Towards patient-tailored surgical treatment in breast cancer
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Towards axilla sparing therapy after neoadjuvant chemotherapy
7.1

Feasibility of FDG-PET/CT to monitor the response of axillary lymph node metastases to neoadjuvant chemotherapy in breast cancer patients

Marieke E. Straver, Tjeerd S. Aukema, Renato A. Valdes Olmos, Emiel J.T. Rutgers, Kenneth G.A. Gilhuijs, M.E. Schot, Wouter V. Vogel, Marie-Jeanne T.F.D. Vrancken Peeters

Submitted
Abstract

Background: The aim of this study was to assess the accuracy of FDG-PET/CT to visualize lymph node metastases before the start of neoadjuvant chemotherapy and to determine how often the visualization is sufficiently prominent to allow monitoring of the axillary response.

Methods: Thirty-eight patients with invasive breast cancer of > 3 cm and/or lymph node metastasis underwent FDG-PET/CT before neoadjuvant chemotherapy. The results of the FDG-PET/CT were compared with those from ultrasonography with FNA-cytology or sentinel node biopsy. Patients suitable for response monitoring of the axilla were defined as having either a SUV_{max} ≥ 2.5 or a tumor-to-background ratio ≥ 5 in the most intense lymph node.

Results: The sensitivity and specificity of FDG-PET/CT in detecting axillary involvement were 97% and 100%, respectively. No difference existed between the SUV_{max} of the primary tumor and that from the related most intense lymph node metastasis. Moreover, the mean tumor-to-background ratio was 90% higher in the lymph nodes compared to the primary tumor (P=0.006). Ninety-three percent of the patients had sufficient uptake in the lymph nodes to qualify for subsequent response monitoring of the axilla. A considerable distinction in metabolic activity was observed between the different subtypes of breast cancer. The mean SUV_{max} in lymph node metastases of ER-positive, triple-negative and HER2-positive tumors was 6.6, 11.6 and 6.6, respectively.

Conclusion: The high accuracy to visualize lymph node metastases and the sufficiently high SUV_{max} and tumor-to-background ratio at baseline suggests that it is feasible to monitor the axillary response with FDG-PET/CT, especially in triple-negative tumors.


INTRODUCTION

The use of neoadjuvant chemotherapy plays an important role in the treatment of breast cancer. One of the major advantages of neoadjuvant chemotherapy is downstaging of the tumor load. As a result, inoperable advanced tumors may become operable and patients with large operable tumors may be offered breast conserving surgery (BCS). Another advantage of neoadjuvant chemotherapy is the possibility to monitor the response of the primary tumor to chemotherapy. Interim evaluation during neoadjuvant chemotherapy creates the opportunity to switch to another regimen in case of unfavorable response. Furthermore, response monitoring enables and moderates translational research which provides more insight in the behavior of different tumor subtypes and predictive factors.

Response monitoring is crucial in the neoadjuvant setting, as it defines the criteria to switch the chemotherapy regimen or to perform early surgery in insufficiently responding tumors at an early time point. After the administration of chemotherapy, response monitoring enables the evaluation of the extent of the residual tumor to select patients for breast conserving therapy. The axillary response after neoadjuvant chemotherapy yields prognostic information. Rouzier et al. showed that after neoadjuvant chemotherapy, a complete remission of nodal metastases was a strong predictor of disease free survival (48.7 versus 73.5% after 5 years). Early knowledge of the response of axillary lymph node metastases may therefore be helpful to tailor the systemic treatment upon individual response. Furthermore, a reliable method to evaluate the axillary tumor response is essential in order to reduce the rate of unnecessary axillary lymph node dissections (ALND), without compromising oncological safety. Patients who achieve a complete remission of their lymph node metastasis could potentially be treated with radiation therapy only. Unfortunately, to date it is not possible to reliably identify these patients. Physical examination and conventional imaging techniques, like MRI and ultrasound, do not have the ability to evaluate the response of axillary lymph node metastasis. Furthermore, the accuracy of the sentinel node procedure after neoadjuvant chemotherapy in patients with proven lymph node metastases remains questionable. At present, the most accurate assessment of the axillary tumor response is an ALND and therefore ‘axilla-conserving therapy’ is not yet among the benefits of neoadjuvant chemotherapy. Some evidence exists that positron emission tomography (PET)/CT using 18-flourdeoxyglucose (FDG) may successfully monitor the response of the primary tumor to neoadjuvant chemotherapy. The potential value of FDG-PET/CT to assess the response of axillary lymph node metastases to neoadjuvant chemotherapy is, however, currently unknown. The primary aim of this study was to assess the accuracy of FDG-PET/CT to visualize lymph node metastases before the start of neoadjuvant chemotherapy. The second aim was to determine how often the visualization is sufficiently prominent to allow monitoring of the axillary response with FDG-PET/CT.
Material and methods

Patients

Women who presented with invasive breast cancer larger than 3 cm in diameter and/or at least one tumor-positive axillary lymph node were scheduled to be treated with neoadjuvant chemotherapy in our institute. Since September 2007 patients were asked to participate in a pilot study to assess the value of FDG-PET/CT in the neoadjuvant setting. This study was approved by the institutional ethical committee and informed consent was obtained from all patients.

We analyzed 38 patients who had a FDG-PET/CT scan before the administration of neoadjuvant chemotherapy. All breast cancers were initially diagnosed by fine-needle aspiration. Core biopsy was used to determine hormone receptors and HER2 status, and to obtain tumor tissue for expression microarray analysis. The tumor size was assessed with ultrasound, mammography and MRI. Axillary staging was primarily done with ultrasonography and fine-needle aspiration (FNA) of suspect lymph nodes. In patients with negative lymph nodes (ultrasound and/or FNA-cytology negative) a sentinel node biopsy (SNB) procedure was performed prior to neoadjuvant chemotherapy. To assess the presence of distant metastases liver ultrasound, bone scintigraphy and chest X-ray were performed in all patients. Neoadjuvant chemotherapy was followed by breast-conserving surgery or mastectomy. All patients with proven axillary lymph node metastases prior to neoadjuvant chemotherapy underwent an ALND after neoadjuvant chemotherapy. Patients undergoing breast conserving surgery received radiation to the breast. The indication for loco-regional radiation therapy (chest wall and regional nodal basins) was based on the original staging before neoadjuvant chemotherapy. Hormone receptor-positive patients received adjuvant endocrine treatment for at least five years and HER2-positive patients received trastuzumab for one year.

FDG-PET/CT

The FDG-PET/CT scan was performed after conventional imaging of the breast and the axilla. Patients were prepared with a fasting period of 6 hours, and 10 mg valium per os 10 minutes before FDG administration to avoid brown fat activation. Blood glucose levels were required to be <10 mmol/l. Patients received 180-240 MBq FDG intravenously. The interval between FDG administrations and scanning was 60 minutes +/- 10 minutes. A whole body PET/CT scanner (Gemini TF, Philips, Eindhoven, The Netherlands) was used. FDG-PET image acquisition was managed via standardized acquisition procedures. Low-dose CT images (40 mAs, 5 mm slices) without oral or intravenous contrast were acquired for anatomical reference and attenuation correction. First, a 2 mm high resolution PET/CT was performed of the thorax (including breasts and axilla) with the patient in prone position with hanging breasts, and 3:00 minutes per bed position. Second, a
whole body PET/CT was performed from the skull base to the groins, with 1:30 minutes per bed position and standard image resolution. The administered activity, time of FDG administration, and body weight on the day of scanning was recorded for calculation of the maximum tumor standardized uptake value ($SUV_{\text{max}}$).

**Image interpretation**

All FDG-PET/CT-scans were read in consensus by three experienced readers. Visual assessments of locations, extent, and intensity of FDG uptake patterns were made. The FDG-uptake in the primary tumor and lymph node metastases was analyzed semi-quantitatively using the $SUV_{\text{max}}$, and the ratio of the $SUV_{\text{max}}$ to the mean activity in an adjacent sufficiently large area of normal surrounding tissue (tumor-to-background ratio, TBR). Patients suitable for response monitoring of the axilla were defined as having either a $SUV_{\text{max}} \geq 2.5$ or a tumor-to-background ratio $\geq 5$ in the most intense lymph node in the axilla.

**Statistical analysis**

The accuracy of FDG-PET/CT to visualize lymph node metastasis was evaluated by comparing the results of the FDG-PET/CT with the pathological results (tumor positive cytology or SNB results). The pathologist was blinded to the FDG-PET/CT results. The results were classified as true positive (TP), true negative (TN), false positive (FP) and false negative (FN). The evaluation of the results was based on the sensitivity; $\frac{\text{TP}}{\text{TP} + \text{FN}}$, specificity; $\frac{\text{TN}}{\text{TN} + \text{FP}}$ and overall accuracy; $\frac{\text{TP} + \text{TN}}{\text{all cases}}$.

The differences in $SUV_{\text{max}}$ and tumor-to-background ratios between the primary tumor and the axillary lymph node metastases were calculated using the paired $t$ Test and the Wilcoxon Signed Ranks Test. Differences in $SUV_{\text{max}}$ between the different tumor types were calculated using the one-way ANOVA test.

**Results**

A total of 38 patients were included in this study. Patient and tumor characteristics are outlined in table 1. The mean age of the patients was 49 (range 30-68) years. Most patients had T2 (n=23) and ductal tumors (n=31). Primary tumors were estrogen receptor (ER)-positive, triple negative (estrogen-, progesterone receptor and HER2 negative) and HER2-positive in 19, 7 and 11 patients, respectively. Twenty-nine patients had suspect lymph nodes on ultrasound with tumor positive FNA-cytology. In one patient only a suspect lymph node in the internal mammary chain was palpable (cN2b) and one patient had a positive subclavicular lymph node (cN3), both confirmed by ultrasound guided FNA-cytology.
Table 1 patients and tumor characteristics (total n=38)

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<tr>
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</table>

Abbreviations: NS (not specified) ER (estrogen receptor), PR (progesterone receptor) HER2 (human epidermal growth factor receptor 2)

Positive FDG PET

FDG-PET/CT showed pathological axillary lymph nodes in 30 patients (86%). In 27 of these 30 patients lymph node metastasis had also been detected with ultrasound and cytology. In one patient with a negative initial ultrasound examination, FDG-PET/CT was positive. Ultrasound was subsequently repeated with FNA-cytology, confirming a tumor-positive axillary lymph node (figure 1, pag. 153). In two patients with negative ultrasound and a positive FDG-PET/CT a sentinel node biopsy (SNB) was done. In one patient the SNB revealed macrometastases in 3 out of 4 sentinel nodes. In the other patient four lymph nodes were removed during the SNB procedure and they were all tumor-negative. However, follow-up FDG-PET/CT images strongly suggested that the suspect lymph nodes were not located in the area of the excised sentinel nodes, and had most likely been missed. (Figure 2, pag. 153). We excluded this patient from our accuracy analysis because of uncertainty about the initial lymph node status.
**Negative FDG-PET**

FDG-PET/CT showed negative axillary lymph nodes in 8 patients. In one patient the FDG-PET/CT was false-negative because she had a proven axillary lymph node metastasis by ultrasound guided FNA-cytology. Seven patients had a negative ultrasound examination. Four of these patients also had a tumor negative SNB. In three patients a SNB was not performed. After neoadjuvant chemotherapy, none of these patients had axillary lymph node metastases in the axillary lymph node dissection specimen, but we excluded them from our accuracy analysis because of uncertainty about their initial lymph node status.

**Feasibility to monitor the axillary response**

The results are summarized in figure 3. Excluding the cases outlined above, 34 patients could be evaluated. The sensitivity to visualize axillary lymph node metastasis was 97% (29/30, 95%CI: 0.83-0.99), the specificity was 100% (4/4, 95%CI: 0.51-1.0) and the overall accuracy was 97% (33/34). Ninety-three percent (29/31) of the patients had sufficient uptake in the lymph nodes to qualify for subsequent response monitoring of the axilla; a $\text{SUV}_{\text{max}} \geq 2.5$ or a tumor-to background ratio $\geq 5$.

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Figure 3 shows the accuracy of FDG-PET/CT to detect lymph node metastases. The sensitivity to visualize axillary lymph node metastasis was 97% (29/30, 95%CI: 0.83-0.99), the specificity was 100% (4/4, 95%CI: 0.51-1.0) and the overall accuracy was 97% (33/34).

*This patient most likely had a false negative sentinel node and was excluded from the analysis together with the three patients with negative ultrasound guided cytology and no sentinel node biopsy (SNB).
Extra-axillary lymph nodes

In 6/38 patients (16%) FDG-PET/CT showed suspicious uptake in extra-axillary lymph nodes that were not detected by conventional imaging (table 2). One patient had two FDG-PET-suspect lymph nodes, one intrapectoral and one subclavicular, which were not detected by ultrasound examination. In three patients FDG-PET/CT indicated additional lymph nodes metastases in the internal mammary chain, a region that is not routinely assessed with ultrasound. In one patient FDG-PET/CT revealed an unknown subclavicular lymph node metastasis and one patient showed pathological uptake in contra-lateral axillary lymph nodes.

<table>
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<th>Conventional imaging (US/FNA positive)</th>
<th>FDG-PET/CT positive</th>
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<tr>
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<td>4</td>
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<tr>
<td>Subclavicular</td>
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<td>3</td>
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<tr>
<td>Intra-pectoral</td>
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<td>1</td>
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<tr>
<td>Contra-lateral</td>
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<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>2 (5%)</td>
<td>9 (24%)</td>
</tr>
</tbody>
</table>

Abbreviations: US (ultrasound), FNA (Fine Needle Aspiration)

Differences between the primary tumor and the lymph node metastasis

The primary tumor could be visualized in 37 of 38 patients. One patient presented with a palpable axillary lymph node and an occult primary tumor that could not be visualized on either FDG-PET/CT or MRI. The mean $SUV_{max}$ of the primary tumors was 6.8 (range 1.6 -18.8, 95%CI 5.1-8.0), with a mean tumor-to-background ratio of 11.3 (range 2.1 to 36.0, 95% CI 8.3-14.3). Positive lymph nodes (axillary or extra-axillary) were visualized in 31 of the 38 patients. The mean $SUV_{max}$ of the most active lymph nodes was 7.7 (range 2.3-26.2, 95%CI 5.8-9.6). The mean background-to-tumor ratio was 23.7 (range 4.0-93.0, 95%CI 15.2-32.1). Paired evaluation of the $SUV_{max}$ of the primary tumor and the related most active lymph node in 30 patients with an active primary tumor and lymph node metastasis showed no difference. Paired evaluation of the tumor-to-background ratio showed a significant higher ratio (2.0) for the lymph nodes (P=0.006), explained by higher background uptake in the breast due to surrounding glandular tissue. A considerable distinction in the $SUV_{max}$ of both the primary tumor and the lymph node metastases was observed between the different subtypes of breast tumors. In ER-positive, triple negative and HER2 positive tumors the mean $SUV_{max}$ was 5.4 (95%CI 3.7-7.1), 11.1 (95%CI 6.0-16.1), and 5.9 (95%CI3.3-8.6), respectively (P=0.01). In the lymph node metastases the mean $SUV_{max}$ of ER-positive, triple-negative and HER2-positive tumors was 6.6 (95%CI 4.7-8.4), 11.6 (95%CI 5.0-18.2) and 6.6 (95%CI 2.7-10.6), respectively (p=0.07).
**Discussion**

This report describes the accuracy of FDG-PET/CT to visualize lymph node metastases in breast cancer before the start of neoadjuvant chemotherapy. The overall accuracy in this selected patient population was high; 97%. In 93% of the patients the lymph nodes showed adequate uptake for subsequent response monitoring of the axilla. Accurate visualization of lymph node metastasis with sufficient signal intensity prior to the administration of neoadjuvant chemotherapy is the first requirement to monitor the axillary response with FDG-PET/CT imaging. With this study we showed that it is reasonable to use FDG-PET/CT to assess the axillary response.

In our series the sensitivity of FDG-PET/CT to visualize lymph node metastases prior to neoadjuvant chemotherapy was 97%.[20,21] The sensitivity reported by others varies widely (20%-94%).[19] Studies including patients with more advanced primary tumors, similar to our population, showed a sensitivity of 85% and higher.[16, 20] This high sensitivity can be explained by the high a-priori likelihood of (voluminous) lymph node metastases. The sensitivity of FDG-PET/CT is lower in patients with early breast cancer and a clinically negative axilla. In a prospective study Veronesi et al. compared preoperative FDG-PET imaging with sentinel node biopsy (SNB) and reported a sensitivity of only 37%.[21] This finding can be explained by the relatively low spatial resolution of PET imaging, that does not allow detection of the micrometastases found using serial sectioning and immunohistochemistry at pathological assessment of sentinel nodes.

Other factors that could influence the sensitivity of FDG-PET are intrinsic tumor characteristics like grade and type. There is evidence that sensitivity of FDG-PET is higher in certain subgroups. Gil Rendo et al. showed a sensitivity of 100% to detect lymph node metastasis in a group of patients with grade III malignancy and a SUVmax higher than 3.5 of the primary tumor.[22] Moreover, Veronesi et al. used FDG-PET alone while integrated FDG-PET/CT can provide both anatomic and metabolic information, which in general provides a better accuracy.[23] Several studies report the superiority of FDG-PET/CT compared with that of PET alone.[24-26]

In our series the specificity was 100%. This is confirmed in other studies consistently reporting a high specificity ranging from 85% to 100%.[19] These findings suggest that a SNB procedure prior to the start of neoadjuvant chemotherapy may not be necessary if FDG-PET/CT shows a positive axillary lymph node, at least in the absence of an apparent inflammatory cause of lymph node activation such as non-malignant mastitis. By omitting the SNB procedure prior to neoadjuvant chemotherapy, the positive axillary lymph node will remain in situ, which will create the opportunity to monitor the axillary response in these patients. In addition, the patient is spared an invasive surgical diagnostic procedure.

Fuster et al. reported that FDG-PET/CT is a valuable tool to exclude unsuspected extra-axillary lymph
node and distant metastases in patients with large breast cancer.\textsuperscript{27} They showed that FDG-PET/CT led to a change in initial staging in 42% of patients, although it is critical to note that they did not perform ultrasound assessment combined with cytology of the axillary lymph nodes. In our series, 16% of the patients had suspect extra-axillary lymph node metastases by FGD-PET/CT that were not visualized by conventional imaging (ultrasound examination and sentinel node biopsy). A limitation of our study is that no biopsies were taken of these suspect extra-axillary lymph nodes, but the probability of nodal involvement is high based on the reported specificity of FDG-PET/CT.

FDG-PET/CT may successfully monitor the response of the primary tumor to neoadjuvant chemotherapy. Quantitative FDG PET scans of primary breast cancers showed a rapid and significant decrease in tumor glucose metabolism after effective treatment was initiated, and the reduction in metabolism antedated the decrease in tumor size.\textsuperscript{11, 17} Several studies investigated the accuracy of FDG-PET to monitor the response to neoadjuvant chemotherapy in breast cancer with reported sensitivity and specificity of 84%-100% and 74%-94%, respectively.\textsuperscript{9, 10, 12-16} These studies are difficult to compare since they use different time points in the course of chemotherapy and different cut-off points in the reduction of the standard uptake value (SUV) to distinguish responders from non-responders. Overall, it appears that the accuracy of FDG-PET to monitor response prediction is high early in the course of therapy. These studies focused on response monitoring of the primary tumor. No results have been presented yet regarding the value of FDG-PET to assess the early response to neoadjuvant chemotherapy of axillary lymph node metastases.

To monitor the early response of axillary lymph node metastases using FDG-PET/CT it is crucial to have a sufficiently high baseline $SUV_{\text{max}}$ or tumor to background ratio at baseline. A low baseline $SUV_{\text{max}}$ can underestimate the subsequent tumor response.\textsuperscript{13} McDermott et al. demonstrated that only tumors with an initial tumor to background ratio of greater than 5 showed a difference between response categories.\textsuperscript{13} Three patients in our study had a $SUV_{\text{max}}$ lower than 2.5 in the axillary lymph nodes, and three patients had a $SUV_{\text{max}}$ lower than 2.5 in the primary tumor. The mean tumor-to-background ratio was 90% higher in the axilla than in the primary tumor of the breast, which might facilitate the evaluation of the tumor response in the axillary lymph nodes compared to the primary tumor. The baseline $SUV_{\text{max}}$ is higher in triple negative tumors, which will make them more suitable for response evaluation with FDG-PET than ER-positive and HER2-positive tumors.

Early axillary response monitoring may have several clinical implications. It seems reasonable that the response of axillary lymph node metastases to the chemotherapy is highly indicative for the efficiency of chemotherapy, particularly, because the aim of chemotherapy is to eliminate micrometastatic disease. The ultimate goal is to adjust the treatment to the early response of the axillary lymph node metastases. Furthermore, knowledge regarding the axillary response might be used to tailor the surgical treatment of...
the axilla. FDG-PET/CT might select patients with a favorable axillary response for more axillary conserving therapies, like axillary radiation therapy.

In conclusion, this study shows that lymph node metastases can be visualized using FDG-PET/CT with good sensitivity and specificity. In addition, pathological lymph nodes have a sufficiently high baseline SUVmax and tumor to background ratio at baseline to enable response monitoring, especially in triple negative tumors. Consequently, monitoring the response of axillary lymph node with FDG-PET/CT during the early course of treatment may be feasible. Future research will focus on establishing of a cut of point in the therapy-induced change of SUV$_{\text{max}}$ to discriminate nodal responders from none-responders.
CHAPTER 7.1  The role of FDG-PET/CT in assessing the axillary response

Figure 1 FDG-PET scan of a patient without suspect axillary lymph nodes on initial ultrasound examination and a clear tumor positive lymph node on FDG-PET (arrow). Repeated ultrasound examination with cytology guided by FDG-PET/CT images revealed a tumor-positive axillary lymph node.

Figure 2 shows the FDG-PET/CT scan of a patient with two FDG-PET positive lymph nodes prior to neoadjuvant chemotherapy. Subsequently, the patient had a negative sentinel node biopsy (2a). After the administration of chemotherapy (2b) the lymph nodes show complete metabolic response but are still clearly visible on the low-dose CT, and were most likely not removed at SNB. These findings suggest that the patient could have a false negative SNB procedure or a false-positive initial FDG-PET/CT.
Reference List


Novel surgical technique to selectively remove metastatic axillary lymph nodes in breast cancer patients after neoadjuvant chemotherapy

Marieke E. Straver, Claudette E. Loo, Jelle Wesseling, Harry Maessen, Marcel Steggerda, Tanja Alderliesten, Marjo J. Holtkamp, Emiel J.T. Rutgers, Marie-Jeanne T.F.D. Vrancken Peeters

Submitted
Abstract

Background: An important benefit of neoadjuvant chemotherapy (NAC) is the increase in breast-conserving surgery. At present the response of axillary lymph node metastases to chemotherapy cannot be accurately assessed. Therefore axilla-conserving therapy is not yet a benefit. We aimed to assess a new surgical method to evaluate the axillary response.

Method: Prior to NAC, proven tumour-positive axillary lymph nodes were ultrasound guided localized with Iodine-125 seeds in 15 patients. After NAC, the marked lymph nodes were selectively removed with the use of a gamma-detection probe. A complementary axillary lymph node dissection was performed to assess if pathological response in the marked node was indicative for the pathological response in the additional lymph nodes.

Results: Tumour-positive axillary lymph nodes were successfully localized with an Iodine-125 seed in all patients. The marked lymph node was surgically detected and selectively removed after NAC in all 6 patients undergoing surgery. In these patients the pathological response in the marked lymph node was indicative for the overall response of the lymph node metastases.

Conclusion: This study shows that marking and selectively removing metastatic lymph nodes after neoadjuvant chemotherapy is feasible. The tumour-response in the marked lymph node may be used to tailor further axillary treatment.
INTRODUCTION

Neoadjuvant chemotherapy, or primary systemic therapy, is the standard of care in patients with locally advanced breast cancer and is increasingly being used in the treatment of large operable breast cancer. The major clinical benefit of neoadjuvant chemotherapy is downstaging of the primary tumour. In a recent meta-analysis Mieog et al. showed that the increase in breast conserving therapy was 25.6%. However, not only the primary tumour shows a response to neoadjuvant chemotherapy. A pathological complete remission (pCR) of axillary lymph node metastases is described in 22-38% of the patients with proven metastases prior to neoadjuvant chemotherapy. These patients might be treated more conservatively by axillary radiotherapy instead of an axillary lymph node dissection or a wait and see policy. Physical examination and ultrasonography do not have the ability to accurately evaluate the response of axillary lymph node metastases to chemotherapy. Due to this inability, a more axilla conserving approach is not yet a recognized benefit of neoadjuvant treatment. Furthermore, the accuracy of the sentinel node biopsy procedure after neoadjuvant chemotherapy remains questionable, especially in patients with proven lymph node metastases prior to the start of chemotherapy. In the present study we assessed the feasibility of a novel surgical method to evaluate the axillary response to neoadjuvant chemotherapy. Tumour-positive axillary lymph nodes were marked under ultrasound guidance, prior to neoadjuvant chemotherapy. After neoadjuvant chemotherapy, the marked lymph nodes were detected and selectively removed. In this way, histopathological evaluation of response of proven positive lymph nodes was obtained. Iodine-125 seeds were used to mark tumour-positive lymph nodes, therefore, we introduced the acronym MARI for this procedure (Mapping of the Axilla with Radioactive Iodine-125 seeds).

Patients and Methods

From 17 October 2008 until 22 April 2009, 15 patients with proven lymph node metastases who were scheduled to undergo neoadjuvant chemotherapy participated in this study. The study was approved by the institutional ethical committee and informed consent was obtained from all patients. All breast cancers were initially diagnosed by fine-needle aspiration and core biopsy was obtained to determine hormone-receptor and HER2-status. A marker was placed under ultrasound guidance in the primary tumour to facilitate the surgical excision and to optimally examine the tumour bearing area in the surgical specimen after neoadjuvant chemotherapy. Axillary staging was done with ultrasonography and in suspect lymph nodes fine-needle aspiration (FNA) cytology was performed. The treatment regimen depended on the presence or absence of HER2 amplification. Preoperative chemotherapy for HER2-negative tumours
CHAPTER 7.2 The MARI-procedure: Mapping of the Axilla with Radioactive I-125 seeds

Figure 1 shows an I-125 seed (STM1251, Bard Brachytherapy Inc., Carol Stream, IL, USA). The gold radiopaque marker (yellow) is covered by an aluminium layer, a copper coating (red) connected to the Iodine-125 layer (white) and covered with a titanium tube.

Figure 2 shows the positioning of an Iodine-125 seed in an axillary lymph node. The tip of an 18-gauge needle is occluded with sterile bone wax (B) and the I-125 seed is placed in the needle. The lymph node (hypoechoic) is visualized by ultrasonography (C) and the tip of the needle (black arrow) is inserted in the lymph node (D). The I-125 seed (with arrow) was moved through the bone wax and into the lymph node using a stylet (E).
employed one of the following regimens: dose dense (dd) AC (6 cycles of doxorubicin and cyclophosphamide) or the combination of 3 cycles of ddAC and 3 cycles of CD (capecitabine and docetaxel). Patients with HER2-positive tumours were treated with PTC (paclitaxel, trastuzumab and carboplatin). Neoadjuvant chemotherapy was followed by breast conserving surgery or mastectomy. Axillary treatment included the MARI procedure and a complementary axillary lymph node dissection.

Figure 3 shows the surgical removal of the marked lymph node. The point with greatest activity is detected (A) and marked at the skin (B). The surgical incision is made on the planned incision site of the mastectomy (C). Guided by the handheld gamma probe (D) the marked lymph node is detected (E) and removed. The removal of the I-125 seed is assured by detecting the Iodine-125 source of activity in the excised lymph node (F).

Figure 4 shows the finding and removal of an I-125 seed (black arrow) at the pathology department. The I-125 seed had not migrated outside the lymph node.
**MARI procedure- Iodine-125 seeds (Figure 1)**

A Iodine-125 (I-125) seed is a source of photon radiation with an average energy of 27 keV.\(^{11,12}\) The titanium encapsulation of the seeds are 4.5 mm by 0.8 mm and are available with a minimal apparent activity of 0.2 millicurie (mCi) (STM1251, Bard Brachytherapy Inc., Carol Stream, IL, USA). The half-life time is 59.6 days. The dose rate constant is 1.018 cGy h\(^{-1}\) U\(^{-1}\).\(^{13}\) Seeds arrived in a single vial and were stored at the nuclear medicine department. At the moment of the implantation, the I-125 seeds had an apparent activity varying from 0.04 to 0.19 mCi (1.6-7.0MBq). After pathological confirmation of the suspect axillary lymph node by cytology, a single I-125 seed was used to localize this lymph node.

**MARI procedure-radiological positioning (Figure 2)**

The I-125 seed was placed within an 18 gauge needle after occluding its tip with sterile bone wax. The positive lymph node was visualized with ultrasound to determine the appropriate place in the axilla to approach the lymph node. Local anaesthesia was given and a small incision in the skin was made. The needle tip was guided by ultrasound to the lymph node and a stylet was used to displace the radioactive seed through the bone wax and into the lymph node. The needle was withdrawn and the position of the I-125 seed was confirmed by ultrasonography and a scintillation detector. Marking of the positive lymph node was performed directly after marking of the primary tumour, for which we also used I-125 seeds. Six different radiologists performed the MARI-procedure. The marked lymph node is further referred to as the MARI-node.

**MARI-procedure- surgical excision (Figure 3)**

After completion of neoadjuvant chemotherapy, surgery to the breast and axilla was planned in the same session. A gamma-probe (neoprobe®, Johnson & Johnson Medical, Hamburg, Germany), adjusted to detect the photon energy of I-125 source, was placed over the surface of the axilla. The point of greatest activity was marked on the skin with ink. In patients who underwent a mastectomy, the incision was made in the area of the planned incision of the mastectomy close to the point of greatest activity. A separate incision was made close to the point of greatest activity in patients who underwent breast conserving surgery. The gamma probe was used to guide the excision of the marked lymph node. Removal of the correct lymph node was ensured by detecting the I-125 source of radioactivity within the lymph node and by detecting the absence of radiation within the area of the excision. The MARI-node was stored in a lead container posted with a caution sign for radioactive material, and transported to the pathology department. After removal of the MARI-node, an axillary lymph node dissection and surgery to the breast was performed. MARI-procedure-pathological assessment (Figure 4). The MARI-node was bisected
Results

Fifteen patients with proven axillary lymph node metastases and breast tumours varying from 2.0 to 9.0 cm (mean 4.5 cm) who were scheduled to undergo neoadjuvant chemotherapy were included in this study. The mean age was 53 years (range 38-66 years). Thirteen patients had a palpable axillary lymph node prior to neoadjuvant chemotherapy while two patients had a non-palpable tumour-positive axillary lymph node assessed by ultrasound and FNA-cytology.

MARI procedure- radiological positioning

The I-125 seeds were successfully positioned in the proven positive axillary lymph nodes of all 15 patients. None of the 6 different radiologists experienced difficulties during the positioning of the I-125 seeds within the axillary lymph nodes. No complications occurred during any of the procedures.

MARI-procedure- surgical excision

At the time of writing this paper, the surgical procedure was performed in 6 patients who completed their neoadjuvant chemotherapy regimen. The other 9 patients were still receiving their neoadjuvant chemotherapy. The median time between marking of lymph nodes prior to the start of neoadjuvant chemotherapy and surgery was 110 days (range 88-183 days). While 5 of the 6 patients had palpable nodes prior to the administration of neoadjuvant chemotherapy, none of the patients had palpable lymph nodes in the axilla after neoadjuvant chemotherapy. The localization of the previously tumour-positive lymph node could, however, easily be detected on the skin with the use of the gamma-probe in all patients. In 5 patients the incision was made in the planned incision site of the mastectomy and in 1 patient a separate incision was made. The marked lymph nodes were preoperatively detected with use of the gamma probe and separately removed in all patients (Table 1). The mean time of the surgical procedure was 10 minutes (range: 6 -13). At the time of surgery the I-125 seeds had an apparent activity varying from 0.02 to 0.06 mCi (0.7-2.1 MBq) and could be...
easily detected using the gamma probe. Four different surgeons performed the procedure straightforward and no complications occurred.

Table 1 shows that the marked lymph node (MARI node) was successfully detected in all 6 patients that undergo surgery. Abbreviations: ln; lymph node

### MARI-procedure-pathological assessment

The I-125 seeds were found within the lymph node in all patients during the pathological assessment, thus no migration of the I-125 seeds was observed. Pathological assessment showed residual metastases in all marked lymph nodes (Table 2). Macrometastases were observed in 4 patients and isolated tumour cells were observed in 2 patients. The patients with macrometastases in the MARI-node had respectively 1, 2, 4 and 14 macrometastases in their additional axillary lymph node dissection specimen. The other 2 patients showed clusters of residual tumour cells in the MARI-node within a greater area of reactive fibrosis, possibly representing the previously tumour-bearing area. In the complementary axillary lymph node dissection, residual micrometastases in an area of reactive fibrosis were found in respectively 1 and 2 lymph nodes.

Table 2 shows that the pathological outcome of the MARI node was indicative for the presence of additional metastases in the complementary axillary lymph node dissection in all six patients. Abbreviations: ln; lymph node
Discussion

In this report we show that it is feasible to mark tumour-positive axillary lymph nodes with I-125 seeds before neoadjuvant chemotherapy and to selectively remove them after neoadjuvant chemotherapy in order to determine the response to neoadjuvant chemotherapy.

This is the first study describing the feasibility to mark lymph nodes with I-125 seeds. Gray et al. previously reported the use of I-125 seeds to localize non palpable tumours in the breast in order to facilitate surgical localization of the tumor.\textsuperscript{14-16} Radiation exposure to the patient and staff was found to comply with radiation exposure regulations.\textsuperscript{17} Safe handling and documentation by radiologist, surgeons and pathologists is required to limit the risk of damage to and lost of the I-125 seeds. Maaskant et al. reported the application of the I-125 seeds to localize breast tumours prior to the start of neoadjuvant chemotherapy to facilitate breast surgery in patients with a clinical complete response of the breast tumor.\textsuperscript{18}

We show that the application of I-125 seeds can be expanded to localize axillary lymph nodes.

Although marking a lymph node in the axilla was a new technique, the six different radiologists experienced no difficulties during this procedure, even in smaller lymph nodes. All procedures were performed without complications. The four surgeons, who are very experienced in the sentinel node biopsy procedure, had no difficulties in detecting and removing the MARI-node with a handheld-gamma probe. They subjectively described the procedure no more difficult than to retrieve a radioactive sentinel node. Moreover, they found it easier because there was no background radiation compared to the sentinel node biopsy procedure.

Besides the practicality of the MARI-procedure, the response to chemotherapy in the marked lymph node seems to be indicative for the response to chemotherapy in the additional axillary lymph node metastases. In the patients with residual macrometastases in the MARI-node, macrometastases were also found in the additional lymph nodes. In patients with signs of regression and only residual micrometastases, similar signs of regression were also found in the additional lymph nodes. Unfortunately in this pilot study we were not able to investigate patients with a pathological complete remission in the MARI-node. To accurately assess the predictive value of the tumour response in the MARI-node we need to include 52 patients with residual axillary disease after neoadjuvant chemotherapy. For this power calculation we hypothesized that the expected true positive rate would be 95% and that the observed true positive rate will be above a minimally acceptable limit of 90% with a 1-sided 95% confidence interval.

Since the marked lymph nodes were easily detected during the surgical procedure, we decided to half the radiation activity of the I-125 seeds at the time of implantation. This has two advantages. Firstly, it reduces the radiation exposure to the patient and staff, although this is negligible.\textsuperscript{17} Secondly, it decreases the risk
on cell eradication through the radiation effects of the Iodine-125 seeds. Of course, we would not like the I-125 seeds to have an effective dose, because this would limit the negative predictive value of the MARI procedure. When tumour cells are eradicated by the radiation instead of the chemotherapy, the response of the tumour cells in the marked lymph node will not be indicative for the response to chemotherapy in the additional lymph nodes. However, we did not observed radiation effects at pathological examination in the first patients of this study. If the accuracy of the MARI-procedure is proven, the discussion remains which patients could be offered a more axilla conserving therapy (i.e., axillary radiation therapy instead of an axillary lymph node dissection). Two patients described in this article had only residual clusters of tumour cells in the MARI-node. In respectively one and two additional lymph nodes a similar small tumour burden was observed. These patients may be treated sufficiently with axillary radiation therapy. Otherwise, it could be argued that only patients with an axillary complete remission in the MARI-node should be treated with axillary radiation therapy or a wait and see policy. Further research will focus on these questions. Another possibility to determine the response of axillary lymph node metastases is to perform a sentinel node biopsy after neoadjuvant chemotherapy. The main principle of the sentinel node procedure is to detect the initial node upon which the primary tumour drains. In patients presenting with a clinically negative axilla the sentinel node procedure could be helpful after neoadjuvant chemotherapy. However, in patients who present with a clinically positive lymph node, marking this proven lymph node before the administration of neoadjuvant chemotherapy is far more logical than detecting the first lymph node upon which the meanwhile treated tumour drains after the administration of chemotherapy. Moreover, because tumour presence in the lymphatic channels may obstruct flow or alter drainage patterns, the false negative rate of the sentinel node could be higher in these circumstances.19 We systematically reviewed all studies separately reporting the sentinel node biopsy results after neoadjuvant chemotherapy in patients with proven metastases (table 3).20 Nineteen studies were identified which included a total of 793 patients.7-10;19;21-34 The pooled identification rate was 85% (range 68-100%) and the pooled sensitivity was 89% (67-100%). Because of the low identification rate and the reported wide ranges in sensitivity and specificity we do not recommend performing a sentinel node procedure in cN1 patients.

In conclusion, in this study we report that marking tumour-positive lymph nodes before neoadjuvant chemotherapy and selectively removing them after neoadjuvant chemotherapy is feasible. Future research will determine the accuracy of this method to predict the pathological response in additional lymph nodes and decide which patients can be offered axillary-conserving therapies.
<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Journal</th>
<th>No. of Patients</th>
<th>SNB Identification Rate</th>
<th>No. of Patients with Positive SNB</th>
<th>False Negative Rate</th>
<th>Axillary pCR</th>
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<td>93.7% (44)</td>
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<td>29.6 (8)</td>
<td>17/44(39%)</td>
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<tr>
<td>Tausch 2008</td>
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<td>Ann Surg Oncology</td>
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<td>12% (3)</td>
<td>21/46 (46%)</td>
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<td>Hino 2008</td>
<td>2008</td>
<td>Surg Today</td>
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<td>77% (17)</td>
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<td>Total weighted for N</td>
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<td></td>
<td>793</td>
<td>85% (68-100)</td>
<td>401</td>
<td>11% (0-33)</td>
<td>35% (5-50)</td>
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Table 3: The accuracy of the SNB procedure after neoadjuvant chemotherapy in patients with initially proven lymph node metastases.
CHAPTER 7.2  The MARI-procedure; Mapping of the Axilla with Radioactive I-125 seeds

Table 3  No. of Patients cN1 prior to PST

<table>
<thead>
<tr>
<th>Identification</th>
<th>No. of patients</th>
<th>with positive SNB</th>
<th>False negative rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nason 2000</td>
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<td>Hino 2008</td>
<td>22</td>
<td>77% (17)</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Total weighted for N 793 85% (68-100) 401 11% (0-33) 35% (5-50)

Reference List


