THE SENTINEL LYMPH NODE IN BREAST CANCER PATIENTS: AN EVALUATION OF NEW DEVELOPMENTS IN CLINICAL PRACTICE

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Contents

Chapter 1

INTRODUCTION AND OUTLINE OF THE THESIS

Introduction

The incidence of breast cancer is increasing worldwide. The yearly incidence in the Netherlands is rising to 127 new breast cancer patients per 100.000 women, or 11.700 patients in 2002. At the moment, one in nine women in the Netherlands has a lifetime risk for developing breast cancer. The mortality rate amounts to 3500 patients and decreases yearly with 1%. In recent years, the 5-year survival rate has improved to 80% for patients of all ages[1,2].

In breast cancer patients, the histologic status of the axillary lymph nodes is the most important prognostic factor[3]. To guarantee appropriate staging and indicate the need for adjuvant treatment, an axillary lymph node dissection (ALND) has long been the golden standard[4-6]. Other reasons to perform an ALND were enhanced regional control and survival benefit[7-12]. However, an ALND often implicates morbidity in terms of lymphedema, seroma formation, reduced shoulder motility and chronic pain[13-16]. This might lead to a reduction in quality of life in exchange for the advantages.

In the seventies, the sentinel lymph node concept was developed by Cabañas in patients with penile cancer[17]. The sentinel lymph node (SLN) is the first lymph node to receive lymph drainage from a primary tumour and is therefore the first node to contain metastatic disease if lymphatic metastasis occurs. Cabañas localised the SLN by anatomical landmarks. Morton transferred this SLN concept to melanoma patients for staging in 1992[18]. He localised the SLN by visualisation of the lymphatic duct leading to the SLN with blue dye. One year later a radioactive tracer and use of the gamma probe was added to facilitate pre- and intraoperative identification of the SLN[19]. The same year the first publication appeared on application of the SLN concept in breast cancer patients[20].

As in melanoma, many validation studies were performed in breast cancer patients. During these studies, all SLN biopsies were routinely followed by a completion ALND. The histological status of the SLN appeared to be predictive for the remaining axillary lymph nodes in patients with clinical T1-2N0 breast cancer. It is therefore a reliable alternative to the traditional ALND and an accurate staging procedure in breast cancer patients[16,21-23]. If metastatic disease is found in the SLN, a completion ALND is recommended to optimise regional control, complete staging and enhance survival[4,11]. When the SLN is tumour free, it was hypothesised patients can be spared an ALND and its concomitant morbidity.

The procedure: the triple technique of the sentinel lymph node biopsy (SLNB). The SLN mapping was performed using 60 MBq of technetium-99m nanocolloid as a radioactive tracer and 1 cc blue dye (Bleu patente V; Guerbet, Aulnay-sous-Bois, France) for lymphatic mapping. Both were injected into breast parenchymal tissue surrounding the tumour or biopsy cavity. The tracer will search its way through draining lymph vessels to the first receiving lymph node. This SLN can be identified and harvested by use of the following triple technique: the lymphoscintigraphy with skin marking of the presumed location of the SLN, pre- and intraoperative use of the gamma probe (Neoprobe, Johnson&Johnson Medical, Hamburg, Germany) to detect radioactivity and intraoperative detection of the blue lymphatic vessels[24,25].

Outline of the thesis

The main objectives of the studies of this thesis were to investigate the possibilities to improve clinical practice and to reveal some of the consequences of the introduction of the SLN procedure.

Intraoperative examination of the sentinel lymph node

After introduction of the SLNB, the biopsy is traditionally combined with the lumpectomy or mastectomy of the diseased breast. A disadvantage of this procedure is the need for a completion ALND as a delayed procedure, if metastatic disease is found in the SLN. Intraoperative examination might reveal the metastatic disease, allowing immediate ALND. Techniques already in use are imprint cytology and frozen section: each having its advantages and disadvantages. The frozen section procedure is time consuming and might give rise to artefacts, but also offers good visualisation. Imprint cytology offers cytological detail and preserves tissue for definitive histopathology. A disadvantage is the higher chance for equivocal results. In chapter II, scrape cytology is introduced as a new technique. It combines the tissue preservation and speed of cytology with a larger yield of cells for examination compared with imprint cytology. The scrape cytology results were compared with the definitive histopathologic results of the SLN in early breast cancer patients. These results were further compared with the available published results of intraoperative frozen section and imprint cytology examination.

Sentinel lymph node biopsy under local anaesthesia

The intraoperative examination of the SLN, however, introduced substantial disadvantages, when compared to the certainties of the ALND-era. No single technique revealed all metastatic disease; accuracy varies from 78-98%. As a consequence, reoperation for the completion ALND was necessary in 19% of the patients with negative intraoperative examination results. Another disadvantage was the time needed for intraoperative examination and the possible ALND in 30-40% of the patients, which extended general anaesthesia duration and hampered operation room planning. Most of these problems could be tackled by performing the SLNB under local anaesthesia. That way both clinician and patient are provided with a histologic diagnosis before definitive breast and possible axillary surgery.
The aim of the study presented in chapter III was to evaluate feasibility of the SLNB performed under local anaesthesia by comparing, among others, the SLN detection rate under local and general anaesthesia. The technical addendum more extensively describes the steps in the procedure of SLNB under local anaesthesia.

Axillary recurrence after a negative sentinel lymph node biopsy

The presentation of an early breast cancer patient with regional recurrence after a negative SLNB in the outpatient clinic presented a new problem. Several validation studies of SLNB all followed by an ALND published false-negative findings varying from 0-22%. A meta-analysis of 13 studies reported a false-negative rate of 5.1%. So, once the validation phase was completed, an unknown number of patients with not-revealed tumour-positive nodes at SLNB did not undergo an ALND. Undetected tumour-positive nodes of clinical importance are those leading to axillary recurrence. The clinical consequences of axillary recurrence after a negative SLNB are not clear yet. In the ALND-era, 30% of the patients with axillary recurrence presented with simultaneous locoregional or systemic metastasis, 50% would develop distant metastasis in the future. Chapter IV describes the incidence of axillary recurrence in current practice and literature. Prognostic features of patients with an axillary relapse after a negative SLNB were analysed.

Predicting the likelihood of non-SLN metastases utilizing a nomogram

Contrary to the problem of possible false-negative SLNB results, possibly leading to axillary recurrence, the investigator finds oneself confronted with overtreatment of the positive SLNB group. Several publications revealed that only in approximately 50% of these patients, additional nodal metastases are detected in the completion ALND. The SLN procedure already caused a 60% decrease of the number of ALNDs in case of a negative SLN. However, a further reduction through selection of a subset of patients with low suspicion for non-SLN metastases after a positive SLNB seems possible.
In order to quantify an individual patient's risk for non-SLN metastases, a nomogram was developed by the Breast Service of Memorial Sloan-Kettering Cancer Center, New York. The nomogram was created using prognostic variables assessed with multivariate logistic regression analysis. The outcome of the nomogram is the predicted probability for the presence of non-SLN metastases. To stimulate use in daily practice, the predicted probability can be calculated by a website and a PDA compatible application. The aim of the study presented in chapter V was to test the accuracy of the nomogram on a general population of breast cancer patients in the Netherlands.

Likelihood for non-SLN metastases: doctors versus numbers

The MSKCC nomogram was developed to identify the patient's individual risk for non-SLN metastases. In daily practice, clinicians raised objections against use of the nomogram for several reasons. Patient's age, as parameter to decide on performing of a completion ALND or not, is not included in the nomogram, since it is not a prognostic factor for non-SLN metastasis. However, age is felt by many surgical oncologists to be a determining factor. Further a low probability for non-SLN metastasis after a positive SLNB makes a completion ALND dubious. This contradicts the nowadays golden standard of a completion ALND after a positive SLNB.
It is clear doctors can not balance all prognostic factors, each to their own weight, and incorporate the results in their clinical decision making. It may therefore be interesting to know how clinical predictions compare to the nomogram results and how these nomogram results influence decisions clinicians take. Medical literature on both subjects is rare. The aim of the study presented in chapter VI was to establish accuracy of surgical oncologists' estimations for non-SLN metastases compared with the MSKCC nomogram. Further the influence of the nomogram on clinical decision-making was examined.

No evidence for increased angiogenesis in lymph node metastases after earlier surgical resection of a primary breast cancer

The phenomenon of accelerated growth of metastases after resection of the primary tumour has been described in animal models and patients. This observation has been attributed to a changing balance between activators and inhibitors of angiogenesis in favour of the first. Contributing factors are, among others, mediators of normal wound healing. The aim of this study was to assess, whether delayed SLNB after earlier resection of the primary tumour in breast cancer patients would lead to increased angiogenesis of the SLN metastases. To this purpose lymph node metastases of patients earlier operated on for primary breast cancer, were compared for mean vascular density and proliferating endothelial cells with a control, one-stage procedure, group. Further the fraction of proliferating cells in tumour and metastasis was examined. The results of this study are presented in chapter VII.

In chapter VIII results and conclusions are summarised.

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Chapter 2

INTRAOPERATIVE SCRAPE CYTOLOGY OF THE SENTINEL LYMPH NODE IN PATIENTS WITH BREAST CANCER

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Abstract

Background: Intraoperative examination of the sentinel lymph node (SLN) may detect metastatic disease, allowing immediate axillary lymph node dissection and therefore avoiding the need for reoperation. The aim of this study was to evaluate the accuracy of scrape cytology of the SLN in patients with early breast cancer.

Methods: Sentinel node biopsy was performed in 148 patients with clinical T1-2 NO breast cancer. After harvesting, the SLN was bisected and cells from both halves were scraped with a scalpel blade on to a slide and stained with a Papanicolaou and Giemsa stain. Scrape cytology results were compared with the results of paraffin sections stained with haematoxylin and eosin and with immunohistochemistry.

Results: The intraoperative diagnosis was correct in 126 patients (85%). Sensitivity and specificity were 67 and 98% respectively; positive and negative predictive values were 95 and 81%.

Conclusion: Scrape cytology is a useful method for intraoperative evaluation of the SLN in patients with breast cancer.

Introduction

The histological status of the axillary lymph nodes remains one of the most important prognostic indicators in patients with breast cancer[1]. The sentinel lymph node (SLN) has proven to be a reliable predictor of the histological status of the remaining axillary lymph nodes[2-6]. SLN biopsy has the advantage of decreased postoperative morbidity compared with complete axillary lymph node dissection (ALND). A disadvantage is the need for reoperation if metastatic disease is found in the SLN.

Intraoperative examination of the SLN may detect metastatic disease, allowing immediate ALND. Two techniques already in use for intraoperative examination are frozen sectioning and imprint cytology. Frozen sectioning offers visualization of architecture of the lymph node, but is tissue consuming and causes artefacts to definitive pathology[7,8]. Imprint cytology offers cytological detail and preserves the entire lymph node for definitive histopathology. Disadvantages include the small number of cells sampled and the higher chance of indeterminate results caused by atypical cells[8,9].

Scrape cytology of the SLN is an alternative that combines the tissue preservation of cytology with the advantage of a larger number of cells for examination[8,10,11].

The aim of this study was to ascertain the value of intraoperative scrape cytology in determining the histological status of the SLN in patients with early breast cancer.

Patients and methods

Between June 2000 and July 2001, 168 patients had a SLN biopsy for clinical stage T1-2 NO breast cancer. In three patients the SLN procedure was not successful and an ALND was performed in the same session. In 17 patients the SLNs were examined only after operation. In the remaining 148 patients the SLN was examined during the operation by scrape cytology. The median age of these patients was 56 (range 27-95) years. The pathological median tumour size was 16 (range 0-80) mm. The tumour stage was Tis in three patients (2%), T1 in 86 patients (58%), T2 in 51 patients (34%), T3 in one (1%) and TO in seven patients (5%). In 26 patients an excision biopsy was performed before the SLN procedure.

Of the 148 patients, 109 (74%) had invasive ductal cancer (IDC), three (2%) had in situ ductal cancer, 18 (12%) had invasive lobular cancer (ILC) and the remaining 18 (12%) had intracystic papillary cancer, tubular cancer, metaplastic cancer, Paget's disease, medullary cancer or fibroadenoma.

SLN biopsy was performed according to Dutch guidelines using technetium-99m

nanocolloid as a radioactive tracer and Patent blue dye (Bleu patente V; Guerbet, Aulnay-sous-Bois, France) for lymphatic mapping; both were injected around the tumour or around the scar when there had been previous excision biopsy[3,12,13]. At operation a maximum of three sentinel nodes was sent to the pathology department for examination by scrape cytology. If more SLNs were identified, only the most suspicious were examined by scrape cytology. Each SLN was bisected, after which cells from both sides were scraped with a scalpel blade on to a slide. The material was fixed in 96% alcohol or air dried and subsequently stained with a Giemsa and Papanicolaou stain. The slides were first screened by a cytological technician and then reviewed by an experienced pathologist. The whole procedure, including communication of the results, took about 20 min (figure 1).

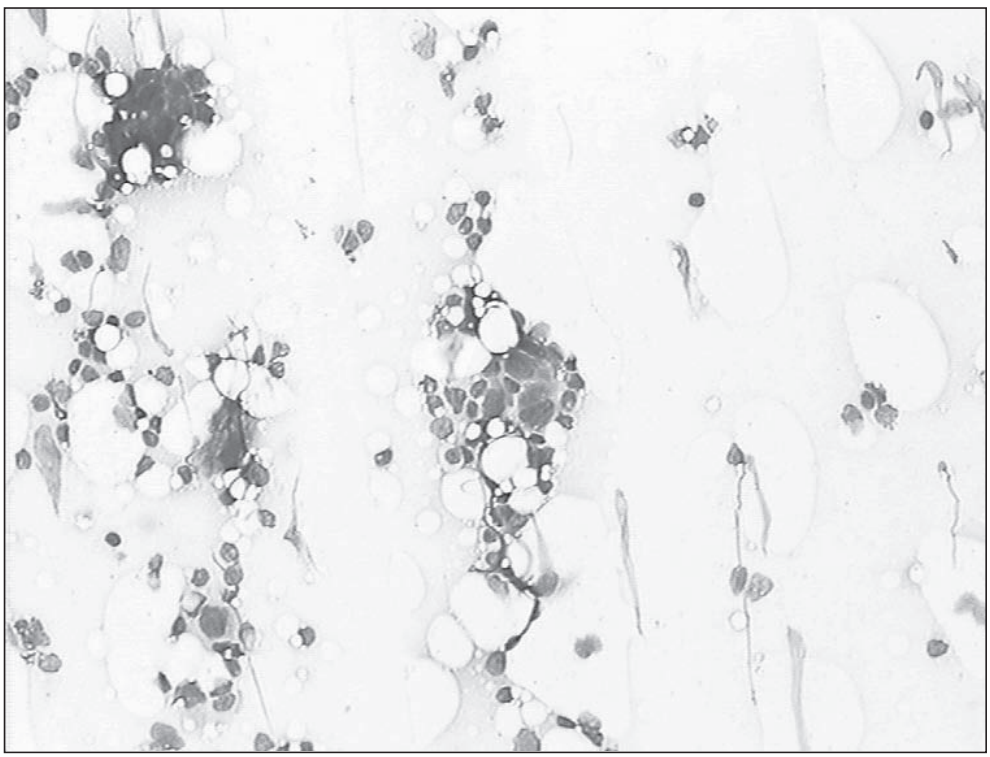

Figure 1 Scrape cytology: the enlarged cells are the tumour cells. Giemsa staining, magnification 200x.

The remaining node halves and other sentinel nodes were examined routinely by embedding in paraffin, step sectioning at 500-µm intervals at three levels, and staining with haematoxylin and eosin. If the SLN was negative on haematoxylin and eosin staining, immunohistochemical staining at the three levels was performed (figure 2). Cam5.2 is a monoclonal antibody directed against cytokeratin

8 localized in the cytoplasm of the tumour cells (Becton Dickinson, San Jose, California, USA).
Scrape cytology results were compared with routine and immunohistochemical staining. Diagnostic accuracy, sensitivity and specificity, and positive and negative predictive values were calculated.

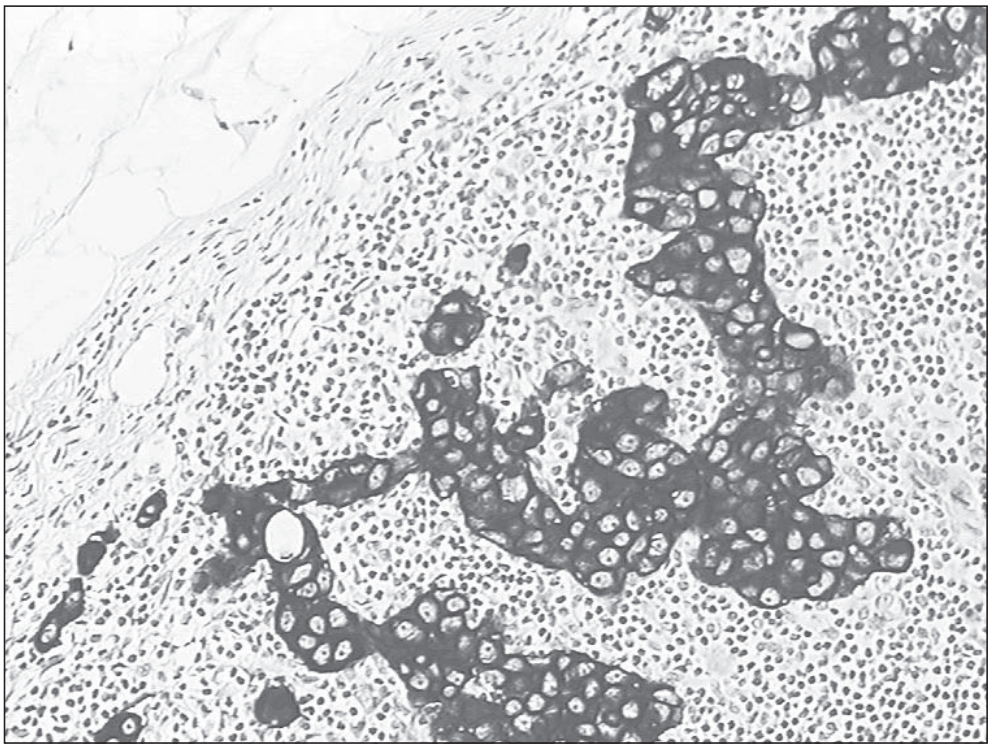

Figure 2 The histology of a ductal carcinoma metastasis in a lymph node. Immunohistochemical stained with CAM 5.2. Magnification 100x.

Results

A total of 268 SLNs were biopsied from 148 patients, a median of 2 (range 1-5) per patient. Sixty patients (41%) were found to have metastatic involvement.
When evaluating the data per patient, 42 patients had at least one positive SLN with scrape cytology. In two patients the SLN was positive on scrape cytology and negative on histopathology. Both of these patients had a T1 tumour and IDC. In 40 patients with positive histopathology, scrape cytology of at least one SLN was positive (sensitivity 67%). The specificity of scrape cytology of the SLN was 98%.

The positive and negative predictive values were 95 and 81% respectively. The overall accuracy of the scrape cytology procedure per patient was 85%. Results per node were not calculated, because the consequences of scrape cytology are taken at a patient level.
In 20 patients scrape cytology gave a false-negative result. This occurred in 14 patients with IDC (13% of all patients with IDC), four patients with ILC (22% of all patients with ILC), and two patients with medullary and tubular cancer (11% of all patients with other types of cancer). Ten of 20 patients with false-negative scrape cytology results actually had micrometastasis in the SLN (defined as a focus of 2 mm or less), as did seven of the 40 patients with positive scrape cytology and histopathology findings. At subsequent ALND only four of 20 patients had additional positive nodes: one extra, twice and two extra, twice. The mean number of positive nodes found with ALND was 0.2 for the group with negative scrape cytology and positive histopathology, compared with 2.5 for the group with positive scrape cytology and positive histopathology.
Micrometastases were identified in 17 (28%) of 60 patients with metastatic disease[14]. Seven of these were found with scrape cytology and confirmed in the haematoxylin and eosin-stained slide. In three patients, metastatic disease was discovered only by immunohistochemistry.

Discussion

Several authors have already reported the results of intraoperative frozen sectioning and imprint cytology of the SLN in patients with breast cancer. The mean accuracy for frozen sectioning in the literature is 89%, with a range from 83 to 96%[2,3,7,8,14,15]. The mean accuracy for imprint cytology ranges from 78 to 98%, with a mean of 91%[15-18]. The results of the various studies are difficult to compare. They might be influenced by the lack of additional immunohistochemical staining in three of ten studies and routine ALND in six of ten studies[2,6,9,15,17,18]. Only two authors compared imprint cytology and frozen sectioning; they came to different conclusions[15,16](table 1). In the present study the accuracy of scrape cytology per patient was 85%.
The distribution of metastases throughout the SLN is not uniform. At least 30% of the metastases in a SLN might be missed with intraoperative examination by scrape cytology after bisection of the SLN. In the present study metastatic disease was diagnosed during operation in 40 patients (67%), which corresponds with the findings of other authors. Two false-positive scrape cytology results were found, both in patients with a T1 tumour and IDC. In both patients this was caused by an interpretation error in the early phase of the trial[19].

Reference	Year	Technique	Accuracy (%)	No. of patients	Routine ALND	IHC
Motomura[15]	2000	FS	88	101	+	+
		IC	96	101	+	+
Veronesi[2]	1997	FS	83	107	+	+
Flett[6]	1998	FS	95	56	+	-
Ratanawichitrasin[17]	1999	IC	98	55	+	-
Rubio[9]	1998	IC	98	55	+	-
Cserni[18]	2001	IC	78	60	+	+
Van Diest[16]	1999	FS	96	54	-	+
		IC	82	54	-	+
Weiser[22]	2000	FS	89	890	-	+
Turner and Giuliano[8]	1998	FS	93	225	-	+
Rahusen[3]	2000	FS	84	100	-	+
Present series	2002	SC	85	148	-	+

Table 1 Review of publications concerning intraoperative sentinel lymph node examination (Abbreviations: FS-frozen sectioning, IC-imprint cytology, SC-scrape cytology, ALND-axillary lymph node dissection, IHC-immunohistochemical staining)

In the present study, 28% of the patients with metastatic disease had micrometastases in the SLN, similar to the results of other authors, who describe incidences ranging from 12 to 51%[5,8,9,19-21]. The percentage of micrometastases found during operation by frozen sectioning or imprint cytology in the literature varies widely from 17 to 100%[8,9,22]. Rubio et al.[9] reported micrometastases in only 12% of all positive SLNs, all of which were found by imprint cytology. These results may have been influenced by the lack of additional inununohistochemical staining and routine ALND, which may influence a pathologist in describing `suspicious' slides[8,9]. In the present study scrape cytology revealed seven of 17 cases of micrometastatic disease during operation. Half of all false-negative results were due to micrometastatic disease.

Finding a metastasis depends on its distribution and site in the SLN. The results of scrape cytology might be improved by making further cross-sections of the SLN (mean size 11 mm)[15], although this will have limitations of thickness of the slide (3-4 mm). This procedure would be time consuming and might destroy the morphology of the SLN. Immunohistochemistry takes too long for an intraoperative diagnosis.

In conclusion, scrape cytology yielded results similar to those of imprint cytology and frozen sectioning of the sentinel node, and could be used for intraoperative analysis of the SLN in patients with breast cancer.

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Chapter 3

SENTINEL LYMPH NODE BIOPSY PERFORMED UNDER LOCAL ANAESTHESIA IS FEASIBLE

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Abstract

Background: A sentinel lymph node (SLN) biopsy in breast cancer patients, performed under local anaesthesia (LA), could have advantages such as more efficient use of operating room and pathologist's time. It also provides a histologic diagnosis before definitive breast surgery. The aim of this study was to assess feasibility by comparing the results of SLN procedures performed under LA versus general anaesthesia (GA).

Methods: The SLN procedure was performed in 50 consecutive outpatients and 167 inpatients with clinical T1-2N0 breast cancer, while they were under LA and GA respectively. The SLN detection rate, a comparison of mapped and harvested SLNs, was compared for both groups. The duration of the SLN biopsies performed under LA was measured.

Results: For both groups a median of two SLN/patient was harvested. The detection rate was 1.00 for the LA group and 0.99 for the GA group. The learning curve for SLN procedures under LA shows a decrease in duration for the consecutive months (not significant).

Conclusion: SLN biopsy can be safely and adequately performed under LA. It allows early diagnosis of the lymph node status, acquired on an outpatient basis, with minimal discomfort to the patient. The learning curve demonstrates that the LA procedure can quickly be mastered if the surgeon is experienced in performing SLN biopsies.

Introduction

The histologic status of the axillary lymph nodes is the most important prognostic indicator in patients with breast cancer[1]. Sentinel lymph node (SLN) biopsy is a reliable technique for axillary staging in clinical stage T1-2N0 breast cancer[2-6]. An important advantage of SLN biopsy is its decreased risk of postoperative morbidity compared with axillary lymph node dissection (ALND). Currently, the SLN biopsy specimen is mostly examined intraoperatively by frozen section or imprint cytology; if metastatic disease is identified, ALND is performed in the same session.

Intraoperative SLN examination carries substantial disadvantages. No single technique discloses all metastatic disease. As a consequence, reoperation is necessary in 4 to 15% of patients with negative intraoperative examination results[7-11]. Another disadvantage is that the time needed for intraoperative examination extends the duration of general anaesthesia (GA) and, by consequence, the use of the operating room[12-15]. Also, patients undergo surgery unsure of the exact diagnosis and the procedure that will be performed[12,15]. In theory, these problems could be negated by performing the SLN biopsy with the patient under local anaesthesia (LA). The outpatient procedure must meet the same standards of quality as the operation it replaces[14,16-18].

Outpatient SLN biopsy has possible advantages. A precise histologic diagnosis, instead of the unsure result of intraoperative cytology or frozen section, can be presented to the patient before definitive breast surgery is undertaken. Furthermore, valuable operating room time is saved because SLN only requires an outpatient surgical unit. Time constraints do not present an issue for the pathologist with regard to the intraoperative diagnosis. Finally, approximately 15% of all patients will be spared a second episode of GA[12-15,17-19].

Still, one could also imagine some procedure-related complications. Patients may experience discomfort as a result of the use of LA. The amount and type of local anaesthesia used could also cause reactions and complications[20]. Theoretically, injection of a local anaesthetic into tumour-bearing axillary tissue could introduce tumour cells into the circulation[12-14].

The present prospective study aims to evaluate the feasibility of the SLN biopsy performed under local anaesthesia. For this purpose the results of 50 consecutive SLN biopsies performed under LA and 167 consecutive SLN biopsies performed under general anaesthesia were compared.

Patients and methods

Between February and June 2002, 50 consecutive patients underwent SLN biopsy under LA in an outpatient setting (group LA). This group was compared with a group of 167 inpatients, in which SLN biopsy was performed under GA from June 2000 until May 2001 (group GA). The first SLN procedure had already been performed in May 1997. All patients presented with T1-2N0 breast cancer.

Sentinel lymph node biopsy performed under local anaesthesia

The SLN procedure was performed using 60 MBq technetium-99m nanocolloid as a radioactive tracer and 1 mL blue dye (Bleu patente V; Guerbet, Aulnay-sous-Bois, France) diluted with 1 mL lidocaine (0.5%) for lymphatic mapping. In the GA group, both were injected into breast parenchymal tissue surrounding the tumour or biopsy cavity. In the LA group, the radioactive tracer was injected periareolar in the diseased breast quadrant, and the blue dye was injected into the tissue surrounding the tumour or biopsy cavity[21].

In the LA patients, the SLN biopsy was performed in the outpatient operating rooms.

Patients were admitted 1 hour before the start of the procedure. No premedication was administered. Although the location of the SLN was marked on the skin using the gamma probe (Neoprobe, Johnson&Johnson Medical, Hamburg, Germany), the incision was standard, i.e., from the anterior to the dorsal axillary line, just caudal to the axillary hairline, with an average length of 5 cm. Local anaesthesia was administered by subcutaneous infiltration of lidocaine (0.5%) with adrenaline (1:200.000). If needed, more anaesthetic was injected during surgery, but it was always far less than the recommended safe dose[20]. The SLN was identified and harvested intraoperatively guided by the lymphoscintigraphy, the blue lymphatic vessels, and the detection of radioactivity by the gamma probe. Haemorrhage was no problem because adrenaline and haemoclips were used[22]. All patients left the hospital within 30 minutes after termination of the procedure. A prescription for paracetamol was routinely given, but no patient made use of it.

Histologic examination of the sentinel lymph node

The SLN was bisected, after which both halves were embedded in paraffin. Each part was step-sectioned at 500-micrometer intervals at 3 levels and stained with

haematoxylin and eosin as well as immunohistochemical staining with Cam5.2. Cam5.2 is a monoclonal antibody directed against cytokeratin 8, which is localized in the cytoplasm of the tumour cells (Becton Dickinson, San Jose, California, USA).

To assess the feasibility of SLN biopsy performed under LA, its results were compared with those obtained after procedures performed under GA. The following parameters were taken into consideration: detection rate of the SLNs, comparison of the number of SLNs harvested and detected by lymphoscintigraphy, number and type of complications, rate of lymph node metastases, and number of lymph nodes detected in the complementary ALND if metastatic disease was found in the SLN.

Results

The results of 50 consecutive patients undergoing SLN biopsy under LA in an outpatient setting were compared with the results of 167 inpatients undergoing SLN biopsy under GA. In 19 patients in the GA group and 3 patients in the LA group, an excisional biopsy was performed before the SLN procedure.

The triple technique, consisting of static lymphoscintigraphy and intraoperative use of the gamma probe and blue dye, was not complete in 25 GA patients. In 3 of these patients and 2 LA patients, the procedure was not successful because the SLN was not visualised on lymphoscintigraphy; ALND was performed instead. The median age of the patients of both groups was 57 years. The median histological tumour size was 16 mm for both patient groups (table 1).

Characteristics	LA	GA
No. of patients	50	167
Median (range) age (years)	57 (31-84)	57 (27-95)
Tumour size (range) (mm)	16 (0-65)	16 (0-80)
Tumour stage (%)		
T0	0	7 (4%)
Tis	2 (4%)	4 (2%)
T1	25 (50%)	98 (59%)
T2	19 (38%)	57 (34%)
T3	4 (8%)	1 (1%)

Table 1 Patient characteristics for LA and GA group (Abbreviations: LA - local anaesthesia, GA - general anaesthesia)

For both groups, a median of two SLNs/per patients (range 1-4) was harvested. All SLN biopsies in the LA group were performed or supervised by one surgeon. The 2 participating surgical oncologists supervised the SLN procedures under GA. In the 48 patients in the LA group (100%), all mapped SLNs were harvested. In 99% of the GA patients (139 patients), all mapped, and in some cases more, SLNs were found (not significant).

The SLN procedure under LA took a median of 30 minutes (range 15-95). In the first month however, median duration was 42 minutes (range 25-95). The last studied month showed a decrease in length of procedure to a median of 26 minutes (range 15-50). This decrease was not significant (P = 0.35, nonparametric independent sample test-medians Fisher's exact test). Only 1 LA procedure was complicated by difficulty in identifying the SLN, but it resulted eventually in a successful biopsy (4 SLNs) within 95 minutes. None of the LA patients had to be admitted as a consequence of complications, and no GA biopsy was complicated by technical difficulties (figure 1).

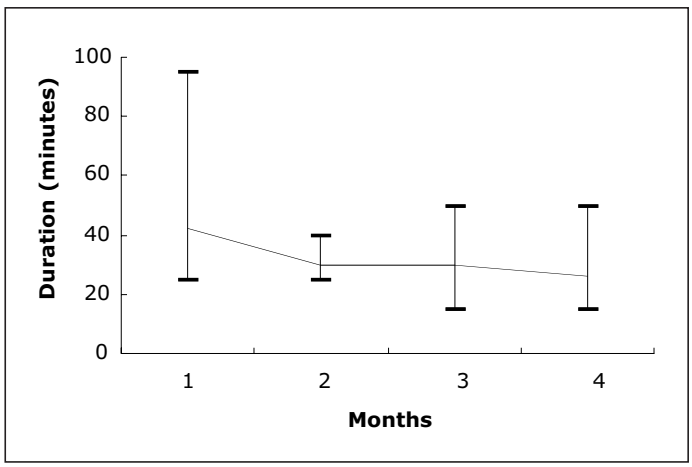

Figure 1 Learning curve of SLN biopsy performed under local anaesthesia: median and range of procedure duration are shown for four consecutive months

Metastases were identified in the SLNs of 12 LA patients (25%). In 7 patients (15%) the outcomes demonstrated micrometastases, 58% of all metastatic disease. The SLNs from the GA group contained metastatic disease in 56 patients (39%). This involved micrometastases in 16 patients, 29% of all metastatic disease. Complementary ALND for both procedures contained a median of 11 lymph nodes (range 3 to19) in GA patients and 12 (range 5-20) in LA patients (table 2). The type of surgery used for procedures performed under GA was for 49 patients

a simple mastectomy and was for 93 patients breast conserving surgery. The numbers for LA patients were comparable (table 3).

Results	LA	GA
SLN metastasis (%)	25	39
Micrometastasis (%)	58	29
Median no. of excised ALNs	12 (5-20)	11 (3-19)

Table 2 Results of the SLN and ALND procedures
(Abbreviations: ALN - axillary lymph node, ALND – axillary lymph node dissection, GA – general anaesthesia, LA – local anaesthesia)

Surgery	LA	GA
BCS (%)	29 (60)	93 (65)
Simple mastectomy (%)	19 (40)	49 (35)

Table 3 Breast surgery after SLN biopsy under LA or simultaneous with SLN biopsy under GA
(Abbreviations: LA - local anaesthesia, GA - general anaesthesia, BCT - breast conserving therapy)

Lymphoscintigraphy of the LA group patients showed extra-axillary lymph nodes in 5 subjects (10%); in 4 patients this involved an intramammary node, in 1 patient it involved an internal mammary node. One out of these 4 nodes located in the breast parenchyma was harvested. In 10 patients (7%) of the GA group, extra-axillary nodes were visualised on the lymphoscintigraphy. These included 3 intramammary nodes and 7 internal mammary nodes. Two of the nodes located in the breast and 2 of the internal mammary nodes were identified.

Discussion

Few investigators have reported on SLN biopsy under LA. Fenaroli et al. presented a successful feasibility study with 14 consecutive patients having early breast cancer[14]. Alex and Krag performed a SLN procedure under LA in a patient with a melanoma of the thigh[17]. Glass et al. and von Smitten mentioned the possibility with both breast cancer and melanoma patients[16,18].

This feasibility study shows similar results for both groups concerning the number of harvested SLNs and the ratio comparing mapped and identified SLNs. The operation time could be measured only for the LA procedures because the GA biopsies were always combined with breast surgery. The learning curve for SLN procedures under LA shows a progressive decrease in duration with cumulative experience expressed for consecutive months, although this was not significant. However, it demonstrates that the LA procedure can quickly be mastered if the surgeon is experienced in performing SLN biopsies. Except for 1 long procedure (resulting in 4 SLNs) in the early phase, no complications occurred.

A SLN procedure under local anaesthesia leads to more efficient use of the operating room, for one does not have to take into account the additional time possibly needed for an ALND. Further it saves the pathologist time otherwise consumed by intraoperative examination. It saves 15% of all patients a second episode of GA and gives a histologic diagnosis instead of the uncertain result of intraoperative examination[14,16-18]. Publications on the results of breast conserving surgery under local anaesthesia report similar advantages[12,13].

Adverse reactions from infiltration of the anaesthetic were not encountered, nor has this problem been mentioned in the literature[12,13,15,19]. In our experience, additional sedative was unnecessary. The amount of local anaesthetic used was always far less than the recommended safe dose, and patients never used the prescribed paracetamol. Because sedatives were not used, patients were able to leave the hospital shortly after termination of the procedure. Additionally, the possibility exists of introducing cancer cells into either the venous or lymphatic circulation by injection of the local anaesthetic. Extrapolating from insights into lumpectomy under LA and intratumoural injection of SLN tracers, leads one to consider infiltration of the axilla a safe technique[12].

SLN biopsy under LA proved no more difficult than a procedure performed under GA. However, performing complementary ALND after SLN biopsy done under LA was more difficult. This was not reflected in the number of lymph nodes detected in the ALND.

The radioactive tracer was injected in the LA group in the subareolar lymphatic plexus. This injection mode assures a higher success rate of identifying a SLN by lymphoscintigraphy, whereas less extra-axillary SLN are mapped scintigraphically[21].

However, in 10% (5 patients) in the LA group, extra-axillary nodes were mapped, but only 1 was harvested. In this patient it did not change adjuvant treatment policy.

In conclusion, SLN biopsy can be safely and adequately performed under LA on outpatient basis. It allows early diagnosis of the lymph node status with minimal discomfort to the patient. Further research concerning patient satisfaction and cost issues is warranted.

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Chapter 4

AXILLARY RECURRENCE AFTER A NEGATIVE SENTINEL NODE BIOPSY FOR BREAST CANCER: INCIDENCE AND CLINICAL SIGNIFICANCE

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Abstract

Background: Sentinel lymph node biopsy (SLNB) carries the inherent risk of false-negative sampling. Undetected tumour-positive nodes of clinical importance are those that lead to axillary recurrence. This survey aims at clarifying the extent of this problem in current practice and literature.
Methods: In a regional teaching hospital, 696 consecutive breast cancer patients underwent SLNB between January 1998 and December 2003, and data were entered in a prospective database. Thirteen studies dealt with the follow-up of a cohort of sentinel lymph node (SLN)-negative patients or presented a case report.
Results: The SLN identification rate was 97.1%. The SLN was tumour free in 439 (65%) of the 676 patients. After a median follow-up of 26 months, axillary recurrence was detected in 2 of 439 patients, 4 and 27 months after the SLNB. The incidence of clinically apparent false-negative SLNB is 0.46%. The systematic review resulted in 3184 SLNB-negative patients with a median follow-up of 25 months. Axillary recurrence occurred in 8 patients after a median of 21 months. The axillary recurrence rate in the literature is 0.25%. One third of these patients presented with synchronous systemic metastases.
Conclusion: Axillary recurrences after a negative SLNB occur, but at a much lower rate than would be expected on the basis of historical figures and the false-negative SLN findings. The natural history of axillary relapse after negative SLN biopsy resembles the locoregional recurrence of breast cancer.

Introduction

The histological status of the axillary lymph nodes is the most important prognostic factor in patients with breast cancer[1]. The sentinel lymph node biopsy (SLNB) has proved to be a reliable alternative to the traditional axillary lymph node dissection (ALND) with regard to predicting the histological status of the remaining axillary lymph nodes in clinical T1-2N0 breast cancer[2-6]. The SLNB has the advantage of reduced postoperative morbidity compared with ALND[7]. In case of a positive sentinel lymph node (SLN), a complementary ALND is recommended to maximise regional control and complete axillary staging.

Several validation studies of SLNB's followed by ALND in breast cancer patients have been published. All these studies report the risk of false-negative sampling, with rates varying from 0% to 22%[2,6,8,-12]. A meta-analysis of 13 studies including 912 patients reported a false-negative rate of 5.1%[13]. Once the validation phase is completed, an unknown number of patients with undetected tumour-positive nodes at SLNB do not undergo an ALND. Undetected tumour-positive nodes of clinical importance are those that lead to axillary recurrence[14].

Several questions arise considering axillary relapse. In the setting of a negative SLNB, it would be interesting to identify prognostic factors for the incidence of axillary relapse, especially regarding prevention. The clinical consequences for the patient are unclear, and the nature of subsequent therapy is still open for debate.

The aim of this study was to identify the extent of this problem in current practice. The clinical consequences for the patients with recurrent axillary disease were clarified. Furthermore, a systemic review of the literature was performed to determine incidence, patient and tumour characteristics, and subsequent therapy.

Patients and methods

Between January 1998 and December 2003, 696 consecutive patients had a SLNB for clinical T1-2N0 breast cancer in a regional teaching hospital. After a validation phase, in which 20 patients underwent SLNB with an ALND in the same procedure, patients with a tumour-free SLN did not undergo an ALND. In any case of tumour involvement of the SLN, an ALND was performed. The follow-up consisted of a physical examination every 3 months during the first 2 years and subse-

quently every 6 months. All data were collected in a prospective database. The median age of these patients was 57 years. The median tumour size was 16 mm. The primary tumour was Tis in 22 patients (3%), T1 in 390 patients (56%), and T2 in 243 patients (35%). The histological tumour type was invasive ductal cancer in 73% and invasive lobular cancer in 13% of the patients (table 1).

Variable	Data
Median age, y (range)	57 (20-95)
Tumour size, mm (range)	16 (0-65)
Primary tumour	
T0	16 (2%)
Tis	22 (3%)
T1	390 (56%)
T2	243 (35%)
T3	19 (3%)
T4	6 (1%)
Histological type	
IDC	506 (73%)
ILC	90 (13%)
Other	100 (14%)
Grade	
1	100 (17%)
2	282 (47%)
3	217 (36%)

Table 1 Patient and tumour characteristics of 696 SLNB patients
(Abbreviations: SLNB – sentinel lymph node biopsy, IDC - invasive ductal cancer, ILC - invasive lobular cancer, Grade - Nottingham combined histological grade)

Lymphatic mapping and operative procedures

The SLN procedure was performed with 60 MBq of technetium-99m nanocolloid as a radioactive tracer and 2 mL of blue dye (Bleu patente V; Guerbet, Aulnay-sous-Bois, France) for lymphatic mapping. The SLN was identified and harvested during surgery guided by lymphoscintigraphy, the blue lymphatic vessels, and detection of radioactivity by the gamma probe.

Pathologic examination of the SLN

The SLN was bisected, after which both halves were embedded in paraffin. Each part was step-sectioned at 500-micrometer intervals at three levels and stained with haematoxylin and eosin. Immunohistochemical staining was performed with Cam5.2 (Becton Dickinson, San Jose, California, USA).

Review of the literature

To determine the axillary relapse rate after a negative SLNB for breast cancer, a systematic review of the literature was performed. Pubmed and the Cochrane library were searched with the use of the Medical Subject Heading terms 'breast neoplasms' and 'sentinel lymph node biopsy'. This pair was linked to the terms 'neoplasm recurrence', 'treatment outcome' and 'diagnostic errors'. This search strategy resulted in 221 titles. Only 11 studies dealt with follow-up of a cohort of SLN-negative patients or a case report on axillary relapse after negative SLNB in breast cancer patients. Two other studies were found through links and references.

Results

At least 1 SLN could be identified in 676 of 696 patients (97.1%). The median number of harvested SLNs was 2 (range 0-9). In 237 of the 676 patients (35%) the SLN contained metastatic disease. In 86 patients, this concerned micrometastases. In 6 of these 86 patients with micrometastatic disease, an ALND was omitted.

After a median follow-up of 26 months (range 1-90), an axillary recurrence was detected in 2 patients out of 439 with a negative SLNB. The incidence of axillary relapse after tumour-negative SLNB was therefore 0.46%.

In one patient, physical examination revealed axillary lymph node recurrence 4 months after the SLNB. The ALND specimen contained two tumorous lymph nodes. The patient received an aromatase inhibitor. In a second patient, axillary relapse was detected by routine physical examination 27 months after the SLNB. She underwent an ALND and ovariectomy and received tamoxifen. The SLNs of these two patients were re-examined but did not reveal any metastasis. Patient and tumour characteristics concerning these two patients are summarized in

table 2. In a third patient with a 0.2 mm micrometastasis in the SLN, ALND was omitted. Axillary recurrence was resected 22 months after the SLNB. Tumour was found in the axillary fat and was not related to any preexistent lymph node structure. No technical problems were met during the ALND.

Study	Age (y)	Primary tumour and therapy						Axillary recurrence and therapy		
		Primary tumour	Type	Grade	Hormonal receptor	Primary surgery	Adjuvant therapy	Axil.recur (mo)	Syst.recur (mo)	Systemic therapy
Salmon[21]	47	T1a	Medullary	2	Er-Pr-	BCT	Rt	19	No	Rt + Ct
Blanchard[22]	NS	NS	NS	NS	NS	MST	Ct	41	No	NS
Loza[23]	41	T1a	Idc	2	Er+Pr-	BCT	Rt + Ht	28	No	Rt + Ct
Chung[24]	45	T1c	NS	NS	NS	NS	NS	4	No	NS
	51	T1c	NS	NS	NS	NS	NS	11	Yes	Ct
	48	T1a	NS	NS	NS	BCT	Ct	40	Yes	NS
Roumen[25]	46	T1b	Idc	2	NS	MST	No	14	Yes	Rt + Ht + ovariectomy
Estourgie[26]	44	T1c	Idc	2	NS	BCT	Rt	21	No	Rt + Ht
Yen[27]	57	T1c	Ilc	NA	Er+Pr-	MST	Ct	24	No	Ct
Smidt	75	T2	Idc	3	Er+Pr-	MST	Ht	4	No	Ht
	35	T1c	Idc	3	Er+Pr-	MST	No	27	No	Ht + ovariectomy

Table 2 Patient and tumour characteristics of all patients in the literature with recurrent axillary disease
(Abbreviations: Er - estrogen receptor, Pr - progesterone receptor, BCT - breast-conserving therapy, MST - mastectomy, Idc - invasive ductal cancer, Ilc - invasive lobular cancer, Rt - radiotherapy, Ct - chemotherapy, Ht - hormonal therapy, NS - not stated, NA - not applicable).

Pubmed and the Cochrane library search resulted in 10 studies concerning the follow-up of a cohort SLN-negative patient with breast cancer and in 3 case reports on axillary recurrence. The results of a total number of 3184 patients (including the present series) with a median follow-up of 25 months (range 16-46) were pooled. In eight patients, an axillary relapse was diagnosed. This resulted in an axillary recurrence rate of 0.25% (table 3). Axillary relapse after negative SLNB of all 11 published cases occurred after a median of 21 months. The data concerning these patients are listed in table 2.

Discussion

Axillary recurrence after a negative SLNB in breast cancer patients is, at 0.46%, rare in this group. The mean axillary relapse rate in comparable studies is equally low at 0.25%. These rates are far lower than would be expected if compared to the false-negative rates of the SLNB in the validation phase; a meta-analysis reported a false-negative rate of 5.1%[13].

Study	Year of publication	No. patients	Study period	Follow-up (mo)	Axillary recurrence (% incidence)
Reitsamer[28]	2003	116	5/99-2/01	Mean 22 mnth	0
Badgwell[29]	2003	159	4/98-10/99	Median 32 mnth	0
Estourgie[26]	2003	353	1/97-11/01	Median 16 mnth	1 at 21 mo (.3%)
Veronesi[7]	2003	167	3/98-12/99	Median 46 mnth	0
Schrenk[19]	2001	83	6/96-9/00	Median 22 mnth	0
Chung[24]	2002	206	1/98-12/01	Median 26 mnth	3 at 4/11/31 mo (1.5%)
Giuliano[30]	2000	67	10/95-7/97	Median 39 mnth	0
Roumen[25]	2001	100	12/97-6/00	Median 24 mnth	1 at 14 mo (1%)
Dessureault[31]	2000	809	4/94-4/99	Mean 20 mnth	0
Blanchard[22]	2003	685	10/97-8/01	Median 29 mnth	1 at 41 mo (.1%)
Present series	2004	439	5/97-12/03	Median 26 mnth	2 at 4/27 mo (.5%)_

Table 3 Incidence of axillary recurrence after negative sentinel lymph node biopsy in breast cancer patients (No. patients - cohort of negative SLN patients)

These results are supported by follow-up studies of clinically node-negative breast cancer patients in whom surgical axillary staging was omitted. A population-based study showed that 34% of the axillary lymph nodes of clinical stage I breast cancer patients contain metastases[15]. In contrast with these findings, Greco and Fisher demonstrated that only 6.7% to 17.8% of the patients without ALND developed axillary recurrence after a follow-up period of 5 to 10 years. Axillary relapses were detected after a median period of 14.7 to 31 months[16,17]. Hence, substantially fewer clinical recurrences were observed than would be expected on the basis of data reported in literature.

Several factors can explain the difference between the false-negative rate of the SLNB in the validation phase and the axillary relapse rates, as well as the lower than expected axillary recurrence rate after omitting ALND. According to the studies by Greco and Fisher, axillary relapse is to be expected, if it occurs, after a median of 14.7 to 31 months at a follow-up of 63 to 126 months. The follow-up period of the studies in the series in table 3 amounted to a median length of only 16 to 46 months and might therefore be too short to lead to comparable results[16,17].

In contrast to earlier series, most patients currently receive adjuvant systemic treatment because of tumour and patient characteristics. Adjuvant chemotherapy has proved to destroy metastases in tumour-bearing axillary nodes and therefore can be expected to decrease axillary relapse rates[18].

Another cause for the low relapse rate might be the decreasing incidence of failure to identify the SLN after the learning phase. A study with a longer validation

phase shows an increase in identifying the SLN from 67% with 18 patients to 96% with 177 patients[19]. The false-negative rates from the published studies always represent the validation phase. The studies reporting on the follow-up of SLN negative patients have always passed this phase.

The young age of the patients with axillary recurrence is remarkable; almost all patients in literature are younger (median 46 year) than the median age in this series (median 57 year). This corresponds with the median age of 48 years of patients with axillary relapse after ALND[20].

The clinical consequences for patients with axillary relapse after a negative SLNB are yet unclear, but similarities to patients with axillary recurrence after ALND are hard to overlook. In both groups, approximately 30% of the patients with axillary recurrence present with simultaneous locoregional or systemic failure. Approximately 50% of the patients with axillary relapse after ALND develop distant metastatic disease. This suggests an ominous prognosis for patients with axillary relapse after a negative SLNB[20].
It is therefore tempting to consider axillary relapse as a presentation of formal locoregional recurrence. A patient with an axillary recurrence should therefore receive therapy for locoregional failure.

In conclusion, axillary recurrences after negative SLNB occur, but at a much lower rate than would be expected on the basis of historical figures and false-negative SLN findings. Considering the similarities to axillary relapse, subsequent therapy should be aimed at locoregional and systemic control.

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Chapter 5

CAN THE MSKCC NOMOGRAM PREDICT THE LIKELIHOOD OF NON-SLN METASTASES IN BREAST CANCER PATIENTS IN THE NETHERLANDS?

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Abstract

Background: According to Dutch guidelines, an axillary lymph node dissection (ALND) is recommended whenever a sentinel lymph node (SLN) contains metastatic disease. However, only in approximately 50% of patients with metastatic disease in the SLN are additional nodal metastases detected in the completion ALND. To identify the individual patient's risk for non-SLN metastases, a nomogram containing eight predictors was developed by the Breast Service of Memorial Sloan-Kettering Cancer Center (New York, NY). The aim of this study was to test the accuracy of the nomogram on a population of Dutch breast cancer patients.

Methods: Patient, tumour and SLN metastasis characteristics were collected of 222 consecutive patients who underwent a completion ALND. The data of the index and test population were compared. A receiver operating characteristic curve was drawn, and the area under the curve was calculated to assess the discriminative power of the nomogram.

Results: Even though our patient population differed in many respects from the source population, the area under the receiver operating characteristic curve amounted to 0.77, a value very much comparable to the one found in the source population.

Conclusion: The nomogram provides a fairly accurate predicted probability for the likelihood of non-SLN metastases in a general population of breast cancer patients at a regional teaching hospital in The Netherlands. This suggests that the nomogram's originally calculated predictive accuracy may be valid for patient populations that differ considerably from the population in which it was developed.

Introduction

The histological status of the axillary lymph nodes is the most important prognostic indicator in patients with breast cancer[1]. The sentinel lymph node (SLN) procedure is a reliable technique for assessing axillary lymph node involvement in clinical T1-2N0 breast cancer[2-7]. The morbidity of an axillary lymph node dissection (ALND) can be avoided in case of a histopathologically negative SLN[8]. In case of a SLN containing metastatic disease, however minimal, prevailing Dutch guidelines recommend an ALND.
A completion ALND is performed for staging, achieving regional control, and improving survival[9-13]. Accurate staging requires information on the total number of nodes involved[14]. Opponents of a routine ALND after tumour-positive SLN biopsy (SLNB) argue that the added therapeutic benefit is low[15]. In earlier articles, only in approximately 50% of patients with metastatic disease in the SLN were additional nodal metastases detected in the completion ALND[16]. A study on the follow-up of 31 SLN-positive patients who declined a completion ALND, showed no axillary recurrences after a mean follow-up of 30 months. All patients had received adjuvant chemotherapy and breast and chest wall radiation[17]. This is in accordance with the present guidelines, which recommend that virtually all SLN-positive patients should receive adjuvant chemotherapy. This, in combination with radiotherapy, might eradicate residual metastatic disease[18].

After the introduction of the SLNB, several studies addressed the predictors of non-SLN metastases after a positive SLNB. To identify the individual patient's risk for non-SLN metastases, a nomogram was developed by the Breast Service of Memorial Sloan-Kettering Cancer Center, New York (figure 1). A nomogram is a graphical tool to depict a complicated calculation[19]. The association of prognostic features with the likelihood of non-SLN metastases was assessed by multivariate logistic regression analysis of a retrospective group of 702 patients. The nomogram was created using pathologic size, tumour type and nuclear grade, lymphovascular invasion, multifocality, estrogen receptor status, method of detection of the SLN metastases, number of positive SLNs and number of negative SLNs. The outcome of the nomogram is the predicted probability of non-SLN metastases. This predicted probability can be determined graphically or, to facilitate use in daily practice and with greater accuracy, can be calculated by the Web site www.mskcc.org/nomograms or through a personal digital assistant-compatible application. The nomogram was validated in a prospective set of patients from MSKCC. It reasonably accurately predicted the probability of non-SLN metastases for an individual breast cancer patient with a positive SLN at MSKCC.

The aim of this study was to assess the nomogram's generalisibility, by testing its predictive accuracy in a population of breast cancer patients from a regional teaching hospital in The Netherlands.

Patients and methods

Between January 1998 and December 2003, 696 consecutive patients underwent a SLNB for a clinical T1-2N0 breast cancer in a teaching hospital. Data concerning these patients were prospectively collected in a breast cancer database. The SLNB revealed metastatic disease in 229 patients (33%). Almost all (n=222) of these patients underwent a completion ALND. To be included in the study population, patients had to meet the following selection criteria: (1) the patient had to undergo an operation for a primary invasive breast cancer, without neo-adjuvant therapy; (2) the SLNB had to be successful, and the SLN had to contain any amount of metastatic disease; and (3) the total lymph node count of SLNB and ALND had to be at least 10. From 47 (24%) of the 222 patients, fewer than 10 lymph nodes were retrieved.

SLN identification

The SLN was identified by using 60 MBq of technetium-99m nanocolloid as a radioactive tracer before surgery and 2 ml of blue dye (Bleu Patente V; Guerbet, Aulnay-sous-Bois, France) for lymphatic mapping. During surgery, the SLN was harvested guided by the triple technique consisting of preoperative lymphoscintigraphy, blue lymphatic vessels, and detection of radioactivity by gamma probe (Neoprobe; Johnson&Johnson Medical, Hamburg, Germany).

SLN histopathological examination

The SLN was bisected, after which both halves were embedded in paraffin. Each part was stepsectioned at 500-micrometer intervals at three levels and stained with haematoxylin and eosin (H&E), and immunohistochemical (IHC) staining with Cam5.2. Cam5.2 is a monoclonal antibody directed against cytokeratin 8, which is localized in the cytoplasm of the tumour cells (Becton Dickinson, San Jose, California, USA). The nomogram differentiates in three or four methods of detection of metastatic disease in the SLN: IHC only, serial, routine H&E and possible frozen-section. Since SLNs in this study were retrieved with use of local

anaesthesia, the nomogram without the frozen-section method of detection was used. No distinction was made in the database between serial and routine H&E, and therefore all slides were reviewed by an experienced pathologist. If the first slide was positive, it was judged as routine H&E positive. Metastases found on further slides were judged as serial H&E or IHC positive, depending on the method of detection.

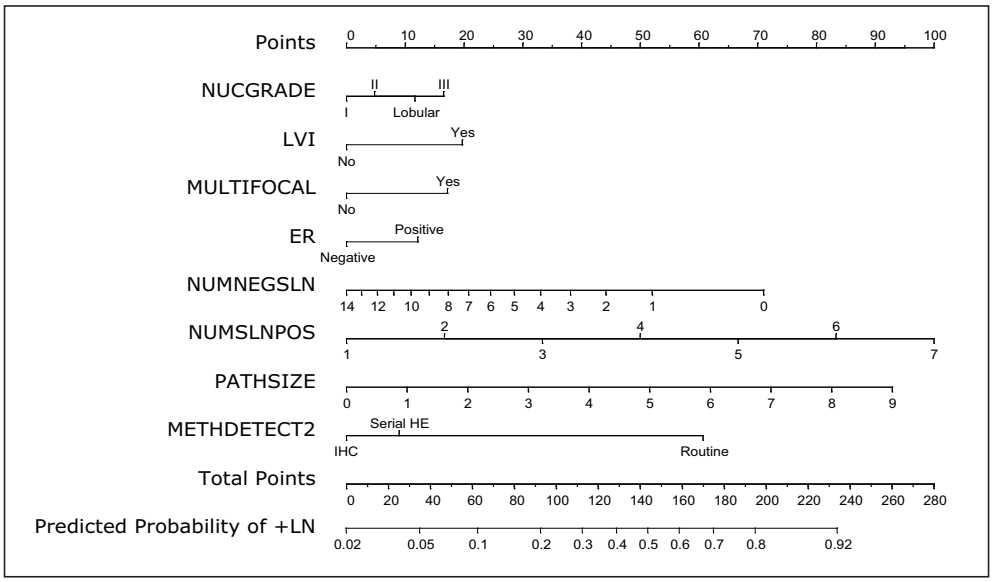

Figure 1 The nomogram to predict the likelihood of non-SLN metastases after a positive SLN biopsy, as developed by MSKCC. NUCGRADE, tumour type and nuclear grade (ductal, nuclear grade I; ductal, nuclear grade II; ductal, nuclear grade III; lobular); LVI, lymphovascular invasion; MULTIFOCAL, multifocality of primary tumour; ER, estrogen-receptor status; NUMNEGSLN, number of negative SLNs; NUMSLNPOS, number of positive SLNs; PATHSIZE, pathological size in cm; METHDETECT, method of detection of SLN metastases (routine H&E, serial H&E and IHC). The first row (POINTS) is the point assignement for each variable. Row 2-9 represent the variables included in the model. For an individual patient, each variable is assigned a point value (uppermost scale, POINTS) based on the histopathological characteristics. A vertical line is made between the appropriate variable value and the POINTS line. The assigned points for all eight variables are summed and the total is found in row 10 (TOTAL POINTS). Once the total is located, a vertical line is made between TOTAL POINTS and the final row 11. Row 11 presents the predicted probability for non-SLN metastases after a positive SLN biopsy.

Data analysis

Patient and tumour characteristics were collected from the prospective database for each variable of the MSKCC nomogram, including tumour type (ductal vs. lobular carcinoma), pathological size (cm), and nuclear grade, presence of lymphovascular invasion, multifocality, estrogen receptor status, method of detection of the SLN metastasis (routine histopathology, serial H&E or IHC) and number of

	Prospective Group CWH (N = 222) N	%	Prospective Group MSKCC (N= 373) N	%	P-value
Age					
≤ 50	78	35.1	157	42.1	≤ 0.11
> 50	144	64.9	216	57.9	
Pathologic Size (cm)					
≤ 0.5	1	0.5	13	3.5	≤ 0.01
0.6-1.0	13	5.9	49	13.1	
1.1-2.0	90	40.5	166	44.5	
2.1-3.0	69	31.1	93	24.9	
3.1-5.0	40	18.0	41	11.0	
≥5.1	9	4.0	11	2.9	
Tumour type and nuclear grade					
Ductal, I	30	13.5	11	2.9	≤ 0.001
Ductal, II	78	35.1	175	46.9	
Ductal, III	65	29.3	129	34.6	
Lobular	49	22.1	58	15.5	
Lymphovascular invasion					
No	150	67.6	219	58.7	≤ 0.039
Yes	72	32.4	154	41.3	
Multifocal					
No	167	75.2	241	64.6	≤ 0.010
Yes	55	24.8	132	35.4	
Estrogen-receptor status					
Negative	32	14.4	83	22.3	≤ 0.025
Positive	190	85.6	290	77.7	
Method of detection					
IHC Only	49	22.1	18	4.8	≤ 0.001
Serial H&E	38	17.1	40	10.7	
Routine H&E	135	60.8	296	79.4	
No. of positive SLNs					
1	163	73.4	265	71	≤ 1.0
2	48	21.6	75	20.1	
3	7	3.1	21	5.6	
4	3	1.4	8	2.1	
5	0	0	3	0.8	
6	1	0.5	0	0	
7	0	0	0	0	
≥8	0	0	1	0.3	
No. of negative SLNs					
0	131	59	132	35.4	≤ 0.001
1	64	28.8	79	21.2	
2	19	8.6	72	19.3	
3	7	3.1	41	11.0	
4	0	0	22	5.9	
5	1	0.5	7	1.9	
6	0	0	10	2.7	
7	0	0	2	0.5	
≥8	0	0	8	2.1	

Table 1 Comparison of descriptive characteristics of both prospective patient populations of the CWH and MSKCC. (Abbreviations: CWH – Canisius Wilhelmina Hospital, MSKCC – Memorial Sloan-Kettering Cancer Center, SLN - sentinel lymph node, IHC – immunohistochemical staining, H&E - hematoxylin and eosin)

positive and negative SLNs (figure 1). These characteristics were compared with the prospective MSKCC data and tested for significance (table1).
By using the MSKCC Web site, the predicted probability was calculated for each patient. The 222 patients were grouped in deciles (groups of 22 patients) according to their predicted probabilities. For each group, the actual probability was calculated by assessing the incidence of additional non-SLN metastases. A calibration plot was drawn showing for each decile the actual versus predicted probability. To measure the discrimination of the nomogram, a receiver operating characteristic (ROC) curve was constructed and the area under the curve was calculated. Calculations were performed for the entire cohort (n = 222) and for the cohort excluding patients for whom fewer than 10 lymph nodes could be retrieved (n = 167). All analyses were conducted with SPSS 11.0 (SPSS Inc., Chicago, IL)

Results

Patient and tumour characteristics were collected and compared with the MSKCC data. All variables differed significantly except age and the number of positive SLNs (table 1). Among the 47 patients from whom fewer than 10 lymph nodes were retrieved, the median number of lymph nodes was 8.

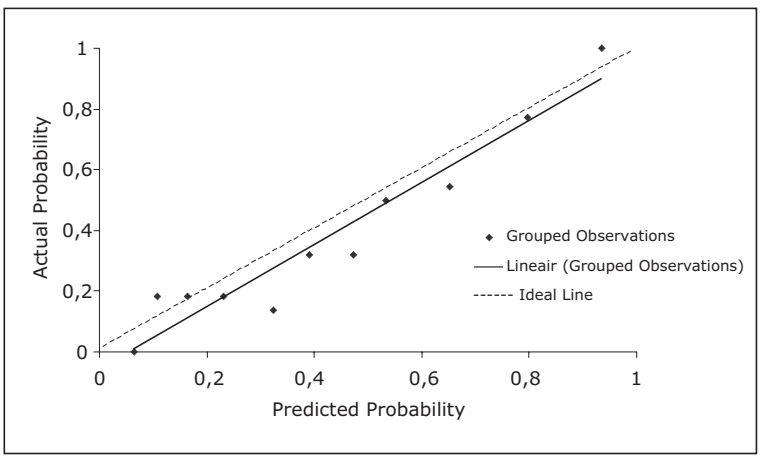

Figure 2 Calibration plot for the nomogram. The entire cohort of 222 patients was classified in deciles according to their predicted probabilities. For each group, the actual probability (incidence of additional non-SLN metastases) was calculated. A calibration plot was drawn showing for each decile the actual versus predicted probability.
(Abbreviations: non-SLN – non sentinel lymph node)

To assess the accuracy of the nomogram, actual probabilities were plotted against the calculated predicted probability for each decile of patients. The trend line shows almost complete concordance, with the ideal line with a slope of 1 (figure 2). A ROC curve was drawn to assess the discrimination of the nomogram (figure 3).

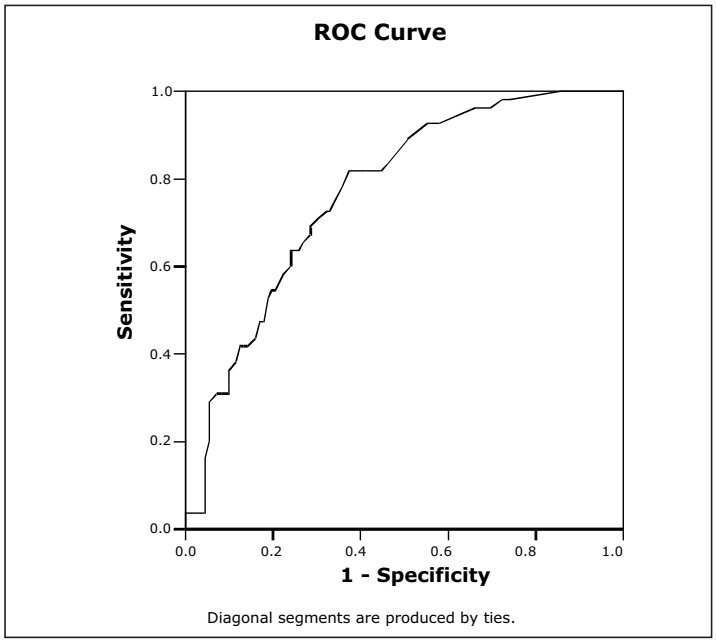

Figure 3 A receiver operating characteristic curve assessing the discrimination of the nomogram. The area under the ROC curve is 0.76.

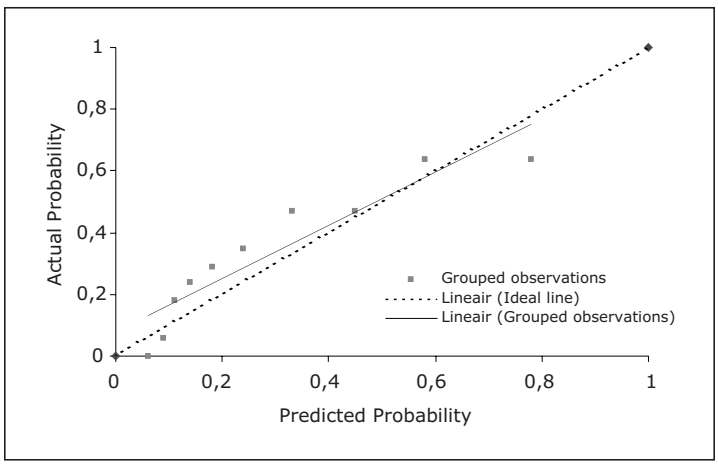

Figure 4 Calibration plot for the nomogram. The cohort of 167 patients in whom 10 or more retrieved lymph nodes could be retrieved were classified in deciles according to their predicted probabilities. For each group, actual probability (incidence of additional non-SLN metastases) was calculated. A calibration plot was drawn showing for each decile actual versus predicted probability (Abbreviations: non-SLN – non sentinel lymph node)

The area under the ROC curve (AUC) was 0.76 (95% confidence interval, 0.69-0.83). Both graphics and statistical analysis were repeated after exclusion of the patients in whom fewer than 10 lymph nodes were recovered. As a result, the calibration plot shifted to the left, and the AUC amounted to 0.77 (95% confidence interval, 0.70-0.84) (figure 4).

Discussion

Soon after the validation phase of the SLN concept, several articles were published concerning prognostic factors for non-SLN metastatic disease after a positive SLNB. Various predictors were identified, such as a palpable breast mass, tumour size, histological grade, lymphovascular invasion, SLN micrometastasis or size of the nodal metastasis, extranodal extension, and more than one positive SLN[16,20-31]. The MSKCC nomogram combines these prognostic factors, revealed by multivariate analysis. The nomogram is a tool that provides a risk estimate for the likelihood of non-SLN metastases and can be used in current practice.

Because the nomogram was developed in MSKCC, which is a tertiary referral center, the question arose whether it would be applicable to the population of a Dutch regional teaching hospital. The difference of the studied populations was reflected in significantly deviating patient and tumour characteristics. The area under the curve, however, amounted to 0.76 in the Dutch population for the entire group and to 0.77 for the group comparable to the source group. For the New York group, the area under the curve was 0.78. The scale of the area under the curve ranges from 0.5 for a test as good as the toss of a coin, to 1.0 which makes a perfect test. An area under the curve of 0.77 is therefore a reasonably accurate predictive test.

According to the MSKCC inclusion criteria, at least 10 lymph nodes need to be retrieved. This criterion was not met in 24% of the Dutch patients. The likelihood for detecting a non-SLN containing metastatic disease increases with the number of examined nodes. A higher yield of nodes per patient would therefore result in a shift of the calibration plot to the left. Our data suggest, however, that the influence of the number of retrieved lymph nodes is minimal and should not preclude use of the nomogram.

Recently a study was presented that compared surgeons' predictions of the probability of non-SLN metastatic disease with the performance of the MSKCC nomogram. The surgeons' predictive accuracy corresponded with an area under the curve of only 0.54. Clearly, the MSKCC nomogram outperformed expert judgment, thus emphasising its clinical utility[32].

The observed differences in patient populations can be explained by the function of the hospitals: MSKCC is an oncologic referral center and the Canisius Wilhelmina Hospital is a regional teaching hospital for the general population. The higher incidence of smaller tumours at MSKCC could be the result of annu-

al screening for breast cancer in all patients, compared with the Dutch screening scheme, which consists of 2-yearly screening of women aged 50 to 75 years. Approximately 30% of the study patients had a screening-detected tumour. Except size, all other tumour characteristics had a better profile in the Dutch population, which corresponds to a more general patient population mix.

A subset of 40%, of the 50% of the breast cancer patients with a SLN containing metastatic disease, will have non-SLN metastases. Consequently, a cutoff value of 10% predicted probability would concern 2.0% of all patients. According to the predicted probability of 10%, only 0.2% would be at risk for residual metastatic disease in non-SLNs. Axillary recurrence rates after negative SLNB are so far lower than expected. This may be explained by the increasing number of patients in this group receiving adjuvant therapy[18,33]. Patients with a SLN containing metastatic disease will always be considered for adjuvant therapy. As a consequence, axillary recurrence rates will even be lower than expected, and a cutoff point of 10% seems therefore reasonable and defensible. In current practice, the nomogram was used to avoid completion ALND in several patients with a low likelihood (<10%) for non-SLN metastases. The Dutch guidelines, however, recommend a completion ALND for all patients in the presence of only one prognostic factor, a positive SLN. This nomogram enables the formation of an individualized risk estimate for the probability of non-SLN metastases based on eight predictors. The nomogram does not make a statement on treatment recommendations. Its results can support the multispecialty oncologic team as well as doctor or patient regarding the choice of whether an ALND is desirable, taking into account, for instance, patients' preferences and risk aversiveness. Further research on estimates for non-SLN metastases by oncologists versus the MSKCC nomogram is warranted.

In conclusion, the nomogram provides a fairly accurate prediction of the probability of non-SLN metastases in a general population of breast cancer patients, differing from the population in which the nomogram was originally developed. The resulting risk estimate can help to individualise a patient's treatment.

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Chapter 6

CAN SURGICAL ONCOLOGISTS RELIABLY PREDICT THE LIKELIHOOD FOR NON-SLN METASTASES IN BREAST CANCER PATIENTS COMPARED WITH THE MSKCC NOMOGRAM?

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Submitted

Abstract

Background: In approximately 40% of the breast cancer patients with sentinel lymph node (SLN) metastases, additional nodal metastases are detected in the completion axillary lymph node dissection (cALND). The MSKCC nomogram can help to quantify a patient's individual risk for non-SLN metastases with fairly accurate predicted probability.

The aim of this study was to compare the predictions of surgical oncologists for non-SLN metastases with nomogram results and to clarify the impact of nomogram results on clinical decision making.

Methods: Questionnaires were sent to surgical oncologists involved in breast cancer care. It contained 10 scenarios presenting female breast cancer patients with a positive SLN and relevant prognostic features. The surgeon was asked to predict the probability for non-SLN metastases for the first 5 scenarios. For the remaining scenarios, the patient's actuarial likelihood, calculated by the nomogram, was supplied. The surgeon was asked whether or not (s)he would perform a cALND. The type of hospital and the surgeon's experience were registered.

Results: 107 Questionnaires were returned, coming from 8 out of 10 academic or cancer centres, 29 out of 41 regional teaching hospitals and 19 out of 61 local hospitals. The concordance-index amounted to 0.78, indicating moderate concurrence. The intersurgeon variation was important. About 25% of the surgeons was influenced by nomogram information and decided in one or more patients to abandon the cALND. Neither the type of hospital nor experience influenced predicting abilities or the clinical decision making process.

Conclusion: Individual predictions of surgical oncologists for non-SLN metastases do not correlate well with the MSKCC nomogram. The distribution between intersurgeon predictions for one scenario is important. Therefore the nomogram is superior to clinical estimations for predicting the likelihood for non-SLN metastases.

Introduction

The sentinel lymph node biopsy (SLNB) is now widely accepted as an accurate staging procedure in breast cancer patients[1-4]. If any amount of metastatic disease is found in the sentinel lymph node (SLN), a completion axillary lymph node dissection (cALND) is recommended according to the Dutch guidelines to optimize ultimate staging, regional control and survival enhancement[5-7]. When the SLN is tumourfree, patients can be spared a cALND and its morbidity in terms of lymphedema, seroma formation, reduced shoulder motility and chronic pain[8,9].

In approximately 40% of the breast cancer patients with SLNs containing metastatic disease, additional nodal metastases are detected in the cALND[10-23]. The SLN procedure already caused a decrease of the number of ALND in case of a negative SLN. A further reduction could be achieved through selection of a subset of patients with low suspicion for non-SLN metastases after a positive SLNB. Many authors determined factors that attempt to predict the presence of non-SLNs containing metastatic disease. The most frequently identified predictors are the size of the primary tumour (size in general[12,16,22-24] and size larger than 2cm[14,17,19,20]) and SLN metastasis (size in general[10,12,16,17,23] and macrometastasis[13,14,18-20]), extranodal growth[10,13,15,24] and lymphovascular invasion[10,14,20-22,24]. Some authors identified subsets with such a small risk for non-SLN metastases that an ALND could safely be omitted, but all concerned small studies and subset groups of patients[24]. Several groups emphasize the importance of the ongoing trial of the American College of Surgeons (Z0011), in which patients with a positive SLN are randomized to ALND or no further axillary treatment. The aim of this trial is to reveal a subset of patients in which an ALND can be omitted.

The MSKCC breast cancer group used the relevant predictors to develop a nomogram to help quantify a patient's individual risk for non-SLN metastases (figure 1). It provides a reasonably accurate predicted probability and was validated for a general population of Dutch breast cancer patients[11,22].

For daily practice, however, it is essential to know how clinical predictions compare to the nomogram results and how these nomogram results influence decisions clinicians take. Comparisons of clinical versus computer-aided decision making are rare in medical literature. One author examined prostate nomogram results against urologists' predictions. He concluded, that nomogram results could be of significant benefit in certain settings of clinical decision making[25]. Almost no literature could be found about the influence (nomogram) results have on clinical decision making[26]. To this purpose a systematic search in Pubmed

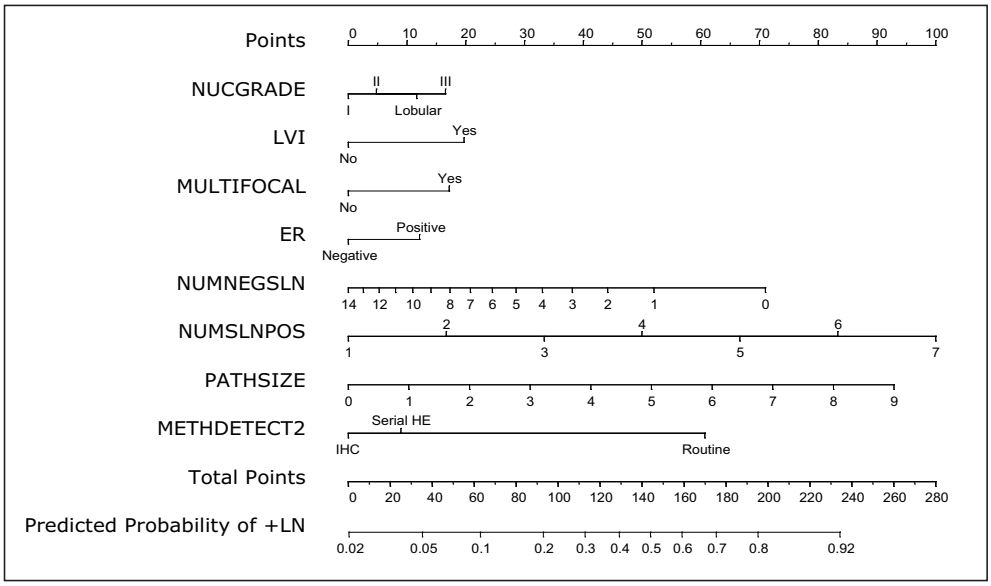

Figure 1 The nomogram to predict the likelihood of non-SLN metastases after a positive SLN biopsy, as developed by MSKCC. NUCGRADE, tumor type and nuclear grade (ductal, nuclear grade I; ductal, nuclear grade II; ductal, nuclear grade III; lobular); LVI, lymphovascular invasion; MULTIFOCAL, multifocality of primary tumor; ER, estrogen-receptor status; NUMNEGSLN, number of negative SLNs; NUMSLNPOS, number of positive SLNs; PATHSIZE, pathological size in cm; METHDETECT, method of detection of SLN metastases (routine H&E, serial H&E and IHC). The first row (POINTS) is the point assignement for each variable. Row 2-9 represent the variables included in the model. For an individual patient, each variable is assigned a point value (uppermost scale, POINTS) based on the histopathological characteristics. A vertical line is made between the appropriate variable value and the POINTS line. The assigned points for all eight variables are summed and the total is found in row 10 (TOTAL POINTS). Once the total is located, a vertical line is made between TOTAL POINTS and the final row 11. Row 11 presents the predicted probability for non-SLN metastases after a positive SLN biopsy.

under the MeSH-terms ”forecasting, outcome assessment and breast neoplasms” and the heading “clinical decision making” was performed.

The aim of the first part of this study was to establish the surgical oncologists’ estimate or “guesstimate” for axillary lymph node involvement after a positive SLN biopsy in breast cancer patients. These findings were compared with the MSKCC nomogram results. The second part of this study should clarify to what extent the additional information provided by the nomogram, influences clinical decision making.

Patients and methods

To examine clinical predictive ability, a questionnaire was sent to all surgical oncologists involved in breast cancer care in the Netherlands. It contained ten

scenarios presenting female breast cancer patients with a positive SLN and added relevant prognostic features. An example of a scenario is presented here.

"What is the likelihood for residual disease in the axilla in a 50 year old female with a 3 cm centrally located multifocal tumour. Tumour characteristics are invasive ductal cancer, nuclear grade II, estrogen and progesteron receptor negative and no sign of lymphovascular invasion. 1 out of 2 SLNs is positive by immunohistochemical staining."

In addition to the data required to use the nomogram, age, location of the tumour and progesteron-receptor status were supplied. The surgeon was asked to estimate the probability for non-SLN metastases for each of the first five scenarios. The accuracy of the surgical oncologists' prediction was established by comparing the results with the nomogram findings.

For the second five similar scenarios, the patient's individualized predicted probability for metastatic disease in non-SLNs, calculated by the nomogram, was supplied. To clarify the impact of the nomogram results on clinical decision making, the surgeon was asked whether or not (s)he would perform a cALND, not taking into account the Dutch breast cancer guidelines[5,27].
Clinicians completing the questionnaire provided the type of hospital they worked at: academic or cancer centre, regional teaching or local hospital. Surgical experience -expressed in years after graduation from medical school- of each surgeon was registered[28]. To examine the influence of experience on predicting abilities, years were transposed to a number of decades.
An experienced statistician performed all statistical analyses with help of the statistical package SAS for Windows release 8.02.

Results

107 Questionnaires were returned, coming from 8 out of 10 academic or cancer centres, 29 out of 41 regional teaching hospitals and 19 out of 61 local hospitals.

The nomogram predicted probability for the first five consecutive scenarios was 4, 10, 10, 32 and 43%. The median clinical guesstimate was 10, 10, 15, 30 and 30% (figure 2). A concordance-index was calculated to demonstrate concordance or discorcordance between the sequence of nomogram results and clinical estimates. The c-index scale varies from 0.5, which represents any toss of a coin, to 1.0, which represents perfection. The c-index in this study amounts to 0.78 indi-

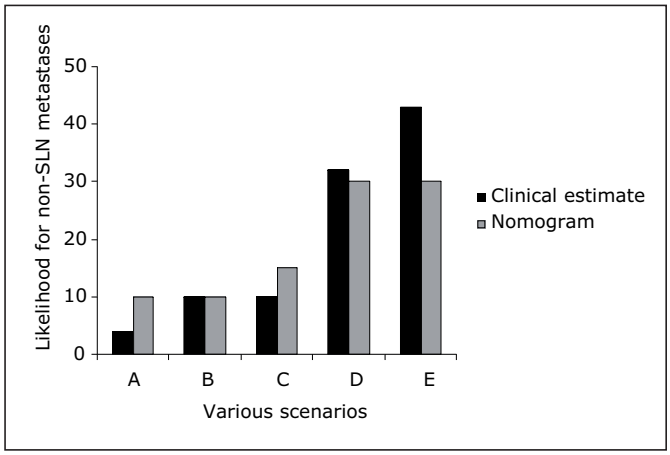

Figure 2 Likelihood for non-SLN metastases for the various scenarios: the bar heights represent the nomogram results versus the median clinical estimate for the various scenarios.

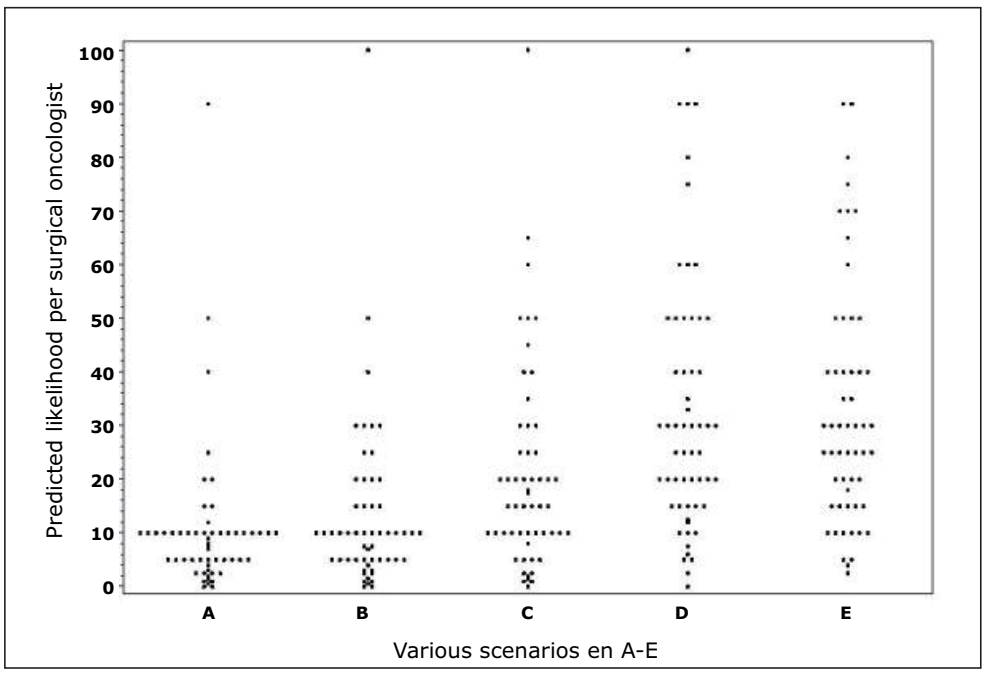

Figure 3 Distribution of the clinical estimates for non-SLN metastases for the various scenarios drawn as a jitplot. One dot represents two surgical oncologists. The actuarial predictions amounted to 4, 10, 10, 32, 43 for the consecutive scenarios.
(Abbreviations: non-SLN – non sentinel lymph node)

cating moderate concurrence. The variation between the predictions of the individual surgeons for each scenario was important, minimum and maximum values varied from 2,5-90 to 0-100% (figure 3). There is no significant difference between the predictions of surgical oncologists of the various types of hospitals.

Experience did not make a difference in clinical predicting abilities either. Several models were used to test both relationships but no correlation could be found (P>0.20, always).

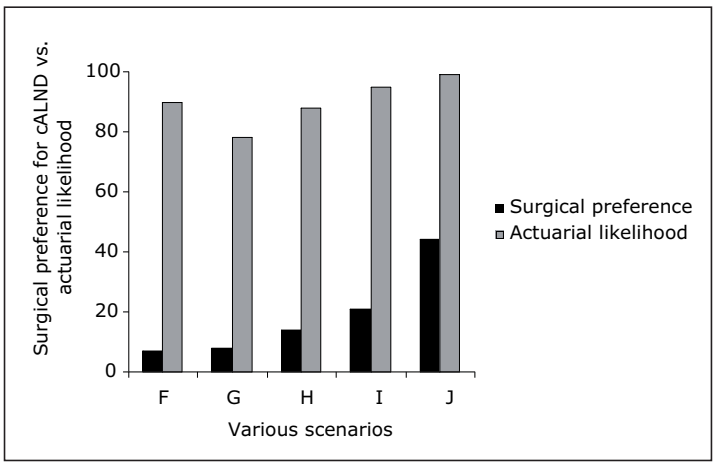

Figure 4 Surgical preference for cALND versus actuarial likelihood for non-SLN metastases for the various scenarios. (Abbreviations : cALND – completion axillary lymph node dissection, non-SLN – non sentinel lymph node)

In the second part of five scenarios, the surgical oncologist determined on the basis of the clinical data and the supplied nomogram results, if (s)he would perform a cALND. The likelihood for non-SLN metastases was 7, 8, 14, 21 and 44%. Surgical oncologists considered the need for a cALND 90, 78, 88, 95 and 99% (figure 4). About 25% of the surgeons was influenced by the information supplied by the nomogram and decided in one or more patients to abandon the cALND despite the positive SLN. The type of hospital, a surgeon works at, or the experience, did not seem to influence the clinical decision making process (P>0.20, always).

Discussion

Clinical and actuarial predictions concerning non-SLN metastasis after a positive SLN biopsy in breast cancer patients do not correlate well. The distribution of clinical predictions of the individual surgeon per scenario is important as shown in figure 3.

The similarity of the bar heights in figure 2 is striking in scenario B, C and D. The 'median' surgical oncologist can discriminate the likelihood for non-SLN metastases for the various scenarios. When compared with figure 3, however, the inter-observer variability is as striking. The individual surgical oncologist, how experi-

enced (s)he may be, is not able to make a solid prediction of the need for a cALND. The type of neither hospital nor the clinical experience influenced predicting abilities.
For some scenarios, the difference between clinical and actuarial prediction is more than mean. This cannot be explained by the age or other variables of the patients in question.

In the second part of the questionnaire, the influence of the actuarial probability on clinical decision making was determined. Of all surgeons, about 25% appears to be influenced by the nomogram results. This could not be attributed to the type of hospital or clinical experience.

The bar heights lines in figure 4, presenting the actuarial likelihood for non-SLN metastases and the percentage surgical oncologists abandoning the cALND, have a similar upward course. For the first scenario, however, the clinical considered need for a completion ALND and actuarial likelihood for non-SLN metastases deviate widely. This is probably caused by the young age of this patient, 28 years, compared to the mean of 51 years of the other patients in this part of the questionnaire.
One author described several studies comparing actuarial and clinical predictions[26]. Actuarial predictions always exceeded the mean accuracy of the clinicians and even the single best clinical prediction. Providing of the actuarial prediction resulted in clinicians' improvement, but never matched the calculated prediction. Exceptions occur in case of very rare events. In a scenario of this questionnaire, the young age of one of the patients may have been regarded by various surgical oncologists as rare. None of the studied articles, however, judged age as a predictive variable for non-SLN metastasis[10,12-16,18-23]. Human judgement is coloured by many factors. Fatigue, recent experience, changes in order of information, overconfidence in one's clinical judgement, inability to distinguish between valid and invalid variables and the weight of various variables influence clinical prediction. Further a tendency exists to overrate information consistent with one's hypothesis and ignore contradictory information[26,29].

Experience of clinicians influences diagnostic and therapeutic performance. A study on diagnostic skills of general practitioners in the first moment of consultation demonstrated a strong correlation between experience and diagnostic performance[30]. Another study proved an inverse relationship between experience expressed as the number of years since graduation of medical school and performance of internists[31]. A third author determined that time in practice as well as type of hospital had an influence on physician performance. A physician would perform optimal between six and 15 years after graduation in a large, multispe-

cialty group[32]. A recently published systematic review reported a decrease in performance with increasing experience in more than half of the examined studies[28]. A possible drawback in this study could be that experience in treating a specific condition may not be necessarily correlated to the number of years after graduation. The present study, however, could not detect any relation between predicting abilities and clinical experience, nor the type of hospital, where the interviewee worked.

Concluding, it is stated that the individual predictions of surgical oncologists for non-SLN metastases do not correlate well with the MSKCC nomogram. The distribution between predictions of individual surgeons for one scenario is important. Therefore the nomogram outperforms expert judgement.

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Chapter 7

NO EVIDENCE FOR INCREASED ANGIOGENESIS IN LYMPH NODE METASTASES AFTER EARLIER SURGICAL RESECTION OF A PRIMARY BREAST CARCINOMA

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Submitted

Abstract

Background: The phenomenon of accelerated growth of metastases after resection of the primary tumour has been described in animal models and patients. It has been attributed to a change of balance between activators and inhibitors of angiogenesis in favour of the first. Other contributing factors are mediators of normal wound healing. The aim of this study was to assess, whether delayed sentinel lymph node (SLN) biopsy after earlier resection of the primary tumour in breast carcinoma patients would lead to increased angiogenesis of the SLN metastases.

Methods: From 222 patients operated on for breast carcinoma between January 2000 and July 2001, 5 patients underwent a diagnostic excision biopsy at first and a SLN biopsy later, which revealed macrometastases. To quantify angiogenesis in the SLN metastases, the density of microvessels (MVD) and percentage proliferating vascular endothelial cells was determined in all patients by sequential double immunohistochemistry. Further the proliferating index for primary tumour and SLN metastases was established. These parameters were compared to matched controls.

Results: The median delay for SLN biopsy was 35 days. No statistically significant difference was identified in the comparison of the endothelial proliferating index and MVD between the matched pairs of patients. Also no significant difference was found in a comparison of the proliferating index of the primary tumour with the lymph node metastases for both groups of patients.

Conclusion: These data cannot objectify an increase of angiogenesis or tumour proliferation of the lymph node metastases after resection of the primary tumour. Since a high MVD is related to decreased overall survival, this might suggest that prognosis was not influenced.

Introduction

Patients with breast carcinoma sometimes require multiple operations; especially if the first operation is a diagnostic excisional biopsy in case of low suspicion for malignancy. They might even have to suffer from preoperative waiting lists, influencing the length of the period in between the subsequent operations. Little is known about the effect of the combination of primary resection and delay on growth rate of lymph node metastases on short term.

Angiogenesis, the sprouting of new capillaries from existing vessels, is a complex process and essential to the growth of primary and metastatic tumour beyond the diameter of 1-2 mm^3 [1]. Angiogenesis is a result of an imbalance between pro- and anti-angiogenic factors in favour of the first, released by both tumour and host cells[1-4]. Some activators of angiogenesis have been extensively studied like fibroblast growth factor, vascular endothelial growth factor-b and -c. Examples of inhibitors of angiogenesis are thrombospondin-1, angiostatin and endostatin[4]. An increase in vascularisation also increases the possibility for tumour cells to enter the circulation and give rise to metastases. Weidner et al. reported a method to quantificate angiogenesis. In early breast carcinoma, the density of microvessels was determined in the primary tumour in areas of most intense revascularisation, so-called hot spots. It appeared to be a significant prognostic indicator for metastases, relapse free and overall survival[5-8]. The presence of neovascular hot spots in axillary lymph node metastases is also associated with a significant reduction of disease free and overall survival[9].

Surgical resection of a primary tumour may lead to enhanced angiogenesis leading to accelerated growth of metastases. First of all, elements from normal wound healing, like fibroblasts, enhance tumour invasiveness and development of metastases in mouse models[10-12]. Also mediators of wound healing, like fibroblast growth factor, increase local and distant tumour growth in a melanoma mouse model[13].

Further, the resection of the primary tumour may lead to growth of its metastases by changing the balance in angiogenesis factors, with a possible consequence of loss of inhibitors or rise of activators. An earlier study reported acceleration in residual tumour growth after resection of a second tumour in mice[14-18]. Moreover, reduction of human breast carcinoma and lung carcinoma in a mouse model was reported after systemic administration of angiostatin or endostatin[19,20]. These and more studies suggest a tumour growth related role, which may be determined by examining tumour related endothelial cells[21-23].

Occasionally, the phenomenon of accelerated tumour growth after tumour resection has been described in patients. Resection of bulky non-seminomatous germ-cell testicular carcinoma was followed by a dramatic relapse[24]. A similar event was reported on colorectal liver metastases; an enhancement of vascular density of the liver metastases was described after removal of the primary tumour[25]. In case of renal cell carcinoma, opinions differ: in metastasized renal cell carcinoma, regression after nephrectomy is a well known but rare phenomenon[26]. And a recent EORTC study shows a better median survival in metastatic renal cell after immunotherapy in combination with radical nephrectomy compared to immunotherapy alone[27]. Thus for different tumour types in model studies and in humans, an effect of surgery on remaining tumour has been shown.

The aim of this study was to assess, whether delayed removal of lymph node metastases after earlier resection of the primary tumour in breast carcinoma patients would have an effect on angiogenesis in the lymph node metastases. To this purpose lymph node metastases of patients earlier operated on for primary breast carcinoma, were compared for MVD and proliferating endothelial cells with a control, one-stage procedure, group. Further the fraction of proliferating cells in tumour and metastases was examined.

Patients and methods

Patients

Between January 2000 and July 2001, 222 patients were operated on for breast carcinoma at a regional teaching hospital. In 33 low suspicion for malignancy patients, the first procedure was a diagnostic excisional biopsy confirming the diagnosis breast carcinoma. Afterwards a SLN biopsy was performed. The time between both operations was recorded. Patient characteristics of the remaining 189 patients and the 33 low suspicion for malignancy patients are collected in table 1. From 11 of the 33 patients, at least one SLN was tumour positive. In 6 out of these 11 SLN's, metastases with a diameter larger than 3 mm were found, beyond the diameter where angiogenesis becomes essential for further growth[1]. Two of these 6 patients were reoperated on because DCIS, but no invasive carcinoma, was revealed in the resection margins of the excisional biopsy.

For each of the 6 patients a control patient was sought in the remaining group of 189 patients, who underwent the excision of the primary tumour and SLN biopsy simultaneously. Patients were matched for age (maximum range 8 years), tumour

Variable	189 patients	33 patients
Median age, y (range)	57 (27-95)	59 (41-81)
Tumour size, mm (range)	18 (0-80)	11 (1-32)
Primary tumour		
T1	102 (54%)	30 (90.9 %)
T2	69 (36.5%)	3 (9.1%)
Miscellaneous	18 (9.5%)	
Histologic type		
IDC	134 (70.9%)	24 (72.7%)
ILC	24 (12.7%)	4 (12.1%)
Miscellaneous	31 (16.4%)	5 (15.2%)
Median tumour grade	2.0	2.0
SLN tumour positive	78 (41.3%)	11 (33.3%)
Micrometastases	21/78 (26.9%)	5/11 (45.5%)

Table 1 Comparison of patient characteristics of the group, who underwent a diagnostic excisional biopsy before the SLN procedure and the remaining patients (Abbreviations: SLN - sentinel lymph node, IDC - invasive ductal carcinoma, ILC - invasive lobular carcinoma, miscellaneous - intracystic papillary carcinoma/tubular carcinoma/medullary carcinoma/apocrinal carcinoma, n.s. - not significant, Grade - Nottingham combined histological grade).

stage, type, grade and positive histopathology of the SLN. A program designed in MS Excel 2000 for Windows performed the matching procedure. In 5 out of 6 patients the program succeeded in finding at least one matching patient. If more than one matching patient was available, the patient with the highest tumour load left in the SLN was chosen as a match. That would leave the highest probability of tumour in the remaining SLN parts, necessary for additional staining.

Immunohistochemistry

Of the SLN of these 10 patients, histologic sections were stained in a double indirect sequential immunohistochemical staining procedure as described before[28]. First MIB-1 antibody (Neomarkers, USA) was incubated and after peroxidase labelled on the second layer, stained brown with diaminobenzidine. Subsequently, the CD 31 antibody (DAKO, Denmark) was incubated and after alkaline phosphatase labelling on the second layer, stained with new fuchsine. MIB-1 is a monoclonal antibody directed against the nucleus of endothelial cells,

which are at the G2M-phase of cell proliferation. CD31 is a monoclonal antibody directed against the cytoplasm of endothelial cells.

Assessment of vascular density and (endothelial) proliferating index

Microvessel density (MVD) was quantified according to Weidner in the lymph node metastases. With low magnification neovascular hot spots were identified. Counts were made on a 200X field; the result was the highest count at any field at this magnification[8]. Further, proliferative and non-proliferative endothelial cells in the lymph node metastatic area were determined by visual counting (figure 1). The number of MIB1 positive nuclei endothelial cells was divided by the total number of endothelial cells in the metastatic area and expressed as a percentage, the endothelial proliferating index. All slides were viewed and quantified by the author and an experienced pathologist (FT), blinded for patients' characteristics.
The proliferating index was established by counting the amount of MIB1 positivity in tumour cell nuclei in primary tumour and SLN metastases.

Statistical analysis

An experienced statistician performed statistical analysis of the data with SPSS statistical software package for Windows. Significance for patient characteristics was calculated with Mann Whitney U and chi square test. Univariate analysis of variance with ANOVA was performed to compare endothelial proliferating index and MVD of the SLN metastases for all matched pairs. Further the proliferating index of primary tumour and lymph node metastases were compared.

Results

Of 189 of the 222 patients, resection of the primary tumour and SLNB were performed simultaneously. For the remaining 33 patients, the median period between the diagnostic excisional biopsy and SLN procedure was 37 days (range 15-100) (figure 2). Patient- and tumour characteristics of the one and two stage procedure patients were collected in table 1. Note that in the group with delayed SLNB, the fraction of patients with lower T-stage and smaller tumour size is higher than in the group with simultaneous resection. Length of the time interval is not related to the existence or size of lymph node metastases

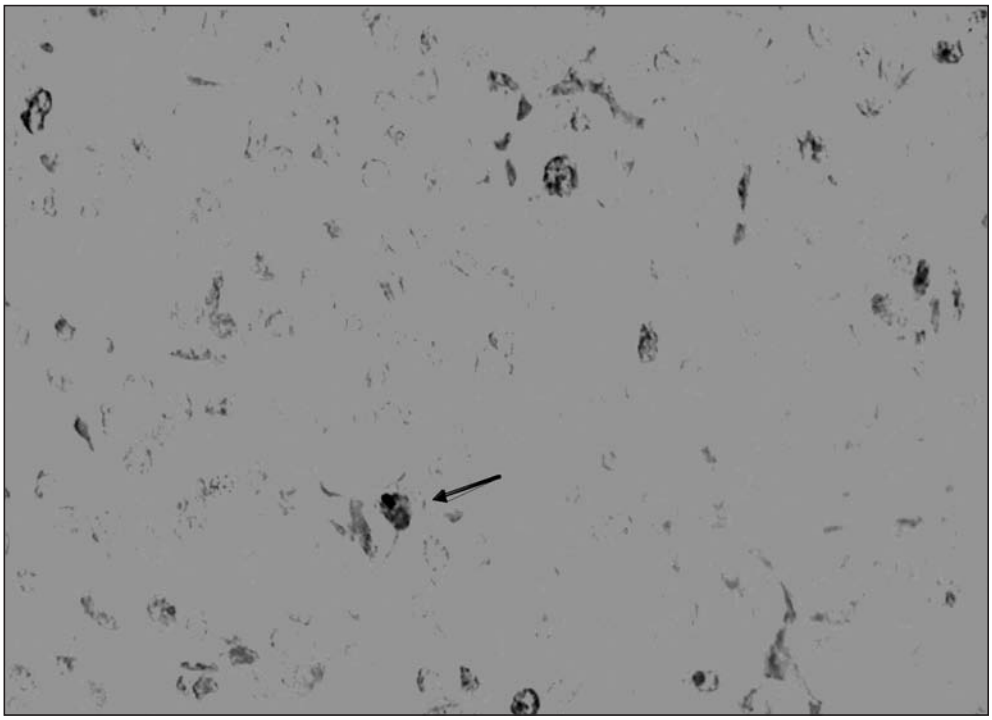

Figure 1 An endothelial cell with a nucleus at the G2M-phase of cell proliferation appears as a cell with red cytoplasm and a dark brown nucleus as a consequence of the double immunohistochemical staining. Any other cell at the G2M-phase of cell proliferation is pictured as a cell with a dark brown nucleus (MIB-1). Any other endothelial cell not at the G2M-phase is a cell with red cytoplasm (CD31). The figure contains a proliferating endothelial cell at the arrow, which is occasionally present.
Magnification: x10 ocular and x40 objective

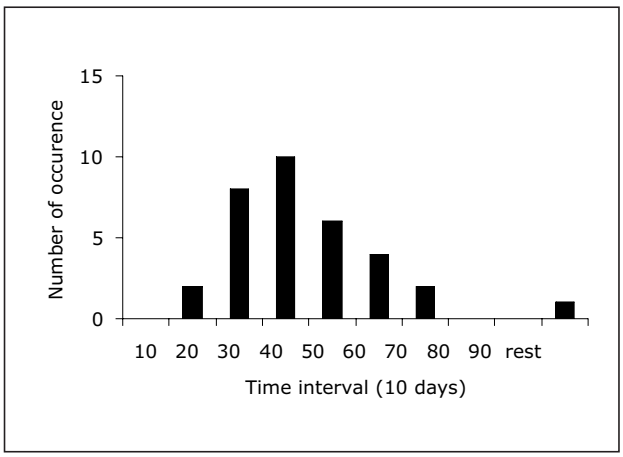

Figure 2 Histogram of delay between subsequent operations in breast carcinoma patients in 2000

	Age	Tumour size (mm)	Tumour grade	Proliferative index			MVD
				Primary carcinoma	Sentinel lymph node		
					Epithelial cells	Endothelial cells	
Case 1	51	20	1	1%	1%	0/266	1
Control 1	51	15	1	>1%	2%	3/127	9
Case 2	46	15	2	>2%	6.5%	1/408	37
Control 2	42	10	3	35%	12%	0/23	3
Case 3	59	6	2	7.5%	10%	3/462	10
Control 3	56	19	3	5%	25%	2/239	1
Case 4	49	25	3	30%	10%	0/302	18
Control 4	56	24	3	72%	30%	0/232	8
Case 5	73	15	2	3.5%	17.5%	0/25	5
Control 5	77	20	2	15%	5.5%	1/397	27
Cases							
Mean(±STD)	56	16	2	8.8 ± 12.1	9.0 ± 6.0	0.0016 ± 0.0012	14.2 ± 14.2
Controls							
Mean(±STD)	56	17	2	25.6 ± 29.1	14.9 ± 12.2	0.0073 ± 0.0047	9.6 ± 10.3

Table 2 Patient characteristics of the matched pairs (Abbreviation: MVD=mean vascular density, STD=standard deviation).

Pathological findings of the five cases and matched controls are presented in table 2. For these five patients, the median delay between both procedures amounted to 35 days (range 15-66). The tumour type of all patients consisted of invasive ductal carcinoma. No statistically significant difference was identified in the comparison of the endothelial proliferating index between cases and controls (p=0.31). The MVD seemed to be slightly higher in cases with delayed SLN biopsy than in controls, mean and STD, 14.2± 14 and 9.6 ± 10.3, respectively. This difference was not significant (p=0.65). Similarly, no significant difference was found in a comparison of the proliferating index of the primary tumour with the lymph node metastases for both groups of patients (p=0.76).

Discussion

The question, whether resection of the primary tumour in breast carcinoma patients would lead to increased angiogenesis of the lymph node metastases, is not affirmed in this study. If an acute effect is taking place, such as in wound healing, an effect on angiogenesis is likely to be present well within a period of 35 days. Nevertheless, the growth of metastases is a more steady process with in time increasing functional disturbances. Possibly a mean delay of 35 days is not

sufficient to detect these differences in metastases. Moreover, no relation is demonstrated between the length of delay in treatment and size of the lymph node metastases. Taking these arguments into account, it is likely that the mean time interval of 35 days seems enough to exclude a major imbalance in angiogenic factors in the lymph node metastases after resection of the primary tumour.

In this study, the setup is delineated by clinical circumstances. The results might be distorted by the fact that all primary tumours in these two stage patients were low suspicion for malignancy at first. Tumour size and stage appeared to be of significant better profile in the two-stage group. Therefore patient pairs (one and two stage procedure) were carefully matched for most significant prognostic factors (age, tumour type, stage and grade), which should nullify this fact. A marginally higher MVD was present in the SLN metastasis of patients with delayed SLN biopsy; this difference was not significant. Moreover, the proliferative fraction of endothelial cells (counted in slides with double immunohistochemical staining) was low and similar in cases and controls. In addition, in the epithelial component of the metastases a similar proliferation fraction was found as in the primary tumour. Although the number of cases is small, an influence of a marked delay in SLN biopsy on the growth of breast cancer metastases does not seem to be present.

In mice models an accelerated growth of metastases was found as a consequence of increased angiogenesis or wound healing mediators after resection of the primary tumour. However, this has not been supported by this study. Nor was an increase seen in proliferating index between the primary tumour and lymph node metastases after resection of the primary tumour, or could any difference in MVD or endothelial proliferating index be demonstrated between the one and two stage procedure patients[14-18]. A high MVD in the SLN is related to a decreased overall survival. The MVD of both patient groups after excision of the primary tumour was low. Therefore no diminishment of overall survival of the two stage patients is to be expected caused by the delay between the subsequent operations[9].

If loss of angiogenesis inhibitors would have a significant impact on angiogenesis, one would expect to find an increase in mitotic activity of the vascular endothelial cells and microvessel density, in spite of the small number of patients[17,18].

No invasive carcinoma was left after the excisional biopsy in any of the patients, which could have influenced the results, by the production of angiogenic activators or inhibitors.

Nowadays, less two or more stage procedures are performed as a consequence of stereotactic biopsies, the use of ultrasound in axillary staging and techniques to perform intraoperative examination of the SLN. Also the delay in between subsequent operations decreased dramatically. Obviously, delay in between operations in breast carcinoma patients is to be avoided. If occurring, no significant effect on angiogenesis was revealed in these patients with these methods. More research to this interesting subject is warranted.

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Chapter 8

SUMMARY & GENERAL CONCLUSIONS

Summary & general conclusions

In **chapter 1**, facts are provided about the lifetime risk for women and mortality rate of breast cancer in the Netherlands. The most important prognostic factor for breast cancer survival, axillary lymph node status, is discussed within the light of the former golden standard for treatment, the axillary lymph node dissection and its morbidity. A résumé is presented on the development of the sentinel lymph node (SLN) concept to the present application in breast cancer patients. A description of the SLN procedure as performed nowadays in most Dutch hospitals, the triple technique, is given. This thesis concentrates on new developments and consequences of the SLN procedure after the introduction and validation of the SLN concept.

The initial experience with a new technique for intraoperative examination of the SLN at a regional teaching hospital is described in **chapter 2**. Scrape cytology appears to be a useful method for intraoperative examination of the SLN. Its accuracy, 85%, is comparable with results of imprint cytology and frozen section as reported in literature. However, as a consequence of a non-uniform distribution of the metastasis in the SLN and the limited time for intraoperative examination a higher accuracy seems not feasible.

In **chapter 3**, the first results of the SLN procedure performed under local anaesthesia are reported, introduced to avoid the disadvantages of the intraoperative examination of the SLN. The detection rates of the SLNs, ratio of mapped and revealed SLNs, performed under local and general anaesthesia were compared and appeared almost identical, respectively 0.99 and 1.0. The learning curve demonstrated that the procedure under local anaesthesia could quickly be mastered if one is experienced with the performance of SLN biopsies. The technical addendum contains a detailed technical description of the SLN procedure under local anaesthesia.

The results of a hospital-based and literature study on regional recurrence after a negative SLN biopsy are presented in **chapter 4**. In contrast to the false negative rates of the validation studies, the incidence of regional recurrence in current practice and literature is much lower, respectively 0.46 and 0.25% after a median follow-up of 26 and 25 months. In a literature review, axillary recurrence occurred in 8 patients after a median period of 21 months. The natural course of axillary relapse after a negative SLN biopsy seems to resemble locoregional recurrence after an ALND. In both groups, about 30% of the patients presented with synchronous systemic metastases. This percentage rises eventually to 50%,

in the group of patients with locoregional failure after an ALND. A patient presenting with axillary recurrence should therefore receive therapy conform locoregional failure, aimed at locoregional and systemic control.

In **chapter 5**, the accuracy of the MSKCC nomogram was tested on a population of breast cancer patients in the Netherlands. This nomogram gives an individualised risk estimate for the probability of non-SLN metastases based on eight predictors. To this purpose, patient, tumour and SLN metastasis characteristics were collected of 222 consecutive patients, who underwent a completion ALND. In spite of the fact that the Dutch and New York patient group differed significantly with regard to almost all predictive variables, the area under the under the ROC curve amounted to 0.77, a value very much comparable to the source population. In consequence, it can be stated that the nomogram provides a reasonably accurate predicted probability for the likelihood of non-SLN metastases in a general population of breast cancer patients of a regional teaching hospital in the Netherlands. This suggests that the nomogram's originally calculated predictive accuracy may be valid for patient populations that differ considerably from the population in whom it was developed. Its result can support both doctor and patient in the choice whether or not an ALND is desirable in individualised circumstances.

In **chapter 6** the accuracy of surgical oncologists in predicting the likelihood for non-SLN metastases compared with the MSKCC nomogram was assessed. Further the influence of the nomogram results on clinical decision making was examined. The concordance-index between clinical estimates and actuarial likelihood amounted to 0.78, indicating moderate concurrence. The intersurgeon variation, however, was important. About 25% of the surgeons was influenced by nomogram information and decided in one or more patients to abandon the cALND. The type of hospital nor the amount of experience influenced predicting abilities or the clinical decision making process. Therefore individual predictions of surgical oncologists for non-SLN metastases do not correlate well with the MSKCC nomogram. The distribution between intersurgeon predictions for one scenario is important. The nomogram is superior to clinical estimations for predicting the likelihood for non-SLN metastases.

In the final study, presented in **chapter 7**, the question is addressed, whether delayed SLNB after earlier resection of the primary tumour in breast cancer patients would lead to increased angiogenesis in the lymph node metastases. Though the median delay for SLN biopsy in the two-stage procedure group amounted to 35 days, no statistically significant difference was identified in the

comparison of the endothelial proliferating index and MVD (mean vascular density) between the matched pairs of patients. Also no significant difference was found in a comparison of the proliferating index of the primary tumour with the lymph node metastases for both groups of patients. Further no relation is revealed between the length of delay and existence or size of the lymph node metastases. Therefore these data cannot objectify an increase of angiogenesis or tumour proliferation of the lymph node metastases after resection of the primary tumour. Since a high MVD is related to decreased overall survival, this might suggest that survival was not influenced by the delay between both procedures.

Samenvatting & algemene conclusies

De inleiding, **hoofdstuk 1**, geeft informatie over de kans die een vrouw heeft gedurende haar leven borstkanker te ontwikkelen en het meest recente sterftecijfer van deze ziekte in Nederland. De belangrijkste prognostische factor, de okselklierstatus, wordt besproken alsmede de voormalige "gouden standaard" behandeling, de okselklierdissectie (OKD) en de mogelijke morbiditeit daarna. Er wordt een samenvatting gegeven van de ontwikkeling van het schildwachtklierconcept tot en met de hedendaagse toepassing ervan bij mammacarcinoompatiënten. De schildwachtklierbiopsie (SWKB), zoals nu in de meeste Nederlandse ziekenhuizen toegepast, wordt beschreven. Dit proefschrift handelt over nieuwe ontwikkelingen en gevolgen van de SWKB na de introductie en validatie van het SWK-concept.

In **hoofdstuk 2** worden de eerste ervaringen met een nieuwe techniek voor intraoperatief onderzoek van de SWK beschreven. Schraapcytologie blijkt een bruikbare methode voor intraoperatief onderzoek. De nauwkeurigheid van deze methode, 85%, is vergelijkbaar met de resultaten van depcytologie en vriescoupeonderzoek, zoals gerapporteerd in de literatuur. Een hogere nauwkeurigheid lijkt niet haalbaar als gevolg van een ongelijkmatige verdeling van metastasen in de SWK en de beperkte tijd die beschikbaar is voor intraoperatief onderzoek.

De bovengenoemde nadelen van intraoperatief onderzoek hebben geleid tot de introductie van de SWKB onder lokale anesthesie. **Hoofdstuk 3** rapporteert de eerste resultaten van deze techniek. De ratio van afgebeelde en ontdekte SWK's, uitgevoerd onder lokale en algehele anesthesie, werd onderzocht en bleek vergelijkbaar (namelijk 0,99 en 1,0). De leercurve laat zien dat de procedure snel te leren is, als men de techniek onder algehele anesthesie beheerst. Het technisch addendum bevat een beschrijving van de techniek van de SWKB onder lokale anesthesie.

De resultaten van een ziekenhuis- en literatuurstudie naar de prevalentie van een regionaal recidief na een SWKB zonder metastase worden gepresenteerd in **hoofdstuk 4**. Het lage aantal regionale recidieven dat in de praktijk wordt gevonden en in de literatuur worden gemeld, te weten 0,46 en 0,25% na een mediane follow-up van 26 en 25 maanden, komt niet overeen met het aantal fout negatieve SWKB uit de eerder uitgevoerde validatiestudies. Een recidief in de oksel trad op bij een totaal van 8 patiënten in de literatuur en praktijk na een mediane periode van 21 maanden na een negatieve SWKB. Het natuurlijk beloop van patiënten met een regionaal recidief na een negatieve SWKB lijkt dat van een

regionaal recidief na een OKD te weerspiegelen. In beide groepen presenteert ongeveer 30% van de patiënten zich met synchrone afstandsmetastasen. Dit percentage stijgt uiteindelijk tot 50% in de groep met een regionaal recidief na een OKD. Dientengevolge zou de behandeling van patiënten met een regionaal recidief na een negatieve SWKB vergelijkbaar moeten zijn met die van patiënten met een regionaal recidief na een OKD, zowel gericht op locoregionale als systemische controle.

In **hoofdstuk 5** wordt de betrouwbaarheid van het Memorial Sloan-Kettering Cancer Center-nomogram getest op een populatie van Nederlandse mammacarcinoompatiënten. Het nomogram, dat is gebaseerd op 8 prognostische factoren, verschaft een individuele risicoschatting van de kans op positieve okselklieren na een positieve SWKB. Van 222 Nederlandse patiënten, die na een positieve SWKB een OKD ondergingen, werden patiënt-, tumor- en SWK-karakteristieken verzameld. Ondanks het feit dat de Nederlandse en Amerikaanse patiëntenpopulatie met betrekking tot vrijwel alle prognostische factoren significant verschilden, bedroeg de "area under the curve" 0,77. Dit getal is vergelijkbaar met de oorspronkelijke Amerikaanse waarde. Dientengevolge kan men stellen dat het nomogram een redelijk voorspellende waarde heeft voor de kans op positieve okselklieren na een positieve SWKB voor een algemene populatie van mammacarcinoompatiënten van een perifeer opleidingsziekenhuis in Nederland. De uitkomsten suggereren dat het nomogram van toepassing kan zijn op populaties die significant verschillen van de oorspronkelijke Amerikaanse groep patiënten. De nomogram uitkomsten kunnen zowel arts als patiënt ondersteunen bij de beslissing, of een OKD al dan niet wenselijk is in bepaalde omstandigheden.

In **hoofdstuk 6**, wordt de nauwkeurigheid van de risicoschatting op positieve okselklieren na een positieve SWKB van chirurgen die dagelijks patiënten met borstkanker behandelen vergeleken met het MSKCC nomogram. Tevens wordt de invloed van nomogramresultaten op klinische besluitvorming onderzocht. De concordantie-index tussen de klinische schattingen en de nomogramuitkomsten bedroeg 0,78, hetgeen duidt op matige overeenstemming. De variatie tussen de schattingen van de individuele chirurg-oncologen was groot. Ongeveer 25% van de chirurg-oncologen lijkt beïnvloed te worden door het nomogramresultaat en besluit bij één of meer patiënten geen OKD uit te voeren. Het soort ziekenhuis of de ervaring van een chirurg-oncoloog heeft geen invloed op zijn of haar vermogen tot risicoschatten of het proces van klinische besluitvorming.
Individuele risicoschattingen van chirurg-oncologen komen dus maar matig overeen met de resultaten van het MSKCC nomogram en de spreiding tussen de individuele risicoschattingen voor een scenario blijken groot. Dientengevolge is

het nomogram superieur aan klinische risicoschatting van de kans op positieve okselklieren na een positieve SWKB.

De laatste studie in **hoofdstuk 7** poogt de vraag te beantwoorden of een verlate SWKB na een voorafgaande excisie van de primaire mammatumor, een toename van angiogenese in de lymfekliermetastase tot gevolg heeft. Alhoewel de mediane vertraging tussen excisie van de primaire tumor en de SWKB 35 dagen bedroeg, werd er geen significant verschil waargenomen bij vergelijking van de endotheelproliferatieindex en gemiddelde vasculaire dichtheid van de SWK met gematchte patiënten, die beide procedures op 1 dag ondergingen. Ook werd er geen significant verschil gevonden bij vergelijking van de proliferatieindex van de primaire tumor met de lymfekliermetastase van beide patiëntengroepen. Er bestaat ook geen relatie tussen de duur van de periode tussen excisie van de primaire tumor en SWKB en het aanwezig zijn van dan wel de grootte van de lymfekliermetastase.
Deze data kunnen derhalve geen toename van angiogenese of tumorproliferatie van de lymfekliermetastase objectiveren na eerdere excisie van de primaire tumor. Omdat een hogere gemiddelde vasculaire dichtheid gerelateerd wordt aan een afname van de overleving, suggereert deze studie dat de overleving niet nadelig wordt beïnvloed door de vertraging tussen beide procedures.

Addendum

SENTINEL LYMPH NODE BIOPSY PERFORMED UNDER LOCAL ANAESTHESIA IN BREAST CANCER PATIENTS: TECHNICAL AND ORGANISATIONAL ASPECTS

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Abstract

A sentinel lymph node (SLN) biopsy in breast cancer patients, performed under local anaesthesia (LA), has advantages such as a more efficient use of operating room and pathologist's time. Additionally, it provides a histologic diagnosis prior to definitive breast surgery. This prevents a decision based on possibly equivocal results of intraoperative examination of the SLN and provides the patient with more specific information. The aim of this report is to present the technical and organisational aspects of a SLN biopsy under local anaesthesia.

Introduction

The sentinel lymph node procedure is a reliable technique for axillary staging in patients with clinical T1-2N0 breast cancer[1]. In most hospitals, the SLN biopsy is combined with the definitive breast surgery. The sentinel lymph node (SLN) is than often examined intraoperatively. If metastatic disease is revealed, an axillary lymph node dissection can be performed in the same session[2-6].

However, this operational sequence has several drawbacks. No single intraoperative technique –frozen section, touch prep or scrape cytology- reveals all metastatic disease. False-negatives lead to reoperation in 11-26% of all patients with negative intraoperative examination results[7-11]. The use of intraoperative techniques also puts the pathologist under time pressure to present a diagnosis[12-14]. Another disadvantage is the time spent waiting for the results, which prolongs the duration of general anaesthesia and the use of the operating room[12-17]. Perhaps the most compelling reason for questioning intraoperative examination of the SLN is the fact that patients enter the operation without knowing the exact diagnosis and the course of the subsequent operation[13,15,16].

In order to deal with the drawbacks mentioned above, a method was developed to perform a SLN procedure under local anaesthesia in breast cancer patients[12,14,17-20]. An earlier study by Smidt et al. already examined and proved feasibility[21]. The aim of this report is to present the technical and organisational aspects of this method as currently performed in our clinics.

Patients and methods

Organisational aspects

Patients with a suspicious breast laesion referred from the breast cancer screening program or by a general physician, are seen by a surgical oncologist and specialised nurse at the breast cancer clinics of both hospitals. Diagnostic imaging and ultrasound guided tumour cytology take place immediately. If unequivocal, the results can be presented to the patient the same day.
If a breast laesion is proven to be malignant, unifocal and clinically T1-2N0, the SLN biopsy is planned. The appointments with the nuclear physician for injection of the radioactive tracer and the static lymphoscintigraphy are scheduled. The outpatient operation room is booked for the SLN procedure performed under local anaesthesia and the patient is provided with a non-steroidal antiflogistic

drug to take, as a pre-emptive analgesia, 4 hours before the SLN biopsy and the day after.
The clinical operation room is booked one week later for definitive breast surgery. Outpatient clinic appointments are scheduled to discuss the histologic diagnosis and subsequent adjuvant treatment.

The sentinel lymph node imaging

The nuclear physician injects the afternoon before surgery the radioactive tracer. As a tracer, 60 MBq of technetium-99m nanocolloid is used, which is injected periareolar in the diseased breast quadrant[22]. A static lymphoscintigraphy is performed at the morning of the day of the procedure. The nuclear physician reviews the images and marks the location of the primary SLN on the skin. If no SLN is visualised, additional tracer (30mBq) will be injected. If even then no SLN can be identified with a static lymphoscintigraphy, the nuclear physician will consult the surgeon. Depending on the patient and pathology the procedure will be continued with blue dye only.

The sentinel lymph node biopsy

The SLN procedure under local anaesthesia is performed in the outpatient operating room. A surgical oncologist and a scrub nurse perform the operation. Another nurse chats informally with the patient during the entire procedure. Background music is chosen in consultation with the patient. The surgical nurses of the outpatient clinic received special training on the subject and equipment. No premedication or intravenous analgesia is administered. Thus, patients are allowed to eat and drink before and immediately after the operation.

A peripheral venous access is established in order to allow a quick response to possible allergic reactions to the blue dye[23,24]. Before desinfecting and draping, 1 ml blue dye (Bleu patente V; Guerbet, Aulnay-sous-Bois, France) diluted with 1 ml lidocaine 0.5% is injected into the tissue surrounding the tumour or biopsy cavity. The lymphatic drainage of the blue dye is then stimulated by massage during three min. The patient is now positioned with the arm at a 90-degree abduction angle or holding the hand behind the head. The nuclear physician already marked the location of the SLN on the skin. The incision is a line from the anterior to the dorsal axillary line, immediately caudal to the axillary hair line, with an average length of 4 cm, somewhat adjusted to the marking of the nuclear

physician. Local anaesthesia is given by subcutaneous infiltration of 10 ml lidocaine 0.5% with adrenaline 1:200.000. As the procedure advances, additional lidocaine -average 5 to 10 ml- is injected on demand. The amount of locally used anaesthesia remains always far below the recommended safe dose[25].

After the skin incision cautery can be useful to provide adequate hemostasis. Further on, the adrenaline induces vasoconstriction. The axillary fascia is entered by scissors dissection. The wound edges are held by Langenbeck retractors. The fatty tissue is now sharp and blunt dissected until a blue vessel, the preferable guide to the SLN, is visible. If none is encountered during dissection to the thoracic wall, the gamma probe (Neoprobe, Johnson&Johnson Medical, Hamburg, Germany) serves as a guide. Harvesting of the node is performed with scissors and haemoclips. Hemostasis is carefully controlled and the skin is closed with intracutaneous Monocryl 4-0. Patients can leave the hospital within 15 min after the biopsy.

The duration of the procedure depends on the habitus of the patient, the experience of the surgeon, the number of SLNs shown on the lymphoscintigraphy and the amount of nuclear uptake. On average the procedure lasts 25 min and the length of the skin incision is four cm[21].

Histologic examination

The SLN is bisected and both halves are embedded in paraffin. Each halve is step-sectioned at 500-micrometer intervals at three levels and stained with both haematoxylin and eosin, and immunohistochemical staining (Cam5.2).

Discussion

Only few authors have reported on sentinel lymph node biopsy under local anaesthesia in patients with breast cancer[12,14,17,21]. All refer to some or all of the advantages mentioned previously. This study reflects the elaborated concept of the SLN biopsy under local anaesthesia based cumulative experience of two breast cancer centres.

In our opinion, the most important advantage is the fact that patients are informed about the histologic diagnosis of the sentinel lymph nodes before the definitive breast surgery. This knowledge might change the mind of a patient concerning

the definitive breast surgery (mastectomy vs. breast-conserving) and, as a consequence, patient satisfaction concerning that decision may increase. One can also spare the patients the distress of consenting to an axillary lymph node dissection, which ultimately proves to be unnecessary[16].

A minor drawback is the additional operation, for under general anaesthesia at least 60% of the breast cancer patients is operated on only once. Further a nurse has to pay full attention to the patient, especially in anxious patients. She/he can also pass the need for additional anaesthesia.

Considerable variety exists among authors in the practice of administration of sedation and intravenous analgesia. To our experience both are unnecessary, if one provides sufficient analgesia and a nurse who can make the patient feel at ease[12,14,17,21].

The radioactive tracer was injected in the subareolar plexus. This injection mode yields a higher success rate of identifying SLNs while less extra-axillary nodes are imaged as compared with peritumoral injection[22]. The discussion on the value of extra-axillary nodes is still ongoing. These nodes seem to have only minor clinical consequences for a small number of patients[26-28].

No difficulties were met during introduction of the SLN under local anaesthesia with respect to organisational and technical aspects. To our opinion the main change is the possibility to present the patient with the definitive histopathologic results of the SLN instead of the equivocal results of intraoperative examination. As a consequence, it creates the chance to inform a patient more specifically and the patient is better able to make a well thought decision concerning the definitive breast and axillary surgery.

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En nu: 'laten we dansen, mijn liefste'.

Curriculum vitae

Marjolein Smidt werd op 19 april 1972 in Geleen geboren. Zij bracht haar jeugd door in Hulsberg. In 1984 ging zij naar het gymnasium van het Bernardinus-college te Heerlen, waar zij in 1990 eindexamen deed. Datzelfde jaar begon zij met de studie Geneeskunde aan de Universiteit Maastricht. Daar had zij een actief studentenleven, met als hoogtepunt het voorzitterschap van de organisatie van de algemene eerstejaars introductie, de Inkom. Later volgde zij diverse chirugisch georiënteerde co-schappen over de grens, onder andere in het Krankenhaus der Stadt Kitzbühel, het Diakonis Krankenhaus in Salzburg, Oostenrijk en het Moi University Teaching Hospital te Eldoret, Kenia. In september 1997 haalde zij haar artsexamen met genoegen.

Na haar artsexamen werkte zij als AGNIO van november 1997 tot augustus 1998 op de afdeling Heelkunde van het Amphia Ziekenhuis, lokatie Langendijk, te Breda. Van augustus 1998 tot december 1999 werkte zij als AGNIO op de afdeling Heelkunde van het Canisius-Wilhelmina Ziekenhuis te Nijmegen (opleider: dr. E.D.M. Bruggink). Daar begon zij de opleiding tot chirurg in januari 2000. In deze periode werd de basis gelegd voor dit proefschrift onder leiding van dr. L.J.A. Strobbe. De opleiding werd vervolgd in het Universitair Medisch Centrum St Radboud te Nijmegen, met als opleider prof.dr. R.P. Bleichrodt. In oktober 2004 ontving zij de Henny C. Dirven prijs voor patiëntgericht schildwachtklieronderzoek bij mammacarcinoompatiënten. De opleiding werd afgesloten met een differentiatiejaar chirurgische oncologie onder leiding van dr. W.B. Barendregt (opleider) en dr. L.J.A. Strobbe (opleider chirurgische oncologie) wederom in het Canisius-Wilhelmina Ziekenhuis te Nijmegen.

Marjolein woont samen met Ivo Panhuizen. Zij werden in augustus 2005 de gelukkige ouders van een wolk van een dochter, Linde.