PET/CT and dedicated PET in breast cancer: Implications for classification, staging, and response monitoring
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Introduction and outline
Breast cancer incidence and mortality
Breast cancer is the most frequent type of cancer in women all over the world. It is estimated to account for 29% of all new cancer cases among women in the US in 2012.\textsuperscript{1} The incidence of breast cancer in the Netherlands is still increasing.\textsuperscript{2} In 2000, 140 new cases per 100,000 women (11,249 new patients) were diagnosed, which increased to 158 new cases per 100,000 women (13,257 new patients) in 2010.\textsuperscript{3} The lifetime probability of developing breast cancer for women in the Netherlands is approximately 12-13% (one in eight). In contrast with the rising incidence, breast cancer mortality has been decreasing consistently since the nineties.\textsuperscript{4} This decrease largely reflects improvements in early detection and adjuvant treatment.\textsuperscript{5,6}

Classification and characterization
Breast cancer is a heterogeneous disease with various morphological appearances, behavior, and response to therapy. It can be subdivided according to several characteristics, yielding different treatment strategies and prognostic groups. The TNM (tumor, node, metastasis) staging system (Table 1) is generally used for this purpose, based on size and invasion of the primary tumor (T-stage), involvement of locoregional lymph nodes (N-stage), and presence or absence of distant metastases (M-stage).\textsuperscript{7}

Based on the T-, N-, and M-stage, different TNM-stages/prognostic groups can be identified:
- Stage 0: TisN0M0
- Stage IA: T1N0M0
- Stage IB: T0-1N1mi
- Stage IIA: T0-1N1 or T2N0
- Stage IIB: T2N1 or T3N0
- Stage IIIA: T0-2N2 or T3N1-2
- Stage IIIB: T4N0-2
- Stage IIIC: Any TN3
- Stage IV: Any TNM1

The five-year relative survival rate of breast cancer patients decreases according to stage at presentation from 99% in node-negative disease, to 84% in node-positive disease, and to 23% in distant disease.\textsuperscript{1}

A second commonly used method for breast cancer classification is immunohistochemical (IHC) analysis, which is routinely used in most diagnostic labs. The estrogen receptor (ER) and progesterone receptor (PR) determine the sensitivity for endocrine (hormonal) therapy,\textsuperscript{8} whereas overexpression of the human epidermal growth factor receptor 2 (HER2) identifies patients sensitive to HER2-targeted therapy.\textsuperscript{9} Based on IHC markers, three clinical subtypes are distinguished: HER2-positive (i.e., HER2 positive, ER and PR may be positive or negative), ER-positive/HER2-negative (i.e., ER positive, HER2 negative, PR may be positive or negative), and triple negative (i.e., ER, PR, and HER2 negative). The prognosis of HER2-positive tumors was initially poor, but has shown a marked improvement after the introduction of targeted HER2-therapy such as trastuzumab.\textsuperscript{9,10}
ER-positive/HER2-negative tumors, the most common subtype, tend to be less aggressive in short term, but are also less responsive to chemotherapy and may recur even after many years.\textsuperscript{11-13} Triple negative tumors are often very proliferative and bear the poorest prognosis, but are frequently more responsive to systemic therapy.\textsuperscript{14}

Also, breast tumors can be classified into subtypes distinguished by pervasive differences in their gene expression patterns, generating five different subgroups: normal breast-like, basal epithelial-like, HER2-enriched, luminal A, and luminal B.\textsuperscript{15,16} In addition, breast cancer can be classified according to histologic type (for instance, ductal, lobular, metaplastic, mucinous),\textsuperscript{17} proliferative activity (Ki-67 labeling index),\textsuperscript{18} grade (assessing tubule formation, nuclear polymorphism, and mitotic count),\textsuperscript{19} and vascular invasion.\textsuperscript{20}

Based on the large variety of human breast tumors and their diversity in natural history and responsiveness to treatment, breast cancer treatment has changed from a generalized concept towards an individualized and patient-tailored approach.

**Table 1** T-, N-, and M-stage according to AJCC cancer staging manual.

<table>
<thead>
<tr>
<th>Site</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T - tumor</strong></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>No primary tumor found</td>
</tr>
<tr>
<td>Tis</td>
<td>Carcinoma in situ</td>
</tr>
<tr>
<td>T1</td>
<td>Carcinoma ≤2 cm in greatest dimension</td>
</tr>
<tr>
<td>T1mic</td>
<td>Micro-invasion ≤1 mm</td>
</tr>
<tr>
<td>T1a</td>
<td>Tumor 2-5 mm</td>
</tr>
<tr>
<td>T1b</td>
<td>Tumor 6-10 mm</td>
</tr>
<tr>
<td>T1c</td>
<td>Tumor 11-20 mm</td>
</tr>
<tr>
<td>T2</td>
<td>Tumor &gt;2 cm and ≤5 cm</td>
</tr>
<tr>
<td>T3</td>
<td>Tumor &gt;5 cm</td>
</tr>
<tr>
<td>T4</td>
<td>Tumor of any size with direct extension in chest wall or skin</td>
</tr>
<tr>
<td><strong>N - regional lymph nodes (clinical)</strong></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>No regional lymph node metastases</td>
</tr>
<tr>
<td>N1</td>
<td>Metastases in mobile ipsilateral axillary nodes (&gt;2.0 mm)</td>
</tr>
<tr>
<td>N2</td>
<td></td>
</tr>
<tr>
<td>N2a</td>
<td>Metastases in clinically fixed or matted axillary nodes</td>
</tr>
<tr>
<td>N2b</td>
<td>Clinically detected ipsilateral internal mammary node metastases without clinically evident axillary lymph node metastases</td>
</tr>
<tr>
<td>N3</td>
<td></td>
</tr>
<tr>
<td>N3a</td>
<td>Subclavicular metastases</td>
</tr>
<tr>
<td>N3b</td>
<td>Metastases in the axilla and internal mammary chain</td>
</tr>
<tr>
<td>N3c</td>
<td>Supraclavicular metastases</td>
</tr>
<tr>
<td><strong>M – distant metastases</strong></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>No distant metastases</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastases</td>
</tr>
</tbody>
</table>
Conventional locoregional and distant staging

The main route of lymphatic drainage of the breast is to the axilla. Therefore, axillary lymph nodes are often the first site of regional metastatic disease in breast cancer. The presence or absence of axillary lymph node metastases remains an important prognostic factor in patients with potentially curably carcinoma of the breast. Knowledge of regional lymph node metastases may dictate indications for surgical treatment, systemic therapy, and/or locoregional radiotherapy. Historically, nodal involvement was determined by complete removal of all axillary lymph nodes (axillary lymph node dissection, ALND). Histopathological staging of the axilla is currently done with a sentinel lymph node biopsy (SLNB). The sentinel lymph node is the initial lymph node upon which the primary tumor drains. Giuliano was the first to describe its use in breast cancer in 1994. The efficacy, safety, and accuracy have been validated and axillary recurrence after a tumor-negative SLNB and omission of ALND was only 0.3% after a median follow-up of 34 months. In case of a tumor-positive SLNB, completion ALND is usually performed.

Currently, axillary staging begins with ultrasound (US) of the axilla. Fine needle aspiration (FNA) of suspect nodes detects approximately 30% of all tumor-positive axillary nodes and reduces the number of SLNBs. However, the accuracy of axillary staging with US is suboptimal and in case of negative FNA an SLNB is required.

An increase in the number of tumor-positive axillary nodes is related to a worsened prognosis, irrespective of primary tumor size. Presence of lymph node metastases in the internal mammary chain (IMC) or periclavicular area reduces survival probabilities even further. Internal mammary node metastases are predominantly detected in case of drainage to the IMC on lymphoscintigraphy, which is seen in 22–24% of patients after intratumoral tracer injection. Although involvement of IMC nodes is of prognostic significance, biopsy and irradiation of these nodes are still controversial because of lack of established survival benefit and increased morbidity. Staging of periclavicular nodes is usually done with US and FNA, although its accuracy in the detection of metastases is limited.

The most common sites of hematologic spread of breast cancer are bone, lung, liver, and brain. Distant metastases at diagnosis are more frequently seen in patients with large tumors or axillary lymph node metastases. Therefore, screening for distant metastases before treatment is advised for patients with locally advanced (stage III) breast cancer and could be considered in stage II disease with positive nodes. Different imaging techniques, such as bone scintigraphy, chest radiography, ultrasound of the liver, and/or computed tomography (CT) scans of thorax and abdomen are advised for this purpose. Because of the low prevalence of metastases, distant staging in stage I breast cancer is not recommended.

Breast cancer treatment

Treatment of breast cancer consists of three aspects. The first is achievement of optimal local control, which is primarily achieved by surgery with or without radiotherapy. In patients with operable breast cancer either a modified radical mastectomy or breast-
conserving therapy with postoperative irradiation of the breast can be performed, with or without direct or delayed reconstruction. No difference in long-term survival was found between both treatment modalities.\(^{40}\)

The second aspect regards regional control. In current clinical practice an ALND is performed in case of node-positive disease. Additional irradiation of chest wall and/or locoregional nodes after surgery is advised in patients at high risk for locoregional recurrence (LRR): \(\geq 4\) tumor-positive axillary nodes, involvement of nodes in the IMC or periclavicular area, T4 tumors, or T3N1 tumors.\(^{41,42}\) The value of chest wall and/or locoregional radiotherapy in patients at intermediate risk for LRR (T1-2 tumors with 1-3 tumor-positive axillary nodes or T3N0 tumors) is uncertain and frequently subject of debate and clinical research.\(^{43-47}\)

The third part of breast cancer treatment aims to prevent the development of distant metastases. Adjuvant systemic therapy (i.e., chemotherapy, hormonal therapy, and/or HER2-targeted therapy) focuses on eliminating micrometastases and circulating tumor cells that might already be present at the time of diagnosis, thereby preventing the outgrowth of metastases and improving survival.\(^{48}\)

**Neoadjuvant chemotherapy**

In contrast with adjuvant chemotherapy (given after surgical removal of the tumor), neoadjuvant chemotherapy (NAC) is administered prior to surgery. Synonymous terms include ‘primary systemic therapy’ and ‘induction chemotherapy’. It was first applied in patients with inoperable breast cancer in order to achieve down staging of the tumor, resulting in the possibility to perform radical surgery in part of them. Currently NAC has become standard practice in inoperable and locally advanced breast cancer and is increasingly applied in large and/or node-positive disease.\(^{49}\) The reduction in tumor load facilitates a higher proportion of breast-conserving treatment (increase 17-32\%) and may allow surgery in patients who were initially unresectable.\(^{50,51}\)

Also, NAC offers an excellent platform for translational research, since the molecular characteristics of the individual tumors can be directly related to sensitivity and resistance for NAC.\(^{12}\) Alternatively, in patients treated with primary surgery and adjuvant systemic treatment, tumor response remains unknown and becomes apparent after development of distant metastases or absence thereof.

The presence of tumor cells in the surgery specimen after NAC has been associated with an unfavorable prognosis, thereby emphasizing the intention of achieving a complete pathological response (pCR), particularly in triple negative and HER2-positive tumors.\(^{52}\) Another advantage of NAC is the opportunity to monitor the response (early) during treatment. In case of an unfavorable response, there is the possibility to switch the chemotherapeutic regimen or perform early surgery.\(^{53}\) In addition, response monitoring could prevent patients from experiencing unnecessary further drug toxicity. Response monitoring with physical examination or ultrasound were imprecise,\(^{54,55}\) but magnetic resonance imaging (MRI) has been shown to be useful; it was especially effective in triple negative and HER2-positive tumors, but was less accurate in ER-positive/HER2-negative tumors.\(^{56}\) Up till now, no benefits in overall and disease-free
Some challenges of neoadjuvant administration of chemotherapy should be noted. First, histopathological information before systemic treatment is based on histological biopsies instead of a complete surgical specimen. Accurate determination of grade and proliferation index is difficult in core biopsies. Further, based on the principle of intratumor heterogeneity, one tumor may consist of different molecular or even IHC-based subtypes, possibly leading to hindered selection of chemotherapeutic regimens. Thus, an important problem with assessing prognostic factors on core biopsy specimens is undersampling of informative areas. Second, because of the administration of chemotherapy before surgical treatment, a different strategy for locoregional nodal staging is required. US with FNA of axillary and periclavicular nodes is routinely performed prior to NAC, but its accuracy in the detection of (small) metastases is suboptimal. SLNB before NAC can accurately stage axillary and IMC nodes and may enable determination of axillary response after NAC, but it requires an additional surgical procedure. Further, if a tumor-positive pre-chemotherapy SLNB is routinely followed by post-chemotherapy ALND, overtreatment may occur in patients with a complete response to NAC. An SLNB after NAC determines final axillary status and results in less invasive axillary treatment in patients with a complete axillary response. However, the identification rate is suboptimal (81-94%) and the false negative rate is relatively high (14-20%), especially in (clinically) node-positive patients. Also, uncertainty remains for the other axillary nodes, as they receive no further treatment in case of a negative post-chemotherapy SLNB, although they might have been tumor-positive initially. A third challenge of NAC is that planning of postoperative irradiation of chest wall and/or locoregional nodes is hampered. Because of pretreatment with NAC, the pathological T-stage and the number of tumor-positive axillary nodes, important indicators for postoperative irradiation, remain unknown. Especially the value of radiotherapy in complete responders remains unknown.

**Individualized breast cancer treatment**

Both under- and overtreatment of patients, mainly caused by improper patient selection, are undesirable and can be decreased by patient-tailored treatment. Examples hereof are treatment with HER2-targeted therapy in HER2-positive patients and increased use of gene-expression profiles. Treatment with NAC offers an exciting foundation for individualized management. If patients achieving a pCR are discovered at an early stage during NAC, less invasive surgery may be sufficient. Conversely, if patients with an unfavorable response can be adequately identified early during treatment, the chemotherapeutic regimen could be changed or early surgery could be performed. It is essential to select patients optimally benefiting from NAC, but also to exclude those for whom a different treatment strategy is more suitable; patients with multiple distant metastases should be recognized at an early stage, sparing them an intensive treatment (i.e., neoadjuvant chemotherapy, major surgery) and directly assigning the appropriate treatment modality. Detection of responders and/or non-responders and improved patient selection is an important goal of the research as described in this thesis.
Molecular imaging in breast cancer: PET/CT
Positron emission tomography (PET) is a radiotracer imaging technique, in which tracer compounds labeled with positron-emitting radionuclides are injected and visualized. While PET was originally used as a research tool, in recent years it has come to have an increasingly important clinical role, mainly in oncology. The most widely used tracer in oncology is the combination of the isotope fluorine-18 (18F) with the tracer compound fluorodeoxyglucose (FDG). It follows a similar metabolic pathway to glucose, except that it is not metabolized to CO2 and water, but remains trapped within tissue. This is of interest in oncology because proliferating cancer cells have a higher than average rate of glucose metabolism. Therefore, as compared with other imaging modalities as US, CT, and MRI, 18F-FDG PET is a functional rather than an anatomical imaging technique. One of the drawbacks of PET was the lack of anatomical information, limiting its specificity. By combining functional FDG PET with morphological CT in dual-modality PET/CT, a hybrid imaging device with both anatomical and metabolic images is generated. Tumor staging of solid tumors with PET/CT was significantly more accurate than PET alone.

Adapting PET/CT acquisition to specific situation of breasts and regional nodes
During PET/CT acquisition patients normally lie in supine position. In this position the breathing motion of the thorax is ventrally, which results in blurring of the area of interest in breast cancer patients. Also, breast tissue is compressed, hampering local evaluation. In our institute breast cancer patients are scanned in prone position and with hanging breasts on a dedicated breast coil. Scan time for the PET is 3.00 min per bed position, CT slices are 2.0mm, and image reconstruction is to 2x2x2 mm voxels. This approach provides a detailed scan of the thorax without tissue compression and results in improved tumor delineation and less breathing artifacts. Also, it enables image comparison with MRI.

Dedicated PET for hanging breast molecular imaging
Although several papers have shown that PET/CT could be used for distant staging in primary stage II-III or recurrent breast cancer, its use is not advised for the detection or visualization of the primary tumor for several reasons. First, the spatial resolution full width at half maximum (FWHM) of most whole body scanners is limited to approximately 5 mm. Second, the partial volume effect limits precise imaging and quantification of small tumors. Also, the path of the photons from source to detector is long and involves structures of the entire thorax, resulting in increased likelihood of the photons to be absorbed or scattered and signal loss because of attenuation and decreased contrast. Despite these limitations, there is increased demand for accurate tumor visualization with FDG PET and quantification of metabolic activity; it can be of...
value in patients with dense breasts, in whom mammography, US, and MRI have been shown to be less accurate. Further, there is a correlation between the degree of FDG uptake and prognostic factors in breast cancer and promising results have been reported regarding PET and PET/CT response monitoring during NAC. Finally, selective biopsies of certain high metabolic areas of the tumor (FDG-guided biopsies) could improve pretreatment molecular classification and genetic profiling. These considerations have led to the development of dedicated breast PET devices.

**MAMMI PET**

The recently developed high-resolution breast PET (MAMMI, Oncovision, Valencia, Spain) is a dedicated PET for hanging breast molecular imaging (Figure 2). Patients are scanned in prone position, without compression of the breast. Through an opening in the table, a single hanging breast is positioned in the detector ring, which consists of 12 detector modules in dodecagon configuration. The effective field of view (FOV) diameter (breast width) is 170 mm and the coronal FOV (breast length, from pectoral muscle to nipple) can extend to 170 mm by means of precise motion of the detector arm from which the ring extends. The spatial resolution (FWHM) ranges from 1.6 mm in the center of the FOV to 2.7 mm at the edges of the FOV. Voxel size is 1 mm³. Images are reconstructed in 3D using a maximum likelihood expectation maximization algorithm including an attenuation correction through image segmentation and using 12 iterations. The first clinical experiences with the MAMMI PET are reported in this thesis.

![PET/CT scanner at the Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital (Philips Gemini TOF).](image)
RATIONALE AND OUTLINE OF THIS THESIS

The general aim of the research described in this thesis was to improve personalized breast cancer treatment by investigating the use and clinical value of 18F-FDG PET/CT in primary breast cancer patients, both before and during treatment: in the detection of the primary tumor and metastases before therapy and in monitoring the response during treatment with neoadjuvant systemic therapy. Second, we wished to assess the value of 18F-FDG PET/CT and MAMMI PET for improvement of primary tumor characterization.

Part 1 of this thesis focuses on pretreatment classification and staging of primary breast cancer with PET/CT. Chapter 2 of this thesis describes the association of primary tumor FDG uptake with different breast cancer characteristics. Also, it describes in which subgroup of stage II-III breast cancer patients PET/CT represents a reliable tool for staging and response evaluation and for which subgroup of patients a PET/CT could safely be omitted because of breast cancer characteristics with insufficient tumor FDG uptake. In chapter 3 the value of PET/CT in T1 breast cancer is assessed. Locoregional lymph node involvement on PET/CT and the change in nodal stage before treatment with NAC is described in chapter 4 and chapter 5, respectively. Chapter 6 describes the comparison of PET/CT with conventional imaging modalities for the detection of distant metastases before NAC.

Part 2 describes the value of hanging breast 18F-FDG PET/CT for response monitoring of the primary tumor and axillary lymph node metastases during NAC. Chapter 7
demonstrates that response monitoring of breast cancer with PET/CT is feasible, but that it is dependent on the clinical subtype. In chapter 8 the value of response monitoring early during NAC is presented. Two PET/CTs are compared with final pathology in order to select an optimal response monitoring time-point. Further, the value of combined breast and axilla response monitoring is evaluated. The combination of PET/CT and MRI and their complementarity for detection of pCR is presented in chapter 9. Finally, chapter 10 describes the accuracy of axillary response monitoring with PET/CT during NAC in order to select patients achieving axillary pCR, in whom less invasive axillary treatment may be appropriate.

In part 3 the value of hanging breast PET/CT and dedicated breast PET for primary tumor characterization is presented. Chapter 11 describes the correlation of the pretreatment tumor sampling location with the area with highest degree of FDG uptake. Based on the principle of intratumor heterogeneity, it aims to select a subgroup of patients with a clinically relevant distance and difference in FDG uptake between the two areas, in which a PET/CT before tumor sampling may be appropriate for guiding biopsies or in which FDG-guided biopsy could add information. Chapter 12 demonstrates the first clinical experience with the MAMMI PET, a dedicated PET for hanging breast molecular imaging. In chapter 13 a review on currently available dedicated breast PETs is presented. Finally, detection of heterogeneity in breast cancer with MAMMI PET is described in chapter 14.

This thesis ends with a summary, general discussion, and future prospects and a summary in Dutch and Spanish (chapter 15).

**Figure 3** Chemotherapy regimens and timing of PET/CT, MRI, and MAMMI scans in patients receiving NAC. NAC is given to patients with either large (>3 cm) and/or node-positive breast cancer. Patients with HER2-negative tumors are treated with six courses of cyclophosphamide and doxorubicin in a dose-dense schedule (ddAC). PET/CTs are performed after one and three courses (two and six weeks). HER2-positive tumors are treated with three cycles of eight weekly administrations of paclitaxel, trastuzumab, and carboplatin (PTC). In weeks seven and eight only trastuzumab is given. PET/CTs are performed after three and eight courses (three and eight weeks). After completion of chemotherapy, surgery of breast (+ axilla) is performed based on post-chemotherapy MRI evaluation.

* Conform institutional guidelines, based on consensus in a multidisciplinary meeting, chemotherapy in HER2-negative patients could be switched to a (hypothetically) non-cross-resistant regimen (docetaxel and capecitabine; CD). A switch was based on patient’s preference, drug toxicity, or MRI response monitoring (<25% decrease on MRI). Further, inclusion of triple negative tumors in additional chemotherapeutic trials could lead to treatment modification (cyclophosphamide, thiopeta, and carboplatin; CTC).
45 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 b6c randomized trials. Radiother Oncol 2007; 82: 247–53.