET2 performed</td>
<td>35 (78%)</td>
<td>45 (75%)</td>
<td>80 (76%)</td>
</tr>
<tr>
<td>PET3 performed</td>
<td>38 (84%)</td>
<td>46 (78%)</td>
<td>84 (80%)</td>
</tr>
</tbody>
</table>

TN, triple-negative; HER2+, HER2-positive; n, number of patients; PA, pathology; SNP, sentinel node procedure; FNA, fine needle aspiration; ER, oestrogen receptor; PR, progesterone receptor; DC, doxorubicin/cyclophosphamide; PTC, paclitaxel/trastuzumab/carboplatin.

<sup>a</sup>SNP performed before PET1, but remaining positive axillary lymph node in situ outside surgical region

<sup>b</sup>Nineteen patients switched treatment after PET3: six to capecitabine/docetaxel, ten to high-dose carboplatin/thiotepa/cyclophosphamide, three to paclitaxel (+/- carboplatin)

<sup>c</sup>Two patients received paclitaxel/trastuzumab/carboplatin plus pertuzumab, and one patients switched to 5-fluorouracil/epirubicin/cyclophosphamide plus trastuzumab after PET3
### Table 2: Correlation coefficients between the metabolic response in breast and axilla with different SUVmax variables according to subtype

<table>
<thead>
<tr>
<th></th>
<th>TN ((n=45))</th>
<th>HER2+ ((n=60))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>median (\text{IQR})</td>
<td>(r) (\text{median (\text{IQR})})</td>
</tr>
<tr>
<td>SUVmax PET1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>10.7 ((6.5 - 16.5))</td>
<td>0.42</td>
</tr>
<tr>
<td>Axilla</td>
<td>8.0 ((4.9 - 13.8))</td>
<td></td>
</tr>
<tr>
<td>SUVmax PET2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>7.9 ((5.1 - 10.0))</td>
<td>0.36</td>
</tr>
<tr>
<td>Axilla</td>
<td>4.2 ((3.1 - 7.2))</td>
<td></td>
</tr>
<tr>
<td>SUVmax PET3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>3.5 ((2.5 - 5.0))</td>
<td>0.33</td>
</tr>
<tr>
<td>Axilla</td>
<td>2.1 ((1.3 - 3.6))</td>
<td></td>
</tr>
<tr>
<td>ΔSUVmax (% PET1-PET2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>-32% ((-49 - -16))</td>
<td>0.49</td>
</tr>
<tr>
<td>Axilla</td>
<td>-33% ((-58 - -13))</td>
<td>-56% ((-70 - -38))</td>
</tr>
<tr>
<td>ΔSUVmax (% PET1-PET3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>-67% ((-77 - -49))</td>
<td>0.57</td>
</tr>
<tr>
<td>Axilla</td>
<td>-70% ((-84 - -48))</td>
<td>-66% ((-79 - -50))</td>
</tr>
</tbody>
</table>

TN, triple-negative; HER2+, HER2-positive; \(n\), number of patients; IQR, interquartile range; \(r\), Spearman’s correlation coefficient

### Table 3: C-indices (95% confidence interval) for the prediction of pathologic complete response by metabolic response in TN and HER2-positive breast cancer

<table>
<thead>
<tr>
<th>Pathologic complete response</th>
<th>Breast</th>
<th>Axilla</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN: ΔSUVmax PET1-PET3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>0.85 ((0.72 - 0.98))</td>
<td>0.83 ((0.69 - 0.98))</td>
<td>0.82 ((0.66 - 0.98))</td>
</tr>
<tr>
<td>Axilla</td>
<td>0.82 ((0.68 - 0.95))</td>
<td>0.82 ((0.68 - 0.97))</td>
<td>0.83 ((0.67 - 0.98))</td>
</tr>
<tr>
<td>Breast + axilla</td>
<td>0.86 ((0.74 - 0.98))</td>
<td>0.86 ((0.72 - 0.99))</td>
<td>0.85 ((0.69 - 1.00))</td>
</tr>
<tr>
<td>(p)-value*</td>
<td>0.78</td>
<td>0.60</td>
<td>0.63</td>
</tr>
<tr>
<td>HER2-positive: SUVmax PET2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>0.62 ((0.44 - 0.81))</td>
<td>0.65 ((0.47 - 0.84))</td>
<td>0.64 ((0.47 - 0.81))</td>
</tr>
<tr>
<td>Axilla</td>
<td>0.68 ((0.52 - 0.84))</td>
<td>0.77 ((0.62 - 0.92))</td>
<td>0.67 ((0.51 - 0.83))</td>
</tr>
<tr>
<td>Breast + axilla</td>
<td>0.72 ((0.56 - 0.89))</td>
<td>0.78 ((0.63 - 0.92))</td>
<td>0.72 ((0.57 - 0.88))</td>
</tr>
<tr>
<td>(p)-value*</td>
<td>0.11</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

\(p\)-value for the improvement in c-index by the addition of metabolic response in the axilla
Results

Baseline and treatment characteristics
In total 169 patients were included. Sixteen were ineligible because of stage I disease (n=5), stage IV disease (n=3), missing baseline PET/CT (n=4), or no trastuzumab use in case of HER2-positive disease (n=4). Of the remaining 153 patients, 105 had a primary tumour and positive axillary lymph nodes, both pathologically proven and visible on PET/CT. Forty-five patients had TN and 60 HER2-positive disease (supplementary figure 1). Positive nodal status was pathologically proven in all but one patient by fine needle aspiration (table 1). In this one patient lymph node metastases were detected by a pre-treatment SNP, however one positive axillary lymph node remained in-situ and showed 18F-FDG-uptake on PET/CT. Nineteen patients changed treatment after six to eight weeks of therapy (i.e. after PET3). In the TN subgroup, six patients changed because of insufficient MRI response and none of them achieved a pCR breast or pCR axilla. Eleven patients switched therapy according to study protocol (ten with an HRD tumour, and one without), and one patient switched because of patient’s preference. Of these twelve patients eight achieved pCR breast and six pCR axilla and pCR total. In the HER2-positive subgroup one patient changed treatment based on an insufficient MRI response. Neither pCR breast nor pCR axilla was achieved.

Surgery and pathologic response
With the exception of one patient with progressive disease during chemotherapy who refused further treatment, all patients underwent surgery. This patient was classified as having no pCR. Thus, 104 patients underwent breast surgery: 66 breast conserving and 38 ablative surgery. Pathologic axillary lymph node response was assessed by axillary lymph node dissection in 89, MARI-procedure in 13, and post-treatment SNP in two patients.

In TN tumours pCR breast was achieved in 53% (24/45), pCR axilla in 47% (21/45), and pCR total in 40% (18/45). In the HER2-positive subgroup the rate of pCR breast was 65% (39/60), pCR axilla 75% (45/60), and pCR total 57% (34/60). In total 25 patients had a discrepant pathologic response of the breast and axilla: 11 pCR breast/no pCR axilla, and 14 pCR axilla/no pCR breast.

Triple-negative disease
Baseline PET/CT was performed in all 45 patients with TN disease, PET2 in 35, and PET3 in 38. Thirty-two patients underwent three PET/CT-scans. The median time between last chemotherapy and PET2 was 13 days (interquartile range [IQR] 13-14), and between last chemotherapy and PET3 seven days (IQR 7-8). The median SUVmax and ΔSUVmax at the different time points are summarized in table 2, including correlation coefficients between metabolic response in breast and axilla. The best correlation between metabolic response in breast and axilla was found with
ΔSUVmax between PET1-PET3, and although all patients showed a decrease in ΔSUVmax in both locations at PET3 the correlation was moderate (r= 0.57) (supplementary figure 2a).

PCR breast prediction was most accurate using ΔSUVmax breast between PET1-PET3 (c-index 0.85) (supplementary table 1). Likewise, ΔSUVmax axilla between PET1-PET3 was best for pCR axilla prediction (c-index 0.82). The metabolic breast response, using ΔSUVmax between PET1-PET3, was well predictive for pCR total and the addition of metabolic response in the axilla using ΔSUVmax between PET1-PET3 did not further improve pCR total prediction (c-index 0.82 versus 0.85, p=0.63) (table 3).

HER2-positive disease
Baseline PET/CT was performed in all 60 patients with HER2-positive disease, PET2 in 45, and PET3 in 47. Forty patients underwent three PET/CT-scans. The median time between last chemotherapy and PET2 was six days (IQR 5-7), and between last chemotherapy and PET3 12 days (IQR 8-14). The best correlation between metabolic response in breast and axilla was found with SUVmax at PET2, although poor (r= 0.49) (supplementary figure 2b). In addition, an inverse response in terms of an increase in SUVmax in one location and a decrease or no difference in the other was observed in four patients at time of PET2.

The metabolic breast response poorly discriminates pCR from no pCR breast, with the best performance of ΔSUVmax breast between PET1-PET2 (c-index 0.64), although rather similar to the performance of SUVmax breast at PET2 (c-index 0.62) (supplementary table 2). SUVmax axilla at PET2 was best associated with pCR axilla (c-index 0.77). PCR total prediction using SUVmax breast at PET2 is poor but improved, although not statistically significant, to fair by including the metabolic axillary response using SUVmax axilla at PET2 (c-index 0.64 versus 0.72, p=0.06) (table 3).
Discussion

This study shows that the correlation between $^{18}$F-FDG PET/CT responses during NST in breast and axillary lymph nodes is moderate in triple-negative and poor in HER2-positive breast cancer. In TN disease, PET/CT response can be used to predict pCR and the breast response alone suffices to predict pCR total. Conversely, in HER2-positive disease, the accuracy of PET/CT to predict pCR is limited, while incorporating the metabolic response of both the breast and axilla may improve pCR total prediction.

Lymph node involvement at baseline and after NST is an important prognostic factor in non-metastatic breast cancer. Furthermore, pCR defined as no invasive tumour cells in breast and axilla is best related to long-term outcome. Despite this knowledge, many previous PET/CT studies evaluated the metabolic response of the breast alone to predict pCR total, without examining if the metabolic response of the primary tumour and lymph nodes is the same. Adding information about the metabolic response of axilla may aid to predict pCR total. Studies, that did evaluate the metabolic response in breast and axilla, used different strategies to combine response information of both locations to predict pCR total. Some evaluated the response of the baseline lesion with highest FDG-uptake alone and others used $\Delta$SUVmax between the lesion with the highest FDG-uptake at baseline and at the subsequent scan. However, information may be missed if the response differs between both sites or may result in comparing a breast lesion with an axillary lymph node or vice versa if the lesion with the highest FDG-uptake changes during treatment. Dalus et al. found different SUVmax measurements for breast and lymph nodes, possibly reflecting a different biological behaviour in these two sites, which may relate to selection of a sub-clone of tumour cells that spreads to the lymph nodes. Therefore, they proposed to evaluate the response of the primary tumour and axilla separately. We agree with this proposal until a valid combined variable has been established. Only a few studies have described the metabolic response in breast and axilla separately and its respective association with pCR breast and pCR axilla within the same cohort. These studies did not evaluate the correlation between the metabolic response in both locations. Therefore our study is unique and provides important new insights for PET/CT interpretation.

We found a moderate correlation between the metabolic breast and axillary response in TN breast cancer ($r=0.57$) without significant improvement in pCR total prediction with adding the metabolic axillary response to the breast response alone. This suggests that chemotherapy sensitivity in breast and axilla corresponds well. Therefore, the metabolic breast response alone suffices to guide NST decisions. In accordance with this, Groheux et al. did not find a better prediction of pCR total in TN disease if the axillary response was incorporated in addition to the breast response. Koolen et al. previously described a part of our study population and found
the strongest association between the combined metabolic breast and axillary response and pCR total with an AUC of 0.93 versus 0.87 for breast response alone. The statistical significance of this improvement was not tested. With the inclusion of additional patients in the current analysis, the association between the combined metabolic response and pCR total was somewhat weaker, although still good with a non-significant improvement using the combination over the breast alone (c-index 0.85 versus 0.82, p=0.63).

In HER2-positive breast cancer the metabolic responses in breast and axilla correlate poorly (r=0.49). The ability to predict pCR breast, and pCR total by the metabolic breast response was poor (c-index 0.62, and 0.64, respectively). The addition of metabolic response in the axilla improved the pCR total prediction compared to the use of breast response alone, which was statistically near significant (c-index 0.64 versus 0.72, p=0.06). Lack of statistical significance despite a relatively large increase in c-index, might be attributable to the small sample size, and larger studies are needed to determine the added value of including the metabolic response in both locations for pCR total prediction in this subtype. In line with our results, Groheux and colleagues found an improvement in pCR total prediction in node-positive patients if the axillary response was included. These and our findings suggest that if PET/CT is used for response monitoring in HER2-positive breast cancer, it should evaluate both breast and axilla, and we recommend separate evaluation of both sites rather than an unconfirmed combined parameter as described above. The use of targeted therapy in HER2-positive tumours may explain why the different response according to tumour location was more pronounced in this subtype, as it may differentially affect sub-clones with varying HER2-expression. Also, we cannot exclude that in selected cases non-specific 18F-FDG uptake related to regional inflammatory processes or tissue sampling may have contaminated the pathological uptake. Although we recognize this as a limitation of our study the impact on our results will be limited, especially after FNA. Furthermore, non-specific 18F-FDG uptake is likely to have affected both subtypes equally. Lastly, with the relatively small sample size we cannot exclude that the poor and moderate correlation of metabolic responses between locations is due to chance rather than a biological finding. However, despite only four inverse responses in the HER2-positive subtype, in relative terms, this constitutes 9% of HER2-positive cases with a PET2. Additionally, the poor correlation between metabolic and axillary response despite a decrease in both locations seems relevant as it may have implications for defining metabolic responders with different thresholds for different localizations.

In accordance with the literature we found that the best prognostic PET/CT response parameter for both pCR breast and pCR axilla is ΔSUVmax between baseline PET/CT and PET/CT after six weeks in TN tumours and the absolute SUVmax value at PET/CT after three weeks of therapy in HER2-positive tumours.
Our data reinforce that it is important to describe results according to breast cancer subtype due to different tumour behaviour. Subgroup analysis based on hormone receptor status within the HER2-positive cohort would have been valuable, but was not feasible due to the limited number of patients.

The inclusion of patients with sufficiently high baseline FDG-uptake for response evaluation, may have led to selection of relatively aggressive tumour types and an associated higher response rate reflecting the high pCR rate in our study. Nevertheless, sufficient baseline activity is required for PET/CT-evaluation and thus this selection reflects daily practice. Furthermore, a substantial number of patients with TN tumours switched therapy, and PET/CT-scans were only performed during the initially applied regimen. However, switches based on insufficient MRI response are assumed to have had little impact on our results as all these patients remained a pathological non-responder despite the change in treatment and it is unlikely that they would have achieved total pCR if they had continued their initially applied regimen.

Clear definitions of responders and non-responders will aid the clinical use of PET/CT during neo-adjuvant breast cancer treatment. The optimal cut-off value depends on several factors as described by others including treatment regimen, timing of evaluation, breast cancer subtype, and mainly depends on the purpose of the response evaluation: identifying non-responders to change ineffective treatment or identifying responders to reduce overtreatment. Several PET-parameters exist but no superiority of one over the other has been established so far. This study started in 2008 and we used the region with the highest metabolic activity (i.e. SUVmax) instead of the entire metabolically active tumour volume, which has been introduced more recently. However, SUVmax has important benefits as it is convenient to use and has good reproducibility.

PET/CT for response evaluation during NST in breast cancer is not the current standard of care and probably awaits a direct comparison with other imaging modalities. In the current study we focused on the use of PET/CT only and how to optimally use this to predict pCR total. Therefore, we cannot make a statement about the relative value of PET/CT compared to other imaging modalities, but this has been described by others. Nowadays, trastuzumab-labelled PET/CT scans are available with visualisation of HER2-positive lesions. This modality may improve selection of patients for anti-HER2 treatment, but its role in monitoring response is undetermined. Furthermore, trials to confirm the benefit of PET/CT-response-based treatment adaptations in terms of outcome are needed.
Conclusion

Our study demonstrates that the correlation between metabolic response in the breast and axilla is moderate in TN and poor in HER2-positive breast cancer. Furthermore, $^{18}$F-FDG PET/CT can be used to evaluate the response to neo-adjuvant chemotherapy in TN disease. The metabolic breast response alone, using $\Delta$SUVmax between PET/CT at baseline and after six weeks treatment, predicts pCR total well and adding metabolic axillary response has no additional value. In HER2-positive tumours, pCR total prediction by the metabolic breast response alone, using SUVmax at PET/CT after three weeks treatment, is poor. This may be improved by evaluating both the primary tumour and axillary lymph node metabolic response in this subtype, and separate evaluation is recommended.

Declarations

Ethical approval and consent to participate
This study protocol was approved by the institutional review board of the Netherlands Cancer Institute and informed consent was obtained from all individual participants included in the study.

Consent for publication
Not applicable.
Availability of data and material: The dataset generated and analysed during the current study is not publicly available because no informed consent was obtained to share data with third parties.

Competing interests
The authors declare that they have no conflicting interests.

Funding
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Acknowledgements

The authors thank the patients and their families for participating in this study, and the medical doctors and clinical research nurses for their effort and commitment.

No additional data are available upon request as all data are represented in the manuscript.

Supplementary figure 2: Correlation between the metabolic response in breast and axilla in (graph a) triple negative tumours (n=38; ΔSUVmax PET1-PET3) and (graph b) HER2-positive tumours (n=45; SUVmax PET2). LNNs=lymph nodes; pCR total=pathologic complete response in breast and axilla.
**Supplementary table 1: SUVmax variables according to pCR breast and pCR axilla and their prognostic value in triple-negative breast cancer.**

<table>
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<tr>
<th></th>
<th>no pCR breast</th>
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<td>median (IQR)</td>
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<td>median (IQR)</td>
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<tr>
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<tr>
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<tr>
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<td>0.72 (0.56-0.89)</td>
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<td>16</td>
<td>-57% (-66--33)</td>
<td>0.81 (0.66-0.96)</td>
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<td>∆SUVmax PET1-PET3</td>
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<td>-84% (-86--73)</td>
<td>0.82 (0.68-0.97)</td>
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pCR= pathologic complete response; n=number of patients; IQR=interquartile range; 95%CI=95% confidence interval; LNNs=lymph nodes
**Supplementary table 2: SUVmax variables according to pCR breast and pCR axilla and their prognostic value in HER2-positive breast cancer.**

<table>
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<td>7.5 (5.1-9.9)</td>
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<td>30</td>
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<td>-68% (-78--42)</td>
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<td>6.2 (2.8-7.6)</td>
<td>45</td>
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<td>0.51 (0.33-0.69)</td>
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<tr>
<td>SUVmax PET2</td>
<td>10</td>
<td>2.4 (2.2-3.6)</td>
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<td>-67% (-77--46)</td>
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pCR= pathologic complete response; n=number of patients; IQR=interquartile range; 95%CI=95% confidence interval; LNNs=lymph nodes
References


Additional value of 18F-FDG PET/CT response evaluation in axillary nodes during neo-adjuvant therapy


Part III
MAMMI-PET:
A new dedicated imaging modality
Chapter 6
Evaluation of a hanging-breast PET system for primary tumour visualization in patients with stage I-III breast cancer: comparison with standard PET/CT.

Published in Clinical Nuclear Medicine, April 2016
Abstract

Objective
To evaluate the performance of a dedicated PET for breast imaging in hanging breast position (MAMMI-PET) for the visualization of primary breast cancer lesions and compare this with whole body PET/CT images.

Materials and methods
Between March 2011 and March 2014 we prospectively included 230 female patients (age: mean 52 y, range 24-82y) with ≥1 histologically confirmed primary breast cancer lesion (=index lesion). This study was approved by the institutional review board (IRB) and all patients gave informed consent. After injection of 180-240 MBq ¹⁸F- FDG patients were scanned with whole body PET/CT and with MAMMI-PET. Index lesions scored 0, 1 or 2 for FDG uptake relative to background. Detection and FDG uptake were compared with breast length, maximal tumour diameter, affected breast quadrants, tumour grade, histological- and immunological subtypes. Finally, both PET-modalities were compared for the detection of index lesions.

Results
For 234 index lesions (diameter 5-170mm), the overall sensitivity was 88.9% for MAMMI-PET and 91% for PET/CT (p=0.61). MAMMI-PET missed twenty-three (9.8%) index lesions located too close to the pectoral muscle. Reversely, PET/CT missed 20 index lesions. Lesion visibility on MAMMI-PET was influenced by tumour grade (p=0.034) but not by cancer subtype (p=0.83).

Conclusions
Although, in an overall evaluation MAMMI-PET was not superior to PET/CT, the MAMMI-PET does have higher sensitivity for primary breast cancer lesions when located within the scanning range of the device. Optimization of the positioning device might increase the visualization of the most dorsal located lesions.

Key words: dedicated PET, breast cancer, oncology, molecular imaging, PET/CT.
Introduction

The clinical classification of primary breast cancer (cT-stage) is based on imaging modalities that are able to detect anatomical disruptions (mammography and ultrasound) or changes in blood perfusion (magnetic resonance imaging, MRI)\(^1\). Breast mammography is used to provide a first overview of the breast and is particularly sensitive for the detection of micro-calcifications\(^2\). Additionally, ultrasonography is able to distinguish invasive masses from non-invasive benign lesions with high accuracy\(^3\) and to guide biopsies for histopathological classification. MRI has a high sensitivity for breast lesion detection\(^4\,^5\) and is therefore preferred for more precise delineation of the primary tumour, including baseline imaging and response monitoring during pre-operative systemic treatment (PST). Nevertheless, due to its clinical value for many medical conditions and high costs, MRI cannot be part of the standard clinical work-up for breast cancer imaging. Ideally, breast cancer lesions would be detected, classified and biopsied using a single device.

Several molecular imaging devices have been developed over the past years to improve the detection of primary breast cancer lesions. Positron emission tomography with integrated computed tomography (PET/CT), using fluorine-18-fluorodeoxyglucose (\(^{18}\text{F-FDG}\)) is used in oncology for many purposes and is currently recommended for distant metastasis screening in stage II/III breast cancer\(^5\,^6\,^8\,^9\). Following the improved clinical value of prone position MRI imaging of primary breast cancer lesions, the value of prone position was also investigated for PET/CT acquisitions\(^10\,^11\). Prone position imaging has been reported to provide a more correct estimation of avid lesions in the breast but especially to more correctly define the N-stage\(^13\,^14\). Nevertheless, the clinical value of PET/CT imaging for primary breast cancer detection is limited as its sensitivity significantly decreases for tumours smaller than 1 cm\(^16\,^17\).

One of the first dedicated molecular imaging devices for breast imaging was positron emission mammography (PEM)\(^18\,^19\). Based on two-dimensional images this system has a sensitivity of 95%\(^20\) but requires positioning and compression of the breast similar to standard mammography\(^21\), while using dosages of 301-472 MBq of \(^{18}\text{F-FDG}\)\(^19\). Another dedicated imaging modality is breast-specific gamma imaging (BSGI) using \(^{99}\text{Tc-sestamibi}\), but the recommended tracer dosages deliver more radiation doses than to the patient than \(^{18}\text{F-FDG}\) for PET/CT imaging\(^22\). On the other hand, this method results in a sensitivity of around 90%\(^23\,^24\), but principally for lesions >1 diameter\(^25\). Responding to the need of identifying smaller breast cancer lesions with reduced radiation dose to the patient, the MAMMI-PET, a high-resolution full-ring system for dedicated hanging breast imaging without compression, was developed in the context of a EU-founded project to improve the detection of breast cancer. The original prototype, as described by Moliner et al. in 2010\(^26\), was used in a first clinical validation in 35 breast cancer patients\(^27\) and the first
clinical experience with the current, more patient- and user- friendly MAMMI-PET device was described by Martinez et al. and Koolen et al. 27–29.

The aim of the present study was to evaluate the performance of the MAMMI-PET in patients with a histologically confirmed primary breast cancer lesion and to compare results with whole-body PET/CT images. To this aim we performed a prospective trial in two European centres where patients were scanned with both modalities.

**Methods**

To analyse the results obtained with the MAMMI-PET and to later compare them with standard whole-body PET/CT we acquired the data in a standardized method. For the analysis of the MAMMI-PET data we used all information obtained to maintain a more heterogeneous group and for the comparison of both modalities we only used the data of patients that had undergone both scans to facilitate an inter-patient comparison of results.

![Figure 1: Image A](imageA.png)  The MAMMI-PET device with the PET ring down and the acquisition computer turned off. The bed is covered by soft cushions and can be moved up and down for patient comfort. The exact distance between opening of the acquisition ring and the surface of the cushions is 1cm. **Image B**: The MAMMI-PET device in action. Patient positioned with both arms down. Acquisition computer is displaying the details of the patients and the progress of the acquisition. **Image C**: Acquisition ring of the MAMMI-PET showing the transparent silicone sleeve marked every centimeter until 19 cm. This sleeve was used to measure the length of the breast.
**Patient inclusion**
Female patients >18 years with at least 1 pathologically proven breast cancer lesion, scheduled to receive pre-operative (systemic or regional radiation-) treatment and $^{18}$F-FDG PET/CT for distant metastases screening, were prospectively included in two European hospitals. At the Netherlands cancer Institute - Antoni van Leeuwenhoek Hospital (NKI), patients were first included from March 2011 in a MAMMI-PET feasibility study and subsequently, until March 2014, through two prospective trials. One trial included stage II/III patients scheduled to receive pre-operative systemic treatment (PST) and the second trial (PAPBI). In the PAPBI trial (Preoperative Accelerated Partial Breast Irradiation, registered with clinicaltrials.gov under number NCT01024582), women aged ≥60 years with unifocal invasive breast cancer with a maximal diameter of 30 mm are treated with preoperative partial breast irradiation. Prior to the start of radiotherapy a whole body PET/CT is performed to investigate its value for response monitoring after radiotherapy.

The results of the first 35 patients scanned with the MAMMI-PET have been previously reported by Koolen et al. The mentioned article described the heterogeneous $^{18}$F-FDG uptake within the primary tumour whereas in this manuscript we report the visibility and number of tumours that can be discriminated per affected breast. At the General University Hospital of Valencia (ERESA) patients with stage I-III breast cancer were included from December 201 until February 2014.

In both centres the additional MAMMI-PET acquisition directly followed the standard whole body PET/CT for the patients who gave written informed consent. All patients had been imaged with mammography, ultrasound (with ultrasound-guided biopsies), and MRI to characterize the tumour. The maximum diameter of the index lesion and the affected breast quadrants were derived from the modality used for clinical decision-making (MRI, sonography or mammography).

**Pathology analyses**
Biopsies were obtained and analysed within two weeks of PET/CT imaging. The core- biopsies of the primary tumour were used for pathology assessment. Briefly, tumours were classified and graded as described by the WHO: Oestrogen receptor (ER) and progesterone receptor (PR) status were considered positive if 10 % or more tumour cells showed nuclear staining in immunohistochemistry (IHC). HER2 was considered positive if the HER2 encoding gene was amplified as determined by silver in situ hybridization (SISH) after the HER2 IHC revealed a 2+ or 3+ score. Surrogate subtypes were derived from the pathology report according the St. Gallen consensus.

**PET/CT**
As a preparation for the PET/CT acquisitions patients fasted for 6 hours before administration of $^{18}$F-FDG and, following standards at our institute, all patients received oral administration of 10
mg diazepam to reduce uptake of $^{18}$F-FDG in brown fat. Blood glucose levels were required to be lower than 10 mmol/l. A dose of 180-240 MBq of $^{18}$F-FDG was injected intravenously, depending on the body mass index.

After a resting period of 60 ± 10 min PET/CT scans were made for distant metastases screening using a whole body PET/CT scanner (Gemini TF, Philips, Cleveland, Ohio, USA), scanning from skull base to groins in supine position (1.30 min per bed position), combined with a low dose CT scan (5 mm CT slices) for attenuation correction and tumour localization.

At the end of the inclusion period, the protocol of the trial was adjusted when patients for pre-operative radiotherapy were included and only a partial PET/CT acquisition was made using a different acquisition protocol. Therefore a small number of the patients included in the overview could not be added to the comparative analysis of MAMMI-PET with supine whole-body PET/CT.

**MAMMI-PET**

The MAMMI-PET device was provided by Oncovision (Valencia, Spain) on the basis of an unrestricted institutional grant for clinical validation by the participating centres. The participating institutions remained in control of the data registration and the analyses. Approximately 110 minutes after injection an acquisition of the breast was performed using the MAMMI-PET. During acquisition the patient was positioned face down on the acquisition table with the breast containing the tumour hanging freely in the opening of the MAMMI-PET device (Fig 1a).

MAMMI-PET uses 12 scintillation crystals coupled to position-sensitive photomultipliers for single breast imaging in hanging breast position. Beneath the acquisition table the acquisition ring (Fig 1b) is incorporated at an exact distance of 1 centimetre from the top of the opening in the acquisition table.

One ring position scans approximately 40 mm (30 mm not counting the overlap) of the breast (counted from the pectoral muscle) with possible extension of the axial field of view (FOV) to 170 mm. One ring position was programmed to take approximately 5 minutes and up to 6 ring positions (19 cm) are possible to implement, depending on the length of the breast (Fig 1c).

The images were reconstructed in 3D using a maximum likelihood expectation maximization algorithm with 12 iterations. All images were corrected for attenuation through image segmentation, scatter and decay. The acquisitions, reconstruction of the images, and data collection were performed using similar standardized methods.

**Image interpretation**

The proven breast cancer lesion with the highest $^{18}$F-FDG uptake and greatest diameter was classified as “index lesion”.
A consensus reading for both MAMMI-PET and PET/CT images was performed. Image reading was performed by three researchers with at least 2 years of experience (SCT, RSJ, BBK) together with two experienced nuclear medicine specialists (JFR, RVO). All images were initially interpreted by at least one researcher together with one experienced nuclear medicine specialist reaching a conclusion to be presented in a consensus meeting.

MAMMI-PET images were evaluated using orthogonal multi-planar projection (MPR) in axial, coronal, and sagittal views as well as by means of maximum intensity projection (MIP) images covering the whole volume of the tumour. PET/CT images were evaluated displaying the CT, PET and the fusion of images simultaneously using orthogonal multi-planar projection (MPR) in axial, coronal, and sagittal views.

First the index lesion was scored as whether it was visible or not visible. Secondly, $^{18}$F-FDG uptake relative to background of the index lesion was scored as follows (figure 2): 0= similar to surrounding breast tissue or tumour completely outside of the scanning range, 1= slightly more uptake than surrounding structures, and 2= (very) intense uptake. We calculated the tumour-to-background ratio (TBR) by measuring the SUVmax of the primary tumour and the SUVmean in an adjacent sufficiently large area of normal surrounding tissue. We used the TBR to illustrate the visual score as described above.

The position of the primary tumour in the breast (proximal, distal and medial) was visually determined. The distances from pectoral muscle to the nipple and to the centre of the tumour

![Figure 2: Sagittal MAMMI-PET image showing two FDG-avid lesions with different $^{18}$FDG uptake relative to background: score 2 (intense uptake; straight arrow) and score 1 (moderate uptake; dotted arrow). The dotted proximal uptake is due to the noise that is normally seen on a MAMMI-PET scan, at the edge of the acquisition ring.](image-url)
were measured on conventional PET/CT images in prone position. The tumours close to the pectoral muscle were scored as visible if they reached into the scanning field of the MAMMI-PET.

Additional lesions visualized by the MAMMI-PET were generally not specifically biopsied or followed in time as the MAMMI-PET is not yet incorporated into the clinical decision-making and therefore the detection of additional lesions could not have clinical consequences. However, we did investigate whether the additional lesions on MAMMI-PET had been visualized on other modalities and if a biopsy had been taken of the additional avid lesions.

**Statistical analyses**

The influence of the different variables on the visualization scores of the index lesions was analysed including: maximal tumour diameter, molecular and histological subtypes, tumour grade, breast length, affected breast quadrants, position in the breast, distance to the pectoral muscle and whether or not the primary tumour touched the pectoral muscle.

Of additional (non-index) lesions we assessed their visibility score (18F-FDG uptake relative to background) score, the maximal diameter of the lesion, position in the breast and whether they had been found to be malignant by pathology, or visualized on other modalities. The relation to the visibility score of the various variables were tested using the Fisher's exact test for categorical data and the Kruskal-Wallis test for continuous variables. To compare the results of MAMMI-PET and PET/CT we used pathologically confirmed number of lesions per breast as best comparable standard to perform a McNemar’s test. The level of significance used was 0.05 and no adjustments were made for multiple testing.

**Results**

In both centres a total of 230 patients was included, of who 4 presented with bilateral breast cancer. The primary characteristics are displayed in table 1. We found a mean TBR of 4.6 (range 1.18-10.5) for lesions with 18F-FDG uptake score 1 and a mean TBR of 16.4 (range 2.55-34.58) for lesions with score 2. The 234 index lesions scanned with MAMMI-PET included 11 lesions smaller than 10mm.

**Visualization of index lesions on MAMMI-PET**

The MAMMI-PET device missed 26 index lesions (18F-FDG uptake relative to background score=0) of which 23 were not situated within the scanning range and 13 touched the pectoral muscle. The distance from the index lesion to the pectoral muscle was significantly correlated with detection by the MAMMI-PET (p=<0.001). The index lesion closest to the pectoral muscle visualized with
the MAMMI-PET was located at a distance of 8 mm from the pectoral muscle; 80% of the index lesions at a distance of ≤0.8 cm was not visualized. At ≥1.5 cm distance 95% of the index lesions was visualized. Increase in breast length was significantly correlated with improved tumour visibility (P=0.014). The tumours located in the lower medial quadrant were most frequently visualized (p=0.021). The three tumours (1.3%) inside the scanning range but not detected by MAMMI were ER+/HER2-. The overall sensitivity was 88.9% ((234-26)/234) and increased to 98.6% ((211-3)/211) after exclusion of tumours outside the scanning range.

Table 1: Baseline characteristics.

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
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</tr>
</thead>
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<td>NKI</td>
<td>211</td>
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<tr>
<td>NAC trial</td>
<td>178</td>
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<tr>
<td>RTx trial</td>
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<td>ERESA</td>
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<table>
<thead>
<tr>
<th>Tumour diameter in mm</th>
<th>Mean (range) 26 (5-170)</th>
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</thead>
<tbody>
<tr>
<td>Histological subtypes:</td>
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<tr>
<td>Lobular</td>
<td>23 (9.8)</td>
</tr>
<tr>
<td>Ductal</td>
<td>200 (85.5)</td>
</tr>
<tr>
<td>Other</td>
<td>18 (7.7)*</td>
</tr>
</tbody>
</table>

| Molecular subtypes:     |                         |
| ER+/HER2-**             | 145 (61.1)              |
| Her2+                   | 41 (17.5)               |
| Triple-                 | 46 (19.7)               |

| Tumour grade*           | 31       |
| 1                       | 31       |
| 2                       | 131      |
| 3                       | 49       |

<table>
<thead>
<tr>
<th>Scanning bed-positions</th>
<th>Mean (range) 3,7 (1-6)</th>
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</table>

* A few patients had more than 1 diagnosis of the index lesion e.g. infiltrating ductal carcinoma with DCIS.
** One patient had PR positive ER negative and HER2 negative receptor status.*Only patients of the NKI were included.
The correlation of the chosen variables and the \(^{18}\text{F}-\text{FDG}\) score is displayed in Table 2. The maximal diameter of the index lesion was more associated with the visibility of the tumour \((p=0.036)\) than with the \(^{18}\text{F}-\text{FDG}\) uptake relative to background score \((0.055)\). The visibility score of all tumours was 0 in 26 patients \((11\%)\), 1 in 28 patients \((12\%)\) and 2 in 180 patients \((77\%)\) as shown in Table 1. The \(^{18}\text{F}-\text{FDG}\) uptake relative to background score of the index lesions was neither influenced by tumour grade \((p=0.21)\) nor by receptor status \((p=0.71)\) or histological subtype \((p=0.65)\). When considering the group of patients scanned at the NKI the tumour grade did influence the visibility of the index lesion \((p=0.034)\).

<table>
<thead>
<tr>
<th>Tumour characteristics</th>
<th>Score 0 (n\ (%))</th>
<th>Score 1 (n\ (%))</th>
<th>Score 2 (n\ (%))</th>
<th>Total (n\ (%))</th>
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<td></td>
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</tr>
<tr>
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<td>180 (85.3)</td>
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<td>Lobular</td>
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<td>5 (21.7)</td>
<td>17 (73.9)</td>
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<td>Ductal</td>
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<td>22 (11%)</td>
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<td>200 (85.5)</td>
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<td>ER+/Her2-***</td>
<td>15 (10.5)</td>
<td>18 (11.9)</td>
<td>112 (77.6)</td>
<td>145 (61.1)</td>
</tr>
<tr>
<td>Her2+</td>
<td>5 (12.2)</td>
<td>3 (7.3)</td>
<td>33 (80.5)</td>
<td>41 (17.5)</td>
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<tr>
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<tr>
<td>Position***:</td>
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<tr>
<td>Proximal</td>
<td>23 (20.2)</td>
<td>13 (11.4)</td>
<td>78 (68.4)</td>
<td>114 (48) (p&lt;0.001)</td>
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<tr>
<td>Median</td>
<td>9 (5.8)</td>
<td>16 (10.4)</td>
<td>129 (83.8)</td>
<td>154 (65) (p&lt;0.001)</td>
</tr>
<tr>
<td>Distal</td>
<td>1 (2)</td>
<td>7 (14.9)</td>
<td>41 (83.7)</td>
<td>49 (21) (p=0.040)</td>
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</table>
Visualization of additional avid breast lesions in ipsi-lateral breast by the MAMMI-PET

A total of 42 non-index FDG-avid lesions were detected by MAMMI-PET in 35 patients of which 7 had a visual score 1 and 36 a score 2. Six patients had two or more additional avid lesions besides the index lesions and one patient had three additional avid lesions.

Sixteen (39%) of all additional lesions had been pathologically confirmed to be malignant, 15 (36.6%) had been confirmed by other imaging modality of which pathology showed benign disease. 11 lesions (36.6%) were solely detected by MAMMI-PET.
Comparison MAMMI-PET and PET/CT

A total of 206 breast cancer patients underwent both standard whole body PET/CT acquisition and MAMMI-PET acquisition. In this group MAMMI-PET missed 23 index lesions of which 20 were situated outside its scanning range. The PET/CT missed 20 index lesions of which 16 were visualized by MAMMI-PET. We found an overall sensitivity of 89% (c.i. 0.84-0.93) for MAMMI-PET and 91% for PET/CT (c.i. 0.88-0.96); both scans did not perform significantly different from each other (p=0.61).

Of eleven patients who had an index lesion <1 cm in diameter, and where within the scanning range of the MAMMI-PET, only two were not visible. Of 8 patients with an index lesion <1 cm in diameter and scanned with PET/CT only one lesion was visible.

Of 19 patients with at least one additional proven lesion the extra lesion was seen on both scans in 8 patients, only seen on MAMMI-PET in 4 patients and both scans missed the additional lesion in 5 patients. In the remaining two patients PET/CT missed both lesions in one patient and in the other patient PET/CT identified only the index lesion and MAMMI-PET identified neither the index lesion nor the additional proven lesion.

Discussion

MAMMI-PET visualized 98.6% of primary breast cancer lesions when located within the scanning range of the device. Lesion visibility on MAMMI-PET was not evidently correlated with tumour size, histological subtype or hormone receptor status as is described for PET/CT. We found that lobular carcinomas, mostly responsible for false negatives on PET/CT, were just as well visualized by MAMMI-PET as ductal carcinomas. Based on resent studies on PET/CT imaging in breast cancer we already expected that if all index lesions would have been included in the scanning range only a minor percentage of ER+/Her2-lesions would have been missed 30. In this study, however, the number of ER+ tumours not detected by MAMMI-PET might still be explained by mere chance.

The overall visibility score of the index lesions was not influenced by tumour grade. However, more in agreement with recent PET/CT studies 30,34,35 the visibility of the index lesions increased with the tumour grade when only patients scanned at the Netherlands Cancer Institute were considered. This was, however, the only variable with missing data that could influence the result.

We found that a greater breast length enhanced the visibility by ameliorating the tumour background ratio as illustrated in figure 4. This is probably the result of a greater % of the breast consisting of (non-avid) fatty tissue. In one patient the additional FDG-avid lesion that had been classified by MRI as unspecific was visualized with MAMMI-PET and thus suspect to be malignant (Fig. 3). The clinical relevance of this finding, however, needs to be established in a larger series of
patients, preferably by FDG-guided biopsies. Also, lesion FDG-uptake scores should be correlated with FDG-guided biopsies for further characterization.

The present study has some limitations. The most important limitation concerns the missed lesions due to the restricted scanning range. Contrary to the first MAMMI-PET prototype27, the current device has an opening for the breast integrated in the examination table (image 1b) where the acquisition ring can be placed directly underneath. This change resulted in more stability and support for the patient compared to the previously used prototype. In a previous evaluation concerning the first MAMMI-PET prototype, with different patient examination table, tumours located at a distance of at least 3 mm 27 from the pectoral were detected. Consequently, a compromise needs to be found to improve this component of the MAMMI-PET. The examination table needs to be adjusted to obtain a more optimal scanning range while preserving the benefits of the new device. Our findings, however, are in line with other dedicated breast imaging devices, like the positron emission mammography (PEM), where tumours closer than 2 cm to the chest wall usually are not detected 18.

Another important shortcoming of this study is that the inclusion was limited to patients with a confirmed breast cancer lesion. Without the possibility to define false-positives we were not able to calculate the specificity and accuracy of the device. As we performed the inclusion in addition to two ongoing trials we have a smaller number of T1 tumours than can be expected at first diagnosis.

A final limitation of the study was the visualization score, which was merely based on a visual subdivision oriented to characterize malignant lesions, which may be influenced by inter-observer variability. As the score given to the MAMMI-PET images did not have a clinical consequence we did not award more value to visual score 1 in comparison to visual score 2, in our analyses. To support our findings using the visual score we calculated a tumour to background ratio (TBR), where we consistently found an overall higher TBR for visual score 2 in comparison to visual score 1.

Considering that the sensitivity for the primary tumour is similar to that of PET/CT we would like to suggest as a future perspective for MAMMI-PET its clinical use in patients with TxN0 breast cancer, who might benefit from a shorter acquisition and a higher sensitivity for smaller lesions, as is provided by the MAMMI-PET device. These advantages may be particularly useful for response monitoring during systemic treatment in patients without regional lymph node involvement, provided that they have already been investigated for $^{18}$F-FDG PET/CT 36–39, and that the index lesions are sufficiently FDG-avid at baseline36. Another future perspective following the satisfactory sensitivity for primary lesions is the development of a MAMMI-PET device with a module for FDG-guided biopsy enabling tissue sampling of the most FDG-avid parts of the
primary tumour for histopathology. This innovative device is currently being developed in the context of a project funded by the European Commission (http://www.mammocare.eu).

We conclude that the MAMMI-PET, a PET device for dedicated hanging breast single breast imaging without compression, has higher sensitivity for the detection of (small) breast cancer lesions than whole body PET/CT with less dependence of tumour size and tumour subtypes. However, due to technical aspects concerning its limited scanning range the overall detection rate of MAMMI-PET resulted not superior to PET/CT in this study. This technical issue needs to be optimized in order to enable visualization of the most dorsal located breast lesions. Further research should include a more heterogeneous group of patients prospectively to accurately determine the specificity and sensitivity of the MAMMI-PET.
**Figure 3:**
Image (A): Sagittal MAMMI-PET image showing two FDG-avid lesions.
Image (B): The smallest distal lesion (arrow) was classified as benign (BIRADS 2) on MRI.

**Figure 4:**
Sagittal MAMMI-PET images showing FDG-avid lesions in breasts with a maximal length of 4 cm on image (A), and 11 cm on image (B). On image (A) there is more activity visible in the active breast tissue, which is less prominent in image (B).
References


31. Esposito A. Highlights from the 14th St Gallen International Breast Cancer Conference 2015 in Vienna: Dealing with classification, prognostication, and prediction refinement to personalize the


Chapter 7
Dedicated breast PET (MAMMI-PET) in daily clinical practice: implications for radiation safety of nuclear medicine personnel

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Renato A Valdés Olmos, Department of Nuclear Medicine, NKI-AvL
Abstract

Aim
A dedicated breast PET (MAMMI-PET) for prone position imaging was recently introduced for better visualization of small breast cancer lesions. The aim of the present study was to evaluate radiation safety for nuclear medicine technologists (NMTs) for additional MAMMI-PET imaging using daily practice standards.

Methods
Five patients who underwent PET/CT before pre-operative systemic treatment (PST) or radiotherapy and gave informed consent for an additional MAMMI-PET scan between September and November of 2013 were included in this study. The additional scan was acquired between 90 and 120 min after injection of either 180 or 240 MBq $^{18}$F-FDG, depending on body mass index. Radiation doses were measured using a dose rate monitor (DRM) before acquisition and NMTs wore electronic personal μSv dosimeters (EPD) during patient related proceedings.

Results
Using the results of the DRM to predict the radiation dose at 1 m we found on average an increased expected dose during the PET/CT scan (4.4 μSv) compared to the MAMMI-PET scan (2.2 μSv). As a consequence of safety measures during PET/CT, the EPD showed a radiation dose of <1 μSv per PET/CT acquisition, but a mean of 0.6 μSv per MAMMI-PET acquisition.

Conclusions
The additional radiation dose for NMTs following possible implementation of a MAMMI-PET acquisition after PET/CT is expected to remain within levels marked by national and international legislations. Further reduction of this dose may be established with additional safety measurements.

Keywords: dedicated breast PET, $^{18}$F-FDG, breast cancer, radiation safety, personnel dosimetry
Introduction

In current clinical practice, the initial work-up of patients suspected of having breast cancer consists of characterizing the primary lesion with ultrasound and mammography. Ultrasound is frequently used as a guidance for biopsy of a suspicious lesion with fine needle aspiration for cytology. Mammography is the most frequently used modality for first visualization of the suspect breast and low-risk screening, but has a low sensitivity for detection of suspect lesions in dense breasts. With its sensitivity of nearly a 100%, magnetic resonance imaging (MRI) is preferred for the detection of multifocality and multicentricity, as well as for response monitoring during PST. MRI is also preferred for screening of young (high-risk) patients as it does not attribute to the additional radiation dose.

Positron emission tomography combined with computed tomography (PET/CT) using fluorine-18-fluorodeoxyglucose (18F-FDG) is reported to find a higher number of distant metastases when compared to conventional imaging and has therefore been added to the Dutch guidelines as the preferred modality for distant metastases screening in stage II/III breast cancer patients. The ability of 18F-FDG PET/CT to detect primary malignant lesions depends on the maximal diameter of the lesion as, due to the partial volume effect, the sensitivity for such lesions ≤10 mm is less optimal (<60%).

Against this background, the MAMMI-PET, a high-resolution dedicated PET for prone position imaging, was developed in a EU-founded project to overcome this low detection sensitivity. Indeed, this full ring detector device is able to detect smaller breast lesions and may require a lower 18F-FDG dose than regular whole body PET/CT. The radiation dose to the nuclear medicine technologist (NMT) related to an increased use of FDG PET/CT scanning is well known and, regarding literature, we expect the extra radiation doses due to additional imaging for NMT to be limited. However, as the NMT operating the MAMMI-PET device in the current setting needs to be in the same room as the patient during the acquisition, the radiation safety needs to be assessed. The aim of the present study was to evaluate the radiation safety of the possible implementation of the MAMMI-PET in current clinical practice while focusing on the radiation safety for NMTs.
Material and methods

Inclusion
At the Netherlands Cancer Institute (NKI), patients with stage II/III breast cancer receive PST in the context of several prospective studies, and are staged for distant metastases with conventional whole body $^{18}$F-FDG PET/CT as part of standard clinical work-up. Five consecutive patients planned to receive PST at the NKI between the 2nd of September 2013 and the 26th of November 2013, who gave informed consent for an additional MAMMI-PET scan, where included in this study. This study was approved by the local ethics committee.

Patient preparation and PET/CT acquisition
Patients were prepared for the $^{18}$F-FDG PET/CT according to EARL-guidelines\textsuperscript{19}; the patient was required to have fasted for 6 h and drink at least one l of water before the appointment at the nuclear medicine department. Blood glucose levels had to be lower than 10 mmol l$^{-1}$. Ten milligrams of diazepam were administered orally to reduce brown fat uptake of $^{18}$F-FDG\textsuperscript{20}, in consent with local regulations. Depending on body mass index, approximately 180 or 240 MBq $^{18}$F-FDG was administered intravenously using a syringe covered with 8 mm Tungsten, followed by 10 ml of 0.9% saline. The patient was then requested to rest for 60 min in a temperature-controlled room (23°C/73.4 °F).

Before start of the PET/CT acquisition the patient was asked to empty the bladder. The PET/CT acquisitions were made using a whole body PET/CT scanner (Gemini TF, Philips, Cleveland, Ohio, USA). The PET scan was performed from the base of the skull to the upper half of both femora in supine position (78 s per bed position) after a low dose CT scan (5 mm CT slices, 9 or 10 bed positions). Additionally, a prone position PET/CT scan was performed using a stripped MRI coil for hanging breast imaging and dedicated breast-imaging protocol (180 s per bed position, 3 bed positions).

MAMMI-PET
Following the PET/CT, an acquisition of the diseased breast was made using the MAMMI-PET (Oncovision, Valencia, Spain). The MAMMI-PET is a high-resolution dedicated breast PET system for single breast molecular imaging in hanging breast position. The detector consists of 12 monolithic LYSO-detectors\textsuperscript{17} (figure 1) and has a maximum of 6 ring positions, covering an axial field of view of 19 cm. The device was programmed to scan 5 min per ring position.
MAMMI-PET showed no implications for radiation safety of NMTs

Dose exposure measurements
Before each PET/CT or MAMMI-PET acquisition the dose rate emitted by the patient was measured at 1 m distance from the patient using a dose-rate monitor (DRM) in μS/h⁻¹ (RNI 10/SR, Berthold Technologies). The radiation exposure to the NMT during the patient preparation and during the PET/CT and MAMMI-PET acquisitions was measured using three electronic personal dosimeters (EPD) measuring in μSv (DMC 2000 XB, MGP instruments). During the ¹⁸F-FDG injection (by the nuclear medicine physician) and during patient transportation, patient positioning on the scans and during PET/CT and MAMMI-PET acquisitions (by the NMT), the person in charge wore a dosimeter to measure the exposure during these steps. After positioning the patient on the PET/CT bed, the NMT remained at more than 2 m distance from the PET/CT device behind a led shielded window. During the MAMMI-PET acquisition two dosimeters were placed (figure 2): one on the centre of the reconstruction computer (EPD 1), which was located at 1.5 m distance from the patient, and one on the centre of the acquisition computer (EPD2), where the NMT remains <1 min to verify the patient and acquisition-details before data acquisition start. After each patient related action the measured radiation dose, and the corresponding time frame were recorded by the NMT.

Figure 1:
Image (A): The MAMMI PET ring detector for hanging breast imaging with 12 LYSO-crystals
Image (B): the MAMMI PET acquisition computer and patient bed.
Figure 2: Position of the two electronic personal dosimeters (EPD1 and EPD2) in the MAMMI-PET acquisition room used during the scan. EPD1 was placed near the reconstruction PC at 1.5 m distance from the patient, where the NMT remained during the acquisition and EPD 2 was placed on the MAMMI-PET acquisition cart, near the patient.

Measurement analyses

We calculated the expected radiation dose at 1m distance for one MAMMI-PET procedure in comparison to one PET/CT procedure for the NMT in charge, using the results from the DRM and the time in minutes of the corresponding acquisition, in order to compare the estimation with the measured dose.

Figure 3: Cumulative doses per scan per patient. The cumulative doses depicted on the electronic personal dosimeter (EPD). Worn by the nuclear medicine physician during FDG injection, and by the nuclear medicine technologist (NMT) patient handlings and acquisition for the PET/CT (blue) and patient handlings and acquisition for the MAMMI-PET (yellow). Measurements are in μSv. During MAMMI-PET related handlings and acquisition of the fifth patient the measured dose was <1 μSv.
Results

MAMMI-PET scans were acquired approximately 2 h after injection (mean 108.2 min; range 91–120 min). The EPD the NMT was wearing during the FDG injection showed a mean radiation dose of 1.2 μSv per patient.

Total radiation dose NMT
The EPD worn by the NMT during the FDG injection showed a mean dose of 1.2 μSv per patient. The EPD showed a mean value of 0.2 μSv for patient handlings after the FDG injection and before start of the PET/CT acquisition (considered PET/CT related), and a mean dose of 0.8 μSv for patient handlings after the PET/CT and before start of MAMMI-PET acquisition (considered MAMMI-PET related). The accumulated NMT radiation doses measured with the EPD, during the separated patient handlings for both procedures, is shown in figure 3. The cumulative dose measured with the EPD after 5 patients was 7 μSv for the conventional PET/CT procedures (FDG injection, patient handling and acquisition), 1 μSv for the PET/CT acquisitions and handlings without taking into account the 18F- FDG injection and <1 μSv for just the acquisition. The cumulative dose was 7 μSv for the MAMMI-PET patient handlings and acquisitions and 3 μSv for the acquisition only. These cumulative doses resulted in a mean dose per patient, measured on the EPD of the NMT of 0.2 μSv for a PET/CT procedure, without taking into account the FDG injection performed by the nuclear medicine physician and 0.6 μSv for the MAMMI-PET acquisition.

Results DRM
The results of the measurements with the DRM are shown in table 1 under ‘DRM’. The expected doses (Exp) were calculated with the results from the DRM using the formula: Exp = (T*DRM)/60 min. The expected doses were generally higher for the PET/CT scan than for the MAMMI-PET scan (mean 4.4 versus 2.2). The radiation doses were only measurable during the MAMMI-PET acquisitions, but not during PET/CT acquisitions (<1 μSv) due to led shielding during the latter acquisitions. The columns ‘EPD’ correspond to the doses measured with the EPD during the acquisition of either the PET/CT or the MAMMI-PET, respectively.
Table 1: measurements

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<td>Mean</td>
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The radiation dose was measured at 1 m distance with the dose-rate monitor (DRM) in μSv h⁻¹, before start of PET/CT or MAMMI-PET. The expected doses (Exp) were calculated using the formula: \( \text{Exp} = \frac{T \times \text{DRM}}{60} \) min. The Exp was measured in μSv (Exp) during the acquisition time (T) in minutes. During the MAMMI-PET scan both electronic personal dosimeters (EPD1 and EPD2) were placed as depicted in figure 2: EPD1 was placed near the reconstruction PC, where the nuclear medicine technologist (NMT) was sitting, and EPD2 on the acquisition cart, near the patient.

Discussion

Adding a MAMMI-PET acquisition of the affected breast to the clinical protocol after F-FDG PET/CT for stage II/III breast cancer patients before start of PST would result in an additional radiation dose for the NMT of approximately 1.4 μSv per patient. A MAMMI-PET procedure consists of all concerned patient handlings and data acquisition.

When describing the radiation exposure to the NMTs, the patient is considered to be an external source that delivers a homogenous radiation dose from the whole body, as a result the radiation exposure of staff members is a direct consequence of patient handlings. With a dose of 370 MBq Pant et al²¹ found a dose-rate before start of PET/CT of 18.5 ± 3.9 μSv h⁻¹. With an ¹⁸F-FDG injection in the range between 180 and 240 MBq a dose rate of approximately 9.7–13 μSv h⁻¹ might be expected, which is an overestimation of the 8 μSv h⁻¹ we found in daily practice. Still, these differences could also be attributed to, for instance, the positioning of the DRM in relation to certain body parts. For the radiation dose measurements concerning all radiation-based handlings, the most commonly used doses found are around 20–25 nSv per injected MBq²². In our study this estimation would result in doses between 3.6 and 4.8 μSv for an ¹⁸F-FDG PET/CT procedure. As we did not include collecting the radionuclide and the preparation of the injection, the average dose of 1.4 μSv, measured for the NMT, was as expected.

Ultimately, the total exposure for the NMT will particularly depend on workload. In our hospital a total of 330 patients were treated with PST in 2013. The total additional radiation dose of assisting 330 MAMMI-PET patient handlings and acquisitions would be 0.46 mSv yr⁻¹. Dividing 0.46 mSv by 12 NMTs currently working at our department, would result in a total additional dose
of 0.04 mSv per NMT per year, which in itself would not compromise the maximum predefined cumulated allowed yearly dose of 6 mSv for NMTs by the Dutch and European radiation laws\textsuperscript{23,24}. Still, the added radiation dose related to the introduction of new technologies should always be considered in relation to the dose already received by employees, and assessed according the principle ‘as low as reasonably possible’ (ALARA). At our department the highest cumulative dose measured on the personal dosimeters worn by the technologists during work related activities in 2013 was 1.54 mSv.

In the current simulation the dosimeter positioned at 1.5 m from the patient during the MAMMI-PET acquisition showed a higher radiation dose than the dosimeter positioned at the working place of the NMT during the PET/CT acquisition. This implies that a possible further reduction of radiation dose might be achieved by implementing a led-shielded window or wall as is currently used for the PET/CT acquisitions or by increasing the distance between the MAMMI-PET acquisition cart and the desk at which the NMT assists the acquisition.

In this study the MAMMI-PET scans were acquired 2 hs after injection, resulting in an expected dose of approximately 100 MBq, when not considering the dose lost by micturition before start of the PET/CT scan and possibly before start of the MAMMI-PET. If the MAMMI-PET would be implemented as a stand-alone procedure, FDG-dose could be reduced to 100 MBq but acquisition would be made after a 60 min resting period; therefore, the NMT radiation dose is not expected to differ much from the radiation doses measured in the current setting. As the current setting was sufficient to provide good quality images we do not expect that with half a dose and acquisition after 60 min will have a negative effect on the image quality. If 100 MBq could be implemented for MAMMI-PET acquisitions the mean dose of 1.2 μSv found in the current measurements for the nuclear medicine physician responsible for the \textsuperscript{18}F- FDG administration is expected to be halved at least.

The relatively low effective doses received by the NMT when assisting the MAMMI-PET acquisitions are encouraging for the possible implementation of a MAMMI-PET device with a module for precision FDG-guided biopsy, which is currently being developed in the context of a project funded by the European Commission\textsuperscript{25}. The mean doses of 0.6 μSv (reconstruction PC) and 4.4 (next to the patient) found for a MAMMI-PET acquisition of approximately 23 min may result in an estimation in the range of 3.6 to 26 μSv for a 2 hr biopsy procedure. For the radiologist in charge of performing the biopsy, taking into account that he or she remains continuously near the patient, a dose of approximately 26 μSv may be expected. Recent literature on FDG PET/CT guided procedures shows a median effective dose of 20 μSv (0.02 mSv)\textsuperscript{26}, which is in line with our expectations.
Conclusions

In the current clinical setting assisting a MAMMI-PET acquisition, an additional radiation dose for NMTs of approximately 0.6 μSv per patient is expected. Although the expected doses during an acquisition, calculated with results from the DRM before start of an acquisition, are higher for the PET/CT acquisition, only measurable doses were detected during MAMMI-PET acquisitions. Further reduction of the radiation dose for the NMT during MAMMI-PET acquisitions may be established with additional safety precautions.
References


Chapter 8
A novel semi-robotized device for high-precision $^{18}$F-FDG-guided breast cancer biopsy

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Abstract

Purpose
To assess the 3D geometric sampling accuracy of a new PET-guided system for breast cancer biopsy (BCB) from areas within the tumour with high $^{18}$F-FDG uptake.

Materials and methods
In the context of the European Union project MammoCare, a prototype semi-robotic stereotactic prototype BCB-device was incorporated into a dedicated high resolution PET-detector for breast imaging. The system consists of 2 stacked rings, each containing 12 plane detectors, forming a dodecagon with a 186 mm aperture for 3D reconstruction (1mm$^3$ voxel). A vacuum-assisted biopsy needle attached to a robot-controlled arm was used. To test the accuracy of needle placement, the needle tip was labelled with $^{18}$F-FDG and positioned at 78 target coordinates distributed over a 35mm × 24mm × 28mm volume within the PET-detector field-of-view. At each position images were acquired from which the needle positioning accuracy was calculated. Additionally, phantom-based biopsy proofs, as well as MammoCare images of 5 breast cancer patients, were evaluated for the 3D automated locating of $^{18}$F-FDG uptake areas within the tumour.

Results
Needle positioning tests revealed an average accuracy of 0.5 mm (range 0–1 mm), 0.6 mm (range 0–2 mm), and 0.4mm (range 0–2 mm) for the x/y/z-axes, respectively. Furthermore, the MammoCare system was able to visualize and locate small (<10 mm) regions with high $^{18}$F-FDG uptake within the tumour suitable for PET-guided biopsy after being located by the 3D automated application.

Conclusions
Accuracy testing demonstrated high-precision of this semi-automatic 3D PET-guided system for breast cancer core needle biopsy. Its clinical feasibility evaluation in breast cancer patients scheduled for neo-adjuvant chemotherapy will follow.

Keywords: $^{18}$F-FDG-assisted biopsy, breast cancer, tumour characterization, 3D PET-guided tumour location
Introduction

In patients with locally advanced breast cancer, tumour sampling is increasingly used for assessment of tumour subtypes and genetic expression profiles in order to personalize neoadjuvant chemotherapy. Usually, breast needle core biopsies are obtained under image guidance using ultrasound, although stereotactic mammography and MRI are also possible. Ultrasonic-guided core biopsy has been reported to be equivalent to open surgical biopsy for both palpable and non-palpable tumours and its use is standard practice. However, tumour sampling is not just important to increase the likelihood of finding cancer in the delivered samples, but is also essential when vital tumour tissue containing sufficient RNA is required for genetic expression profiles and molecular subtyping. Biopsy success percentages are lower as, for example, found in the validation and clinical utility of the 70-gene prognostic signature where useful RNA only could be sampled from 81% of the core biopsies. Recently, non-correspondence between the core biopsy location, identified by marker placement, and the tumour areas with highest metabolism, as assessed by \(^{18}\text{F-FDG PET/CT}\), has been regularly observed by Koolen et al. This non-correspondence was found in 28 (14%) of 203 tumours in stage II–III breast cancer patients scheduled to receive neoadjuvant chemotherapy and was seen more often in tumours appearing as non-mass enhancement or multifocal disease on MRI, diffuse or multifocal tumours on PET/CT and in lobular carcinomas.

The uptake of \(^{18}\text{F-FDG}\) in breast cancer is based on the principle of augmented trapping of the tracer due to increased glycolysis in malignant tumours. The areas with the highest degree of \(^{18}\text{F-FDG}\) uptake mainly represent the most proliferative parts of the tumour, which has been associated with prognostic characteristics and survival. In theory, visualization of heterogeneity in malignant breast tumours may help to select areas for assessment of core needle biopsy sampling for adequate molecular subtyping and gene expression profiling.

A few years ago, a dedicated single-ring 3D PET scanner (MAMMI-PET) for hanging breast imaging was developed in the context of an European Union project. This device has extensively been validated for primary tumour visualization and has also been able to visualize a higher degree of intratumour heterogeneity in \(^{18}\text{F-FDG}\) uptake than conventional PET/CT scanning in a group of patients with locally advanced breast cancer.

Based on the increased detection characteristics of the MAMMI-PET, in 2013 a second European Union project was started to develop a breast biopsy system prototype guided by a dual-ring PET (MammoCare) aimed at allowing real-time 3D tumour lesion localization and biopsy needle insertion guidance for higher sampling accuracy and efficiency. Herewith, we report the results of the accuracy tests as well as a first technical evaluation of the automated lesion visualization and localization program of the system using a phantom simulation model and the images of a group of breast cancer patients.
Material and methods

In 2013, a consortium constituted by Oncovision GEM Imaging S.A. (Spain), Statice Sas (France), Virtual Angle (The Netherlands), Institute of Biomechanics of Valencia (Spain), Institute for Instrumentation in Molecular Imaging I3M (Spain) and UK Heri (United Kingdom) was formed to develop a high-precision breast biopsy system in the context of the project MammoCare subsidized by the European Union (Project Number: FP7-SME-2013-606017). In this consortium (www.mammocare.eu) the Netherlands Cancer Institute participated as end-user in charge of the clinical tests to validate the feasibility of the device. The MammoCare device was installed and extensively tested at the Netherlands Cancer in July–September 2015.

MammoCare was designed and developed as a semi-robotized stereotactic biopsy device incorporated in a dedicated dual-ring PET-detector for hanging breast imaging (Fig. 1). The PET-detector consists of two stacked rings, each containing 12 plane detectors, forming a dodecagon with a 186 mm aperture. Each detector module consists of a single continuous (nonpixelated) LYSO scintillating crystal (12mm thickness), a position sensitive photomultiplier tube, and an electronic

Figure 1: MammoCare system installation at the Netherlands Cancer Institute showing (on the left) the computerized console of the device and (on the right) the biopsy device. The biopsy device is placed below the bed for prone positioning of the patient. The MammoCare bed can be positioned at a height of 120 cm to facilitate biopsy handling.
board including the high voltage power supply. Images are reconstructed with a 3D maximum likelihood expectation maximization (MLEM 3D) algorithm with a voxel size of 1 mm×1 mm×1 mm. Dead time, scatter, random events, and attenuation correction through image segmentation are used in the reconstruction process.\textsuperscript{10} The detector ring covers an axial field of view of 94 mm, and can be translated axially to cover up to 170 mm for longer breasts. A vacuum-assisted 9 G biopsy needle (Eviva, Hologic, Bedford, Massachusetts, USA) is used on a robot-controlled arm (Fig. 2) enabling translational movement along the x-axis, horizontal movement along the y-axis, and vertical movement along the z-axis. The most upper position of the biopsy needle is at 2

\textbf{Figure 2:} Above, the biopsy needle placed on the robot-controlled arm of the device. The biopsy module has been extended with a holder (red arrow) for eventual marker insertion to mark the biopsy site. Bottom, details of the Eviva 9-gauge biopsy needle without and completed with the piece of plastic material inside the opening of the needle used to test needle positioning accuracy.
mm distance from the upper image field of view border. The position of the sterile needle tip is automatically calibrated using a laser pointer. For biopsy the breast is immobilized using two transparent biocompatible paddles connected to a support base for compression in the range of 70–90 N. The PET-ring can be opened to facilitate handling with the robot-controlled arm and needle insertion. This window enables the intervention team to acquire images while the biopsy needle is located inside the breast.

For biopsy with the MammoCare the working protocol is based on a 5-step procedure: (1) Start with a full breast image acquisition of the uncompressed breast using the closed PET-ring. Image target coordinates (x' y' z') can be selected in the software based on 3 image projection planes: sagittal, transverse, and coronal. The system automatically calculates the shortest needle insertion path towards the selected index lesion (x' y' z') and positions the biopsy needle module in that position. The index lesion is now in the centre of the biopsy range (y = 0 and z = 0). (2) This step concerns scanning a part of the breast including the index lesion, using a closed PET-ring fixed on one ring position, and compressed breast in order to adjust for the 3D lesion location movement due to breast compression. The new index lesion coordinates are then selected. (3)

Subsequently, the PET-ring opens during breast compression and the needle is inserted. (4) A new scan is then obtained with open ring, compressed breast, and inserted needle in order to monitor possible index lesion displacement caused by needle insertion. (5) Finally, the needle position can be adjusted and vacuum-assisted core biopsy is performed manually. The biopsy range is along the whole x-axis, and the needle has a limited adjustment range in the y and z-axis; −12mm<y<12mm, −13mm<z<15mm. The total time of one biopsy procedure is approximately 30 min with a 10-min system setup time based on phantom experiments. The image acquisition using closed and open detector ring in relation to the biopsy needle module is schematically given in Fig. 3.

Closed versus open detector ring acquisitions
Ideally, an open ring acquisition needs to provide identical x' y' z'-coordinates as a closed ring acquisition when a target lesion has not moved. The aim of this validation was to evaluate whether closed and open ring image acquisitions of exactly the same setup would result in identical images. Open and closed ring acquisitions were performed using a $^{22}$Na point source at 15 different locations within the detector field of view, and these image coordinates were compared.

Needle positioning accuracy
To evaluate the needle positioning accuracy a 12 mm × 28 mm piece of plastic has been manufactured with the same dimensions as the aperture of the Eviva vacuum biopsy needle. A 0.8
A novel semi-robotized device for high-precision $^{18}$F-FDG-guided breast cancer biopsy

A 165 mm hole was drilled in the centre of the plastic piece and then it was placed in the opening of a 9-gauge needle (Fig. 2). A drop of radioactivity ($^{18}$F-FDG) was placed inside the hole corresponding with the coordinates of the needle ($x'y'z'$). The biopsy needle was mounted on the MammoCare system and the needle laser calibration was performed following the above-described 5-step procedure. First, a 285 kBq $^{22}$Na point source, simulating an index lesion, was scanned for 30s with closed ring. The acquired image was reconstructed to determine the $x'y'z'$-coordinates of the $^{22}$Na source. Next, the detector ring was repositioned in order to have the shortest needle trajectory towards the target ($^{22}$Na source). A second 30s image was acquired with a fixed ring position covering the $^{22}$Na source in its field of view. Subsequently, the $^{22}$Na source was repositioned at 77 other locations within the biopsy range ($x>0, -12<y<12, -13<z<15$), in order to simulate that the target had been moved. An image acquisition plus reconstruction was performed at each location. In total, 78 MammoCare images were gathered with a different position of the $^{22}$Na

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**Figure 3:** Above on the left, full uncompressed breast image acquisition performed by a closed PET-ring. On the upper right, detector ring is positioned at correct height and angle for shortest needle trajectory towards index lesion. A second scan is performed from part of breast, including the index lesion, using a closed PET-ring and compressed breast. Bottom on the left, detector ring opens and biopsy needle is manually positioned at target coordinates. A third breast scan is performed with open ring, compressed breast, and inserted needle. On the lower right, the needle position can finally be adjusted and vacuum-assisted core biopsy is performed.
source in each image. The $x'y'z'$ -coordinates of the $^{22}$Na source were measured. Subsequently, the $^{22}$Na source was removed from the detector field of view. During the biopsy procedure, the needle coordinates are tracked in real-time and displayed on the screen in the dedicated software ($x'y'z'$). The biopsy needle containing $^{18}$F was then positioned in each of the 78 $^{22}$Na locations (for which $x'y'z'$ equals $x'y'z'$) followed by an open ring image acquisition (steps 3 and 4). This resulted in an additional 78 images in all of which the real location of the needle was detected in the image ($x'y'z'$), thanks to the radioactive drops inside the needle opening. The position of the needle in the image was evaluated using a PET reconstruction with 1 mm$^3$ voxels with open ring. Finally, all 78 measurements were repeated to measure the reproducibility of the system. Needle positioning errors were expressed as:

$$\text{Needle position error} = |x_{syszs} - x_{nynzn}|$$

**Simulated phantom training**

Approximately 1 MBq of $^{18}$F-FDG was injected into a piece of vacuum-packed piece of meat used as a phantom to simulate a breast with an index lesion. Following automated lesion localization, a total of 3 biopsies from the index lesion were taken on the basis of the above-described procedure. Additionally, 2 biopsy samples were taken at a distance of 1.5 cm from the index lesion, in a region considered to be free of or to contain a negligible $^{18}$F-FDG contamination. All 5 biopsies were placed on a Petri dish and scanned with an image acquisition time of 20 s by the MammoCare system.

**Lesion visualization and automated localization**

The dedicated software for automated lesion visualization and localization (steps 1 and 2) was technically evaluated in 5 breast cancer patients, initially scheduled for standard PET/CT for staging, and who all gave informed consent for an additional Mammo-Care image. All patients undergoing PET/CT were prepared with a 6h fasting period. Blood glucose levels were required to be <10 mmol/L. A total dose of 180–240 MBq was given intravenously, depending on the body mass index. Immediately following PET/CT ($100 \pm 10$ min after injection), without the requirement of an additional injection, additional MammoCare imaging was performed.
Results

Closed versus open detector ring acquisitions
Comparison of closed versus open ring acquisitions revealed an average image coordinate difference of 0.3 mm (range 0–1 mm), 0.4 mm (range 0–1 mm), and 0.1 mm (range 0–1 mm) for the x/y/z-axes, respectively. This resulted in average absolute deviations of 0.5 mm (range 0–1.6 mm).

Needle positioning accuracy
Needle positioning tests revealed an average accuracy of 0.5 mm (range 0–1 mm), 0.6 mm (range 0–2 mm), and 0.4 mm (range 0–2 mm) for the x/y/z-axes, respectively. This resulted in average absolute errors of 1.1 mm (range 0–2.8 mm). Reproducibility tests of the needle positioning in all 78 image coordinates demonstrated an average deviation between the first and second test of 0.2 mm (range 0–1 mm), 0.4 mm (range 0–1 mm), and 0.3 mm (range 0–1 mm). The average absolute error in the reproducibility test was 0.9 mm (range 0–1.4 mm). These small deviations were noticed when the coordinates of the needle were derived from the open ring reconstructions.

Simulated phantom training
The piece-of-meat-phantom training demonstrated that the contaminated target, with compression, did displace up to 3 mm due to biopsy needle insertion. In Fig. 4 the biopsy experiments using the piece-of-meat-phantom containing a radioactive area to simulate an ¹⁸F-FDG-avid lesion is shown. This figure also illustrates the 3 biopsy specimens obtained from

<table>
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<th>Patient</th>
<th>Tumour size on MRI (mm)</th>
<th>Size (mm)</th>
<th>Size (mm)</th>
<th>Size (mm)</th>
<th>Distances between intratumoural regions (mm)</th>
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<tr>
<td></td>
<td>intratumoural region 1 (x,y,z coordinates)</td>
<td>intratumoural region 2 (x,y,z coordinates)</td>
<td>intratumoural region 3 (x,y,z coordinates)</td>
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<tr>
<td>1</td>
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<td>12 (34,20,2)</td>
<td>16, a 23, b 32c</td>
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<tr>
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<td>6 (−8,8,3)</td>
<td>5 (0,24,3)</td>
<td>47, a 18, b 37c</td>
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<tr>
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<td>7 (−21,12,−17)</td>
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<td>12a</td>
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<tr>
<td>5</td>
<td>33</td>
<td>12 (−36,−10,50)</td>
<td>6 (−30,−1,50)</td>
<td>5 (−24,−7,51)</td>
<td>11, a 9, b 12c</td>
</tr>
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a=absolute distance in mm between intratumoural region 1 and intratumoural region 2. b=absolute distance in mm between intratumoural region 2 and intratumoural region 3. c=absolute distance in mm between intratumoural region 1 and intratumoural region 3.
the simulated “index lesion” showing high radioactivity levels. The other 2 biopsy specimens obtained from the proximity of the “index lesion” show only minor levels of radioactivity, which is probably due to diffusion of $^{18}$F-FDG from the injection site to the periphery of the piece-of-meat-phantom. These same results were subsequently reproduced by two radiologists and one nuclear physician after training with the system.

Figure 4: Above, a biopsy proof using a piece of meat containing a radioactive area to simulate a $^{18}$F-FDG-avid lesion. Bottom, a Petri dish with 5 biopsy samples placed in the scanner (left image). Biopsy specimens 1–3 were taken from the highest radioactive region, while biopsy specimen 4–5 were taken 1.5 cm further on from a low radioactive region. The ex vivo MammoCare image of all 5 biopsy specimens can be seen on the right.
Lesion visualization and automated localization

The MammoCare system was able to visualize small (<10 mm) intratumoural regions with a high $^{18}$F-FDG uptake (Fig. 5). As shown in Table 1 distances between intratumoural $^{18}$F-FDG-avid regions varied from 7mm to 47mm (median 23 mm).

**Figure 5:** Top, coronal slice showing a multifocal 18 F-FDG avid tumour with two small regions of 10 and 12 mm (red arrows) and a sagittal slice showing one region of 31 mm of patient 2. Below, coronal and sagittal plane of patient 1 with a heterogeneous $^{18}$F-FDG avid tumour with a total diameter of 94 mm (red dashed line) of which the posterior tumour part shows more $^{18}$F-FDG uptake than the anterior tumour region.

Discussion

In this first technical evaluation of the MammoCare device designed for $^{18}$F-FDG-guided biopsy of breast cancer lesions, accuracy testing resulted in high precision scores for both needle positioning and automated lesion localization. Needle positioning tests revealed an average absolute error of 1.1mm (range 0–2.8 mm). These deviations might be explained partly by coordinate differences between open ring and closed ring reconstructions of up to 1.6 mm. Furthermore, the location of the needle derived from the image was measured manually with a 1mm accuracy due to the
voxel size reconstruction. However, these small deviations in needle positioning are within the acceptance range of biopsy sampling following use of 9 G needles, especially since the biopsy needle itself has a diameter of 3 mm and obtains biopsy specimens of 2.8 mm × 12 mm when used with a vacuum-assisted device. This kind of device was incorporated into the MammoCare prototype because a vacuum-assisted large bore needle approach allows to obtain multiple core biopsies of tissue without removing the needle from the breast. We believe that lesions of 5 mm or larger are feasible for PET-guided biopsy using MammoCare by taking into account the needle positioning errors and the size of the 9 G biopsy needle.

Adequate tumour sampling is not just important to increase the likelihood of finding cancer in the delivered samples, but becomes crucial when molecular diagnostics is required. For tumour subtype analysis and RNA-based gene expression profiling tests, specific demands have been formulated for tumour tissue sampling. Molecular diagnostics was initially validated on the basis of surgical excisional biopsy which enables pathologists to work with representative tissue samples. In early breast cancer, due to its high concordance with excisional biopsy, core needle biopsy has been found to be reliable for HER2 determination, while for assessment of grade, PgR and ER concordance is lower and results need to be considered with caution. In patients with larger breast tumours the possibility to deliver biopsy samples containing stroma, inflammatory cells and fatty tissue in addition to malignant cells increases and may be a source of failure in RNA-based diagnostics. In 203 evaluated primary tumours, correspondence between the site of the marker placed after core needle biopsy and the tumour area with the highest 18F-FDG uptake, reached only 55% with a significant 14% non-correspondence of ≥2 cm. This non-correspondence was seen more often in tumours appearing as non-mass enhancements and multifocal masses on MRI, diffuse or multifocal tumours on PET/CT, and in lobular carcinomas.

Against this background the possibility to develop a functional approach for core needle biopsy to optimize sampling becomes very attractive. Other investigators have used molecular imaging to guide breast biopsy but its use has been limited to tissue sampling for conventional histopathology in suspected breast lesions. In addition to this possibility the MammoCare device, as technically evaluated in the present study, is also oriented to obtain tumour tissue samples matching the requirements for RNA-based molecular diagnostics. As shown in the phantom biopsy experiment, the future use of the MammoCare device may facilitate not only the selection of vital tumour areas for sampling, but also check correct deliverance of these samples for subsequent molecular analysis; to accomplish this, post-biopsy specimens can be checked on radioactivity, which reflects 18F-FDG avid sampled tumour tissue, in the MammoCare scanner. This feature of the device can contribute to optimize core needle sampling for RNA-based tools in breast cancer diagnostics. For instance, one of the most used tests, the MammaPrint, demands samples taken from regions clear of both necrotic and stromal tissue with delivered specimens.
containing at least 30% malignant cells on haematoxylin and eosin staining to be eligible for analysis. 17

The PET-detector of the MammoCare system is equipped with two stacked rings. This significantly reduces whole breast acquisition times in comparison with the original MAMMI-PET, which is equipped with just a single ring. 18 The system necessarily uses breast fixation with transparent paddles to facilitate biopsy but compression in the 70–90 N range is appreciably lower than the 130–200 N compression force usually used for mammography. Finally, ergonomics of MammoCare has been designed for prone position of the patient; the bed can be positioned at a height of 120 cm allowing sufficient space for the intervention team to adequately prepare breast compression and subsequently to carry out the biopsy procedure in a continuous concept. Lesion displacement due to biopsy needle insertion was observed in the piece-of-meat-phantom training. Therefore, step 4 of the procedure, a control image with the needle in place, is essential for breast biopsy procedure.

In conclusion, the first technical evaluation of the MammoCare device has led to promising results concerning core biopsy precision and automated lesion localization. Thanks to its specific approach based on tumour uptake of 18F-FDG, the device offers potential to optimize core needle biopsy for both conventional histopathology and RNA-based molecular diagnostics. Phantom experiments show that the 5-step PET-guided biopsy procedure takes 30 min, which is similar to MRI-guided breast biopsy in our institute. Further clinical validation in breast cancer patients is needed.

Conflict of interest

Jorge Alamo is actually employed for Oncovision (General Equipment for Medical Imaging S.A., Valencia, Spain) but his participation in this study was realized in the framework of the European Union project MammoCare. Besides his affiliation to the Netherlands Cancer Institute Daan Hellingman is working as independent contractor for Oncovision. None of the other authors declare conflict of interest.

Acknowledgement

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References


Summary in English
Summary

Whole body positron emission tomography coupled with computed tomography (PET/CT) using 18-fluoro-deoxy-glucose (¹⁸F-FDG or simply FDG) is increasingly being used in oncology since its introduction in 1998. FDG PET/CT imaging relies on the glucose metabolism of malignant cells, that show an elevated absorption of glucose together with the coupled FDG.

PET/CT was introduced in The Netherlands Cancer Institute in 2006 for clinical research on its possible role for breast cancer imaging. Recent studies have shown that PET/CT imaging has additional value over conventional imaging for the detection of distant metastases in stage II and III breast cancer patients. Consequently the breast cancer guidelines of the Netherlands added a recommendation in 2013, stating that PET/CT has superior value for distant metastases screening in stage III breast cancer patients and may be considered in stage II patients. The same recommendation states that for clinically suspected stage IV patients (recurrence or distance metastases) PET/CT may be considered as additional imaging. The reason why PET/CT is not yet accepted worldwide for distant metastases screening is due to higher costs compared to conventional imaging (bone scan, liver sonography and chest x-ray in Europe or bone scan and a CT of the chest and abdomen in the US).

For local regional breast cancer imaging, prone position PET/CT imaging was introduced after the successful introduction of prone position MRI for imaging local regional disease. Prone position MRI has a sensitivity of up to 100% for detecting primary lesions in the breast, compared to 67 - 98% for PET/CT. Although, standard supine PET/CT (face up) does not have a current position in clinical imaging for the visualisation of the tumour in the breast (primary lesion), with the introduction of prone position PET/CT imaging, however, the sensitivity for lymph node metastasis identification in stage III breast cancer patients increased from 70% to 82%. Consequently a few first reports on the additional value of PET/CT to adequately stage lymph node metastases in stage IVIII breast cancer patients have been published.

In the present thesis we have explored the additional value of PET/CT to guide breast cancer treatment; both in the diagnostic phase and in the treatment phase. We particularly aimed at further exploring how PET/CT might improve personalized treatment in a selection of breast cancer patients.

In the current chapter, a summary of our main findings is provided followed by future prospects on relevant aspects of clinical practice and its consequences for future research related to the use of dedicated PET/CT scan for breast cancer diagnosis and treatment.
PART 1 PET/CT: Improved baseline visualization

The first part of this thesis describes the technical improvements of PET/CT scanning for visualisation of the primary tumour and lymph node metastases in patients with primary breast cancer. Supine position, in which PET/CT is originally performed, is not an optimal imaging position for detecting malignant lesions in the breast. By scanning in prone position the breathing motion of the front thorax is reduced, resulting in less anatomical mismatch between the fused PET (FDG uptake) and CT images (anatomical structure)\(^{17}\). As a result smaller tumour locations (= lymph node metastases) are correctly identified.

To further improve primary breast tumour visualisation with PET/CT and to enable direct comparison with MRI prone positioning with hanging breasts was introduced for PET/CT using a coil similar to the one used for (prone position) breast imaging with MRI. As mentioned in Chapter 1 several studies have described that PET/CT in prone position may improve the detection and extension of the primary tumour in the breast \(^{18,19}\) and has added value for the detection of lymph node metastases\(^ {14,20} \). In Chapter 2 we present new data, currently representing the largest patient series on the additional value of prone position PET/CT imaging of the primary tumour in the breast and of lymph node metastases in patients that are to be treated with primary systemic treatment. Patients received a single dose of 180 to 240 MBq of \(^{18}\)F-FDG intravenously, depending on their body mass index. After a resting period of 60 ± 10 minutes, PET/CT studies were acquired using a whole body PET/CT scanner (Gemini TF, Philips, Cleveland, OH). First, a PET/CT of the thorax was performed in prone position, using a specific coil, followed by a standard supine PET/CT acquisition. In this series we also investigated the additional value of improving CT reconstruction for the standard supine PET/CT imaging: 2mm slices compared to the original 5 mm slices. Of 198 patients studied, 201 images were collected of which the first 100 supine images where fused with the standard 5mm CT slices and the consecutive 101 supine images with the improved 2mm slice reconstruction.

After both univariate and multivariate analyses prone position PET/CT showed more primary tumour multifocality (p<0.001), a higher number of axillary lymph node metastases (p<0.001) and a higher number of total lymph node metastases (p<0.001). Therefore we may conclude that prone PET/CT imaging is a valuable addition to more accurately assess loco-regional staging of breast cancer before start of PST. For supine PET/CT imaging the standard 5mm CT slice reconstruction brought about an increase in anatomical mismatch between PET and CT for axillary lymph node metastases (p=0.004) compared to the improved 2mm CT reconstruction.

Prone position PET/CT imaging may also supplement prone position MRI imaging for baseline primary tumour and nodal staging and response monitoring. In our hospital all stage II/III breast cancer patients undergo supine and prone position PET/CT imaging before start of PST. We
recommend to centres that perform supine position PET/CT imaging in primary breast cancer patients to add a prone acquisition when possible since the extra acquisition takes 10-15 minutes and provides a better delineation of the primary tumour and lymph node metastases.

As mentioned before recent studies have reported that prone position PET/CT detects more lymph node metastases than conventional imaging\(^2,15,16\) with a positive predictive value of up to 100\(^{\%}\).\(^{12,21}\) In Chapter 3 we analysed the effect of nodal upstaging by prone position PET/CT on short-term (3 year) outcome in the group of stage II/III breast cancer patients as previously presented by Koolen et al\(^16\). These 278 primary breast cancer patients had been classified into three groups based on the risk for regional recurrence: low-risk (T2N0), intermediate-risk (T0-2 N1 and T3N0) and high-risk (T0-3N2-3, T3N1, T4) after staging with conventional imaging. After staging with conventional imaging, 43 patients were upstaged by PET/CT to the high-risk group of which 42% from the low-risk group, 28% from the intermediate-risk group, additionally new lymph node metastases were identified in 13 high-risk patients (30%) requiring additional fields for radiotherapy.

The group of patients upstaged with PET/CT showed a worse progression free survival compared to patients in which PET/CT showed the same (low intermediate or high) stage as in conventional imaging. This finding could be explained by the fact that lesions with less favourable characteristics show higher FDG uptake\(^22\) and therefore are more prone to be detected by PET/CT. Upstaging with PET/CT did not show an improvement in short-term survival but it did keep patients, which had actually a higher nodal stage than expected after conventional imaging, from receiving insufficient additional treatment.

The most important drawback for broader implementation of PET/CT imaging in breast cancer are the additional costs; currently PET/CT imaging is (three to five times) more expensive than conventional imaging, due to the use of FDG. Please find in the addendum a manuscript on this subject.

PART II: Response monitoring

Pre-operative systemic treatment (PST) is increasingly being used in stage II/III breast cancer patients. PST has the potential to tailor the treatment to response of the primary tumour and lymph node metastases instead of solely relying on a biopsy of the primary tumour. As mentioned before, MRI is the golden standard for the planning of the operative treatment as it has a sensitivity of 93% for detecting multicentric disease.\(^{23}\) Also for response monitoring during PST, MRI is most commonly used.
Response monitoring of the primary tumour with MRI is based on tumour extent, morphology and relative enhancement during initial enhancement (90 seconds) and late enhancement (450 seconds) measured before and during PST. PET/CT can also be used to aid in response monitoring and in this respect two methods have been described in literature: 1. Using relative change in FGD uptake (ΔSUVmax) of the primary tumour or regional lymph nodes and 2. Using the absolute FDG uptake (SUVmax) at the different time points (before and during PST).

In **Chapter 4** we describe the comparison of response monitoring during PST treatment by MRI alone, by PET/CT alone and by a combination of both modalities. Primary endpoint was pathological complete response (pCR) defined as no or only small numbers of scattered invasive tumour cells. MRI and PET/CT, both in prone position, were performed at the start of PST (baseline) and during PST (interim). MRI was performed using a 3.0-T scanner (Achieva, Philips, Best, The Netherlands). Response on MRI was calculated by relative changes in LD (=largest diameter primary tumour) of baseline and interim, separately for LD on initial and LD on late enhancement. On the PET/CT images made at baseline and during PST we measured the SUVmax and calculated ΔSUVmax in a 3D region of interest containing the primary tumour. We included 188 women with stages II and III breast cancer and divided the group into the three main subtypes of breast cancer as obtained by immunohistochemistry (55 triple negative breast tumours, 35 HER-2 positive tumours and 87 ER positive tumours).

Response monitoring of HER-2 positive tumours was most accurately accomplished using MRI alone (Area Under the Curve; AUC= 0.735). For triple negative tumours, monitoring of response was not significantly different when comparing MRI to PET/CT (AUC 0.855 and 0.844). The combination of MRI and PET/CT did not increase the accuracy of response monitoring for the triple negative and Her-2 positive subtypes of breast cancer. Where our group previously reported that MRI is less accurate in response monitoring of the ER positive primary tumour after PST we found that using PET/CT together with MRI may be of additional value in this group (AUC for MRI + PET/CT= 0.818) versus MRI alone (AUC: 0.742) or PET/CT alone (AUC: 0.791).

Most previous studies evaluating PET/CT as stand-alone device for early response monitoring focused on the metabolic response of the primary tumour in the breast as we did in chapter 4. Substantially fewer studies have evaluated the early metabolic response of the axillary lymph nodes, of the combined response in primary tumour and lymph node metastases or the concordance or discordance between both. In **Chapter 5** we assessed the early and mid-regimen response with PET/CT of the breast tumour and involved lymph nodes in stage II/III breast cancer patients. Overall 134 patients were included in the study but only 45 patients with triple negative and 60 patients with HER2-positive primary breast cancer were included in the analyses. We did the analyses on patient who had a primary tumour in the breast and axillary
lymph node metastases. In the analyses no ER positive patients were included since only 2 had a pCR in the surgery specimen. pCR in this study was defined as no residual invasive tumour cells irrespective of in-situ lesions.

We evaluated the therapy response found on PET/CT with pCR of the primary tumour in the breast and of the lymph node metastases in the axilla, found in the surgical specimen. A PET/CT was performed at baseline (PET1) after two to three weeks of treatment (PET2) and after six to eight weeks of treatment (PET3). All scans were qualitatively assessed for sufficient FDG-uptake (=SUVmax) for visual distinguishing the primary tumour and lymph node metastases from surrounding tissue. We measured the SUVmax of the primary tumour and most avid lymph node metastasis in the axilla at the different time points (PET1/PET2/PET3) and the difference in SUVmax between the time points (ΔSUVmax) to find the best prediction for pCR. The association of SUVmax at different time points with pCR and of ΔSUVmax (between PET2 and PET1 and PET3 and PET1), with pCR were tested using logistic regression analyses and presented as the c-index (equivalent of the area under the curve [AUC] in ROC analyses).

For triple negative breast cancer we found that the ΔSUVmax of the tumour in the breast between PET1 and PET3 was best to predict pCR of the tumour in both breast (c-index 0.85) and axilla (c-index 0.82). In HER2-positive tumours, the best performance for prediction of pCR was the ΔSUVmax of the tumour in the breast between PET1 and PET2 (c-index 0.64), although rather similar to the performance of the absolute SUVmax of the tumour in the breast at PET2 (c-index 0.62). We also found that, in total, 25 patients had a discrepant pathologic response of the breast and axilla: 11 patients had pCR of breast but not of the pCR axilla and 14 patients had pCR of the axilla but not of the breast.

This study shows that the correlation between PET/CT responses during PST in breast and axillary lymph nodes is moderate in triple-negative and poor in HER2-positive breast cancer. We recommend performing response monitoring with PET/CT for primary tumour and lymph node metastases separately and we emphasize the need to describe results according to breast cancer subtype due to different tumour behaviour.

**PART III. A new dedicated imaging modality**

Mammography is the most frequently used modality for visualization of suspect lesions in the breast and is the current tool for primary breast cancer lesion screening. The drawback of mammography is its low sensitivity for the detection of suspect lesions in dense breasts. Ultrasonography and MRI are currently widely being used for diagnostic reasons in addition to mammography.
As mentioned in part I, PET/CT imaging is currently mainly used for distant metastases screening and staging of regional disease in case of a primary tumour and for response monitoring when PST is being administered. For the detection of primary lesions in the breast PET/CT imaging is not widely being used yet, mainly because of the high sensitivity of MRI.

Recently a new single-ring PET system for dedicated breast imaging has been developed; the MAMMI-PET which enables high-resolution hanging breast imaging without compression. In Chapter 6 we compared MAMMI-PET to supine position PET/CT for baseline visualization of histologically proven primary breast cancer lesions. Supine position PET/CT was chosen as comparison since it is the standard position advised by the Dutch breast cancer guidelines for distant metastases screening\textsuperscript{10} and because the coil for prone position PET/CT imaging is not yet commercially available. Of the 230 included patients with proven primary breast cancer 204 patients (total of 206 affected breasts) underwent a supine position PET/CT acquisition and MAMMI-PET acquisition. The diameter of the included primary tumours (measured on MRI) ranged from 6 to 170 mm.

In 23 out of 206 MAMMI-PET missed the primary lesions located too close to the pectoral muscle. In comparison PET/CT missed 20 lesions. This resulted in an overall sensitivity of 89\% for MAMMI-PET and 91\% for PET/CT. Of the 20 lesions missed by PET/CT in supine position 16 were detected by MAMMI-PET. The greatest advantage of MAMMI-PET was seen in the group of eight patients with an index lesion <10 millimetres in diameter, MAMMI-PET visualized all of them and PET/CT only one.

With the expanding interest of PET and PET/CT modalities for different indications we have to consider the effect of implementing MAMMI-PET in clinical practice on total radiation dose for Nuclear medicine technologists (NMTs). When measuring radiation exposure to the NMTs during clinical work the patient is considered to be an external source delivering a homogenous radiation dose from the whole body after being injected with FDG. Thus, the additional radiation exposure of NMTs due to MAMMI-PET use needs to be measured during the acquisitions and patient handlings.

In Chapter 7 we assessed the radiation dose expected for the implementation of MAMMI-PET into daily practice as an addition to regular PET/CT use. We measured the radiation dose during the tracer injection and patient transport using the standard procedures for PET/CT. PET/CT acquisitions were made 60 minutes after FDG administration and the MAMMI-PET acquisitions were made between 90 and 120 minutes after injection. The radiation doses of the different patient handlings, as used for PET/CT and MAMMI-PET, were measured for 12 consecutive patients. During PET/CT acquisitions the NMTs were positioned behind a thick lead wall. Complying with international radiation safety standards\textsuperscript{34,35}, during MAMMI-PET acquisitions, radiation exposure was measured at 1 meter distance from the patient.
The additional radiation dose to NMTs attributed to MAMMI-PET use in the current set-up, would consist of 1.4 μSv per patient scanned. In 2012 a PET/CT scan was made of 330 stage II/III breast cancer patients before start of PST. If an additional MAMMI-PET acquisition had been made of these patient this would have resulted in a total additional dose of 0.46 mSv per year per patient for all the NMTs of the department. Our department currently consists of 12 NMTs, which reduced the additional dose to 0.04 mSv per year per NMT. A radiation dose in this range would not compromise the predefined maximum radiation dose allowed by the Dutch and European radiation laws yearly dose of 6 mSv for NMTs.\textsuperscript{34,35}

During the measurements of the current study the high-resolution MAMMI-PET acquisitions were made up to 2 hours after the FDG injection. Therefore, if MAMMI-PET would be incorporated as a stand-alone procedure, we expect to need half to two thirds of the current FDG dose but would still recommend additional radiation safety measures as currently applied for PET/CT imaging.

In 2013, a consortium (www.mammocare.eu) constituted by Oncovision GEM Imaging S.A. (Spain), Statice Sas (France), Virtual Angle (The Netherlands), Institute of Biomechanics of Valencia (Spain), Institute for Instrumentation in Molecular Imaging I3M (Spain), UK Heri (United Kingdom) and The Netherlands Cancer Institute as end-user was formed to develop a high-precision breast biopsy system subsidized by the European Union; the MammoCare system. In Chapter 8 we reported a first technical evaluation of the automated PET-based biopsy device. The MammoCare system was developed on the detection characteristics of the MAMMI-PET but with a dual-ring PET-detector; the possibility to compress the breast and a semi-robotized stereotactic biopsy device using a vacuum-assisted 9 Gauge biopsy needle (Eviva, Hologic, Bedford, Massachusetts, USA).

For the evaluation of MammoCare imaging we included 5 stage II/III breast cancer patients. Comparison of closed versus open ring acquisitions revealed an average absolute deviation of 0.5 mm (range 0–1.6 mm). When the coordinates of the needle were derived from the open ring reconstructions deviations with a maximum of 1 mm where seen in needle position reproducibility. Closed PET rings of the MammoCare resulted in the best resolution and are recommended to detect the tumour and let the computer calculate the coordinates of the lesion. By inserting the found coordinates into the biopsy system software, the biopsy needle is positioned by the computer (translational movement along the x-axis, horizontal movement along the y-axis, and vertical movement along the z-axis) after which the PET ring opens to allow a physician to manually take the biopsy. After manually positioning the needle, with the tip of the needle visible inside the lesion using a static image, the position of the tip of the needle may be evaluated using a PET reconstruction with open ring. The open ring reconstructions show less resolution but are
sufficient to detect the lesion and the tip of the needle. If the position of the tip of the needle then is unsatisfactory, the needle can be moved manually.

For the evaluation of the biopsy device approximately 1 MBq of $^{18}$F-FDG was injected into a piece of vacuum-packed of meat used as a phantom to simulate an index lesion within a breast. Following automated lesion localization a total of 3 biopsies from the index lesion were taken using a biopsy needle with a diameter of 3 mm that obtains biopsy specimens of 2.8 mm × 12 mm, and at the same time administering compression on the phantom. The phantom study demonstrated that the contaminated target inside of the phantom displaced up to 3 mm due to biopsy needle insertion, which is acceptable, considering the diameter of the needle. As an additional verification of correct deliverance of biopsy samples the MammoCare device may also facilitate imaging of the taken specimen to identify present radioactivity.

In conclusion this first technical evaluation of the MammoCare showed promising results concerning automated lesion localization and core biopsy precision, although further clinical validation in breast cancer patients is needed. Following this technical evaluation FDG-guided biopsies may be utilized to further investigate the clinical value of tumour heterogeneity.

In summary we have showed in the different chapters in this thesis the additional value of PET/CT in breast cancer diagnosis and treatment. As a general recommendation, we would like to state that PET/CT deserves a role as additional imaging modality for primary breast cancer patients to improve baseline staging before start of PST.

MAMMI-PET is still under development but has shown promising results in detecting primary lesions compared to the more common supine position PET/CT. Also the first technical evaluation of the MammoCare system offers promising results.

In the next subsection we will enlighten our point of view regarding the possible role for PET/CT and breast dedicated PET in breast cancer imaging for the near future.
References


Future prospects
Future prospects

In this thesis we have presented the additional value of prone position PET/CT for visualisation of the primary tumour and lymph node metastases. We also explored the role of PET/CT for response monitoring during pre-operative systemic treatment (PST). Finally we evaluated the potential clinical value of MAMMI-PET and performed the first technical evaluation of MammoCare, a PET-based biopsy system.

On the basis of these items and our general appreciation of PET/CT scanning in breast cancer, we conclude that the added value of a PET-based approach in breast cancer imaging should further be developed in three general directions:
1. Improvement of systemic breast cancer localisation by fusion of imaging techniques (PET/MRI)
2. Improvement of primary tumour characterisation using FDG guided biopsy
3. Improvement of response monitoring using tumour subtype specific tracers

Improvement in systemic breast cancer localisation by fusion of imaging techniques (PET/MRI)

In the last decade MRI has been found to have high sensitivity for primary tumour delineation in stage II/III breast cancer before start of PST\textsuperscript{1–3}. More recently PET/CT has shown additional value to assess regional staging\textsuperscript{4–6} and metastases at distance\textsuperscript{7–11} in the same category of patients. Combining both modalities into one system therefore appears to be attractive and may constitute a step further in breast cancer imaging as it would combine the unique tissue characterization of MRI with the quantifiable functional and molecular information provided by PET. The combination of both modalities has the potency to improve breast cancer imaging for tumour delineation\textsuperscript{12}, regional staging, response monitoring\textsuperscript{13,14} and distant metastases staging, specially in soft tissue (brain and liver\textsuperscript{15}). Recent small studies have shown potential additional value of combining PET and MRI results for response monitoring in breast cancer patients\textsuperscript{16,17}.

Currently, two modalities of PET/MRI acquisition have been developed for clinical use\textsuperscript{18}. The first system is based on separate PET and MRI gantries in the same room, with a table that moves on a single track between the two systems (e.g. Ingenuity TS PET/MR, Philips). This approach potentially offers a better registration and less time difference between the acquisitions. Ideally would be an integrated hardware system would be the best option presenting methodological advantages such as intrinsic motion compensation, partial volume correction and model-based attenuation correction.
This brings us to the most attractive system design in which the PET detector is placed inside the MRI gantry (e.g. Biograph mMR, Siemens). Unfortunately, in order to achieve a successful integration of both systems, smaller PET detectors are used, limiting optimal PET detection. Additionally, attenuation correction with this system is not optimal as MRI-based correction is still under development.

Although the integration of PET with MRI assumes to reflect the best of both worlds for breast cancer imaging, further development of the PET/MRI modality needs to be supported by an extensive clinical validation in breast cancer patients before implementation in daily practice.

**Improvement of primary tumour characterisation using FDG guided biopsy**

Suspect primary breast cancer lesions are in general primarily evaluated with ultrasound, followed by core biopsy. Ultrasound-guided core biopsy has been reported to be of equivalent value to open surgical biopsy for either palpable or non-palpable tumours and its use is standard practice\(^9\). A recent study by Koolen et al., however, has shown that in 199 stage II/III patients the correspondence between radiological markers placed after core biopsy and the area with the highest SUVmax was only 55%, with a 14% at a distance of 2cm or more away from the FDG uptake\(^20\). These findings suggest that the molecular subtyping following these biopsies may not represent the complete tumour correctly.

An approach based on high precision biopsy, as technically evaluated in this thesis with the dedicated PET device MammoCare, may improve the selection of vital tumour areas for sampling and subsequent molecular analysis. Additionally, the resolution of the MammoCare could be valuable in patients with dense breasts, after previous (breast-conserving) surgery, in larger (heterogeneous) tumours or if the lesion appears to be occult on conventional modalities\(^21\).

Further clinical validation is necessary to prove if FDG-guided tumour sampling is able to improve characterization of primary breast tumours in the context of neo-adjuvant systemic therapy.
Improvement of response monitoring using tumour subtype specific tracers

A less invasive method to better identify the biological functional activity of a given receptor in the primary breast cancer is currently being developed: subtype-specific tracers.

Using targeting molecules such as antibodies used in Her2 targeted therapy (trastuzumab and bevacizumab) subtype specific tracers can be developed for PET/CT imaging provided they can be coupled with radioactive isotopes. When tracers are coupled with radioactive isotopes that have a short half-life, such as $^{68}$Ga, $^{18}$F, $^{64}$Cu, and $^{76}$Br, PET/CT imaging can be performed on the day of injection, minimizing the required dose of radioactivity. However, for adequate evaluation of uptake variations in the tumour the tracer needs to be coupled with radioactive isotopes with a longer half-life, such as $^{89}$Zr, $^{124}$I, and $^{86}$Y, where images need to be retrieved up to several days after tracer injection.

The PET-tracers $^{89}$Zr-Trastuzumab and $^{64}$Cu- Trastuzumab have recently been investigated in small studies including breast cancer patients with distant metastases. Gaykema et al. presented a case of a young woman with multiple metastases after being treated for a triple negative and a Her2+ breast cancer lesion in the same breast. One of the lesions was biopsied, but with inconclusive results. After PET/CT imaging most metastatic lesions showed $^{89}$Zr-Trastuzumab uptake while under hormonal treatment (tamoxifen), therefore tamoxifen treatment was temporaraily discontinued, and chemotherapy was initiated including Her2-specific treatment (trastuzumab).

Following the promising results of the recent smaller studies a large prospective clinical study, the ZEPHIR trial, was set up to explore HER2 specific imaging as a clinically useful predictive biomarker in breast cancer patients with distant metastases of Her2-positive primary breast cancer. Patients were included in Belgian and Dutch centres and had been previously treated with several cytotoxic drugs, such as trastuzumab and lapatinib, and may benefit form another more expensive Her2 specific drug: T-DM1. Pre-treatment and early treatment imaging with $^{89}$Zr-Trastuzumab PET/CT, $^{18}$F-FDG PET/CT and their combination are compared for the identification of lesions with sufficient expression of Her2 receptors to benefit from T-DM1. Preliminary data of this study showed that when PET/CT images showed no $^{89}$Zr-Trastuzumab uptake before and after one cycle of systemic treatment this had a 100% negative predictive value. The ZEPHIR trial will end in 2018 and will show the final value of Her2 specific imaging in metastasised patients that have been treated with specific agents.

The most promising field, in which subtype specific traces may have additional value in the future is in stage II/III breast cancer patients, to predict the response of subtype specific therapy.
When subtype specific tracers would be implemented in early stage breast cancer patients new research possibilities may also be explored for PET/MRI, MAMMI-PET and MammoCare.

Concluding remarks

Recent studies have reported promising results regarding detection of lymph node metastases and distant metastases with supine position PET/CT. The implementation of prone position PET/CT further improves staging of regional lymph node metastases prior to PST and the detection of additional primary lesions within the breast compared to supine position PET/CT.

The complementary value of prone position PET/CT with MRI imaging in the pre-treatment phase reinforced the development of an integrated PET/MRI system. Further improvement of the existing integrated systems may improve visualisation of soft tissue tumours and their distant metastases.

The additional value of PET/CT imaging for response monitoring needs further investigation. Subtype specific tracers are under development but may be a valuable aid for treatment planning in the future.

Using dedicated PET imaging may improve the detection of smaller lesions and can safely be implemented into daily practice. The MammoCare system additionally offers the possibility to perform high-precision PET-guided biopsies, which may improve both tumour sampling and molecular characterization compared to current clinical practice.
References


Summary in Dutch
**Samenvatting**

Positron emissie tomografie van het gehele lichaam in combinatie met computer tomografie (PET/CT) met 18-fluoro-deoxy-glucose (18F-FDG of simpelweg FDG) wordt in toenemende mate toegepast in de oncologie sinds de introductie in 1998. FDG PET/CT detecteert maligne laesies door middel van glucose metabolisme van de laesies, doordat de glucose in verhoogde mate wordt opgenomen in de cellen samen met de gekoppelde FDG\(^1\).

In 2006 werd PET/CT geïntroduceerd in het Nederlands Kanker Instituut voor klinisch onderzoek naar de mogelijke rol bij de diagnostiek bij mammacarcinoom patiënten. Recente studies hebben aangetoond dat PET/CT aanvullende waarde heeft voor de detectie van afstandsmetastases in stadium II en III mammacarcinoom patiënten\(^2,9\) ten opzichte van conventionele beeldvorming. Dientengevolge is in 2013 een aanbeveling toegevoegd aan de landelijke richtlijn “mammacarcinoom”\(^10\) die luidt dat PET/CT voor stadium III mammacarcinoom superieur is voor het screenen van afstandsmetastasen en dat tevens overwogen kan worden PET/CT aan te wenden voor dit doel in stadium II mammacarcinoom patiënten. Dezelfde aanbeveling stelt dat PET/CT overwogen kan worden als aanvullende beeldvorming bij patiënten met klinische verdenking op stadium IV (recidief of afstandsmetastases). De reden waarom PET/CT nog niet wereldwijd geaccepteerd is voor afstandsmetastase screening is vanwege de hogere kosten ten opzichte van conventionele beeldvorming (botscan, echo lever en X-thorax in Europa\(^2\) of botscan en CT-thorax/abdomen in de VS\(^11\)).

Voor locoregionale beeldvorming bij mammacarcinoom patiënten is de PET/CT in buikligging geïntroduceerd na de succesvolle introductie van MRI in buikligging voor hetzelfde doeleinde. De sensitiviteit van MRI in buikligging voor de detectie van primaire laesies in de mamma benadert de 100%\(^3\) vergeleken met 67 tot 98% voor PET/CT\(^3,12,13\). Hoewel standaard PET/CT in rugligging nog geen actuele rol vervult voor klinische beeldvorming van de primaire tumour in de mamma is de sensitiviteit voor de identificatie van lymfkliermetastasen in stadium II/III mammacarcinoom patiënten gestegen van 70%\(^2\) naar 82% met de introductie van PET/CT in buikligging\(^14\). Hierop volgend zijn reeds enkele eerste artikelen gepubliceerd over de toegevoegde waarde van PET/CT voor adequate stadiering van lymfkliermetastasen in stadium II/III mammacarcinoom patiënten\(^2,15,16\).

In dit proefschrift is de toegevoegde waarde van PET/CT onderzocht om de behandeling van mammacarcinoom beter te leiden; zowel in de diagnostische- als in de therapeutische fase. Met name is geëxplorieerd hoe PET/CT het aanpassen van de behandeling op maat in een geselecteerde groep mammacarcinoom patiënten kan verbeteren. In het huidige hoofdstuk wordt een samenvatting aangeboden van onze voornaamste bevindingen.
DEEL 1 PET/CT: Verbetering van baseline visualisatie

Het eerste deel van dit proefschrift omschrijft de technische verbeteringen van beeldvorming met PET/CT voor de visualisatie van de primaire tumour en lymfkliermetastasen in patiënten met primair mammacarcinoom. Oorspronkelijk wordt PET/CT beeldvorming in rugligging verricht, welke geen optimale houding is voor het detecteren van maligne laesies in de mamma. Door te scannen in buikligging worden de ademhalingsbewegingen van de thorax gereduceerd, met als gevolg verbeterde overlap tussen de gefuseerde PET (FDG opname) en CT beelden (anatomische structuur)\textsuperscript{17}. Als gevolg hiervan worden kleinere tumour laesies (= lymfkliermetastasen) correct geïdentificeerd.

Om de visualisatie van primair mammacarcinoom met PET/CT verder te verbeteren en om directe vergelijking met MRI mogelijk te maken werd positionering in buikligging met hangende mammoe geïntroduceerd voor PET/CT door middel van een houder (coil) gelijkend op de coil die voor MRI in buikligging wordt gebruikt. Zoals besproken in Hoofdstuk 1 is in meerdere studies reeds beschreven dat PET/CT in buikligging de detectie en het weergeven van de uitgebreidheid van de primaire laesie in de mamma kan verbeteren\textsuperscript{18,19} en toegevoegde waarde heeft voor de detectie van lymfkliermetastasen\textsuperscript{14,20}. In Hoofdstuk 2 presenteren we nieuwe data, momenteel de grootste serie patiënten, over de toegevoegde waarde van PET/CT in buikligging, voor de visualisatie van de primaire laesie in de mamma en lymfkliermetastasen in patiënten die zullen worden behandeld met neo-adjuvante systemische therapie (NST). Bij de patiënten werd intraveneus 180 tot 240 MBq \textsuperscript{18}F-FDG toegediend, afhankelijk van BMI. Na 60±10 minuten zijn de PET/CT acquisities gemaakt met een total-body PET/CT scanner (Gemini TF, Philips, Cleveland, OH). In eerste instantie is een acquisitie gemaakt in buikligging met de specifieke coil, gevolgd door een standaard PET/CT acquisitie in rugligging. In deze serie hebben we tevens de toegevoegde waarde onderzocht van het verbeteren van de CT reconstructie voor beeldvorming met de standaard PET/CT in rugligging: 2mm plakjes vergeleken met de originele 5 mm plakjes. Van de 198 bestudeerde patiënten zijn 201 images verzameld waarvan de eerste 100 beelden in rugligging gefuseerd zijn met de standaard 5mm CT plakjes en de opeenvolgende 101 beelden in rugligging de verbeterde 2mm plakjes (reconstructie).

Na univariate en multivariate analyses toonde PET/CT in buikligging meer primaire tumour multifocaliteit (p<0.001), een groter aantal axillaire lymfkliermetastasen (p<0.001) en een groter totaal aantal lymfkliermetastasen (p<0.001). Derhalve kunnen we concluderen dat beeldvorming met PET/CT in buikligging een waardevolle aanvulling is voor de nauwkeurige bepaling van locoregionale stadiëring van mammacarcinoom patiënten voor start met NST. Voor PET/CT acquisities in rugligging toonde de nieuwe reconstructie met 2mm CT plakjes verbeterde overlap.
tussen PET en CT voor axillaire lymfkliermetastasen (p=0.004) vergeleken met de standaard 5mm CT reconstructie.

PET/CT in buikligging kan mogelijk ook van aanvullende waarde zijn bij MRI in buikligging voor stadiering van primaire tumour en lymfkliermetastasen en voor het monitoren van therapie respons tijdens NST. In ons ziekenhuis worden alle stadium II/III mammacarcinoom patiënten gescand met PET/CT in buikligging en in rugligging voor start met NST. We bevelen centra die PET/CT beeldvorming in rugligging gebruiken bij primaire mammacarcinoom patiënten aan om een aanvullende scan te maken in buikligging waar mogelijk. De extra scan neemt 10 tot 15 minuten in beslag en verschaf een betere omschrijving van de primaire tumour en lymfkliermetastasen.

Zoals eerder genoemd hebben eerdere studies aangetoond dat PET/CT in buikligging, met een positief voorspellende waarde die de 100% benadert, meer lymfkliermetastasen detecteert dan conventionele beeldvorming. In Hoofdstuk 3 hebben we het effect onderzocht van de verbeterde detectie van het aantal aanwezige lymfkliermetastasen (=upstaging) door PET/CT in buikligging op de korte-termijn overleving (3 jaar) in de groep stadium II/III mammacarcinoom patiënten die eerder beschreven zijn door Koolen et al. Deze 278 primaire mammacarcinoom patiënten waren in drie groepen verdeeld gebaseerd op het risico op een regionaal recidief: laag-risico (T2N0), mid-risico (T0-2 N1 en T3N0) en hoog-risico (T0-3N2-3, T3N1, T4) na stadiëring met conventionele beeldvorming. Na de stadiëring met conventionele beeldvorming werden 43 patiënten verplaatst naar de hoog-risico groep na stadiering met PET/CT, waarvan 42% van laag-risico groep en 28% van de mid-risico. Voor 13 patiënten (30%) in de hoog-risico groep werd een hoger aantal lymfkliermetastasen gevonden waarvoor aanvullende radiotherapie velden werden toegevoegd aan de behandeling.

De groep patiënten die in de hoog-risico classificering terecht kwam door de aanvullende PET/CT toonde een verslechterde overleving zonder het ontwikkelen van een recidief of afstands metastase (=progression free survival) vergeleken met de patiënten waar PET/CT geen verschil maakte voor de stadiering ten opzichte van conventionele beeldvorming. Deze bevinding kan worden verklaard doordat laesies met minder gunstige eigenschappen een toegenomen FDG opname tonen en daardoor een grotere kans maken te worden gedetecteerd met PET/CT. Het vinden van aanvullende lymfkliermetastasen met PET/CT toonde geen voordeel voor de korte termijn overleving maar voorkwam wel dat patiënten, die eigenlijk een hoger stadium mammacarcinoom hadden dan aanvankelijk gedacht na stadiering met conventionele beeldvorming, onvoldoende werden behandeld.

Het grootste bezwaar om PET/CT beeldvorming in toenemende mate in te voeren voor beeldvorming bij mammacarcinoom zijn de aanvullende kosten; op dit moment is PET/CT beeldvorming (twee tot vijf keer) duurder dan conventionele door het gebruik van FDG. Een manuscript over dit onderwerp is toegevoegd als een addendum in dit proefschrift.
DEEL II: Responsmonitoring

Neoadjuvante systemische therapie (NST) wordt in toenemende mate toegepast in stadium II/III mammacarcinoom patiënten. Door NST toe te passen kan het effect van de behandeling op de primaire tumour en lymfkliermetastases worden vervolgd (responsmonitoring) en kan de therapie eventueel aangepast worden in plaats van enkel te vertrouwen op de biopsie van de primaire tumour in de mamma voor aanvang van NST. Zoals eerder vermeld is MRI de gouden standaard voor het plannen van de operatieve behandeling met een sensitiviteit van 93% voor het ontdekken van multifocale ziekte\textsuperscript{23}. Ook voor responsmonitoring tijdens NST wordt MRI het meest ingezet.

Responsmonitoring van de primaire tumour met MRI is gebaseerd op de uitgebreidheid van de tumour, morfologie en relatieve aankleuring tijdens de vroege aankleuring (scan na 90 seconden) en late aankleuring (scan na 450 seconden), gemeten voor en tijdens NST\textsuperscript{24}. PET/CT kan tevens worden ingezet als aanvulling bij responsmonitoring\textsuperscript{25} en hiervoor zijn twee methodes omschreven in de literatuur: 1. Het relatieve verschil in FGD opname (\(\Delta SUV\text{max}\)) van de primaire tumour\textsuperscript{25–27} of regionale lymfkliermetastasen\textsuperscript{26–29} en 2. De absolute opname van FDG (SUV\text{max})\textsuperscript{27,29} in de maligne laesies op de verschillende analyse momenten (voor en tijdens NST).

In Hoofdstuk 4 wordt de vergelijking van responsmonitoring tijdens behandeling met NST door alleen MRI, alleen PET/CT en door een combinatie van beide modaliteiten omschreven. Het primaire eindpunt was een pathologisch complete respons (pCR) gedefinieerd als geen of enkele verspreide maligne tumour cellen in het chirurgie preparaat. MRI en PET/CT, beiden in buikligging, werden verricht voor start van NST (baseline) en tijdens NST (interim). MRI acquisities werden gemaakt met een 3.0-T scanner (Achieva, Philips, Best, Nederland). Respons op MRI werd berekend door relatieve verandering van de LD (=langste diameter van de primaire tumour) bij baseline en interim, afzonderlijk voor de LD op de vroege en late aankleuring. Op de baseline en de interim beelden PET/CT beelden werd de SUV\text{max} gemeten en de \(\Delta SUV\text{max}\) berekend vanaf een 3D regio welke de primaire tumour bevatte (region of interest of ROI). In totaal zijn 188 vrouwen geïncludeerd met stadium II en III mammacarcinoom en is de groep verdeeld volgens de drie mammacarcinoom subtypes zoals omschreven door immuunhistochemie (55 triple negatieve mammacarcinomen, 35 HER-2 positieve tumouren en 87 ER positieve tumouren).

Responsmonitoring van HER-2 positieve tumouren werd het meest nauwkeurig bereikt door MRI alleen (Area Under the Curve; AUC= 0.735). Voor triple negatieve tumouren was er geen verschil tussen responsmeting door MRI of PET/CT (AUC 0.855 en 0.844). De combinatie van MRI en PET/CT verbeterde de nauwkeurigheid van responsmonitoring van triple negatieve en Her-2 positieve mammacarcinoom patiënten niet. Onze onderzoeksgroep heeft eerder beschreven dat MRI minder accuraat is voor responsmonitoring van ER positieve primair mammacarcinoom
patiënten tijdens NST. De hierboven beschreven studie toonde dat samenwerking tussen PET/CT en MRI mogelijk de nauwkeurigheid verbetert van responsmonitoring in deze groep (AUC voor MRI + PET/CT= 0.818) ten opzichte van alleen MRI (AUC: 0.742) of alleen PET/CT (AUC: 0.791).

De meeste voorafgaande studies die PET/CT hebben onderzocht als een op zichzelfstaande modaliteit voor responsmonitoring kort na de start van NST hebben zich gericht op de metabole respons van de primaire tumour in de mamma, net als omschreven in hoofdstuk 4. Aanzienlijk minder studies hebben de vroege responsmonitoring onderzocht van de axillaire lymfkliermetastasen, van de gecombineerde response in de primaire tumour en lymfkliermetastasen of de overeenkomst of tegenstrijdigheid van beiden. In Hoofdstuk 5 hebben we de vroege en de mid-therapie response met PET/CT onderzocht van de primaire tumour in de mamma en de aangedane lymfeklieren in stadium II/III mammacarcinoom patiënten. In totaal werden 134 patiënten geïncludeerd in deze studie, hiervan werden 45 patiënten met triple negatief en 60 patiënten met HER2-positief primaire mammacarcinoom geïncludeerd in de analyses. De analyses zijn uitgevoerd bij patiënten met een primaire tumour in de mamma en minimaal 1 axillaire lymfkliermetastase. In de analyses zijn geen patiënten geïncludeerd met ER positieve tumouren, gezien slechts 2 een pCR hadden in het definitieve operatie preparaat. pCR in deze studie is gedefinieerd als het aanwezig zijn van enkel in-situ laesies en geen invasieve cellen.

De therapierespons op PET/CT beelden is vergeleken met de pCR van de primaire tumour in de mamma en van de axillaire lymfkliermetastasen. Een PET/CT scan werd verricht bij baseline (PET1) na twee of drie weken behandeling met NST (PET2) of na zes tot acht weken behandeling met NST (PET3). Alle scans werden kwalitatief onderzocht voor voldoende FDG-opname (=SUVmax) voor het visueel onderscheiden van de primaire tumour en de lymfkliermetastasen van het omliggende weefsel. De SUVmax van de primaire tumour werd gemeten alsmede de meest avide lymfkliermetastase in de axilla tijdens de verschillende tijdstippen (PET1/PET2/PET3) en het verschil in SUVmax (ΔSUVmax) in beide laesies tussen de genoemde tijdstippen (Tussen PET2 en PET1 en tussen PET3 en PET1), om de beste voorspelling te vinden voor pCR in het uiteindelijke preparaat. De relatie tussen SUVmax op de verschillende tijdstippen en pCR en van ΔSUVmax tussen de verschillende scans en pCR werd getest middels een logistische regressie analyse en gepresenteerd als c-index (equivalent van area under the curve [AUC] in ROC analyses).

In de groep met triple negatieve mammacarcinoom patiënten werd gevonden dat de ΔSUVmax van de primaire tumour in de mamma tussen PET1 en PET3 de beste was voor de voorspelling van pCR van de tumour in zowel de mamma (c-index 0.85) als de axilla (c-index 0.82). In de groep HER2-positieve tumouren was de beste voorspaning van pCR de ΔSUVmax tussen PET1 en PET2 van de mamma (c-index 0.64), echter met een soortgelijke prestatie van de absolute SUVmax.
van de tumour in de mamma bij PET2 (c-index 0.62). We hebben ook ontdekt dat bij totaal 25 patiënten een discrepantie werd gezien tussen pCR van mamma en axilla: bij 11 patiënten werd een pCR van de mamma gevonden maar niet van de axilla en bij 14 patiënten werd een pCR van de axilla gevonden maar niet van de mamma.

Deze studie toonde dat de correlatie tussen PET/CT respons tijdens NST in mamma en axilla matig is in triple-negatieve en zwak in HER2-positieve mammacarcinoom patiënten. We bevelen aan de responsmonitoring met PET/CT voor de primaire tumour en lymfkliermetastasen gescheiden te verrichten en we benadrukken de noodzaak om resultaten per subtype te omschrijven gezien het verschillende tumourgedrag.

DEEL III: Een nieuwe specifieke modaliteit

De mammografie is de meest gebruikte modaliteit voor het visualiseren van verdachte laesies in de mamma en is op dit moment de modaliteit voor mammacarcinoom screening. Het belangrijkste nadeel van de mammografie is de lage sensitiviteit voor de detectie van verdachte laesies in mamma’s met dicht klierweefsel. Echografie en MRI maken momenteel deel uit van de aanvullende diagnostiek bij mammacarcinoom patiënten.

Zoals genoemd in deel I, wordt PET/CT momenteel voornamelijk gebruikt voor afstandsmetastasen screening en stadiëring van regionale ziekte bij primair mammacarcinoom en voor responsmonitoring tijdens NST. Voor het detecteren van primaire laesies in de mamma wordt PET/CT nog niet uitgebreid ingezet, voornamelijk als gevolg van de hoge sensitiviteit van de MRI.

Onlangs is een nieuwe enkel-rings PET systeem ontwikkeld voor specifieke beeldvorming van de mamma: de MAMMI-PET, welke beeldvorming van de mamma mogelijk maakt met hoge resolutie en hangende mamma’s zonder compressie. In Hoofdstuk 6 hebben we MAMMI-PET met standaard PET/CT in rugligging vergeleken voor de baseline visualisatie van met histologie aangetoonde primaire mammacarcinoom laesies. Standaard PET/CT in rugligging is gekozen voor deze studie als vergelijking, aangezien het de standaard positie is om PET/CT te verrichten en welke wordt geadviseerd te gebruiken voor afstandsmetastasen screening door de Nederlandse richtlijnen voor mammacarcinoom10; daarbij is de coil voor PET/CT in buikligging beeldvorming nog niet commercieel verkrijgbaar.

Van de 230 geïncludeerde patiënten met bewezen primair mammacarcinoom werd bij 204 patiënten (totaal 206 aangedane mammæ) een PET/CT acquisitie in rugligging en een MAMMI-PET acquisitie gemaakt. De diameter van de geïncludeerde primaire tumoren (gemeten op MRI) varieerden tussen de 6 en 170 mm.
Van de 206 scans heeft MAMMI-PET 23 primaire laesies gemist die zich te dicht tegen de musculus pectoralis bevonden. PET/CT had daarentegen 20 laesies gemist. Als gevolg van deze bevindingen werd voor MAMMI-PET een sensitiviteit van 89% gevonden en voor PET/CT van 91%. Van de 20 laesies die gemist werden door de PET/CT heeft MAMMI-PET er 16 gedetecteerd. Het grootste voordeel van de MAMMI-PET werd gezien in de groep van patiënten met laesies die een diameter hadden van <10 mm, MAMMI-PET heeft alle laesies gedetecteerd en PET/CT slechts 1.

Met de toenemende belangstelling voor PET en PET/CT modaliteiten voor verschillende indicaties moest het gevolg voor de radiatie dosis van de medisch nuclear werkers (MNWers) van het implementeren van de MAMMI-PET in de klinische praktijk worden beschouwd.

Voor de blootstelling aan straling voor de MNWers tijdens werkzaamheden wordt de patiënt beschouwd als een externe bron die een homogene stralingsdosis afgeeft vanuit het gehele lichaam, nadat de patiënt met FDG is geïnjecteerd. Daarom is het noodzakelijk de toegevoegde blootstelling aan straling door de MNWers ten gevolge van gebruik van de MAMMI-PET te meten tijdens de acquisities en patiënt gerelateerde werkzaamheden. In Hoofdstuk 7 hebben we de stralingsdosis geëvalueerd voor de implementatie van de MAMMI-PET in de dagelijkse klinische praktijk als aanvulling op het standaard gebruik van de PET/CT. We hebben de stralingsdosis gemeten tijdens de FDG injectie, patiënt transport en acquisities, waarbij de we standaard procedures toepassen voor PET/CT beeldvorming. Hiervoor dienden we intraveneus 180-240 MBq FDG toe per patiënt. PET/CT acquisities werden 60 minuten na de FDG toediening verricht en de MAMMI-PET acquisities werden verricht tussen 90 en 120 minuten na injectie. De stralingsdosis van de patiënt gerelateerde werkzaamheden, voor de PET/CT en MAMMI-PET, werden gemeten bij 12 opeenvolgende patiënten. Tijdens de PET/CT acquisities verbleven de MNWers achter een dikke loden muur. Ter naleving van internationale richtlijnen voor stralingsveiligheid34,35 werd blootstelling aan straling tijdens de MAMMI-PET acquisities gemeten op 1 meter afstand van de patiënt.

De stralingsdosis toegekend aan het MAMMI-PET gebruik voor de MNWers in de huidige set-up heeft geleid tot een aanvullende dosis van 1.4 μSv per gescande patiënt. In 2013 werd in ons ziekenhuis een PET/CT acquisitie gemaakt bij 330 stadium II/III mammacarcinoom patiënten voor het starten met NST. Indien een aanvullende MAMMI-PET zou zijn gemaakt bij deze patiënten zou dit geleid hebben tot een totale aanvullende dosis van 0.46 mSv per jaar per patiënt voor alle MNWers. Momenteel werken er 12 MNWers op onze afdeling. Dit zou de aanvullende stralingsdosis reduceren naar 0.04 mSv per jaar per MNWer. Deze aanvullende stralingsdosis zou de vastgestelde maximum jaarlijkse stralingsdosis van 6 mSv voor MNWers, zoals vastgesteld door de Nederlandse en Europese stralingsrichtlijnen, niet overschrijden34,35.
Tijdens de metingen van de huidige studie zijn de MAMMI-PET acquisities gemaakt met hoge resolutie, maximaal 2 uur na de FDG injectie. Indien de MAMMI-PET zou worden opgenomen als een opzichzelfstaande procedure verwachten we dat slechts de helft tot twee derde van de huidige FDG dosis nodig zou zijn. Echter zijn aanvullende maatregelen, zoals worden toegepast voor beeldvorming met PET/CT, noodzakelijk.

In 2013 is een consortium samengesteld (www.mammocare.eu), bestaand uit Oncovision GEM Imaging S.A. (Spanje), Statice Sas (Frankrijk), Virtual Angle (Nederland), Institute of Biomechanics of Valencia (Spanje), Institute for Instrumentation in Molecular Imaging I3M (Spanje), UK Heri (Verenigd koninkrijk) met als end-user het Antoni van Leeuwenhoek Ziekenhuis om een zeer nauwkeurig biopsie apparaat te ontwikkelen voor mammacarcinoom patiënten, gesubsidieerd door de Europese Unie: het MammoCare systeem. In Hoofdstuk 8 rapporteerden we de eerste technische evaluatie van het geautomatiseerde PET-geleide biopsie systeem.

Het MammoCare systeem was ontwikkeld met de eigenschappen van de MAMMI-PET. De MammoCare is echter uitgerust met een dubbel-rings PET-detector; de mogelijkheid mammacompressie toe te passen en een semi-gerobotiseerd stereotactisch biopsie systeem met een vacuüm-ondersteunde 9 Gauge biopsie naald (Eviva, Hologic, Bedford, Massachusetts, VS).

De vergelijking tussen acquisities met open en gesloten ringen toonden een gemiddelde absolute deviatie van 0.5 mm (range 0–1.6 mm). Wanneer de coördinaten van de naald werden afgeleid van de reconstructies met open detector ringen werden deviaties van maximaal 1 mm gezien bij de reproduceerbaarheid van de naald positie. De MammoCare toonde op reconstructies van acquisities met gesloten PET detector ringen de beste resolutie, derhalve wordt beeldvorming met gesloten ringen aanbevolen voor het detecteren van de tumour waarna de computer de coördinaten van de tumour kan berekenen. Wanneer de gevonden coördinaten werden ingesteld in het biopsie systeem programma plaatst de computer de naald (translatiebeweging langs de x-as, horizontale beweging langs de y-as en verticale beweging langs de z-as) waarna de PET ringen openen om het de arts mogelijk te maken een manueel biopt te nemen. Na de manuele plaatsing van de naald, met de tip van de naald zichtbaar in de laesie (op een statisch beeld), kan de positie van de tip van de naald worden geëvalueerd middels een PET acquisitie en reconstructie met open PET ringen. De resolutie van een reconstructie van een open PET ring acquisitie is minder accuraat, maar voldoende om de tumour coördinaten te detecteren. Indien de positie van de tip van de naald niet juist is geplaatst kan de naald nog manueel worden verplaatst.

Voor de evaluatie van het biopsie systeem werd circa 1 MBq $^{18}$F-FDG geïnjecteerd in een verpakt stuk vlees hetgeen diende als fantoom om een maligne laesie in de mamma de simulieren. Na de geautomatiseerde lokalisatie van de tumour werden in totaal 3 biopten genomen van de gesimuleerde laesie met een biopsienaald van 3 mm doorsnee die biopten neemt van 2.8 mmx12 mm, waarbij gelijktijdig compressie werd toegepast op het fantoom. Deze fantoomstudie toonde
dat de tumoursimulatie in het fantoom maximaal 3 mm verplaatste ten gevolge van de insertie van de naald, wat acceptabel is gezien de diameter van de naald en de mogelijkheid om de naald te herpositioneren. Het MammoCare systeem kan als aanvullende controle van het correct nemen van de biopsie ook een scan maken van het genomen biopt om radioactiviteit te identificeren. We hebben 5 stadium II/III mammacarcinoom patiënten geïncludeerd om beeldvorming met MammoCare te evalueren.

Concluderend toont deze eerste technische evaluatie van MammoCare veelbelovende resultaten wat betreft de automatische lokalisatie en de nauwkeurigheid van het core biopt van de primaire tumour. Verdere klinische validatie in mammacarcinoom patiënten is echter noodzakelijk. Als vervolg op deze technische evaluatie zouden FDG-geleide biopten kunnen worden ingezet om verder onderzoek te doen naar de klinische betekenis van tumourheterogeniteit.

Samenvattend hebben we in verschillende hoofdstukken in dit proefschrift de aanvullende waarde van PET/CT getoond voor de diagnostiek en behandeling van het mammacarcinoom. Als een algemene aanbeveling willen we stellen dat PET/CT een rol verdient als aanvullend onderzoek van patiënten met primair mammacarcinoom met als doel de baseline stadiëring te verbeteren voor start van NST.

MAMMI-PET is in ontwikkeling maar toont veelbelovende resultaten wat betreft het detecteren van primaire mammacarcinoom laesies vergeleken met de bekendere standaard supine PET/CT. De eerste technische evaluatie van het MammoCare systeem toont tevens veelbelovende resultaten.
References


**Summary in Dutch | 207**
Summary in Portuguese
**Resumo**

Tomografia Computadorizada por emissão de Positrões (PET/CT) de corpo inteiro com fluorodesoxiglicose marcada com Flúor-18 (FDG-\(^{18}\)F ou simplesmente FDG) está a ser mais frequentemente utilizada no âmbito da oncologia desde a sua introdução em 1998. As lesões malignas são detetáveis nas imagens de FDG PET/CT devido ao elevado metabolismo da glicose, que é absorvida nas células juntamente ao FDG acoplado\(^1\).

PET/CT foi introduzida no centro oncológico da Holanda (NKI-AvL) em 2006 para facilitar investigação clínica sobre o seu possível papel como modalidade de imagem para pacientes com cancro da mama. Como resultado de estudos sobre o valor adicional de PET/CT para pacientes com cancro da mama em estádio II e III e comparando com modalidades de imagem mais convencionais \(^2\)-\(^9\), as recomendações nacionais Holandesas foram atualizadas em 2013\(^10\) onde foi acrescentado que deve ser efetuado um scan com PET/CT para os pacientes de estádio III, tendo valor superior sobre modalidades convencionais de imagem. Em pacientes com estádio II um scan com PET/CT pode ser considerado pelo mesmo motivo. Na mesma recomendação foi acrescentado que o PET/CT pode ser considerado como modalidade de imagem adicional em pacientes em que exista suspeita de um estádio IV (recorrente ou metastático). A razão pela qual PET/CT ainda não é aceite mundialmente para detecção de metástases à distância é devido aos custos elevados quando comparados com as modalidades convencionais de imagem (cintigrafia óssea, ecografia do fígado e radiografia do tórax na Europa\(^2\) ou cintigrafia óssea e CT abdominal e do tórax nos Estados Unidos\(^11\)).

PET/CT em decúbito ventral foi introduzido para visualização de doença loco-regional de cancro da mama após a introdução bem sucedida da mesma posição na ressonância magnética (MRI), técnica utilizada para o mesmo fim. A sensibilidade de MRI em decúbito ventral atinge os 100% para detectar tumores primários na mama, enquanto que a PET/CT tem uma sensibilidade de 67-98%\(^3,12,13\). Embora PET/CT ainda não tenha lugar como modalidade de visualização de tumores primários de mama, a sensibilidade para detecção de metástases nos gânglios linfáticos em pacientes com cancro de mama em estádio II e III aumentou de 70% \(^2\) para 82% \(^14\) com a introdução de PET/CT em decúbito ventral. Consequentemente foram publicados vários primeiros artigos sobre o valor adicional de PET/CT para detetar metástases adequadamente nos gânglios linfáticos em pacientes com cancro de mama em estádio II e III\(^2,15,16\).

Nesta tese explorámos o valor adicional de PET/CT para guiar o tratamento para pacientes com cancro da mama; tanto na fase do diagnóstico como na fase de tratamento. Pretendemos, particularmente, explorar como PET/CT pode melhorar o tratamento personalizado numa seleção de pacientes com cancro da mama. No presente capítulo, é fornecido um resumo das principais conclusões.
PARTE 1 PET/CT: Melhor visualização antes de terapêutica neoadjuvante

A primeira parte desta tese descreve os aperfeiçoamentos técnicos do PET/CT para melhorar a visualização do tumour primário e a detecção de metástases de gânglios linfáticos e à distância em pacientes com cancro da mama (doença primária) sem diagnostico prévio.

PET/CT é originalmente executado em decúbito dorsal, esta não é a posição ideal para detetar lesões malignas na mama. Em decúbito ventral, o movimento de respiração do tórax frontal é reduzido, resultando em menos incompatibilidade anatômica entre a fusão de PET (absorção de FDG) e as imagens CT (estrutura anatômica)\textsuperscript{17}. Consequentemente tumores menores (metástases de gânglios linfáticos) são identificados corretamente.

Para continuar a aperfeiçoar a visualização de tumores primários da mama com PET/CT e para permitir a comparação direta com MRI, a posição decúbito ventral com mamas suspensas foi introduzida para utilizar com PET/CT utilizando um molde parecido ao que é utilizado para fazer imagens de mama com MRI. Alguns estudos já descreveram que a PET/CT realizada em decúbito ventral pode melhorar a detecção e a extensão de tumores primários na mama\textsuperscript{18,19} e tem valor adicional para detetar metástases de gânglios linfáticos\textsuperscript{14,20}. No Capítulo 2 apresentamos novos dados, o quais representam atualmente a maior série de pacientes investigada sobre o valor adicional de PET/CT em decúbito ventral para visualizar o tumour primário na mama e as metástases de gânglios linfáticos em doentes, antes de receberem terapêutica sistémica neoadjuvante (TSN).

Nesta série de pacientes investigámos também o valor adicional da reconstrução com CT em relação a PET/CT comum em decúbito dorsal: comparámos cortes de 2mm com as cortes originais de 5mm. Dos 198 casos estudados 201 imagens foram realizadas, sendo que as primeiras 100 imagens PET/CT em decúbito dorsal foram reconstruídas com as cortes CT de 5 mm e as restantes 101 aperfeiçoadas com cortes de 2mm.

Depois das análises univariadas e multivariadas, as imagens PET/CT em decúbito ventral mostraram mais multifocalidade do tumour primário (p<0.001), apresentando um numero mais elevado de metástases de gânglios linfáticos da axila (p<0.001) e um número mais elevado de metástases de gânglios linfáticos em totalidade (p<0.001). Portanto podemos concluir que PET/CT em decúbito ventral é uma adição valiosa para estimar de modo exato o estadiamento loco-regional do cancro da mama antes de iniciar TSN. PET/CT em decúbito dorsal reconstruído com cortes CT a 5mm mostrou mais incompatibilidade anatômica entre as imagens PET e CT para as metástases de gânglios linfáticos da axila (p=0.004), quando comparado com as imagens com cortes CT aperfeiçoados e reconstruídas a 2mm.
PET/CT em decúbito ventral também poderá complementar MRI na mesma posição para estimar o estadiamento do tumour primário e das metástases de gânglios linfáticos e para avaliar TSN. No nosso hospital é obtido um PET/CT em decúbito dorsal e decúbito ventral em todos os pacientes com cancro da mama em estádio II e III antes de iniciar TSN. Recomendamos aos centros hospitalares que realizam PET/CT em decúbito dorsal de pacientes com cancro da mama, que acrescentem uma aquisição em decúbito ventral quando possível, uma vez que a obtenção da imagem dura apenas 10 a 15 minutos e fornece uma melhor estimativa do tumour primário e das metástases de gânglios linfáticos presentes.

Como mencionado à priori, estudos prévios descreveram que PET/CT em decúbito ventral deteta mais metástases de gânglios linfáticos relativamente às modalidades de imagem convencionais (MRI e biopsia guiada por sonografia)\(^{12,15,16}\), tendo um valor preditivo positivo aproximando os 100\(^{12,21}\). No Capítulo 3 analisámos o impacto do PET/CT na sobrevivência a curto prazo (3 anos) e na optimização do estadiamento das metástases de gânglios linfáticos no grupo de pacientes com cancro da mama em estádio II e III como previamente apresentado por Koolen et al\(^{16}\). Os 278 pacientes com cancro da mama como doença primária foram previamente divididos em três grupos baseados no risco de desenvolver recorrência regional: baixo-risco (T2N0), risco-intermédio (T0-2 N1 e T3N0) e alto-risco (T0-3N2-3, T3N1, T4) depois de serem estadeados com modalidades de imagem convencionais.

Depois de um novo estadiamento com PET/CT, 43 pacientes mudaram para o grupo alto-risco, 42\% tinham sido previamente colocados no grupo baixo-risco e 28\% tinham sido previamente colocados no grupo de risco-intermédio. Em 13 pacientes (30\%) do grupo alto-risco foram descobertas metástases de gânglios linfáticos adicionais, com necessidade radioterapia em áreas linfáticas adicionais. Os pacientes que subiram para o grupo alto-risco devido ao estadiamento com PET/CT mostraram pior sobrevivência a curto prazo sem desenvolvimento de recidiva ou metástase (progression free survival) quando comparados com pacientes que se mantiveram no mesmo grupo de risco após PET/CT. Este resultado poderá ser explicado pelo facto das lesões malignas com características menos favoráveis mostrarem mais absorção de FDG\(^{22}\), aumentando a probabilidade de serem detetadas com PET/CT. Mudar os pacientes para o grupo alto-risco devido ao estadiamento com PET/CT não melhorou a sobrevivência a curto prazo, no entanto foi evitado que pacientes que tendem a metastizar gânglios linfáticos adicionais, recebam terapia adicional insuficiente.

A mais importante razão pela qual PET/CT ainda não foi implementada globalmente como modalidade de visualização para pacientes com cancro da mama são os custos adicionais. Atualmente aquisições com PET/CT têm custos (três a cinco vezes) mais elevados que modalidades de imagem convencionais pelo facto de usar FDG. No apêndice podemos encontrar mais informações.
PARTE II: Avaliação de terapia neoadjuvante

Terapêutica sistémica neoadjuvante (TSN) é cada vez mais utilizada em pacientes com cancro da mama em estádios II e III. Com TSN é possível adaptar o tratamento ao efeito que tem sobre as metástases em vez de confiar somente na biopsia do tumour primário. Como mencionado antes, a MRI é o gold standard para o planeamento do tratamento cirúrgico, tendo uma sensibilidade de 93% para detetar tumores multicêntricos\(^\text{23}\). É também modalidade mais utilizada para avaliar o efeito de TSN durante o tratamento.

Avaliação do tumour primário durante TSN com MRI é baseada na extensão do tumour, morfologia e realce relativo do tumour durante o realce com contraste inicial (90 segundos) e realce tardio (450 segundos) avaliados antes e durante TSN.\(^\text{24}\) PET/CT também pode ser utilizada para ajudar na avaliação do tratamento\(^\text{20}\) e para este objetivo foram relatados dois métodos na literatura: 1. Utilizar a alteração de absorção de FDG (\(\Delta\text{SUVmax}\)) do tumour primário\(^\text{25–27}\) ou metástases de gânglios linfáticos regionais\(^\text{26–29}\) e 2. Utilizar a absorção absoluta de FDG (SUVmax)\(^\text{27,29}\) nos diferentes intervalos (antes e durante TSN). No Capítulo 4 descrevemos a comparação da avaliação do tratamento durante TSN entre utilizar somente MRI, somente PET/CT ou combinando os resultados de ambas as modalidades. O ponto de referência era a Reação Completa Patológica (pCR) definida como restando nenhuma ou somente poucas células dispersas de tumour invasivo no preparado pós-cirurgia.

Incluímos 188 pacientes com cancro da mama em estádios II e III e dividimos o grupo conforme os três subtipos principais obtidos com imuno-histoquímica (55 tumores triplos negativos, 35 tumores Her2neu positivo e 87 tumores RH-positivos). A MRI e PET/CT foram executados antes de iniciar TSN (avaliação inicial) e durante TSN (avaliação interim).

O efeito da terapia foi calculado utilizando as alterações relativas de MD (maior diâmetro do tumour primário) na avaliação inicial e interim. O MD foi medido separadamente durante o realce com contraste inicial e tardio. Nas imagens PET/CT obtidas na avaliação inicial e na avaliação tardia medimos o SUVmax numa região 3D que continha o tumour primário. Também as alterações relativas do SUVmax do tumour primário entre a avaliação PET/CT inicial e avaliação tardia foram calculadas.

A avaliação de terapia no grupo com tumores Her2neu positivos foi realizado com mais precisão utilizando somente MRI (Area Under the Curve; AUC= 0.735). Para tumores triplos negativos a avaliação de terapia com somente PET/CT ou MRI não mostrou diferença comparando ambos (AUC 0.855 e 0.844). A combinação de MRI e PET/CT não melhorou a avaliação de terapia para tumores triplos negativos ou Her2neu positivos. Pelo que o nosso grupo previamente apresentou resultados que demonstram que MRI é menos exato para avaliar o efeito de TSN em tumores RH-positivos (receptores hormonais). Concluímos neste estudo que PET/CT possivelmente tem
valor adicional neste grupo (AUC para combinação de MRI e PET/CT= 0.818) comparado com somente MRI (AUC: 0.742) ou somente PET/CT (AUC: 0.791).

A maioria de estudos previamente publicados sobre o possível valor de PET/CT para avaliar o efeito inicial de TSN focaram-se na avaliação do tumor primário na mama\textsuperscript{9,27,29,30} como descrevemos no capítulo 4. Existem substancialmente menos estudos que avaliaram o efeito inicial de TSN sobre o tumor primário na mama e as metástases de gânglios linfáticos\textsuperscript{27,31} ou a conformidade entre ambos\textsuperscript{32}. No Capítulo 5 avaliamos o efeito de TSN inicial e interim do tumor primário e, quando presentes metástases de gânglios linfáticos, em pacientes com estádio II e III de cancro da mama. Comparámos o efeito de terapia dos tumores, visualizado nas imagens PET/CT, com o resultado na patologia final do tumor primário e dos gânglios linfáticos da axila.

Em total incluímos 135 pacientes neste estudo: 45 pacientes com tumores triple negativos e 60 pacientes com tumores Her2neu positivos nas análises. Todos os pacientes incluídos nas análises tinham um tumor primário e metástases de gânglios linfáticos, não foram incluídos pacientes com tumores RH-positivos porque somente 2 pacientes tinham uma pCR no preparado pós-cirurgia. pCR neste estudo foi definido como restando nenhuma células de tumor invasivo (excluindo tumores in situ)\textsuperscript{33}.

As imagens PET/CT foram obtidas: antes de iniciar o TSN (PET1); entre duas e três semanas após inicio de TSN (PET2) e entre 6 e 8 semanas após iniciar TSN (PET3). Todas as imagens foram avaliadas qualitativamente para verificar se os tumores mostravam absorção de FDG suficiente para o tumor primário e as metástases linfáticas serem distinguidos do tecido circundante. Medimos o SUVmax do tumor primário e da metástase linfática axilar mais ávida nos diferentes intervalos (PET1/PET2/PET3) para encontrar a melhor predição para uma pCR. A associação entre o SUVmax nos diferentes intervalos e pCR e do ΔSUVmax entre as imagens e pCR foram testados usando uma análise de regressão logística apresentada como índice-c (equivalente de Area under the curve [AUC] nas análises de ROC).

Para os tumores triple negativos descobrimos que o ΔSUVmax do tumor primário entre PET1 e PET3 resultava na melhor predição para o pCR do tumor primário na mama (índice-c 0.85) e as metástases linfáticas na axila (c-index 0.82). Nos tumores Her2neu positivos a melhor predição de pCR resultou do ΔSUVmax do tumor primário na mama entre PET1 e PET2 (índice-c 0.64), apesar do resultado ser idêntico à predição do SUVmax absoluto no PET2 (índice-c 0.62). Descobrimos que um total de 25 pacientes tinha discrepância entre a pCR do tumor primário na mama e as metástases linfáticas axilares: 11 pacientes tinham uma pCR do tumor primário mas não da axila e 14 pacientes tinha pCR da axila e não no tumor primário.

Este estudo mostra que a correlação entre a avaliação do tumor primário e das metástases linfáticas com PET/CT durante TSN com o pCR obtido é moderada em tumores triplo negativos e fraco em tumores Her2neu positivos.
Recomendamos a avaliação com PET/CT do tumor primário na mama e das metástases linfáticas axilares separadamente e acentuamos a necessidade de descrever os resultados por subtipo devido ao comportamento distinto dos tumores.

PARTE III: Uma nova modalidade dedicada ao cancro da mama

A mamografia é a modalidade mais frequentemente utilizada para visualizar lesões suspeitas na mama e é o teste de referência para triagem de cancro da mama. Uma desvantagem da mamografia é a sensibilidade inferior para detetar lesões suspeitas em mamas densas. A sonografia e o MRI são amplamente utilizados a complementar a mamografia.

Como mencionado na parte I, atualmente PET/CT é principalmente utilizado para triar metástases à distância e estadear doença regional para doentes com tumores primários e para avaliar terapia quando TSN é administrada. No entanto não é provável que a PET/CT seja amplamente utilizada no futuro para detetar tumores primários na mama, principalmente devido à elevada sensibilidade do MRI.

Recentemente uma nova modalidade PET com anilho único foi desenvolvido para obtenção de imagens dedicadas da mama: o MAMMI-PET, o qual permite aquisições com alta-resolução de mamas suspensas sem compressão. No Capítulo 6 comparamos MAMMI-PET com PET/CT em decúbito dorsal para visualização inicial de tumores primários da mama comprovados histologicamente. A PET/CT em decúbito dorsal foi a técnica escolhida como comparação, uma vez que é a posição comum aconselhada pelas recomendações nacionais Holandesas para triar metástases à distância e devido ao molde para PET/CT em decúbito ventral ainda não estar disponível no mercado. Dos 230 pacientes incluídos neste estudo com cancro da mama comprovado, 204 pacientes (206 mamas com um tumour) foram scaneados com PET/CT em decúbito dorsal e com MAMMI-PET. O diâmetro dos tumores incluídos (medidos em MRI) variam entre 6 e 170 milímetros.

Das 206 lesões, a MAMMI-PET não localizou 23, devido às lesões estarem localizadas demasiado perto do músculo peitoral. Em relação ao PET/CT, esta técnica não localizou 20 lesões. Resultando assim em sensibilidades de 89% para a MAMMI-PET e 91% para a PET/CT. Das 20 lesões que a PET/CT não localizou, MAMMI-PET localizou 16. O maior benefício da utilização da MAMMI-PET ocorreu no grupo que incluía lesões de diâmetro <10 milímetros, enquanto que a MAMMI-PET visualizou todas as lesões, a PET/CT detectou somente 1.

Para além do elevado interesse nas modalidades PET e PET/CT nas diversas indicações, deve também ser considerado o efeito da dose total de radiação para os técnicos do serviço de medicina nuclear (TMN) ao implementar MAMMI-PET na prática clínica. Quando a exposição de
radiação dos TMN é medida durante trabalho clínico, o paciente é considerado uma fonte externa de radiação fornecendo uma dose de radiação homogénea do corpo total após administração intraveneosa de FDG. Portanto, quando os TMN são expostos a radiação adicional devido à técnica MAMMI-PET, esta deve ser medida durante a aquisição de imagens, durante transporte e outras atividades que envolvam os pacientes após administração de FDG.

No capítulo 7 avaliamos a dose de radiação expectável para a implementação de MAMMI-PET na prática clínica, como técnica adicional à PET/CT. Medimos a dose de radiação durante a administração de FDG e transporte do paciente, utilizando os procedimentos regulares para a realização PET/CT e administra-se de 180 a 240 MBq de FDG por paciente. As imagens de PET/CT foram obtidas 60 minutos após a administração de FDG.

A dose de radiação atribuída à prática clínica, tanto para PET/CT como para MAMMI-PET, foi medida em 12 pacientes consecutivos. Durante as aquisições PET/CT, os TNM protegiam-se através de uma parede de chumbo de espessura aumentada. Conforme as normas internacionais de segurança de radiação, os TNM mediram a exposição de radiação a 1 metro de distância do paciente.

A radiação adicional para o TNM, atribuído ao uso de MAMMI-PET na atual configuração, consistiria em 1.4 μSv por paciente scaneado. Em 2012 foram realizadas PET/CT a 330 pacientes com cancro da mama em estádio II e III antes de iniciar TSN. No caso de ter sido utilizada também a MAMMI-PET, teria resultado numa dose adicional total de 0.46 mSv por ano por paciente, para todos os TNM do serviço em conjunto. No nosso departamento trabalham 12 TNM, reduzindo a dose de radiação adicional para 0.04 mSv por ano, por TNM. Uma dose de radiação como esta não compromete a dose máxima predefinida de 6 mSv permitida para um TNM pelas leis Holandesas e internacionais.

Durante este estudo as aquisições MAMMI-PET de alta-resolução foram adquiridas no máximo 2 horas após administração intraveneosa de FDG. Portanto, se a MAMMI-PET fosse incorporada como um procedimento independente, seria expectável necessitarmos de metade a dois terços da dose atual de FDG. Todavia são recomendadas medidas de segurança de radiação como as aplicadas atualmente para na realização de PET/CT.

Em 2013 foi formado um comité (www.mammocare.eu) constituído por Oncovision GEM Imaging S.A. (Espanha), Statice Sas (França), Virtual Angle (Países Baixos), Institute of Biomechanics of Valencia (Spain), Institute for Instrumentation in Molecular Imaging I3M (Espanha), UK Heri (Reino Unido) e The Netherlands Cancer Institute como usuário final, para desenvolver um sistema de biopsias de alta-precisão subsidiado pela União Europeia: o sistema MammoCare. No Capítulo 8 descrevemos a primeira avaliação técnica do dispositivo de biopsia automatizado baseado na PET. O sistema MammoCare foi desenvolvido a base das características de detecção de MAMMI-PET mas com anilho duplo de detetores PET, incluindo a possibilidade de comprimir
a mama e um dispositivo de biopsia estereotáxica semi-robotizado, o qual utiliza uma agulha de biopsia de vácuo de 9 Gauge (Eviva, Hologic, Bedford, Massachusetts, Estados unidos).

A comparação de aquisições de anilho aberto comparado com anilho fechado revelou um desvio médio absoluto de 0.5 mm (alcance 0–1.6 mm). Quando as coordenadas da agulha foram calculadas através de imagens obtidas com anilho fechado mostraram desvios de 1 mm no máximo na reconstrução da posição da agulha. Imagens adquiridas com anilho fechado mostraram a melhor resolução e são portanto recomendadas para detetar o tumor e deixar o computador calcular as coordenadas da lesão. Após introduzir as coordenadas no software do sistema de biopsia, a agulha de biopsia é posicionada pelo computador (movimento translacional ao longo do eixo x, movimento horizontal ao longo do eixo y, e movimento vertical ao longo do eixo z), posteriormente o anilho PET abre e o médico tem oportunidade de realizar a biopsia manualmente. Após posicionar manualmente a agulha com a ponta visível dentro da lesão, utilizando uma imagem estática, a posição da ponta da agulha pode ser avaliada com a aquisição de imagem com anilho aberto. A resolução da imagem adquirida com anilho aberto é menos elevada, mas o suficiente para detetar a lesão e a ponta da agulha. Se a posição da ponta da agulha não for idónea, a agulha pode ser movimentada manualmente.

Para avaliar o sistema de biopsia utilizou-se aproximadamente 1 MBq de FDG-18F injetado num pedaço de carne embalado a vácuo para servir de fantoma, simulando lesão maligna dentro da mama. Após a localização automática da lesão foram adquiridas 3 biopsias utilizando a agulha de biopsia com um diâmetro e 3 mm, que obtém amostras de biopsia de 2.8 mm × 12 mm, e ao mesmo tempo administrando compressão no fantoma. O estudo de fantoma demonstrou que o alvo contaminado no interior do fantoma deslocou no máximo 3 mm devido à inserção da agulha, o que é aceitável, considerando o diâmetro da agulha. Após a administração das biopsias o sistema é possível verificar a presença de radioatividade nas amostras fazendo imagens adicionais das amostras com o sistema MammoCare. Para avaliar as imagens adquiridas com MammoCare incluímos 5 pacientes com estádio II e III de câncer da mama.

Concluindo, esta primeira avaliação técnica de MammoCare mostrou resultados promissores relativamente à localização automatizada e precisão de biopsias, embora seja necessária validação clínica adicional em pacientes com câncer da mama. Na sequência desta avaliação técnica as biopsias guiadas por FDG podem ser utilizadas para investigar o valor clínico de heterogeneidade de tumores.

Resumindo demonstrámos em diversos capítulos desta tese o valor adicional de PET/CT para o diagnóstico e o tratamento de pacientes com câncer da mama. Como recomendação geral gostaríamos de afirmar que PET/CT poderá ter um papel de relevo como modalidade de imagem em pacientes com câncer primário da mama para melhorar o estadiamento inicial antes de iniciar TNS.
A MAMMI-PET continua em desenvolvimento, tendo no entanto revelado resultados promissores na detecção de lesões primárias quando comparado com a PET/CT em decúbito dorsal. De igual forma a primeira avaliação técnica do sistema MammoCare mostrou resultados promissores.
References


Summary in Portuguese | 221
Appendix
"18F-FDG PET/CT for distant metastasis screening in stage II/III breast cancer patients: A cost-effectiveness analysis from a British, US and Dutch perspective"
Abstract

Purpose
18F-FDG PET/CT (PET/CT) is more accurate than conventional imaging (CI) in detecting distant metastasis (DM) in primary stage II/III breast cancer patients. As PET/CT comes at high costs, we estimated its added value from a perspective of the UK, the US and the Netherlands.

Patients and methods
A Markov model compared costs, life years (LYs), quality-adjusted LYs (QALYs), and cost-effectiveness (incremental net monetary benefit, iNMB) of DM screening with PET/CT vs. CI (according to European and US standards) from a hospital perspective over a 5-year time horizon in four breast cancer subtypes (classified by ER and HER2 status). Imaging performance, systemic, and local treatment data stemmed from the Netherlands Cancer Institute. Epidemiological, survival and utility data were derived from recent literature. Costs (2013) derived from national tariffs (UK/NL)/Centres for Medicaid and Medicare Services (US). One-way sensitivity analysis identified the ceiling PET/CT costs to achieve cost-effectiveness per country.

Results
PET/CT was more sensitive (92% vs. 13%) and specific (98% vs. 94%) than CI. Gains in LYs (0.007±0.0001) and QALYs (0.002±0.0001) were similar across subtypes. Largest cost savings were in ER-positive/HER2-negative patients (incremental costs NL/UK/US=€447/€1100/-€1461) and least in ER-positive/HER2-positive (€1739/€4382/€2662). PET/CT was expected cost-effective with high certainty in HER2-negative patients of the US (iNMB 2 range=€1089/€1571, 83-97%). Ceiling PET/CT costs for ER-positive/HER2-negative and ER-negative/HER2-positive were $1000 (US)/€600 (NL)/£500 (UK). For the remaining subtypes, this was conditional to additional cost-reductions in trastuzumab (US), or trastuzumab plus paclitaxel (NL/UK).

Conclusions
PET/CT adds value if it reduces costly palliative treatment. So far, this is only achieved with high certainty in the HER2-negative subtypes of the US. Reductions in PET/CT and palliative treatment costs are warranted to attain cost-effectiveness in the NL and UK.
Introduction

Preoperative systemic treatment (PST) is becoming treatment of first choice in breast cancer as it facilitates breast conservation and has positive influence on survival. Breast cancer patients receiving PST require prior distant metastases (DM) screening. Currently, this is performed by bone scan, plus liver sonography and chest X-ray in Europe, or plus CT thorax/abdomen in the US. Recently, positron emission tomography with integrated low-dose computed tomography (PET/CT), using fluorine-18 fluoro-deoxy-glucose ($^{18}$F-FDG) has shown to be of additional value to detect DM. In a series of 167 patients recruited in a comprehensive cancer centre (Netherlands Cancer Institute–Antoni van Leeuwenhoek Hospital; NKI), an overall sensitivity of 100% was found for PET/CT, compared to 57.9% for conventional imaging (CI). These findings lead to new recommendations in the ‘Dutch guidelines for breast cancer diagnostics and treatment’ stating that “$^{18}$FDG PET/CT can replace conventional staging methods for DM screening and is, therefore, advised for stage III and can be considered in stage II primary breast cancer”.

PET/CT is also able to better detect metastatic lesions in an earlier stage than CI. If DM lesions are limited in number (max 3 or 5), so-called “oligometastatic lesions”, the patient can be treated with curative intent. The clinical adoption of PET/CT is thus expected to improve survival outcomes in breast cancer patients. However, PET/CT comes at significant additional cost. Its actual implementation will therefore depend on the extent to which these costs are justified by the incremental health benefits achieved, as well as by the potential cost savings attained in other parts of the patient pathway.

To estimate added value of implementing PET/CT for DM screening in stage II/III breast cancer patients generalizable to the current clinical setting, we conducted a model-based cost-effectiveness analysis (CEA) using patient data from the NKI. As PET/CT is a potentially relevant application in a variety of countries for this purpose, we conducted this analysis from a perspective of the Netherlands (NL), the United Kingdom (UK) and the United States (US). Furthermore, we explored the ceiling PET/CT costs to achieve cost-effectiveness in each country.

Patients and methods

We developed a Markov model to compare health economic consequences of DM screening by ‘full body $^{18}$FDG PET/CT’ or by ‘CI’ in four cohorts of stage II-III breast cancer (ER-negative HER2-positive, ER-positive HER2-positive, ER-negative HER2-negative, and ER-positive HER2-negative) scheduled for PST. CI was modelled according to European and US standards. For technical details of PET/CT and CI see supplementary material. The CEA was performed from a hospital perspective.
perspective of the NL, the UK and the US (annual discount rates per country were of 4% for costs and 1.5% for effects\textsuperscript{12}; 3.5% for both\textsuperscript{13}; 3% for both respectively)\textsuperscript{14} over a 5-years’ time horizon.

Imaging performance, systemic and local treatments, and patient baseline characteristics (stage II/III breast cancer, post-menopausal status, 50 years old) were derived from patients treated at the NKI from 2007 to 2013. Epidemiological, survival and utility data were derived from recent literature or expert assumptions. Costs (2013) were obtained from national tariffs (UK and NL), and the Centres for Medicaid and Medicare Services (US).

**Markov model**
The Markov model has eight mutually exclusive health-states reflecting the natural history of the disease (Figure 1). Patients entered the model classified with respect to the presence of DM at imaging, based on the PET/CT or CI strategy, as true-positive (TP), false-positive (FP), true-negative (TN) or false-negative (FN). DM lesions were grouped into single lung, single bone, single liver or multiple. Patients were classified as positive following a tumour-positive biopsy, or if no biopsy was taken, by confirmation on another imaging modality. Patients were classified as negative based on survival at 6 months after the PET/CT was made. Specific definitions for TP, FP, TN and FN are shown in table 1.

Transition of a patient from one health-state to another was defined in yearly cycles for a time horizon of 5-years. A description of the course that patients followed in the model as well as the assigned health-state costs and utilities are presented in the supplementary material.

**Figure 1:** Decision tree and Markov model of distant metastasis screening with PET/CT vs CI in four subtypes of stage II/III breast cancer patients. Two strategies are presented: DM screening with PET/CT vs. DM screening with CI (chest X-ray, liver sonography plus bone scan (UK/NL) and CT-thorax-abdomen plus bone scan (US)). In the first year of the model, simulated by the decision tree, all patients incur the costs of DM screening and primary breast cancer treatment. Furthermore, in the case of true- and false- positive patients, they also incur the additional cost of biopsy, plus DM treatment (true positives) and imaging (false positives), and in the case of false- negative patients, additional costs of biopsy plus imaging and DM treatment. The quality-of-life of patients in this first year will mainly be determined by the presence or absence of DM. The last square of the tree represent the health-state of Markov model were patients enter in the 1st year, either stable or DM health-state. The Markov model simulates the disease progression of the patients, were costs and quality of life are accumulated at the time horizon of 5-years.

Abbreviations: DM= distant metastases; Tx=treatment; L=local, PBC= primary breast cancer treatment

NOT INCLUDED.
Table 1: Definitions, survival, costs and quality of life associated assumptions regarding true-positive, false-positive, true-negative and false-negative patients.

<table>
<thead>
<tr>
<th>Definition</th>
<th>Survival</th>
<th>Costs</th>
<th>Quality of life</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imaging reveals metastasis and is confirmed by biopsy or additional imaging</td>
<td>++</td>
<td>+++</td>
<td>++ (Presence DM and Palliative)</td>
</tr>
<tr>
<td>(early detection DM)</td>
<td></td>
<td>(biopsy and DM&lt;sub&gt;i&lt;/sub&gt;)</td>
<td></td>
</tr>
<tr>
<td><strong>FP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imaging reveals metastasis but the presence of metastatic disease is not confirmed by biopsy or additional imaging</td>
<td>+++ (no DM)</td>
<td>++ (biopsy and confirmation scans)</td>
<td>+++ (PBC&lt;sub&gt;fin&lt;/sub&gt;)</td>
</tr>
<tr>
<td><strong>TN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imaging reveals no metastasis and this is confirmed by “6 months follow-up”</td>
<td>+++ (no DM)</td>
<td>+ (none)</td>
<td>+++ (PBC&lt;sub&gt;fin&lt;/sub&gt;)</td>
</tr>
<tr>
<td><strong>FN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imaging reveals no metastasis but metastatic disease is present at “6 months follow-up”</td>
<td>+ (late detection of DM)</td>
<td>++++ (biopsy, confirmation scans and DM&lt;sub&gt;i&lt;/sub&gt;)</td>
<td>+ (painful DM and Palliative)</td>
</tr>
</tbody>
</table>

*As all patients in our database were scanned by CI and PET/CT, when calculating the performance of CI the following had to be assumed: patients that were negative under the conventional strategy but that were treated as positive at the discretion of the physician after PET/CT discovered DM were included in the false negative (FN) group. These patients were assigned the same costs, utilities and transition probabilities as the remaining FNs.

Abbreviations: DM= distant metastases; Tx=treatment; L=local, PBC= primary breast cancer treatment

Model input data

*Clinical database*

We retrospectively collected data from 545 stage II/III breast cancer patients who underwent CI and PET/CT to detect distant dissemination before start of PST in the NKI from 2007 to 2013. From this database, we derived imaging performance (PET/CT and CI) and data on primary breast cancer treatment (PST, breast surgery, adjuvant chemotherapy and breast radiotherapy). Performance data was obtained from 413 patients (supplementary table 2). Data on primary
A cost-effectiveness analysis from a British, US and Dutch perspective

breast cancer treatment came from 157 patients treated in 2013 only (supplementary table 3). As this was the most recent data, it was expected to most adequately represent current treatment.

Pre-treatment core biopsies of the primary tumour were classified according to the conventional criteria of the World Health Organization\textsuperscript{15} to determine breast cancer subtypes. After pathology assessment, but prior to PST initiation, patients were scanned with CI and PET/CT. Next, the reports of PET/CT and CI were discussed in a multi-disciplinary meeting where the nuclear physician and radiologist gave their advice, and whether further investigations were desirable, so consensus could be reached.

The treatment for patients with DM was assumed, as only nine patients in our dataset developed a metastasis. A patient with a single metastasis received local treatment consisting of surgery for metastases in liver and lung, and radiotherapy for lesions in the bone. Furthermore, patients with bone DM were treated with zometa (bisphosphonate). Multi organ metastasis were assumed to always include a bone lesion, and were treated with one line of systemic treatment, (according to Dutch guidelines)\textsuperscript{7}. If DM lesions were detected prior to start of treatment, patients received anastrozole plus zometa for 5-years (ER+/HER2-), trastuzumab plus paclitaxel until death (ER+/HER2+, ER-/HER2+) or Paclitaxel monotherapy until death (ER-/HER2-). If multi DM lesions where detected during treatment, regimens were capecitabine (ER+/HER-, ER+/HER2+, ER-/HER2-) and trastuzumab plus paclitaxel (ER-/HER2+) (Dosages and treatment time per systemic regimen in supplementary table 1).

Data derived from literature

Epidemiological data (i.e., common types and sites of metastasis per subtype, and frequencies of chemotherapy-related toxicities) and survival data (i.e., per site of metastasis) were derived from recent literature. Epidemiology data came from studies with similar subtype and DM sites classification as our model. While frequencies on the types of DM (multi or single) were derived from a Finish cohort study on 2.032 invasive operable breast cancer\textsuperscript{16} with similar frequencies as our database (22% multiple vs. 78% single), frequencies on the DM sites (lung, liver, bone and multiple) came from a cohort of 531 U.S citizens with distant metastatic disease from breast cancer\textsuperscript{17}, in accordance with results of similar recent literature\textsuperscript{18-21}. Short-term chemotherapy-related adverse-events included vomiting, neutropenia, hand-food-syndrome, thrombocytopenia, mucositis and cardio-toxicities (symptomatic, class II-IV from the NYHA\textsuperscript{22}). These were included in the model if prevalence $\geq$10% and if classified as related to anthracyclines, taxanes, anthracyclines plus taxanes, anthracyclines plus Trastuzumab and paclitaxel plus Trastuzumab (supplementary table 4).

The data on breast cancer mortality came from a Norwegian study on the survival of 304 metastasized breast cancers\textsuperscript{18}. Survival was assigned based on first site of metastasis: bone (bone
DM), visceral (liver and lung DM) or ‘bone plus visceral’ (multi organ DM). Survival rates in years 4 and 5 were assumed equal for patients with ‘visceral’ and ‘bone plus visceral’ lesions. This was decided upon the low patient numbers in these years, generating unexpectedly different survival rates between these groups. In FN patients, the probability of breast cancer death was simulated higher than in FP, as metastases are detected with a delay and there is a lower likelihood of cure. The applied factor was estimated from our database, where a 1.8 higher probability of breast cancer death was observed in FNs vs FPs, which was corroborated by an experienced surgical oncologist. The probability of dying from a non-breast cancer related event was derived from the Dutch cancer registry.

The costs of systemic treatments and of the treatment of adverse events were derived from Dutch published literature, except vomiting where we used data from Canada due to the lack of a Dutch estimator, NHS reference costs and average selling prices from CMS and literature. All costs were inflated to 2013 values using the Consumer Price Index and transformed to euros.

Utility estimates were obtained from the review of Peasgood et al or from the CEA registry. When multiple utilities were identified, we prioritized those reflecting the patient’s perspective using the EQ-5D profile. Biopsy was assumed 100% accurate and that induces no QALY decrement.

Supplementary table 4 summarizes all model parameters and its sources.

Model outcomes
Outcomes were 5-years’ incremental effects (FN and FP prevented, TP and TN gained, and life years (LY) and quality-adjusted-life-years (QALYs) gained), incremental costs (2013, reported in country-specific currencies and euros) and incremental net monetary benefit ratio (iNMB) of DM screening with PET/CT minus DM screening with CI. Cost-effectiveness of PET/CT was assumed if iNMB>0 and no cost-effectiveness if iNMB<0.

Cost effectiveness analysis
A probabilistic sensitivity analysis (PSA) with 10,000 Monte Carlo simulations was undertaken for each breast cancer subtype, using the costs of each country (NL, UK and US). Each model parameter was assigned a probability distribution: Dirichlet for performance, beta for effectiveness and utilities, and gamma for costs parameters (supplementary table 4). By randomly drawing a value for each input parameter from the assigned distribution, the PSA quantifies the joint decision uncertainty in model outcomes. This is summarized in cost-effectiveness acceptability curves (CEACs) that represent the probability that, given a certain threshold of willingness to pay for a QALY, the intervention is cost-effective. The iNMB (i.e., cost-effectiveness) was determined
using the prevailing threshold for cost-effectiveness in each country ($\lambda = €80.000/QALY$ in the Netherlands, £30.000/QALY in the UK and $50.000/QALY$ in the US). CEACs were presented per country and subtype.

**One-way sensitivity analysis**

One-way sensitivity analysis (SA) was conducted to all model parameters to determine to which parameters each model was most sensitive. This was performed from a US and NL perspective, but not the UK model, as this is expected to behave similar to the NL model. Furthermore, by using each parameter mean from the PSA, we determined the upper margin of costs of PET/CT that warrant the PET/CT strategy to become cost-effective per country.

**Results**

Sensitivity and specificity were 13% and 92% for CI, and 94% and 98% for PET/CT respectively. The PET/CT strategy prevented FNs and FPs by 0.89 and 0.65 respectively, while increasing TN and TP by 1.04 and 8.3 respectively. Subtypes with higher probability to develop bone DM (ER-positive/HER2-positive and ER-positive/HER2-negative) had higher LYs, as these lead to longer short-term survival as compared to visceral DMs. Subtypes with high frequency of multiple DMs (ER-negative/HER2-negative and ER-positive/HER2-positive) had lower utility weights resulting in lower QALYs. This lead to $0.007\pm0.0001$ LYS and $0.002\pm0.0001$ QALYs gained, depending on tumour subtype.

An increase in costs by the PET/CT strategy was consistently seen in the UK (range €1100/€4382) and in the NL (€447/€1739), but not in the US (€-1461/€2662). In the UK and the NL, largest cost savings were seen in ER-positive/HER2-negative (€1100/€447), followed by ER-negative/HER2-positive (€1319/€582), ER-negative/HER2-negative (€2629/€1050), and ER-positive/HER2-positive (€4382/€1739). In the US, largest savings were in ER-positive/HER2-negative (-€1461), followed by ER-negative/HER2-negative (-€991), ER-negative/HER2-positive (€133) and ER-positive/HER2-positive (€2662).

The iNMBs were highest in the US (range -€2517/€1571), compared to the NL (€259/-€1560) and the UK (-€4289/-€1003), following the opposite order as incremental costs. In the US, PET/CT became cost-effective in the subtypes with cost savings. The probability that PET/CT was cost-effective was low in the UK (range 0/22%) and the NL (4/31%), dependent on subtype. In the US, this was high for the ER-positive/HER2-negative (97%) and ER-negative/HER2-negative subtypes (83%), but below 50% for the remaining subtypes. Cost-effectiveness results are summarized in table 2 and CEACs are presented in Figure 2.
Table 2: Results from the cost-effectiveness analysis.

<table>
<thead>
<tr>
<th></th>
<th>The US</th>
<th>The Netherlands</th>
<th>The UK</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ Costs</td>
<td>Δ LYs</td>
<td>Δ QALYs</td>
<td>iNMB (pCE)</td>
</tr>
<tr>
<td>ER-positive/HER2-positive</td>
<td>-€1461</td>
<td>0.007</td>
<td>0.002</td>
<td>€1571</td>
</tr>
<tr>
<td>HER2-negative</td>
<td>-€1606</td>
<td>0.007</td>
<td>0.002</td>
<td>€1727</td>
</tr>
<tr>
<td>ER-negative/HER2-positive</td>
<td>€133</td>
<td>0.007</td>
<td>0.002</td>
<td>-€18</td>
</tr>
<tr>
<td>HER2-negative</td>
<td>€146</td>
<td>0.007</td>
<td>0.002</td>
<td>-€20</td>
</tr>
<tr>
<td>ER-negative/HER2-negative</td>
<td>-€5991</td>
<td>0.007</td>
<td>0.002</td>
<td>€1089</td>
</tr>
<tr>
<td>HER2-positive</td>
<td>-€1090</td>
<td>0.007</td>
<td>0.002</td>
<td>€1197</td>
</tr>
<tr>
<td>ER-positive/HER2-positive</td>
<td>€2662</td>
<td>0.007</td>
<td>0.002</td>
<td>-€2517</td>
</tr>
<tr>
<td>HER2-negative</td>
<td>€2822</td>
<td>0.007</td>
<td>0.002</td>
<td>-€2766</td>
</tr>
</tbody>
</table>

Abbreviations: LY = life years; QALY = quality adjusted life year; iNMB = incremental monetary benefit; pCE: probability of cost-effectiveness. 1 pound = 1.41 euros; 1 dollar = 0.91 euros

Figure 2: Cost-effectiveness acceptability curves per subtype and country (10,000 simulations). In each figure, the bottom curves represent the probability that the PET/CT strategy is more cost-effective than conventional imaging (CI *iNMB>0), at a specific willingness to pay threshold, different per country (marked with a horizontal line).
Results from one-way SA to all model parameters showed that DM screening costs, palliative treatment costs and imaging performance drove cost-effectiveness. These are presented in the supplementary material.

The upper margin of cost of PET/CT that warrant the PET/CT strategy cost-effective in ER-positive/HER2-negative and ER-negative/HER2-positive were $1000(US), €600(NL), and £500(UK) (table 3). Even with these levels, achieving cost-effectiveness in ER-positive/HER2-positive and ER-negative/HER2-negative patients of the NL and the UK, and ER-positive/HER2-positive patients of the US, requires lowering yearly costs of palliative trastuzumab and paclitaxel given to TPs (potential scenarios for the treatment costs are presented in supplementary table 5).

Discussion

Our study reveals that PET/CT outperforms both combinations of CI in detecting DMs in stage II-III breast cancer patients. However, this comes at additional costs of imaging and palliative treatment, so far only outweighed by health benefits in the US. Lowering the costs of PET/CT, possibly with costs of specific palliative treatment, may result in cost-effectiveness in the NL and the UK is to be achieved.

The 8.3x increase in early and 0.89x decrease in late detection of DMs by the PET/CT strategy resulted in LYs and QALYs gains in all subtypes and countries analysed (equal between countries, and similar between subtypes). The observed health gains were, however, modest (0.007 LYs and 0.002 QALYs), as can be expected for the limited survival of metastatic patients.
Incremental costs were mainly driven by costs of DM screening; as these are incurred in the total breast cancer population under study. This trend was noticed in the incremental costs per country; high incremental DM screening costs (the UK) had the highest overall incremental cost per patient. A secondary driver of incremental costs were palliative treatment costs, and their influence was seen in HER2-positive subtypes in the US, where drug prices are high. While in the US most savings were expected for having the lowest increase in DM screening cost, the incremental costs in the HER2-positive subtypes were similar to those in the NL and the UK.

The main driver of incremental cost differences between subtypes was palliative treatment costs, received by TP and FN patients. As PET/CT increased TPs (x8.3) and decreased FPs (x1.04), we observed that ER-positive/HER2-positive patients who needed the most costly TP treatment of all (trastuzumab plus paclitaxel) and a proportionally cheaper FP treatment (capecitabine), had the highest incremental costs in all countries. On the other end of the spectrum, ER-positive/HER2-negative patients had the least incremental costs, as their TP treatment is the cheapest (anastrozole plus zometa), and their FPs treatment is proportionally more expensive (capecitabine).

As health gains were similar across countries and subtypes, but costs differed, the latter drove cost-effectiveness results. Only in the subtypes with cost savings (HER2-negative subtypes in the US), cost-effectiveness was achieved, with high probabilities. In the remaining of cases probability of cost-effectiveness remained below 50% ((Figure 2).

The main driver of cost-effectiveness was imaging performance, followed by DM screening costs or palliative treatment costs depending on subtype. As PET/CT performance is substantially better, courses of action to warrant the PET/CT strategy to reach better cost-effectiveness (vs CI) should focus on alternative drivers. Thus, in determining upper margin costs for PET/CT, we saw that for ER-positive/HER2-negative and ER-negative/HER2-positive cost-effectiveness could be achieved without varying palliative treatment costs, which was not the case for the remaining subtypes, where additional cost-reductions in trastuzumab (US), or trastuzumab plus paclitaxel (NL/UK) would be needed. Additionally, palliative treatment costs could eventually be lowered as DM lesions could be identified in the “oligometastatic” stage, as these patients can be treated with curative intent.

The cost-effectiveness of DM screening with PET/CT in breast cancer has previously been reported from a Dutch perspective. Unfortunately, this study reported incremental costs per saved biopsy70, and can therefore not be compared to our cost/QALY estimates.

One of our study’s limitations is that biopsy performance was assumed perfect, yet false-negative rates reported in literature (0-9%58) make this a fairly feasible assumption. Moreover, the factor applied to lower FNs survival, warrants further research, as despite being derived from our clinical database and confirmed by an experienced surgeon, it is uncertain and a key driver of cost-effectiveness. Yet at the time of study, this was the best available source. Although it is
not well known whether 6-months follow-up is sufficient to capture missed DM at screening, this time frame was chosen in accordance with previously reported results of our institute\textsuperscript{5}. Finally, we assumed primary breast cancer treatment in all countries to be equal of that of the NKI, as we expect treatment guidelines to be similar.

Our study demonstrates that PET/CT adds value in detecting DM in breast cancer if it detects TP patients treated with low-priced palliative treatment, and prevents FNs with low-prognosis. In short, if it reduces costly palliative treatment. So far, this is only achieved in the HER2-negative subtypes of the US. To achieve cost-effectiveness in the NL and the UK, reductions in PET/CT and palliative treatment costs are warranted, as well as the increased detection of ‘oligometastatic lesions’ treated with local procedures and curative intent.

Acknowledgements

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Author Contribution
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Chapter 1: The role of PET/CT for nodal staging in primary stage II/III breast cancer patients
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Manuscript review: all authors

Chapter 2: Additional Prone 18F-FDG PET/CT Acquisition to Improve the Visualization of the
Primary Tumour and Regional Lymph Node Metastases in Stage II/III Breast Cancer
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Chapter 3: PET/CT with 18F-FDG predicts short-term outcome in stage II/III breast cancer patients
upstaged to N2/3 nodal disease
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Data analyses: VvN, ST
Manuscript writing: ST
Manuscript editing: ST, BK, EP, WV, JW, MS, MVP, ER, RVO
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**Chapter 4**: Monitoring Primary Tumour Response to Neoadjuvant Chemotherapy using MRI and 18F-FDG PET/CT in Breast Cancer Subtypes
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Data acquisition: ST, BK, WV, CL, JW, KP
Data analyses: AS, KG
Manuscript writing: AS, ST, KG
Manuscript editing: all authors
Manuscript review: all authors

**Chapter 5**: Additional value of 18F-FDG PET/CT response evaluation in axillary nodes during neoadjuvant chemotherapy for triple-negative and HER2-positive breast cancer
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Data acquisition: BK, ER, GS, KP, MvR, RVO, SR, ST, WV
Data analysis: GS, MVP, MvR, WV
Manuscript writing: BK, GS, MvR, ST, WV
Manuscript editing: all authors
Manuscript review: all authors
Chapter 6: Evaluation of a Hanging-Breast PET System for Primary Tumour Visualization in Patients With Stage I-III Breast Cancer: Comparison With Standard PET/CT

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Data acquisition: BK, ST, JFR, RSJ, MdP
Data analysis: VnN, ST, RSJ
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Chapter 7: Dedicated breast PET (MAMMI-PET) in daily clinical practice: implications for radiation safety of nuclear medicine personnel

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Data analysis: ST, RVO, LWV
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Manuscript editing: all authors
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Chapter 8: A novel semi-robotized device for high-precision $^{18}$F-FDG-guided breast cancer biopsy

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Manuscript editing: all authors
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Summary of PhD training, teaching and parameters of esteem

Name PhD student: Suzana Cipriano Teixeira
PhD period: September 2012 – September 2017

Name PhD supervisors: Prof. Dr. EJTh Rutgers, Dr. MPM Stokkel, Dr. M-JTFD Vrancken Peeters

Portfolio PhD training

<table>
<thead>
<tr>
<th>Courses</th>
<th>Year</th>
<th>Workload Hours (ECTS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncology school Of Amsterdam (OOA), Amsterdam, The Netherlands</td>
<td>2012</td>
<td>42 (1.5)</td>
</tr>
<tr>
<td>- Basic Medical Statistics / SPSS</td>
<td></td>
<td></td>
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<tr>
<td>12-16 November 2012</td>
<td></td>
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<tr>
<td>Medical Evaluation &amp; Technology Assessment (ME-TA), Antwerp, Belgium</td>
<td>2013</td>
<td>24 (1.0)</td>
</tr>
<tr>
<td>- Training economic evaluations of medical interventions, 6-8 November 2013</td>
<td></td>
<td></td>
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<tr>
<td>Amsterdam Medical Centre</td>
<td>2014</td>
<td>42 (1.5)</td>
</tr>
<tr>
<td>- Scientific Writing in English for Publication.</td>
<td></td>
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<tr>
<td>9 May, 16 May, 20 June and 4 July 2014</td>
<td></td>
<td></td>
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<tr>
<td>- Oral Presentation in English.</td>
<td>2014</td>
<td>22 (0.8)</td>
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<tr>
<td>8 April and 6 May, 2014</td>
<td></td>
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<tr>
<td>Meetings:</td>
<td></td>
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<tr>
<td>Journal club, nuclear medicine department (NKI-AvL)</td>
<td>2012-2015</td>
<td>112 (4)</td>
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<tr>
<td>Monthly sentinel node meeting, nuclear medicine department (NKI-AvL)</td>
<td>2012-2014</td>
<td>72 (3.0)</td>
</tr>
<tr>
<td>Annual breast cancer symposium NKI-AvL</td>
<td>2012-2015</td>
<td>24 (1.0)</td>
</tr>
<tr>
<td>Monthly Wednesday morning section XI meeting</td>
<td>2013-2015</td>
<td>24 (1.0)</td>
</tr>
<tr>
<td>Regular attendance of DOo Rolopende Nascholing Medische Oncologie DONAMO</td>
<td>2012-2015</td>
<td>22 (0.8)</td>
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<tr>
<td>Presentations at (Inter)national conferences</td>
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<tr>
<td>Annual PhD retreat of Oncology Graduate School (OOA), 23-25 October 2012, Ermelo, The</td>
<td>2012</td>
<td>24 (1.0)</td>
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<tr>
<td>Netherlands. (1 poster)</td>
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<tr>
<td>- Screening, staging and response monitoring for patients with breast cancer: How can we</td>
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<td>tailor medical imaging for the individual patient?</td>
<td></td>
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<tr>
<td>Annual congress of the European Association of Nuclear Medicine (EANM) 19-23 October</td>
<td>2013</td>
<td>40 (1.25)</td>
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<tr>
<td>2013, Lyon, France. (1 poster, 1 oral presentation)</td>
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<tr>
<td>- Evaluation of primary tumour and regional lymph node metastases with FDG PET/CT in</td>
<td></td>
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<tr>
<td>breast cancer patients: prone versus supine position, (oral)</td>
<td></td>
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<tr>
<td>12 (0.5)</td>
<td></td>
<td></td>
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</tbody>
</table>

248 | PhD Portfolio
- Evaluation of primary tumour and regional lymph node metastases with FDG PET/CT in breast cancer patients: prone versus supine position. (Poster)
The European Cancer Congress (ECCO) 26 September to 1 October 2013. (2 posters)
- Evaluation of primary tumour and regional lymph node metastases with FDG in breast cancer patients: prone versus supine position.
- Detection of occult N3-disease in breast cancer patients with PET/CT - does it affect survival?
Annual congress of the European Association of Nuclear Medicine (EANM) October 18-22 2014, Gothenburg, Sweden. (3 oral presentations)
- A multivariate analysis to evaluate FDG PET/CT for locoregional assessment in stage II/III breast cancer patients.
- Visualization of histologic proven breast cancer on a dedicated PET for hanging breast imaging: the MAMMI-PET
- Detection of > 4 FDG-avid nodes or occult N3-disease in breast cancer patients with PET/CT - does it affect short-term progression free survival?
Annual PhD retreat of Oncology Graduate School (OOA), 22-24 October 2014, Renesse, The Netherlands (1 oral presentation)
- Detection of >4 FDG-avid nodes or occult N3-disease in breast cancer patients with PET/CT - does it affect short-term progression free survival?
- Visualization of histologic proven breast cancer on a dedicated PET for hanging breast imaging: the MAMMI-PET
San Antonio breast cancer symposium (SABC), December 9-13 2014, San Antonio, Illinois, USA. (3 posters)
- Locoregional assessment by FDG PET/CT in stage II/III breast cancer patients: A multivariate analysis.
- Visualization of histologic proven breast cancer on a dedicated PET for hanging breast imaging: the MAMMI-PET
- Detection of occult N3-disease in breast cancer patients with PET/CT - does it affect survival?
Annual congress of the European Association of Nuclear Medicine (EANM) 10-14 October 2015, Hamburg, Germany. (2 oral presentations, 1 poster)
- Comparison of dedicated hanging breast PET and standard whole body PET/CT for primary tumour visualization in breast cancer patients (oral)
- Monitoring Tumour Response to Neoadjuvant Chemotherapy using MRI and 18F-FDG PET/CT in Breast Cancer Subtypes. (oral)
- \(^{18}\text{F}-\text{FDG PET/CT for distant metastasis screening in stage II/III breast cancer patients:} \)

A cost-effectiveness analysis from a Dutch perspective.

Radiological Society of North America (RSNA), 29 November to 4 December 2015

2015, Chicago, Illinois, USA. (1 oral presentation)

- Evaluation of a dedicated hanging breast PET (MAMMI-PET) for primary tumour visualization in stage I-III breast cancer patients: a comparison with standard PET/CT

2015, 48 (1.5)

2. Teaching

<table>
<thead>
<tr>
<th>Year</th>
<th>Workload Hours (ECTS)</th>
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</thead>
<tbody>
<tr>
<td>2012</td>
<td>8 (0.25)</td>
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<tr>
<td>2013</td>
<td>8 (0.25)</td>
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<tr>
<td>2015</td>
<td>8 (0.25)</td>
</tr>
</tbody>
</table>

3. Parameters of Esteem

Awards

Annual congress of the European Association of Nuclear Medicine (EANM) 19-23 October 2013, Lyon, France:

Eckert & Ziegler Abstract Award, 1000 euro

4. Peer reviewed Publications

In this thesis

- The role of PET/CT for nodal staging in primary stage II/III breast cancer patients. SC Teixeira, M-J TFD Vrancken Peeters, MPM Stokkel, EJTh Rutgers, RA Valdés Olmos, BB Koolen. Breast Cancer Management, June 2015

2015

- Dedicated breast PET (MAMMI-PET) in daily clinical practice: implications for radiation safety of nuclear medicine personnel


2016

- Additional Prone \(^{18}\text{F}-\text{FDG PET/CT Acquisition to Improve the Visualization of the Primary Tumour and Regional Lymph Node Metastases in Stage II/III Breast Cancer.} \)


2016


- Additional value of 18F-FDG PET/CT response evaluation in axillary nodes during neoadjuvant therapy for triple-negative and HER2-positive breast cancer, MS van Ramshorst, SC Teixeira, BB Koolen, KE Pengel, KG Gilhuijs, Jelle Wesseling, S Rodenhuis, RA Valdés Olmos, EJTh Rutgers, WV Vogel, GS Sonke, MJTFD Vrancken Peeters. Cancer Imaging, 2017 May


Other

- Urological leaks after pelvic exenterations comparing formation of colonic and ileal conduits, SC Teixeira, FT Ferenschild, MJ Solomon, L Rodwell, JD Harrison, JM Young, A Vasilaras, D Eisinger, P Lee, C Byrne European Journal of Surgical Oncology, April 2012.

Breast cancer tailored staging using molecular imaging

A warm thank you to Cees A. van der Mey who made this beautiful watercolour specially for the cover of this thesis.

Invitation
to the public defence of my PhD thesis entitled:

Breast cancer tailored staging using molecular imaging

On Thursday 14th of September 2017 at 10:00 in the Agnietenkapel
University of Amsterdam
Oudezijds Voorburgwal 229-231
Amsterdam
Reception/lunch afterwards at Dante Kitchen & Bar
Spuistraat 320, Amsterdam

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