Improvements in locoregional treatment of breast cancer
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RADIOGUIDED OCCULT LESION LOCALIZATION (ROLL) IN BREAST-CONSERVING SURGERY AFTER NEOADJUVANT CHEMOTHERAPY

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**ABSTRACT**

**Background**
An important benefit of neoadjuvant chemotherapy, as compared to adjuvant chemotherapy, in breast cancer patients is down staging of the primary tumour, which allows for more breast-conserving surgery. When a tumour becomes non-palpable after this down staging, precise localisation of the original tumour bed is crucial to be able to perform breast-conserving surgery. Radioguided Occult Lesion Localisation with $^{99m}$Technetium (ROLL-$^{99m}$Tc) is commonly used to perform breast-conserving surgery in patients with non-palpable breast tumours. We modified this technique to use it in the neoadjuvant setting. The present analysis was performed to assess its feasibility and analyse the number of patients in which a mastectomy was correctly withheld using this technique.

**Patients and methods**
A retrospective analysis was performed for all patients who were treated with neoadjuvant chemotherapy between 2007 and 2010 in our institute and underwent breast-conserving surgery with the ROLL-$^{99m}$Tc technique afterwards. The status of the margins and the weight of the resected specimen were assessed.

**Results**
The median weight of the resected specimen in these 83 patients was 53 g (range: 11–204 g). Eleven of the 58 patients with residual disease revealed positive margins at pathological examination. However, in only 5 of those 11 patients a secondary mastectomy was indicated. This means that in 94% of all included patients a mastectomy was correctly withheld.

**Conclusion**
The ROLL-$^{99m}$Tc technique is a feasible technique that can be used to perform breast-conserving surgery after neoadjuvant chemotherapy in a carefully selected group of patients.
INTRODUCTION

Neoadjuvant chemotherapy, also known as primary, induction, or pre-operative chemotherapy, is the standard of care for the management of locally advanced breast cancer and is also increasingly used for women with early stage breast cancer. A proven advantage of neoadjuvant chemotherapy is a reduction in tumour size which enables breast-conserving treatment (surgery + radiotherapy) in 25–40% of the patients who were initially scheduled for mastectomy.2,3

In breast-conserving treatment after neoadjuvant chemotherapy three aspects are important. First, adequately removing all residual tumour cells after neoadjuvant chemotherapy is the best predictive factor for a good prognosis in terms of loco-regional recurrence or ipsilateral breast tumour recurrence.4 Consequently, performing breast-conserving surgery in patients with initially large tumours, can only safely be done in case of a good response to neoadjuvant chemotherapy. Secondly, the predictive value of a radiological response is limited. Therefore, a radiological response should always be confirmed by pathological examination of a lumpectomy taken from the original tumour bed.

Thirdly, pathological complete response rates after neoadjuvant chemotherapy can be as high as 50%, while clinical complete response rates are even higher. In these cases the previously palpable tumour may become non-palpable. Therefore it is crucial to mark the tumour prior to the start of neoadjuvant chemotherapy in order to be able to localise the original tumour bed and perform a lumpectomy afterwards.

Table 1 provides an overview of the different techniques that are used to localise non-palpable breast lesions with or without previous administration of neoadjuvant chemotherapy: implantation of metallic markers with or without additional wire localisation,8-10 skin tattooing,11,12 Radioguided Occult Lesion Localisation with $^{99m}$Technetium (ROLL-$^{99m}$Tc)13,14 and localisation with a radioactive iodine seed ($^{125}$I seed localisation).15,16 When using the ROLL-$^{99m}$Tc technique, a small amount of macroaggregate albumin labelled with radioactive technetium ($^{99m}$Tc) is used to identify the tumour. Under ultrasound guidance, the $^{99m}$Tc is injected into the tumour and intraoperatively a gamma probe is used to detect the $^{99m}$Tc in order to perform a wide local excision around the tumour. If there is an indication for a sentinel node biopsy (SNB) as well, this can be performed in the same procedure after one single intratumoural injection, without compromising the identification rate of the sentinel node.17,18
We have adjusted the ROLL-$^{99m}$Tc technique to use it in the neoadjuvant chemotherapy setting with the addition of a radiopaque twist marker, which is placed in the tumour prior to the start of the neoadjuvant chemotherapy. If neoadjuvant chemotherapy leads to a complete radiological response, this twist marker is still detectable by ultrasound and thereby enables injection of the $^{99m}$Tc at the original tumour site (Fig. 1). In this way a local excision of the original tumour bed can be also be performed in case of a radiological and/or clinical complete response.

### Table 1. Overview of different techniques to localize non-palpable breast tumours after neoadjuvant chemotherapy

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Blunt metallic markers +/- wire</th>
<th>Skin tattoo</th>
<th>$^{125}$I seed localisation</th>
<th>ROLL-$^{99m}$Tc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4 markers in or around the tumour before neoadjuvant chemotherapy. WLE guided by ultrasound or with additional wire localisation.</td>
<td>Marking position of the tumour on the skin before start neoadjuvant chemotherapy. WLE guided by tattoo marks.</td>
<td>Intratumoural iodine seed ($^{131}$I) before start neoadjuvant chemotherapy. WLE guided by gamma probe on I$^{131}$ setting.</td>
<td>Intratumoural twist marker before start neoadjuvant chemotherapy. Intratumoural $^{99m}$Tc injection before surgery. WLE guided by gamma probe on Tc-setting.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of interventions</th>
<th>1 or 2</th>
<th>1</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive procedure</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>yes</td>
</tr>
<tr>
<td>Negative margins at first resection</td>
<td>89%</td>
<td>91%</td>
<td>94%</td>
<td>87%</td>
</tr>
<tr>
<td>Precision of localisation</td>
<td>not precise</td>
<td>not precise</td>
<td>very precise</td>
<td>very precise</td>
</tr>
</tbody>
</table>

Figure 1. Overview of the ROLL-$^{99m}$Tc technique.
A: before the start of the neoadjuvant chemotherapy a twist marker is placed intratumoural. B: pre-operative scintigraphy anterior view. C: The wide local excision is guided by the gamma-probe (Neoprobe®, Johnson & Johnson Medical, Hamburg, Germany).
The aim of the local excision in the neoadjuvant chemotherapy setting is to confirm the response of the tumour to the chemotherapy that was seen on pre-operative imaging. In this way, patients with a large tumour before the chemotherapy and a good response afterwards – which is confirmed at pathological examination – can be spared a mastectomy.

A PubMed search (with terms breast cancer, Radioguided Occult Lesion Localisation, ROLL, neoadjuvant chemotherapy, primary systemic therapy) resulted in not a single study describing the feasibility of the ROLL-\(^{99m}\)Tc localisation technique in the neoadjuvant chemotherapy setting. Therefore we analysed the results of patients who underwent breast-conserving surgery using the ROLL-\(^{99m}\)Tc technique after neoadjuvant chemotherapy.

**PATIENTS AND METHODS**

**Patient and tumour characteristics**

In our institute, patients with invasive breast cancer larger than 3 cm and/or proven involved lymph nodes are eligible for neoadjuvant chemotherapy. The tumour size was assessed with mammography, ultrasound, and contrast-enhanced MRI. Prior to neoadjuvant treatment, 14-gauge biopsies of the breast tumour were taken under ultrasound guidance to determine the histological subtype and receptor status. Tumours were classified according to the standard criteria of the World Health Organization. Estrogen receptor (ER) status and Progesterone receptor (PgR) status were determined by immunohistochemistry and interpreted as positive if more than 10% of the nuclei stained positive. The Her2 status was assessed by scoring the intensity of membrane staining using immunohistochemistry. Tumours with a score of 3+ (strong homogeneous staining) were considered Her2-positive. In case of 2+ scores (moderate homogeneous staining) chromogenic in situ hybridization (CISH) was used to confirm amplification.

Nodal status prior to neoadjuvant chemotherapy was determined by ultrasound-guided fine-needle aspiration or, when negative, sentinel node biopsy prior to the start of chemotherapy.

This study was approved by the institutional ethical committee and informed consent was obtained from all patients.

**Tumour localisation**

Prior to chemotherapy, all patients had a radiopaque O-shaped twist marker (BARD GmbH, Türkenfeld, Germany) inserted centrally in the tumour under ultrasound guidance at the time of the biopsy. After this localisation procedure, a mammography was performed to confirm correct placement of the twist marker.

**Neoadjuvant chemotherapy and response evaluation**

The majority of patients received neoadjuvant chemotherapy in a clinical study in which contrast-enhanced MRI (Philips Medical Systems, Best, the Netherlands) was used for response evaluation. A baseline MRI was made before the start of the chemotherapy.

The treatment regimen was assigned to each patient, consisting of six courses of dose-dense doxorubicin/cyclophosphamide (ddAC) or six courses of capecitabine/docetaxel (CD). If the therapy response was considered unfavourable by contrast-enhanced MRI evaluation after three courses, ddAC was changed to CD or vice versa. Tumour response to neoadjuvant chemotherapy was evaluated as reported previously by our group. Patients with Her2+ tumours received three
8-week courses of trastuzumab, paclitaxel and carboplatin (PTC). Outside the context of this study some other regimens were used.
After completing of the chemotherapy and just prior to surgery, a MRI was performed which was used to decide whether breast-conserving surgery could be offered to the patient. The largest diameter of the tumour (in three planes) was assessed at the initial enhancement series (90 s after contrast injection) and in the late enhancement series (450 s after contrast injection). Patients with no enhancement in the original tumour area after completing the chemotherapy were considered as complete radiological responders.

Planning and accomplishment of breast surgery
Surgery was planned three to four weeks after completion of the neoadjuvant chemotherapy. A multidisciplinary team consisting of breast cancer specialists including surgeons, radiologists, pathologists, radiotherapists and medical oncologists assessed for every individual patient whether breast-conserving surgery was feasible. Patients with a residual tumour area smaller than 3 cm and without multicentric disease and/or extensive DCIS were considered suitable candidates for breast-conserving surgery. To localise the original tumour bed pre-operatively, 0.2 ml nanocolloid (Amersham Cygne, Eindhoven, the Netherlands) labelled with $^{99m}$Tc (average net dose 37 MBq) was administered under ultrasound guidance into the centre of the residual tumour bed or, in case of a complete radiological response, at the site of the twist marker. After this $^{99m}$Tc injection, the patient went to the nuclear medicine department where a scintigraphy with planar anterior and prone lateral images with hanging breast was obtained with a dual-head gamma camera (Siemens, Symbia T, Erlangen, Germany) in order to check the correct placement of the tracer at the site of the injection and to exclude leakage.

After localisation with $^{99m}$Tc and the subsequent scintigraphy, the surgical excision procedure followed the same day ($n = 28$), or the next day ($n = 55$). The wide local excision was guided by the gamma-probe (Neoprobe®, Johnson & Johnson Medical, Hamburg, Germany) at its lowest sensitivity setting. The point of maximum activity was identified with the gamma-probe in the anterior projection, to assess the surgical approach, and in the lateral projection to determine the depth of the lesion. This way, a three dimensional localisation of the $^{99m}$Tc within the breast can be achieved. The wide local excision included the breast parenchyma from the skin to the pectoral fascia. In these patients with a good radiological response, only a limited wide local excision was taken around the marker and the residual tumour. There was no intention to remove the complete volume of the tumour bed as it was defined prior to chemotherapy. Adequate removal of the $^{99m}$Tc-marked tumour bed was confirmed by the absence of background radioactivity in the remaining breast tissue.

In patients with a positive sentinel node (>0.2 mm) or axillary lymph node metastases proven by fine needle aspiration prior to neoadjuvant chemotherapy, an axillary lymph node dissection at levels I and II with level III sampling after neoadjuvant chemotherapy was performed. Adjuvant treatment was given according to the Dutch national guidelines

Pathology examination
Margins of the excised specimen were inked with four different colours. The specimen was sliced in 3-mm sections, fixed overnight in buffered formalin and embedded in paraffin. Perpendicular slices were made on the central peripheral axis. X-rays of all specimens and extensive sampling of the (former) tumour bed, the nearest margins, and any other X-ray abnormalities were performed. Sections were stained with haematoxylin and eosin. The tumour margins were defined as a clear
pathologic margin when no invasive cells or carcinoma in situ cells were present in the margin on microscopic evaluation. Pathological complete response was defined as the absence of any invasive carcinoma in the breast at microscopic examination of the specimen, regardless of the presence of carcinoma in situ.²⁰ Pathological partial response was defined as residual tumour in the specimen at pathological examination. In each specimen the smallest distance from the invasive part of the tumour to the nearest edge was measured. Furthermore the weight of each specimen was recorded as a substitute for the total amount of excised volume

**RESULTS**

**Patient characteristics**

Between January 2007 and December 2010, 405 breast cancer patients were treated with neoadjuvant chemotherapy at our institute. In 205 of these patients a mastectomy was performed after chemotherapy, 178 patients underwent breast-conserving surgery and no breast surgery was performed in 22 patients due to the presence of distant metastasis (n = 15), patient refusal (n = 5), or because of occult breast cancer (only dissection for axillary lymph node metastases, n = 2). In 95 out of the 178 patients who underwent breast-conserving surgery, the tumour was still palpable or a wide local excision was performed using a different localisation method. In the remaining 83 patients a wide local excision was performed with the use of the ROLL-⁹⁹ᵐ{Tc technique. Demographic and clinical characteristics of these 83 patients as well as the neoadjuvant chemotherapy regimen administered, are summarized in Table 2.

**Tumour localisation**

No radiologists experienced any difficulty with placing the twist marker before neoadjuvant chemotherapy. In one patient haemorrhage occurred after the twist marker was placed which was treated conservatively. In 100% of the cases the twist marker was placed in the correct position, as confirmed by mammography

**Radiological response**

Complete radiological response was seen in 51% of the patients, while the remainder of the patients showed a partial or near complete radiological response (Table 3).
Table 2. Patient- and tumour-related characteristics (n=83)
No: no evidence of lymph node metastasis, including a negative ultrasound and sentinel node biopsy negative or only isolated tumour cells; N+: Axillary lymph node metastasis diagnosed by fine needle aspiration (FNA) or metastasis ≥0.2 mm in sentinel node biopsy (SNB); Nx: lymph node status unknown; ER: estrogen receptor; PgR: progesterone receptor; Her2: human epidermal growth factor receptor.
*Receptor-based subtype as established on pre-chemotherapy biopsy.
1Doxorubicine 60 mg/m² and Cyclophosphamide 600 mg/m² q 2 weeks x 6
2Docetaxel 75 mg/m² and Capecitabine 2 x dd 1,000 mg/m² orally during 14 days, q 3 weeks x 6
3Paclitaxel 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.

Surgery
Surgery was performed by an oncologic surgeon specialized in breast cancer surgery or by a fellow oncologic surgeon. The short-term postoperative course was uneventful in 82 patients: one patient had a haemorrhage, which was treated conservatively. No major postoperative complication occurred.
Pathological results

The median weight of the excised specimen was 53 g (range: 11–204 g) and the median of minimal margin of the resected invasive part was 3.5 mm (range 0.4–15.0 mm).

Thirty percent (25 patients) of the 83 patients achieved a pathological complete response after neoadjuvant chemotherapy, leaving 58 patients with residual tumour for further analysis. From these 58 patients, 11 patients displayed positive margins at pathological examination. In five patients the positive margin involved the pectoral fascia or the margin was only focally positive. In those patients a surgical re-excision was not performed and they were treated with adjuvant radiotherapy with a boost dose on the original tumour bed. The remaining six patients needed a second surgical intervention to obtain negative margins: a mastectomy in five cases and an additional local excision in one case (Table 3).

In two of the six re-resection specimen no tumour cells were found, in two specimens residual invasive lobular carcinoma was found and the remaining two patients had an invasive ductal carcinoma. When the pathology results were correlated to the pre-operative imaging in these six patients, the MRI showed a complete response in three of these patients and a partial response in the other three patients. These characteristics are summarized in Table 4.

| Table 3. Outcome parameters (n=83) |

<table>
<thead>
<tr>
<th>Axillary dissection</th>
<th>65 (78%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Radiological response after chemotherapy</strong></td>
<td></td>
</tr>
<tr>
<td>Partial response</td>
<td>41 (49%)</td>
</tr>
<tr>
<td>Complete response</td>
<td>42 (51%)</td>
</tr>
<tr>
<td><strong>Pathological response after chemotherapy</strong></td>
<td></td>
</tr>
<tr>
<td>Residual tumour</td>
<td>58 (70%)</td>
</tr>
<tr>
<td>Complete response</td>
<td>25 (30%)</td>
</tr>
<tr>
<td><strong>Margins</strong></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>72 (87%)</td>
</tr>
<tr>
<td>Positive</td>
<td>11 (13%)</td>
</tr>
<tr>
<td><strong>Minimal margin of resected invasive part</strong></td>
<td></td>
</tr>
<tr>
<td>Median in mm (range)</td>
<td>3.5 (0.4–15.0)</td>
</tr>
<tr>
<td><strong>Weight of resected specimen</strong></td>
<td></td>
</tr>
<tr>
<td>Median in g (range)</td>
<td>53 (11–204)</td>
</tr>
<tr>
<td><strong>Surgical intervention necessary for irreducibility</strong></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6 (7%)</td>
</tr>
<tr>
<td>Re-resection</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Mastectomy</td>
<td>5 (6%)</td>
</tr>
</tbody>
</table>
Table 4. Characteristics of the patients with a surgical re-intervention (n=6)
Abbreviations; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma

<table>
<thead>
<tr>
<th>Patient</th>
<th>Radiological response</th>
<th>Histology</th>
<th>Residual tumour in re-resection specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Complete response</td>
<td>IDC</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Complete response</td>
<td>ILC</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>Complete response</td>
<td>IDC</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Partial response</td>
<td>IDC</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>Partial response</td>
<td>ILC</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>Partial response</td>
<td>IDC</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Follow up**

During the follow up, which ranged from 4.5 to 54.3 months (median, 37.9 months), one local recurrence, one second primary, and seven distant metastases were observed in nine patients and one patient presented with loco-regional recurrence and distant metastasis. Seven women died as a result of their disease recurrence.

**DISCUSSION**

Achieving tumour-free margins in breast surgery after treatment with neoadjuvant chemotherapy is the most important factor to prevent local recurrences. Predicting the residual tumour burden with imaging such as MRI is unreliable.\(^6,7\) This was also seen in the present study: three of the six patients who required additional surgery because of positive margins had complete response on MRI. Therefore it is crucial to precisely localise the tumour prior to starting chemotherapy using a reliable, safe, and patient-friendly localisation technique to enable resection of the original tumour bed after neoadjuvant chemotherapy.

The ROLL-\(^{99m}\text{Tc}\) technique is an easy procedure and incorrect placement of the radiotracer is extremely rare.\(^{14,21}\) In prior studies an average lower weight of the excised specimen (34 and 64 g) has been reported compared to other localisation techniques.\(^{13,22-25}\) Furthermore, this technique has shown excellent results in terms of tumour-free margins after a wide local excision for non-palpable lesions.\(^{23}\) As a result, the ROLL-\(^{99m}\text{Tc}\) technique helps to achieve an overall reduced need for surgical re-interventions with a good cosmetic outcome.

In the present study, breast-conserving surgery after neoadjuvant chemotherapy performed with the ROLL-\(^{99m}\text{Tc}\) technique resulted in 11 of 58 patients with residual tumour who revealed positive margins at pathology. However, the aim of the local excision was to accurately identify the patients with a good response to the chemotherapy that could be spared a mastectomy. When looking to the whole group of treated patients, only small percentage (7%) required a second surgical intervention. Furthermore, an even smaller proportion of these patients (6%) had to be treated with a secondary mastectomy. This means that 94% of the patients with initial large tumours (94% of all patients had initial T2–T3 tumour) had a correct indication for breast-conserving surgery and could thus be spared a mastectomy.

The median weight of the resected specimen was 53 g (range 11–204). It is difficult to put this in
perspective since no comparison could be made with patients who underwent breast-conserving surgery after e.g. the commonly used wire localisation. There are no studies comparing these two techniques in the neoadjuvant chemotherapy setting. However, in a review from Lovrics et al., the radioguided surgery with the ROLL technique or with $^{125}$I seed localisation was compared to wire localisation in primary surgery. From the 5 studies reporting the weight of the specimen, only one study found that the surgical specimens in the group of patients treated with the ROLL technique were significantly smaller compared to the group of patients treated with wire localisation. The weight of the specimens ranged from 34.0 g to 68.1 g in the ROLL group and from 31.0 g to 73.5 g in the wire localisation group. Furthermore, in this systematic review the ROLL technique yielded lower positive margins and re-operation rates and showed improved cosmetic outcomes compared to wire localisation. The authors suggested that radioguided surgery with ROLL or $^{125}$I seed localisation may be the preferred technique to guide surgical resections of non-palpable breast tumours.

In a study from Espinosa et al., two different localisation methods were compared: marking the tumour with either skin tattoo or a metallic marker before the start of neoadjuvant chemotherapy. They reported positive margins in 7 from the 118 patients treated with skin tattoo and in 1 patient from the 31 patients in which the tumour was marked with a metal marker. Since patients who required a secondary mastectomy to obtain negative margins were excluded from this analysis, it is hard to interpret the results.

In patients undergoing neoadjuvant chemotherapy in our institute, the SNB is performed before the start of the chemotherapy. Nevertheless, if there is an indication to perform the SNB after the chemotherapy, this can be done synchronous with the surgery of the breast. Both SNB and probe-guided excision of the breast tumour are feasible with the aid of a single intratumoural tracer administration.

A minor disadvantage of the localisation procedure with $^{99m}$Tc is the relative short half-life time (6 h) of the radioactive labelled technetium. Therefore the $^{99m}$Tc should be injected the same day or the day before the surgical intervention, which can lead to scheduling conflicts. This can be overcome with the use of alternative techniques such as $^{125}$I seed localisation where an $^{125}$I seed instead of a radiopaque twist marker is placed intratumoural (Table 1). Because of the long half-life of the radioactive $^{125}$I seed of 60 days, it can be placed prior to the start of the neoadjuvant chemotherapy and will still be traceable after completion of the chemotherapy regimens. However, the general experience with $^{125}$I seed localisation is limited and thus more research should prove its usefulness. Finally, since it considers radioactive material, the rules and regulations may discourage hospitals to use the $^{125}$I seeds and in that case, the ROLL-$^{99m}$Tc technique provides a good alternative.

**CONCLUSION**

An important benefit of neoadjuvant chemotherapy is a considerable tumour reduction prior to surgery that often allows breast-conserving surgery instead of mastectomy. In the present study we evaluated the feasibility of the ROLL-$^{99m}$Tc technique to perform a wide local excision after neoadjuvant chemotherapy. Based on the low percentage of re-interventions and relative small excision volume, the ROLL-$^{99m}$Tc technique appears to be a valuable option in the neoadjuvant setting as it frequently makes it possible for breast-conserving surgery to be safely performed.
REFERENCES


