Improvements in locoregional treatment of breast cancer
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MARKING AXILLARY LYMPH NODES WITH RADIOACTIVE IODINE SEEDS FOR AXILLARY STAGING AFTER NEOADJUVANT SYSTEMIC TREATMENT IN BREAST CANCER PATIENTS: THE MARI-PROCEDURE


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ABSTRACT

Background
The MARI procedure (Marking the Axillary lymph node with Radioactive Iodine (\(^{125}\text{I}\)) seeds) is a new minimal invasive method to assess the pathological response of nodal metastases after neoadjuvant systemic treatment (NST) in breast cancer patients. This method allows axilla-conserving surgery in patients responding well to NST.

Patients and methods
Prior to NST, proven tumor-positive axillary lymph nodes were marked with a \(^{125}\text{I}\) seed (the MARI-node). After NST, the MARI-node was selectively removed using a gamma-detection probe. A complementary ALND was performed in all patients to assess whether pathological response in the MARI-node was indicative for the pathological response in the additional lymph nodes.

Results
A tumor-positive axillary lymph node was marked with a \(^{125}\text{I}\) seed in 100 patients. The MARI-node was successfully identified in 97 of these 100 patients (identification rate 97%). Two patients did not undergo subsequent ALND, leaving 95 patients for further analysis. The MARI-node contained residual tumour cells in 65 of these 95 patients. In the other 30 patients, the MARI-node was free of tumor, but additional positive lymph nodes were found in five patients. Thus, the MARI-procedure correctly identified 65 out of 70 patients with residual axillary tumor activity (false negative rate 5/70=7%).

Conclusions
This study shows that marking and selectively removing metastatic lymph nodes after neo-adjuvant systemic treatment has a high identification rate and a low false negative rate. The tumor-response in the marked lymph node may be used to tailor further axillary treatment after NST.
INTRODUCTION

Neoadjuvant or primary systemic treatment is increasingly applied in the treatment of operable breast cancer. Down staging of the primary tumor is one of the important goals of neoadjuvant systemic treatment, thereby permitting breast-conserving treatment without affecting the risk for a local relapse.\(^1\)\(^-\)\(^6\) Complete pathological response rates after neoadjuvant systemic treatment vary across histological subtypes and can be over 50% in HER2 positive disease. Down staging of the axilla is also observed in patients initially presenting with metastatic lymph nodes. Complete pathological response rates in the axilla vary between 22-42% in reported series, again depending on tumor subtype.\(^7\)\(^,\)\(^8\)

The therapeutic effect of a complete axillary lymph node dissection (ALND) is limited in case of a complete pathologic response in the axilla. If reliably identified, such patients can be offered a more conservative therapy of the axilla, sparing them the substantial short-and long-term morbidity of an ALND.\(^9\)\(^,\)\(^10\) Physical examination and imaging such as ultrasound or PET-CT have insufficient sensitivity and specificity to discriminate between residual disease and a complete pathological response in the axilla.\(^11\)\(^-\)\(^13\) Post-chemotherapy sentinel (SN) node biopsy (SNB) in patients with proven metastatic lymph nodes prior to NST is under debate since different rates of identification rates (between 68 and 100%) and false-negative rates (between 5 and 30%) are reported.\(^14\)\(^,\)\(^15\) In many of the studies described, the presence of nodal metastasis before the neoadjuvant systemic treatment was a predictive factor for failure of the SNB.

We aimed to develop a new technique to assess the response to neoadjuvant systemic treatment in patients presenting with nodal metastasis. For this purpose, radioactive iodine (\(^{125}\)I) seeds were used. The use of \(^{125}\)I seeds is increasingly applied in breast conserving surgery.\(^16\)\(^,\)\(^17\) Recent studies have described the use of \(^{125}\)I seed localisation to facilitate breast conserving surgery also after neoadjuvant systemic treatment.\(^18\)\(^,\)\(^19\) Before the start of systemic treatment, a \(^{125}\)I seed is placed in the center of the tumor. In case of a good clinical response after systemic treatment, a local excision can be performed around the \(^{125}\)I seed to remove residual disease or confirm the complete response by histological examination.

\(^{125}\)I seed localisation can also be performed in lymph nodes. It has previously been shown in a feasibility study with fifteen patients, that it is technically possible to mark tumor-positive axillary lymph nodes with \(^{125}\)I seeds prior to neoadjuvant systemic treatment and selectively remove them afterwards: the ‘Marking of the Axilla with Radioactive Iodine-125 seeds’ (MARI)-procedure.\(^20\) In this report the final analysis of the predictive value of this novel surgical technique to identify residual axillary lymph node metastasis in a prospective study is presented.

PATIENTS AND METHODS

Patients and systemic treatment regimes

From October 2008 until November 2012, patients with proven axillary lymph node metastases who were scheduled to undergo neoadjuvant systemic treatment were asked to participate in this study. One-hundred-and-three patients agreed and signed informed consent. Prior to the start of neoadjuvant treatment, tumor size was assessed with MRI and biopsies of the breast tumor were performed to determine the histological subtype and receptor status. Axillary staging was performed with ultrasonography and in suspect lymph nodes fine-needle aspiration (FNA) cytology
was performed. Tumors were classified according to the standard criteria of the World Health Organization and the neoadjuvant systemic treatment regimen depended on the presence or absence of HER2 amplification (table 1).  

The institutional ethical committee approved this study. Adjuvant radiotherapy and systemic treatment were given according to Dutch national guidelines.

**Seed localisation**

A detailed overview of the MARI-procedure was given previously. A titanium encapsulated $^{125}$I seed (STM1251, Bard Brachytherapy Inc., Carol Stream, IL, USA) with an average energy of 27 keV and a half-life time of 59.6 days was placed in an 18-gauche needle. Under ultrasound guidance, the $^{125}$I seed was placed in a previous proven metastatic lymph node by one of 15 different radiologists (Figure 1A, B). This marked lymph node is further referred to as the MARI-node. At the moment of the implantation, the $^{125}$I seeds have an apparent activity varying from 0.04 to 0.19 mCi (1.6-7.0 MBq) and the dose rate constant is 1.018 cGy h$^{-1}$ U$^{-1}$. Although the dose of the $^{125}$I seeds used for localization of metastatic lymph nodes is low, it requires authorization by the Dutch government. Furthermore, some safety issues need to be addressed. Radiation safety protocols and detailed documentation regarding the acquisition, handling and storage including guidelines for patients and hospital staff are described previously.

In the same procedure as the $^{125}$I seed localisation in the axillary lymph node, a $^{125}$I seed was placed in the breast tumor, which is in our institute standard practice for patients undergoing neoadjuvant systemic treatment. Afterwards a conventional X-ray was performed to confirm the presence of the $^{125}$I seed in the axilla and in the breast.

**Surgical procedure**

After completion of neoadjuvant systemic treatment, surgery of the breast and axilla was executed in the same session. One of eight different surgeons performed the procedure. The surgical part of the MARI- procedure has been described before in detail. In short: with the gamma-probe (neo2000®, Neoprobe Corporation, Dublin) on the $^{125}$I setting the point of greatest activity was detected on the skin of the axilla. The incision to remove the MARI-node was made in the planned incision for the ALND, close to the point of highest activity. Guided by the gamma probe the MARI node was intraoperatively detected and selectively removed (Figure 1C). Removal of the correct lymph node was ensured by detecting the $^{125}$I source of radioactivity within the lymph node ex vivo and by detecting the absence of radiation in the axilla within the area of the excision. After removal of the MARI-node, an ALND was performed in all patients and consequently surgery to the breast was performed.

**Pathology examination**

The pathologist extracted the $^{125}$I seed from the MARI-node using a gamma-radiation detector. After removal of the seed, the MARI-node was bisected and completely embedded. All paraffin blocks were cut at three levels with minimally 150-µm intervals. The MARI-node was assessed according to the routine pathological assessment in SNB procedure. H&E staining was performed in all cases. Immunohistochemical-keratin staining was only done in lymph nodes with a tumour-negative H&E staining. Lymph nodes in the axillary dissection specimen were evaluated at one level and stained with H&E; immunohistochemical staining was not routinely performed. A specialized breast cancer pathologist (JW) revised all MARI nodes and classified them according the response to the systemic
<table>
<thead>
<tr>
<th><strong>Median age in years (range)</strong></th>
<th>49 (24-67)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Radiological tumor stage prior to systemic treatment</strong></td>
<td></td>
</tr>
<tr>
<td>To</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>T1</td>
<td>24 (23%)</td>
</tr>
<tr>
<td>T2</td>
<td>51 (50%)</td>
</tr>
<tr>
<td>T3</td>
<td>20 (19%)</td>
</tr>
<tr>
<td>T4</td>
<td>7 (7%)</td>
</tr>
<tr>
<td><strong>Clinical lymph node stage prior to systemic treatment</strong></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>62 (60%)</td>
</tr>
<tr>
<td>N2</td>
<td>13 (13%)</td>
</tr>
<tr>
<td>N3</td>
<td>28 (27%)</td>
</tr>
<tr>
<td><strong>Tumor histopathology</strong></td>
<td></td>
</tr>
<tr>
<td>Ductal carcinoma</td>
<td>88 (85%)</td>
</tr>
<tr>
<td>Lobular carcinoma</td>
<td>8 (8%)</td>
</tr>
<tr>
<td>Adenocarcinoma NOS</td>
<td>7 (7%)</td>
</tr>
<tr>
<td><strong>Receptor-based subtype</strong></td>
<td></td>
</tr>
<tr>
<td>ER- / PgR- / Her2-</td>
<td>22 (21%)</td>
</tr>
<tr>
<td>ER+ / Her2-</td>
<td>54 (52%)</td>
</tr>
<tr>
<td>Her2+</td>
<td>27 (26%)</td>
</tr>
<tr>
<td><strong>Neoadjuvant systemic treatment regimen</strong></td>
<td></td>
</tr>
<tr>
<td>ddAC</td>
<td>72 (70%)</td>
</tr>
<tr>
<td>CD</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>PTC</td>
<td>24 (23%)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (4%)</td>
</tr>
</tbody>
</table>

**Table 1.** Patient- and tumor-related characteristics (n=103)

*Receptor-based subtype as established on histological biopsy prior to neoadjuvant systemic treatment.

ER: estrogen receptor; PgR: progesterone receptor; Her2: human epidermal growth factor receptor.

1. Doxorubicin 60 mg/m² and Cyclophosphamide 600 mg/m² q 2 weeks x 6
2. Docetaxel 75 mg/m² and Capecitabine 2 x dd 1,000 mg/m² orally during 14 days, q 3 weeks x 6
3. Paclitaxel 70 mg/m², Trastuzumab 2 mg/m² and Carboplatin 3 AUC mg/ml/min on days 1, 8, 15, 22, 29, 35 q 8 weeks x 3.
treatment. For this study, pathologic complete response was defined as no vital tumor cells present in the lymph node, irrespectively of the response in the breast.

Figure 1. (A) Insertion of a radioactive iodine seed in an axillary lymph node under ultrasound guidance. The black arrow indicates the tip of an 18-G needle through which the iodine seed is inserted in the lymph node. (B) Position of the iodine seed in the lymph node. (C) Excised lymph node with the iodine seed in situ.

Statistical analysis
The primary endpoints used for statistical analysis were the success rate for identifying and selectively removing the MARI node and the correlation of the response observed in the MARI-node on pathology with the ALND specimen.
To calculate the sample size needed for the prediction model of response of the axilla we hypothesized that the expected true positive rate for the localization method 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. Based on these hypothesis 52 patients were required with a tumor-positive lymph after neoadjuvant systemic treatment, so, patients with residual disease.
The identification rate of the MARI node was analysed in all patients who underwent surgical removal of the MARI-node. Correlation between the pathologic responses observed in the MARI-node and in the ALND specimen was estimated using false-negative rates, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy.

RESULTS

Patient characteristics and seed localisation
Table 1 illustrates patient and tumor characteristics of the 103 patients prior to the systemic treatment. The median age at the time of enrollment was 49 years (range 24-67) and most patients had a T1 or T2 tumor. The majority of the patients (85%) had an invasive ductal carcinoma and 52% was estrogen receptor positive.
In all patients an attempt was made to mark one proven pathological axillary lymph node. No complication occurred during the ultrasound guided positioning of the $^{125}$I seed. In two patients misplacement of the seed had occurred during the radiological localisation procedure.
Surgical procedure and identification rate of the MARI-node

In three of the 103 patients selective surgical removal of the MARI-node was not attempted due to the presence of distant metastasis before surgery (n=2) or switch to primary surgery (n=1) (Fig 2). The resulting 100 patients underwent the surgical part of the MARI-procedure. At the time of surgery, the $^{125}$I seed had been in place for a median of 17 weeks (range 9-31 weeks) and had an apparent activity varying from 0.006 to 0.06 mCi (0.2-2.1 MBq). All seeds could be easily detected using the gamma probe. The median time of the identification and excision of the MARI node was 6 minutes (range: 3 -20 minutes). In all 100 patients, the $^{125}$I seed was identified and removed during surgery. In three patients the MARI-node could not be identified since the $^{125}$I seed was not properly located into a lymph node. This resulted in an identification rate of the MARI-node of 97% (97 out of 100 patients; 95%CI: 91-99). In two of these three patients with non-identification, misplacement of the seed had occurred during the radiological localisation procedure. In the third patient, a reason for the presence of the seed outside the lymph node was not found. A complementary ALND was not performed in 2 patients due to the presence of distant metastasis (n=1) and patient refusal (n=1). Thus, the correlation between response in the MARI-node and the additional lymph nodes could be assessed in 95 patients.

![Flow chart for all included patients. ALND = axillary lymph node dissection; pCR = complete pathological response $^{125}$I = radioactive iodine seed](image-url)
Accuracy of the MARI-node

Overall, the neoadjuvant systemic treatment resulted in a pathologic complete response of all axillary lymph nodes (MARI-node + additional nodes) of 26% (25/95).

The correlation between the response in MARI-node and the response in the additional lymph nodes is presented in table 2. The MARI node was tumor positive in 65 patients. Additional positive nodes in the ALND specimen were found in 46 of these 65 patients (71%). The median number of positive additional nodes was three (range 1 - 28).

In the remaining 30 patients with a negative MARI node, residual disease in the additional lymph nodes was found in five patients (negative-predicting value: 83%; 95%CI: 65-94). Of these five patients, the additional nodes contained only isolated tumor cells in two patients and a macrometastasis in three patients.

The MARI node accurately predicted axillary nodal status after neoadjuvant systemic treatment in 90 of the 95 patients (overall accuracy: 95%; 95%CI: 88-95). The false negative rate of the MARI procedure was 5/70 = 7% (95% CI: 2-16).

<table>
<thead>
<tr>
<th>Outcome MARI-node</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>46</td>
<td>19</td>
<td>65</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>44</td>
<td>95</td>
</tr>
</tbody>
</table>

Table 2. Pathology results of the MARI node and additional axillary lymph nodes in patients in who the MARI node was identified (n=95)
False negative rate: 5/70=7% (95%CI: 2-16); overall accuracy: 90/95=95% (95%CI: 88-95); negative predicting value: 25/30=83% (95%CI: 65-94); positive predicting value: 100% (by definition); sensitivity: 46/51=90% (95%CI: 79-97); specificity: 100% (by definition)

DISCUSSION

The MARI-procedure (Marking Axillary lymph nodes with a Radioactive Iodine seed) has a high identification rate of 97% and a low false-negative rate of 7%. It is safe, feasible and promising as a new technique to assess axillary lymph node involvement after neoadjuvant systemic treatment. The present study is the first to investigate the use of $^{125}$I seeds to mark positive lymph nodes before the start of neoadjuvant systemic treatment and selectively removing them afterwards.

Another more commonly method used to assess axillary lymph node involvement after neoadjuvant systemic treatment is the post-chemotherapy SNB. Currently, this method in patients who present with metastatic lymph nodes prior to the start of neoadjuvant systemic treatment is debated since
Chapter 9

The identification rate as well as the false-negative rate of the MARI-procedure appears favorable compared to results of the post-chemotherapy SNB in patients presenting with node-positive breast cancer. The high false-negative rate of the SNB after systemic treatment may be caused by residual tumor cells obstructing the lymphatic channels or altering lymphatic flow. This disadvantage does not exist in the MARI-procedure, since the identification of the affected lymph node is independent of changes in lymphatic flow due to treatment.

The MARI procedure is a safe technique and easy to learn. Radiological insertion can be challenging if the node is small, and therefore confirmation of the localization with ultrasound is necessary. Surgical removal of the MARI-node requires skills comparable to the removal of a SN. The surgeons performing the MARI-procedure in our institute found the technique even easier than removing a SN, since there was no background radiation.

A few limitations of the MARI procedure were identified and modified. First, since the FNA was performed in a separate intervention from the seed localisation, it is very important to document the localisation of the pathological lymph node to avoid seed localisation in a tumour-negative lymph node. Repeating FNA of the node after insertion could confirm localisation in the correct node. Second, ultrasound-guided localisation of the seed in a small lymph node can be difficult, as was illustrated by the two patients in whom the seed was not properly located in the lymph node. Therefore, training of the radiologists and confirmation of the location of the seed by ultrasound is very important. Third, the iodine seed is - however minimal - radioactive and can in theory itself sterilize tumor-positive lymph nodes. We lowered the radiation dose to such an extent that any radiation effect to the lymph node was minimized without the seed getting untraceable. Finally, the iodine seed is a radioactive source and although the dose of these iodine seeds is low, it requires authorization by the government and safety issues need to be addressed. However, when safety protocols are taken into account, the iodine seed is an easy and patient-friendly localisation method. Marking of the axillary lymph node was performed in the same procedure as marking of the breast lesion, so no separate visit had to be scheduled. Furthermore, since the half-life time of the iodine seed is 60 days, there is no strict time frame in which the $^{125}$I seeds have to be placed. This is in contrast with the SNB in which radiocolloid is injected, where the halftime is about eight hours. Therefore the injection of the radiocolloid has to be done one day before or at the same day of surgery, and may cause scheduling conflicts.

With an identification rate of 97% and false-negative rate of 7% the MARI-procedure is a reliable measurement of the axillary response and may be used to tailor further axillary treatment after neo-adjuvant systemic treatment. How to treat the axilla after a negative MARI-node is yet undetermined. Theoretically, the axilla can be left untreated when all nodal metastasis responded
completely to systemic therapy. In primary breast surgery, there is even trend towards omitting axillary treatment in patients with a positive SN because some studies showed no survival benefit of ALND.\textsuperscript{25,26} Furthermore, it has been shown that axillary radiotherapy in patients with primary breast surgery and a positive SNB gives the same rates of axillary recurrences and survival compared to ALND, with less side effects.\textsuperscript{27} Therefore axillary radiotherapy could be a good option in these patients. Whether axillary treatment can be completely omitted in patients with a tumor negative MARI node will be subject of future research.

**CONCLUSION**

It is technically possible to mark tumor-positive axillary lymph nodes with an iodine seed before the start of the neoadjuvant systemic treatment and selectively remove them afterwards. The MARI-procedure is a safe and patient-friendly method with a high identification rate and low false-negative rate. We believe that this method can be used to select patients with a complete pathological response in the axillary lymph nodes and omit an ALND in these patients.
REFERENCES


