Sentinel Lymph Node Biopsy in Patients with Breast Cancer: Comparison of Peritumoral and Periareolar Injection

Introduction: Intradermal periareolar injection technique for sentinel lymph node biopsy (SLNB) may offer an advantage by including multifocal breast cancer as an additional indication. In May 2008 we changed our standard procedure from peritumoral (PT) to periareolar (PA) injection. We compared the results for corresponding periods before and after the change in procedure.

Material and Method: A total of 117 patients (pts.) were investigated the year after we changed our technique; a total of 152 pts were investigated in the reference period 2007. We investigated the identification rates for sentinel lymph nodes (SLN) identified scintigraphically and surgically as well as the rates of metastatic involvement (LN).

Results: After PT injection, scintigraphic detection of SLN failed in 5/152 pts., and in a further 10 pts. SLN was not found at surgery. In 7 of 15 pts. in whom SLN was not detected, histology demonstrated nodal involvement. Metastases were found in the SLN of 28 of 137 pts. with successful detection of SLN; no other lymph nodes were affected in 21 of these pts. (75.0% of pts. with positive SLN detection). With PA injection at least one SLN could always be detected using scintigraphy; only 2/117 SLN could not be found intraoperatively. Metastasis was found in SLN in 34/115 pts.; in 19/34 pts., metastatic involvement was limited to the SLN with no other lymph nodes involved (55.9% of pts. with positive detection of SLN).

Discussion: The detection rate for SLN was significantly higher using PA injection (98.3% vs. 90.1%). As axillary dissection was not done in SLN-negative patients, rates of false-negative detection cannot be determined. PA injection not only results in better detection rates, it also offers the advantage that the technique can be performed correctly regardless of tumour localisation.
Introduction

The status of the axillary lymph nodes is one of the most important prognostic factors for patients with a primary diagnosis of breast cancer. Axillary lymph node involvement is associated with a significantly higher mortality and has such a high risk of recurrence that adjuvant systemic therapy is usually indicated [1–3, 25–28]. Prior to the introduction of sentinel lymph node biopsy (SLNB), radical axillary lymphadenectomy was the standard surgical procedure for the therapy and staging of breast cancer. With the advent of more modern screening methods and as the general population has become better informed, the majority of patients now have a negative lymph node status at the time of diagnosis [4], making radical axillary lymphadenectomy an unnecessarily radical intervention with a high morbidity in these patients [5].

SLNB is a well-established diagnostic procedure for the detection of tumour-draining lymph nodes. It was initially used in the therapy of other tumour types such as malignant melanoma. The diagnostic significance of SLNB is well known and has been extensively investigated [6, 7]. A radioactive tracer is injected into breast tissue to show the first lymph nodes in the lymphatic flow downstream from the primary tumour. These lymph nodes are subsequently removed during surgery and histologically examined for metastasis. A negative nodal status of this first “filter station”, which indicates the absence of lymphogenically metastasised tumour cells, is considered sufficient to exclude axillary metastasis, making the removal of axillary lymph nodes unnecessary in these patients. This prevents the shoulder and arm morbidity associated with axillary lymph node dissection [8–14].

Currently, the standard indication for SLNB is small, unifocal breast carcinomas with diameters of up to 2 cm (T1 tumour stage) and a clinically negative nodal status. The current consensus in Germany is that SLNB can optionally be applied to bifocal tumours or tumours with diameters of between 2 and 5 cm (T2 tumours) [15]. A further expansion of indications is currently being discussed. Other discussions have focussed on the optimal injection method for the tracer. At present, various peritumoral, periareolar and subareolar injection techniques are considered equivalent [16]. However, periareolar injection offers various advantages: the technique is easier to learn, does not require additional efforts for non-palpable tumours, and the procedure is less painful [17–19]. Cheng et al. compiled a recent review of the literature [20] which included all studies comparing injection methods in more than 100 patients.

In May 2008 the Institute for Diagnostic Radiology and Nuclear Medicine of Minden Hospital changed their previous technique of peritumoral injection to periareolar, intradermal injection. The aim of this study was to compare the results for the two methods in clinical practice. The study also focused on the identification rates for SLN using scintigraphy and at surgery.

Table 1 Distribution of tumour stages in the patient collectives with peritumoral and periareolar injection. The difference in the distribution of tumour stages was not statistically significant (p = 0.29).

<table>
<thead>
<tr>
<th>Tumour stage</th>
<th>Peritumoral injection</th>
<th>Periareolar injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of patients</td>
<td>Relative frequency (%)</td>
</tr>
<tr>
<td>Ductal carcinoma in situ</td>
<td>4</td>
<td>2.6</td>
</tr>
<tr>
<td>pT1a</td>
<td>7</td>
<td>4.6</td>
</tr>
<tr>
<td>pT1b</td>
<td>18</td>
<td>11.8</td>
</tr>
<tr>
<td>pT1c</td>
<td>59</td>
<td>38.8</td>
</tr>
<tr>
<td>pT2</td>
<td>62</td>
<td>40.8</td>
</tr>
<tr>
<td>pT3</td>
<td>1</td>
<td>0.66</td>
</tr>
<tr>
<td>pT4a</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>pT4b</td>
<td>1</td>
<td>0.66</td>
</tr>
<tr>
<td>total</td>
<td>152</td>
<td></td>
</tr>
</tbody>
</table>
capillaries [21]. Phagocytosis of the particles occurs in the inter-
mediary lymph nodes by the reticuloendothelial system of the 
lymph nodes, which together with mechanical retention of the 
tracer, is responsible for the concentration of tracer in the first in-
termediary lymph node(s).

Injection methods
In 2007 peritumoral injection was used for detection. After pal-
pation, sonographic visualisation of the tumour and disinfection 
of the skin, fan-shaped peritumoral injection was done at two 
opposing injection sites together with subdermal injection above 
the tumour area, where necessary. After May 2008 the injection method consisted of intradermal, 
periareolar injection of $^{99m}$Tc nano colloids. Four injections of ap-
prox. 0.2 ml of the radiopharmaceutical were done at the transi-
tion area of the areola to the normal cutis in the 3, 6, 9 and 12 
o’clock positions.
The dosage of the radiopharmaceutical depends on whether intra-
operative identification is planned for the same day or for the 
following day. Approx. 50 MBq is administered if surgery is 
planned on the same day, and dosages between 150 and 
200 MBq are administered if surgery is planned for the following 
day.

Scintigraphy
Static images of the chest were obtained 60 to 180 minutes post 
injection with and without lead coverage of the injection sites. 
Imaging was done using a dual-head gamma camera. Mapping 
of the body contours for anatomical orientation was either done 
with a Co-57 flood source phantom or by tracing the contours of 
the body with a tracer-filled syringe. Static images were obtained 
ventrally and laterally with a matrix of 128 × 128. The lymph 
node(s) showing the highest tracer intensity were considered 
the sentinel lymph nodes, although assessment was done based 
purely on visual impressions. The sentinel lymph nodes were 
subsequently tagged with the patient placed in the position for 
surgery. Tagging was done using a radioactive point source with 
camera monitoring of the source where the tracer had accumu-
lated. The sites were then marked ventrally using a water-resis-
tant pen so they could be found during surgery.

Intraoperative detection
Surgery was done in our hospital by one of three experienced 
surgeons; the same surgeons performed all operations in both 
periods of data acquisition. All surgeons were experienced in 
the use of the injection techniques prior to the start of data acquisi-
tion as SLN detection in breast cancer patients has been carried 
out in our hospital since 2003. A gamma probe with a collimator 
was used for SLN detection intraoperatively to reduce interfer-
ence from scattered radiation. Alternatively, we used the C-Trak 
System (Care Wise, Morgan Hill, CA, USA) and the Gamma Finder 
(World of Medicine AG, Ludwigstadt, Germany). Using a trans-
cutaneous approach we searched for maximum activity signal in 
the axilla at the level of the ventral marking. A small skin incision 
was done and further dissection was then performed with little 
loss of blood and minimal trauma with repeated use of the gam-
ma probe. Finally the lymph nodes which had accumulated the 
radionuclide were removed and examined ex vivo for the pres-
ence of nuclide accumulation. If several lymph nodes in close 
proximity to one another showed nuclide accumulation, the 
nodes were removed by en bloc resection. After the resection of 
lymph nodes, the site was examined for any remaining activity
and was palpated by the surgeon. If less than 20% of tracer activ-
ity in the SLN was still present after SLN resection, this measured 
amount was considered negligible and no further lymph node 
dissection was done. If the measured value was higher, additional 
nodes were removed and considered as SLN.
Obtained specimens were examined intraoperatively; histopath-
ological investigation of frozen sections done by the Institute for 
Pathology, Cytology & Molecular Pathology of Minden Clinics. In 
the mean time the primary tumour was resected using either 
mastectomy or breast conserving surgery. If metastasis was 
found in a SLN, additional lymph nodes on the affected side were 
removed. The resected SLN were removed and examined histop-
athologically. If metastasis was found in an SLN, resection of 
axillary lymph nodes was done in a second procedure.

Statistical analysis
Fisher’s exact test was used to determine the distribution of tu-
mour stages between the groups and the differences in detection 
rates. Unpaired t-test was used to determine whether the num-
bers of detected sentinel lymph nodes differed significantly be-
tween groups. A p-value < 0.05 was considered significant. The 
subgroup of multifocal tumours was too small for statistical anal-
ysis. Stat View 5.0 (SAS Institute Inc., Cary, NC, USA) and R 2.15.0 
(The R Foundation for Statistical Computing, Vienna, Austria) 
were used for statistical analysis.

Results

Scintigraphic detection of sentinel lymph nodes
No sentinel lymph node (SLN) was found in 5 women who had 
peritumoral injection (3.3%. Table 2). One SLN was found in 60 
women (39.5%), two SLN were found in 44 women (28.9%) and 
more than two SLN were found in the remaining women (28%). 
Three patients additionally showed parasternal lymphatic flow. 
In the group with periareolar injection one SLN was found in the 
majority of cases (75/117 patients, 64.1%). Two sentinel lymph 
nodes were found in 33 women (28.2%), more than two sentinel 
lymph nodes were only found in 9 patients (7.7%). The mean 
number of SLN detected scintigraphically after peritumoral injec-
tion was significantly higher than after periareolar injection 
(1.9 ± 1.1 vs. 1.4 ± 0.7 SLN, p < 0.001).

Intraoperative detection of sentinel lymph nodes 
and total number of successful SLN biopsies
No SLN were found intraoperatively after peritumoral injection 
in 10 patients despite scintigraphic SLN detection. SLN biopsy 
was successful in 90.1% of patients (Table 2). The remaining 
15 patients underwent axillary lymph node dissection. After

<table>
<thead>
<tr>
<th>Type of injection</th>
<th>Scintigraphic SLN detection</th>
<th>Intraoperative SLN detection</th>
<th>Patients with successful SLN biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritumoral</td>
<td>147/152 (96.7%)</td>
<td>137/147 (93.2%)</td>
<td>137/152 (90.1%)</td>
</tr>
<tr>
<td>Periareolar</td>
<td>117/117 (100%)</td>
<td>115/117 (98.3%)</td>
<td>115/117 (98.3%)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.071 (n.s.)</td>
<td>0.072 (n.s.)</td>
<td>0.0094</td>
</tr>
</tbody>
</table>

n.s. = not significant
periareolar injection, intraoperative detection failed only in 2 patients, resulting in successful SLN biopsy in 98.3% of cases. The difference in the number of successful sentinel lymph node biopsies was significant (p = 0.0094).

In both groups, a mean of 1.9 lymph nodes were considered sentinel lymph nodes intraoperatively and resected (periareolar group 1.9 ± 1.1; periareolar group 1.9 ± 1.3).

Metastatic involvement of sentinel lymph nodes
In the patient collective with peritumoral injection, 28 of 137 patients (20.4%) had metastasis in one or more sentinel lymph nodes. In 21 of these 28 patients (75.0%) subsequent systematic lymphadenectomy showed no metastatic involvement in any other lymph nodes. In the collective with periareolar injection the rate of involvement was 29.6% (35/115 patients), a slightly higher percentage but not statistically significant (p = 0.11). In this collective again metastases were limited to the SLN in the majority of patients (19/34 patients, 55.9%).

Patients with negative SLN biopsy after peritumoral injection
As mentioned above, SLN biopsy was negative in 15/152 patients after peritumoral injection. Eleven of these patients had tumour stage pT2, the others had tumour stage T1. Primary tumours were located in all quadrants, but the majority were in the upper quadrants (in the upper external quadrant in 5 patients and in the upper internal quadrant in 5 patients). Systematic lymphadenectomy in this collective showed lymph node involvement in a high number of cases (7/15 patients, 46.7%). The difference in the rate of involvement of the axillary lymph nodes was borderline significant compared to the total collective with peritumoral injection (p = 0.046).

Patients with multifocal tumours
12 of 152 patients (7.9%) in the group with peritumoral injection had multifocal tumours. The number was higher in the patient collective with periareolar injection (18 of 117 patients, 15.4%). After peritumoral injection SLN biopsy was negative in 2 of 12 patients (16.7%). The rate of successful SLN detection was therefore lower than in the total collective with peritumoral injection (83.3 vs. 90.1%). In the group with periareolar injection SLN biopsy was negative in one of 18 patients with multifocal tumours (successful SLN detection in 94.4% of patients with multifocal tumours compared to 98.3% of the total collective with periareolar injection). No statistical comparison was done due to the limited number of patients with multifocal tumours.

Discussion

The change of injection technique used for sentinel lymph node biopsy in patients with breast cancer was performed in May 2008 in our clinic primarily for practical reasons. One advantage of the periareolar injection technique with regard to small tumours is that the tumour does not have to be palpable or visualised sonographically. Another advantage is that the injection technique remains the same even for multifocal tumours, an important aspect in view of the fact that the numbers of patients with multifocal tumours presenting to our hospital have increased over time. This retrospective analysis aims to investigate whether the change in injection technique has had a negative impact on detection rates and whether the detection rates in our hospital correspond to those reported in the literature.

In our hospital the change of injection method from peritumoral to periareolar injection resulted in a significant improvement in the detection rate for SLN. This is in accordance with the results of the largest multi-centre study on this topic, the FRANSENODE study [18]. The detection rates with a rate of 90.1% for peritumoral injection and of 98.3% for periareolar injection are within the ranges reported in recent studies. In an overview article published in 2011 the reported detection rates ranged from 78 to 99.1% for peritumoral injection and between 92.7 and 100% for periareolar injection [20].

In the group of patients with peritumoral injection it was noticeable that negative SLN biopsy was associated in a large number of cases with axillary lymph node involvement. Out of a total of 15 patients with negative SLN biopsy, 7 (47%) had axillary involvement. This effect was not found in the patients with periareolar injection. In a retrospective analysis published in 2003, Brenot-Rossi et al. also showed that the percentage of patients with axillary lymph node involvement was significantly higher in the group of patients with negative SLN biopsy [22]. It was suggested that the negative SLN biopsy might indicate a blockade of lymph vessels by tumour cells. While insufficient amounts of the tracer pass into the axilla after peritumoral injection, other lymphatic flow paths are available with periareolar injection, so that higher SLN detection rates are possible even with lymph node involvement. However, our patient numbers were not sufficient to allow a more precise analysis. The primary tumours of the patients with negative SLN biopsy were not located in the upper external quadrant in the majority of cases. A masking of axillary lymph nodes by the diffusion of the radiocolloid into tissue, as postulated in other studies for tumours in unfavourable locations in the upper external quadrant [17–19], does not appear to have played any role in our collective.

In both groups the numbers of patients with multifocal tumours were too small for statistical analysis and thus it was not possible to make any definitive statement regarding patients with multifocal tumours. In a retrospective analysis published in 2008, Holl et al. reported a reduced detection rate after peritumoral injection in patients with multifocal tumours [23]. This trend also appears to be detectable in our study; however the numbers of patients are too low for statistical analysis.

It was not possible to determine a false-negative rate in our collectives as no systematic lymphadenectomy was done in patients with negative sentinel lymph node status. Moreover, the follow-up period is still to short to assess the rate of axillary recurrence. The high rate of patients with only SLN involvement may be an indirect indication that the correct lymph node was identified as the sentinel lymph node with both methods. In a recent meta-analysis of studies published between 1993 and 2011 there was no significant difference in the rates of false-negative results after peritumoral and periareolar injection [24].

One of the limitations of the present study is that it is a retrospective data analysis. The patient collectives did not differ significantly with regard to tumour stages; however the study was neither randomised nor were patients assigned according to tumour stage. As this was a retrospective study it was not possible to determine all potential factors which might have influenced successful sentinel detection. Thus, the body mass index (BMI) was not available for all patients, and could therefore not be included in the analysis. As intraoperative detection was done in both groups by the same surgeons, all of whom had several years ex-
perience of SLN biopsy prior to the start of data acquisition, a learning curve which would result in a higher detection rate for the periareolar injection group is unlikely but cannot be completely excluded.

**Conclusion**

Peritumoral and periareolar injection for sentinel lymph node biopsy in patients with breast cancer are currently still considered equivalent. Periareolar injection offers practical advantages: the method is easier to learn, additional imaging of non-palpable lesions is not required, and the intradermal injection technique is less painful. In our collective, periareolar injection additionally proved to result in better SLN detection rates compared to peritumoral injection. Even if our data do not permit determination of the rate of false-negative results, the high number of patients with metastasis in only the SLN indicates that sentinel lymph nodes can be identified correctly with both injection methods.

**Conflict of Interest**

None.

**References**