The evolution of the sentinel node procedure in the treatment of breast cancer

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List of included papers

This thesis is based on the following 10 papers

I. **Tvedskov** TF, Jensen MB, Balslev E, Ejlertsen B, Kroman N. Stage migration after introduction of sentinel lymph node dissection in breast cancer treatment in Denmark: A nationwide study. Eur J Cancer 2011 Apr; 47(6):872-8

II. **Tvedskov TF,** Jensen MB, Kroman N, Balslev E. Iatrogenic displacement of tumor cells to the sentinel node after surgical excision in primary breast cancer. Breast Cancer Res Treat. 2012 Jan;131(1):223-9

III. Damgaard OE, Jensen MB, Kroman N, **Tvedskov TF.** Quantifying the number of lymph nodes identified in one-stage versus two-stage axillary dissection in breast cancer. The Breast 2013, Feb 22(1):44 - 46

IV. **Tvedskov TF,** Jensen MB, Lisse IM, Ejlertsen B, Balslev E, Kroman N. High risk of non-sentinel node metastases in a group of breast cancer patients with micrometastases in the sentinel node. Int J Cancer 2012, Nov 15;131(10):2367-75

V. **Tvedskov TF**, Jensen MB, Balslev E, Kroman N. Robust and validated models to predict high risk of non-sentinel node metastases in breast cancer patients with micrometastases or isolated tumor cells in the sentinel node. Acta Oncol. 2014, Feb;53(2):209-15

VI. **Tvedskov TF,** Meretoja TJ, Jensen MB, Leidenius M, Kroman N. Cross-validation of three predictive tools for non-sentinel node metastases in breast cancer patients with micrometastases or isolated tumor cells in the sentinel node. Eur J Surg Oncol. 2014, Apr;40(4):435-41.

VII. **Tvedskov TF**, Bartels A, Jensen MB, Paaschburg B, Kroman N, Balslev E, Brünner N. Evaluating TIMP-1, Ki67 and HER2 as markers for non-sentinel lymph node metastases in breast cancer patients with micrometastases to the sentinel lymph node. APMIS 2011, Dec;119(12):844-52

VIII. **Tvedskov TF**, Jensen MJ, Balslev E, Garne JP, Christiansen P, Ejlertsen B, Kroman N. Risk of non-sentinel node metastases in patients with symptomatic cancers compared to screen-detected breast cancers. Acta Oncol. 2015, Oct 9:1-5

IX. **Tvedskov TF**, Jensen MJ, Ejlertsen B, Christiansen P, Balslev E, Kroman N. Prognostisk significance of axillary dissection in breast cancer patients with micrometastases or isolated tumor cells in sentinel nodes: A nationwide study. Brest Cancer Res Treat. 2015, Oct;153(3):599-606

X. Uth CC, Christensen MH, Oldenbourg MH, Kjær C, Garne JP, Teilum D, Kroman N, **Tvedskov TF**: Sentinel lymph node dissection in locally recurrent breast cancer. Ann Surg Oncol. 2015, Aug;22(8):2526-31

Study I, IV and VII were included in the PhD thesis entitled: Staging of women with breast cancer after introduction of the sentinel node guided axillary dissection" defended at Copenhagen University, May 2012.

Introduction

The surgical treatment of breast cancer has changed dramatically over the latest centuries and has become increasingly less aggressive. Treatment of the breast tumor has changed from Halsted's mastectomy, where breast, pectoral muscles and regional lymph nodes were removed (1), over modified radical mastectomy to breast conserving surgery supplemented by oncoplastic techniques to improve the cosmetic results (2). Likewise, the treatment and staging of the axilla for lymphatic spread has changed. Axillary lymph node dissection (ALND) was previously the standard procedure for staging of the axilla. This procedure is associated with considerable morbidity (3;4) and is redundant in women without lymph node metastases. In 1994 the sentinel lymph node dissection (SLND) was introduced in the treatment of breast cancer (5) as a procedure to identify patients who could be spared an ALND. In the following years, the use of

the procedure in breast cancer rapidly increased and in 1997 the first breast cancer patient in Denmark was treated by SLND (6). In 2004 the sentinel node procedure was fully implemented in all departments of breast surgery in Denmark (6), and has now replaced ALND as standard procedure for staging of the axilla in clinically node negative primary breast cancer.
Under the sentinel node procedure, the first lymph nodes receiving lymph drainage from the breast is identified, removed and examined (7). Examination is done intraoperatively on frozen sections in the majority of cases, and a supplementary final conventional histological examination is done postoperatively. Only in case of metastatic spread to these first lymph nodes, a completion ALND is performed (8). SLND can accurately stage the axilla by removing in average only two lymph nodes (8), thereby causing less arm morbidity than ALND (4).
Today, more than 3000 sentinel node procedures are performed in Denmark every year, as a part of the surgical treatment of breast cancer. This procedure spares thousands of Danish breast cancer patients the risk of arm morbidity each year.
Worldwide, the SLND is becoming more and more widespread. In a European Work Package run by epidemiologists in Heidelberg, the recent trends in ALND and SLND practices among breast cancer patients in different countries and centers in Europe have been compared (9). Denmark has delivered data for this comparison. The study showed a widespread and increasing use of the sentinel node procedure from 2003 to 2010, but with large differences between countries and a potential for extended use. In 2010, the SLND was offered to 79 – 96% of patients with pT1 tumors and 49 – 92% of patients with pT2 tumors. A decrease in the use of ALND in the same period was seen, but again large differences between countries, in offering ALND to sentinel node positive patients existed. This could probably be explained by important differences in guidelines between countries.
In Denmark, data from all Danish women with breast cancer have been prospectively collected and registered in the Danish Breast Cancer Group (DBCG) database since 1977, including information on several patient, tumor and sentinel node characteristics, as well as adjuvant systemic treatment and radiotherapy, and follow up status (10). Furthermore, the DBCG describes guidelines for the treatment of breast cancer in Denmark (11). The DBCG recommends SLND as standard procedure for patients with primary breast cancer without lymph node metastases verified by ultrasound of the axillary (11). In addition the SLND is recommended to selected patients with ductal carcinoma in situ (DCIS). Today, the DBCG database contains information on more than 100.000 breast cancer patients. Thus, the DBCG database holds data of a unique size for investigating the use of SLND in breast cancer patients.
The majority of studies included in this thesis are based on data from the DBCG database.

Aim

Overtreatment is a major problem in breast cancer. The trend towards less invasive surgical treatment should continue to minimize this overtreatment. Efforts should continue towards an additional reduction in ALND in patients without prognostic benefit from the procedure.

Since the introduction of the SLND in the treatment of breast cancer, the focus has changed from identifying patients who can safely be spared an ALND, to identifying patients who will benefit from, and therefore do need, an ALND. The selection of patients for SLND and subsequent ALND is of utmost importance to secure a tailor-made treatment of the axilla. ALND should be avoided in sentinel node positive patients without prognostic benefit from ALND and the use of the SLND should be extended to groups of breast cancer patients, where SLND is not yet considered as standard procedure, despite some of these patients being without axillary lymph node metastases.

The work included in the present thesis was carried out to optimize the use of the sentinel node procedure in the treatment of breast cancer, to tailor the treatment in a way, so that overtreatment and unnecessary side effects is kept to a minimum.

The aim of this thesis was to:

- investigate the incidence and therapeutic consequences of identification of micrometastatic disease in the sentinel node
- identify patients with metastases in the sentinel node who do not need a completion ALND
- extend the use of SLND beyond patients with primary breast cancer

Incidence and therapeutic consequences of micrometastatic disease in the sentinel node

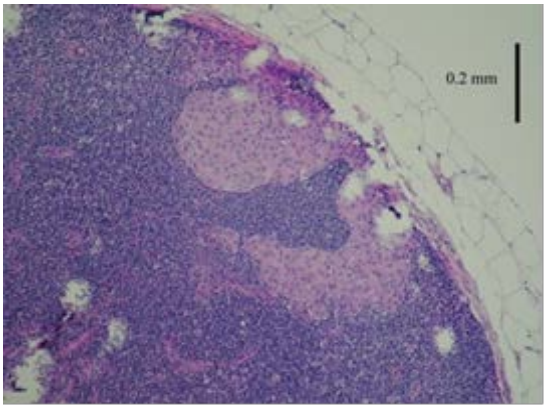
Definition of micrometastases and isolated tumor cells

In average only two lymph nodes are initially removed by the SLND, compared to more than ten lymph nodes by ALND (8). Removing fewer nodes has made more extensive histopathological examinations possible (12) and as a result more metastases are found (13), especially micrometastases and isolated tumor cells (ITC)(Fig 1). The definition and staging of these small metastases has changed along with the increasing evidence of their significance.
When introducing the SLND in the treatment of breast cancer, micrometastases and ITC were classified together as metastatic lesions no larger than 2 mm, according to the fifth edition of the American Joint Committee on Cancer (AJCC) staging manual from 1997. With the increasing identification of small metastases after introduction of the SLND, this definition was changed in the sixth edition of the AJCC staging manual in 2002. Micrometastases were subsequently defined as tumor cell deposits no larger than 2 mm, and ITC were defined as tumor cell deposits no larger than 0.2 mm (14). Patients with micrometastases in the sentinel node were considered node positive (pN1mi) and patients with ITC were considered node negative (pN0(i+)). In Denmark, in addition to size, cell count was used to classify metastases. Metastases between 10 and 100 tumor cells were defined as micrometastases, and single cells or cell clusters of less than 10 cells were defined as ITC (8). The change in definition over years has made comparison of case material from different periods difficult and in some studies re-evaluation of specimens from the early SLND era was necessary (15). In 2010, cell count was added to the AJCC definition (16). Along

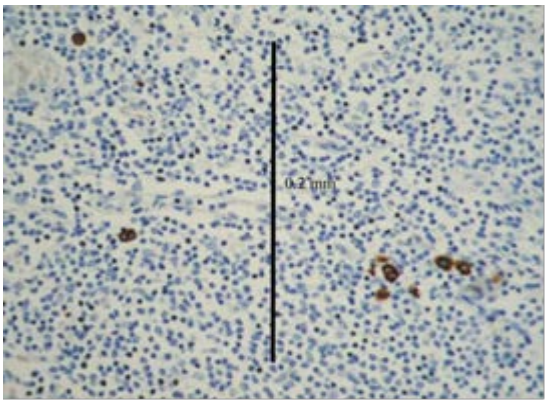
with an increased understanding of the prognostic significance of small metastatic deposits, the staging of these patients is still under debate (17).

Figure 1 (From (18))

a) Micrometastases in the sentinel node



b) Isolated tumor cells in the sentinel node



Stage migration

The use of SLND, with more extensive examination of the removed lymph nodes, has led to identification of more metastases in the axillary lymph nodes. The identification of low volume metastases has increased further with the recent introduction of the one-step nucleic acid amplification (OSNA) in some international centres for examination of the sentinel nodes (19;20). The increased identification of metastases causes stage migration, where patients that were previously regarded as node negative today are staged as node positive, not because of a more advanced stage of the disease but solely due to introduction of a new and more detailed method for lymph node examination (21).
Few studies have investigated the magnitude of stage migration after introduction of SLND and only four have been population-based (22-25). Two studies have investigated the magnitude of stage migration in different parts of the Netherlands. Both studies showed an increase in the proportion of node positive patients mainly caused by an increase in patients with micrometastases (23;24). A previous smaller study including Danish patients from three different counties indicated a similar stage migration in Denmark, but patients with micrometastases and ITC were not addressed separately (22). We have determined the magnitude of stage migration after introduction of the SLND on a national basis in Denmark *(Paper I)* (25), which is the largest study to date on the subject. This nationwide study, based on data from more than 24,000 breast cancer patients from the DBCG database, the proportion of breast cancer patients diagnosed as node positive in 1993 - 1996, before introduction of the sentinel node procedure, has been compared to the proportion of node positive patients in 2005 - 2008, after introduction of the sentinel node procedure (25).

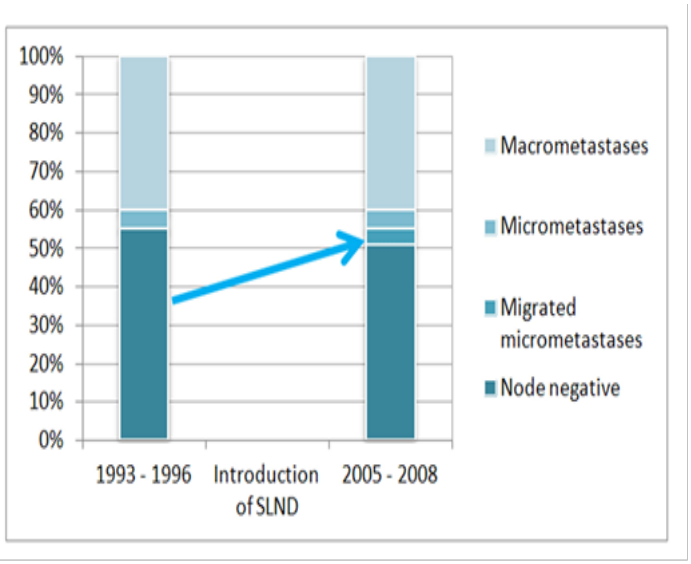


Figure 2: Stage migration after introduction of SLND in breast cancer treatment in Denmark. (From (18))

The study showed that the introduction of the SLND has resulted in an increase from 5% to 9%, in the proportion of breast cancer patients identified with micrometastases in the lymph nodes, and the identification of patients with ITC has increased from basically non-existing to around 2 % (25). There was no difference in the proportion of patients diagnosed with macrometastases (Fig 2).

Iatrogenic displacement

It could be questioned whether all these small tumor cell deposits, like micrometastases or ITC, identified in the sentinel nodes are of clinical significance and are likely to disseminate to other axillary lymph nodes (non-sentinel nodes) (26). During the last decades the theory of cancer stem cells has been introduced (27). According to this theory, breast cancer cells represent a heterogeneous group of cells (28) with different metastatic potential. It has been proposed that some tumor cell deposits in the sentinel node represent insignificant tumor cells, spread from the breast by iatrogenic displacement, and not by cancerogenious spread (29;30). These cells might not have any metastatic potential.
The existence of iatrogenically displaced tumor cells in the needle track after core needle biopsy is well described, and is not related to increased risk of local recurrence (31). The discussion on the existence of a similar iatrogenic displacement of tumor cells to the sentinel node has been going on for years but without concluding evidence (29;32). Studies have shown an increased incidence of ITC in the sentinel node after needle biopsy of the primary tumor. These tumor cells have a different morphology than metastatic tumor cells and are often placed in the subcapsular sinus of the sentinel node. The studies are however small and the clinical significance of this observation is unclear (29;30;32).
In addition to needle biopsy, some breast cancer patients are offered a surgical excision to confirm the cancer diagno-

Table 1: Sentinel node status of 17,374 Danish breast cancer patients operated with SLND according to recent surgical tumor excision. (From *Paper II* (35))

Sentinel node status	Recent surgical tumor excision: Yes No.	Yes %	No No.	No %	Crude OR	95% CI	P-value	Adjusted OR	95% CI	P-value
Negative	265	64.0	9,980	58.9						
ITC	36	8.7	495	2.9	3.17	2.23;4.51	<.0001	3.73	2.57;5.43	<.0001
Micrometastases	62	15.0	2,068	12.2	1.27	0.97;1.67	0.09	1.49	1.12;1.97	0.006
Macrometastases	51	12.3	4,417	26.0	0.40	0.30;0.54	<.0001	0.67	0.49;0.92	0.01
Total	414	100.0	16,960	100.0						

Abbreviations: SLND: Sentinel Lymph Node Dissection, ITC: Isolated Tumor Cells; OR: Odds Ratio; CI: Confidence Interval

sis before final cancer surgery. Studies indicate that this procedure, like a needle biopsy, can cause iatrogenic displacement, but the existence and the magnitude is still

under debate. One study including 663 patients with either fine needle biopsy, core needle biopsy, or surgical excisional biopsy has shown a significantly lower risk of sentinel node metastases after previous surgical excision compared to previous needle biopsy in an adjusted analysis (P=0.04) (33). The difference was however only seen in the group of patients with macrometastases and could represent residual confounding. In another study including 4016 patients, a significantly increasing risk of sentinel node metastases detected by immunohistochemistry (IHC) was seen after previous intervention on the breast; 1.2% after no previous biopsy, 3.0% after previous fine needle biopsy, 3.8% after core needle biopsy, and 4.6% after surgical excisional biopsy (P=0.002)(34). In this study adjustment for tumor size, lymphovascular invasion and location of tumor in the breast was done.

In 2012, we conducted a Danish nationwide study including 17,374 patients from the DBCG database, to investigate whether a surgical excision could lead to iatrogenic displacement of tumor cells to the sentinel nodes *(Paper II)*. The study compared the risk of sentinel node metastases in 414 breast cancer patients with previous surgical excision with 16,960 patients without previous surgical excision. We found that a recent surgical excision resulted in a nearly four-fold increased risk of having ITC in the sentinel node (Odds Ratio (OR)=3.73, 95% Confidence Interval (CI)= 2.57 – 5.43; P<0.0001)(35). The results supported the existence of iatrogenic displacement of tumor cells from the breast to the sentinel node after surgical excision biopsy (Table 1). Patients with diagnostic surgical excision before cancer surgery is a highly selective group of patients where the cancer diagnosis is not evident, and therefore adjustments were made for tumor size, lymphovascular invasion, histological type, and malignancy grade. Despite adjustment for these known confounders, we found an unexplained significantly lower risk of macrometastases in the sentinel node (OR=0.67; 95% CI 0.49-0.92; P=0.001) after previous surgical excision, compared to patients without surgical excision, and some degree of residual confounding might exist.

Like others, we did not find an association between time interval from the previous intervention of the breast to the

sentinel node procedure and the degree of iatrogenic displacement (36).

Despite ITC generally being more common in the sentinel node of patients with lobular carcinomas (37), the displaced tumor cells were especially seen in patients with ductal carcinomas.

None of the 23 patients with ITC in the sentinel node after previous surgical excision had non-sentinel node metastases (Table 2). Despite the small sample size this may indicate that these iatrogenically displaced cells are clinically insignificant and should not lead to a completion ALND.

It is possible that a similar iatrogenic displacement exists in patients with DCIS (29;38). Some of these patients are exposed to several biopsies including stereotactic biopsies and excisional biopsies before the diagnosis is settled. It has been shown that DCIS patients with several interventions before surgery have an increased risk of tumor cells in the sentinel node (39). If tumor cell deposits are found in the sentinel node of patients with DCIS, they are up-staged to invasive carcinomas, and some are offered an ALND.

Further investigation on the existence and significance of iatrogenic displacement of tumor cells in patients with DCIS is warranted.

Axillary staging as a two-stage procedure

In 23 - 52 % of patients with micrometastases or ITC, metastases are not identified on the intraoperative frozen sections of the sentinel node, but found by the final postoperative histopathological examination, even if IHC are used on frozen sections (40;41). These patients were until 2012 offered an additional ALND as a second procedure. This two-stage surgical procedure may potentially further increase the risk of arm morbidity, due to increased formation of scar tissue and risk of nerve damage due to difficult surgical dissection

Table 2: Number of patients with NSN metastases out of 369 Danish breast cancer patients with ITC and 1,793 with micrometastases in the SN, according to recent surgical tumor excision. (From *Paper II* (35))

	Recent surgical tumor excision: Yes	NSN metastases: Yes	NSN metastases: No	Total	No	NSN metastases: Yes	NSN metastases: No	Total	P-value
ITC	0	0%	23	23	41	11.9%	305	346	0.09
Micrometastases	6	11.5%	46	52	330	19.0%	1,411	1,741	0.18

Abbreviations: SN: sentinel node; NSN: non-sentinel node; ITC: isolated tumor cells

in the previously operated axilla, in addition to prolonged hospital stay and increased economic expenses (42;43). Furthermore, it has been hypothesized that fibrosis caused by the primary surgical procedure may hamper surgery and subsequent histopathological examination at the second surgical procedure (42-46), thereby reducing the number of

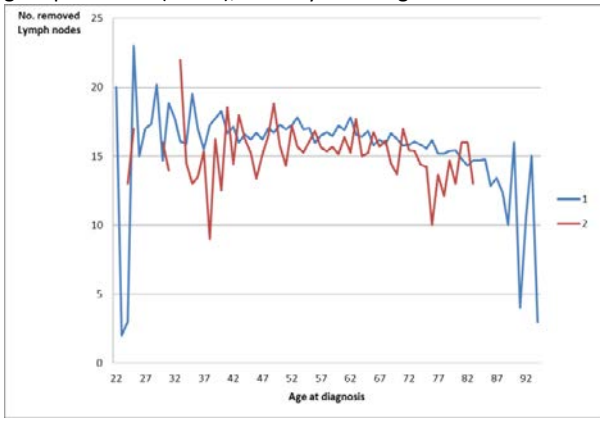


Figure 3:
Number of lymph nodes removed in breast cancer patients according to age and one- or two-stage procedure
1: one-stage procedure; 2: two-stage procedure. (Based on data from *Paper III* (47) by permission from MD Olaf Damgaard)

lymph nodes removed and identified. This will impair locoregional control if metastatic lymph nodes are left in the axilla. In a large nationwide study, including 8257 Danish breast cancer patients treated by SLND and ALND in either a one-stage (7393 patients) or two-stage (864 patients) procedure, we found that the average number of lymph nodes removed was 15.6 in the two-stage procedure compared to 16.6 in the one-stage procedure *(Paper III)* (47) (Fig 3). Due to the large sample size this slight difference was in fact statistically significant ($p<0.0001$), but hardly clinically relevant. This result was in line with the result from an American study including 1003 patients from the ACOSOG Z0010 and Z0011 trials, where in average 14 lymph nodes were removed in patients treated by a one-stage procedure as well as a two-stage procedure (45). Similar results of only a slight difference in the numbers of lymph nodes removed by a one-stage vs. two-stage procedure have been shown in two minor studies (42;48). These results indicate that impaired surgical treatment after a two-stage procedure cannot be supported, and intraoperative identification of metastases in the sentinel node at the primary surgical procedure is not of crucial importance for precise staging and local control.
At the same time a large Danish cohort study, including 2847 patients from the same period and using a detailed questionnaire, showed that there was no statistically significant difference in the morbidity between the two groups treated by one- or two-stage procedures, respectively (49). This further supports the safety of a two-stage axillary procedure.
Metastases not diagnosed at the intraoperative examination on frozen sections are in particular micrometastases and ITC with a low risk of non-sentinel node metastases. Identification of these small metastases on frozen sections could be increased by the use of IHC analyses. In 2012 a Danish study showed that significantly more micrometastases and ITC were identified in the sentinel nodes intraoperatively in a center using IHC analysis on frozen sections compared to a center of similar size using only conventional haematoxylin-eosin staining (40). Accordingly, it was concluded that intraoperative use of IHC on frozen sections would reduce the proportion of patients undergoing a two-stage axillary procedure. Since then, the Danish guidelines for ALND have change and ALND is no longer recommended in patients with only micrometastases or ITC in less than three sentinel nodes (11). Due to this change in guidelines, it is no longer important to identify micrometastases or ITC intraoperatively, and the use of IHC on frozen sections has become redundant. In 2011 the St. Gallen International Expert Consensus Guidelines stated that routine use of IHC analysis on sentinel node specimens was no longer indicated (50).

Influence on adjuvant treatment

In Denmark adjuvant systemic treatment is offered to patients with primary breast cancer and at least one of the following characteristics: tumor size >1 cm, age < 60 years, malignancy grade II or III, negative hormone receptor status, or positive HER2 status (Table 3).
In addition adjuvant systemic treatment is offered in case of axillary metastases, including micrometastases (10). Thus, the increased identification of micrometastases in the sentinel node could potentially result in an increase in the proportion of patients offered adjuvant systemic treatment. However, in the Danish study on stage migration after introduction of the SLND, the increased identification of small metastases did not seem to affect the proportion of patients offered adjuvant systemic treatment by more than 1%, due to the use of several other independent risk factors in stratification for adjuvant treatment *(Paper I)* (25). The increasing tendency towards including new prognostic markers, including genetic subtypes, as high risk criteria (51), will diminish the consequences of stage migration even further, because nodal status is gradually losing its importance as a prognostic marker.

Risk of non-sentinel node metastases

Far from all patients with only micrometastases or ITC in the sentinel node have metastatic spread to non-sentinel nodes. In general, only about 15-20% of patients with micrometastases (52) and 10-15% of patients with ITC (53) in the sentinel node have macrometastatic spread to non-sentinel nodes. Thus, the majority of patients with small metastases in the sentinel node does not benefit from the additional ALND but still run the risk of arm morbidity (3;4). In a Danish study, based on information from more than 1800 patients with micrometastases or ITC in the sentinel node registered in the DBCG database between 2002 and 2008, it was established that 18 % of patients with micrometastases and 9% of patients with ITC had metastases in non-sentinel nodes *(Paper IV)* (15). In other words, ALND was redundant in 82 % and 91 % of these patients, respectively. These results questioned the benefit form ALND in patients with micrometastases or ITC in the sentinel node.
In patients with macrometastases, the risk of non-sentinel node metastases is generally considerably higher; around 40 % (8;54;55).

In conclusion, the introduction of the SLND has increased the identification of micrometastases and ITC. These small metastases are often not identified until the final postoperative histopathological examination, resulting in completion ALND as a two-stage procedure. However, this two-stage procedure does not seem to impair the quality of surgery, and is redundant in a considerable proportion of patients because of a low risk of non-sentinel node metastases, e.g. patients

Table 3: Prognostic groups of breast cancer patients in Denmark according to DBCG guidelines. Only patients allocated to group I are not offered adjuvant systemic treatment (http://www.dbcg.dk)

Age	Tumor size	Positive lymph nodes	Type and malignancy grade	ER status (% positive)	HER2 status	DBCG prognostic group
>/=60 years	</= 10 mm	0	Ductal grade I/unknown Lobular grade I-II /unknown Other type	</=10%/unknown Medullar (neg)	Negative/unknown	I
					Positive	II
				0-9%		II
			Ductal II – III, Lobular III			II
		>/=1				II
	>10 mm					II
< 60 years						II

with iatrogenically displaced tumor cells in the sentinel node. Furthermore, the increased identification of small metastases does not seem to affect the subsequent adjuvant treatment, because other prognostic markers including genetic subtypes, is gradually replacing nodal status as the most important marker.

Prediction of non-sentinel node metastases

Model development

Not all non-sentinel node metastases will become clinical relevant. Despite a false negative rate of the sentinel node procedure at about 5%, the local recurrence rate is less than 1% after eight years of follow-up in patients with a negative sentinel node (56;57). This means that even some patients with non-sentinel node metastases can be spared an ALND without impairment of prognosis.

Due to the low proportion of patients with non-sentinel node metastases when only micrometastases or ITC are found in the sentinel node, it could be questioned whether ALND will improve prognosis in these patients. Still a group of patients with a high risk of non-sentinel node metastases, which will benefit from an ALND, might exist. It would be advantageous to identify this group of patients. This could spare the majority of patients with micrometastass or ITC in the sentinel node a large, unnecessary and often two-staged axillary procedure.

Several studies have tried to identify sentinel node positive patients with no further metastatic spread to non-sentinel nodes. When macrometastases are found in the sentinel node several risk factors have been identified (58-60) and scoring systems have been developed (61-63). Unfortunately these scoring systems are not very well adapted for small metastases (64-66).

Only about 12% of patients with primary breast cancer have micrometastases or ITC in the sentinel node (25) and accordingly a large cohort of patients are needed to get a sufficient sample size for an investigation of risk factors for non-sentinel nodes in these patients. In Denmark, the first SLND was registered in the DBCG database in 2001. Between 2002 and 2008 a total number of 2293 breast cancer patients with micrometastases or ITC in the sentinel node have been registered in the database. This means that the DBCG database can provide a unique sample size for investigating risk factors for non-sentinel node metastases in patients with micrometastases or ITC. 1577 of the patients from the DBCG database had micrometastases in the sentinel node and underwent an ALND, and 304 patients had ITC in the sentinel node as well as an ALND. Based on these two groups of patients, risk factors for non-sentinel node metastases have been identified in a logistic regression model *(Paper IV)* (15). Based on these factors, two models have been developed for prediction of the risk of non-sentinel node metastases; one for patients with micrometastases and one for patients with ITC. The models stratified patients into risk groups of non-sentinel node metastases according to the number of risk factors present. In the model for patients with micrometastases, tumor size, hormone receptor status, lymphovascular invasion, location of tumor in the breast, and proportion of positive sentinel nodes were identified as risk factors for non-sentinel node metastases (Table 4). A group of patients (5%) was identified with 4 or more risk factors present, which had a high risk of non-sentinel node metastases on nearly 40%, comparable to the risk of non-sentinel node

Table 4: Risk factors for NSN metastases in a multivariate analysis of 1521 Danish breast cancer patients with micrometastases and 299 patients with ITC in the sentinel node operated between 2002 – 2008. (From *Paper IV* (15))

Variable	OR	95% CI	P-Value
		Micrometastases	
Tumor size, cm, trend	1.22	1.06-1.39	0.005
Proportion of pos SN, 100% vs. <100%	1.69	1.29-2.21	0.0001
Lymphovascular invasion	1.74	1.18-2.55	0.005
Hormone receptor status, neg vs. pos	1.47	1.00-2.16	0.049
Location of tumor in upper lateral quadrant	1.72	1.30-2.26	0.0003
		Isolated tumor cells	
Tumor size, >2 vs. ≤2 cm	4.21	1.74-10.2	0.001
Age, <40 vs. ≥40 years	3.57	1.11-11.4	0.03
Proportion of pos SN, 100% vs. <100%	2.90	1.27-6.60	0.01

Abbreviations: SN: Sentinel Node, NSN: Non-Sentinel Node, ITC: Isolated Tumor Cells, neg: negative, pos: positive, OR: Odds Ratio, CI: Confidence Interval

metastases in patients with macrometastases in the sentinel node (55). These patients might still benefit from an ALND. A group of patients with a low risk of non-sentinel node metastases less than 10% could not be identified.
In the model for patients with ITC, age at diagnosis, tumor size, and proportion of positive sentinel nodes were identified as risk factors for non-sentinel node metastases (Table 4). Only four patients had all three risk factors present. These patients had a 75% risk of non-sentinel node metastases, but this risk estimate was based on a very small sample size. 32% of patients with ITC had none of the risk factors present and a risk of non-sentinel node metastases at only 2%.

Model validation

Before a predictive model can be taken into clinical use, validation in a new dataset is necessary. Since the development of the two models, registration of patients with micrometastases and ITC in the DBCG database has continued and between 2009 and 2010 another 900 patients have been registered. These patients were used for validation of the two models. The accuracy of the model for patients with micrometastases changed only slightly from an AUC=0.64 in the original cohort to 0.63 in the validation cohort, while the accuracy of the ITC model, based on a minor number of patients and fewer risk factors, dropped from 0.73 in the original cohort to 0.60 in the validation cohort *(Paper V)* (67)(Table 5).
It has been shown, that validation of previously developed predictive models often fails, when tested in a foreign population (61;68). In example, the Tenon score was developed in a French population and worked well in the French validation study (69), but did not perform very well in a Swedish population (70). Likewise, the model from the Memorial Sloan-Kettering Cancer Center (58) worked well in other American populations (71), but was not very precise in a Hungarian population (72). Finally, only two out of twelve tested models worked well in a Chinese population (73). Therefore validation of the models in a Danish dataset could not secure that the models would work well in centers outside of Denmark.
By the same time as the two models were developed in Denmark, development of a predictive model for non-sentinel node metastases in patients with micrometastases or ITC in the sentinel node was done in Helsinki, based on information from 484 Finnish breast cancer patients. In this model the risk of non-sentinel node metastases was associated with tumor size and multifocality, and the AUC was 0.68 (68). The Finnish model had been internally validated on 51 patients with an AUC=0.79.
An agreement was made to test the Danish and Finnish models in the opposite dataset *(Paper VII).* The results showed that the Danish model for micrometatases was accurate when tested in the Finnish cohort, with a slight change in AUC from 0.64 to 0.63. This model was developed based on the largest sample size and included the largest number of risk factors. Thus, the accuracy of the Danish micrometastatic model did not change, under neither internal nor external validation.
The AUC of the Finnish model decreased from 0.68 to 0.58 when tested in the Danish cohort, and the AUC of the Danish model for ITC decreased from 0.73 to 0.52, when tested in the Finnish cohort (74)(Table 5). The lower performance of these two models under cross-validation could be due the development based on smaller sample sizes where fewer risk factors were identified. In addition, demographic differences and differences in diagnosis, surgical techniques, and pathology methods might exist between countries.
Apart from the Danish and Finnish models, a French model exists for the prediction of non-sentinel node metastases in patients with micrometastases in the sentinel node. This model was developed from information on 909 breast cancer patients and uses four different risk factors; tumor size, detection by haematoxylin-eosin vs. IHC, lymphovascular invasion and histological type (75). The AUC for the French model was 0.66 in the initial series. The model was validated in a series of 484 patients but the AUC was not reported (76). The Finnish and the French micrometastatic model have previously been validated on 313 breast cancer patients from 5 different institutions in Europe. The two models did not perform well in this multi-institutional cohort, especially not in predicting patients with high risk of non-sentinel node metastases. The AUC for the two models was 0.58 and 0.56, respectively, at this external validation (77). Both the Finnish and the French model focused on prediction of patients with a low risk of non-sentinel node metastases. Along with the increasing evidence suggesting that ALND can be omitted in patients with only micrometastases or ITC in the sentinel node without impairment of prognosis (78), prediction of patients with low risk of non-sentinel node metastases is no longer important.
The Danish micrometastatic model can be considered as a robust model for prediction of patients with micrometastases in the sentinel node with high risk of non-sentinel node metastases, which might benefit from ALND (74).

Molecular biological risk factors

Despite the extensive validation, the two models do not give a perfect distinction between patients with and without non-sentinel node metastases. Therefore efforts have been made to optimize the models by including additional new risk factors for non-sentinel node metastases.
It is possible that new molecular markers can be used in the prediction of non-sentinel node metastases and be included in and improve existing models. Only few markers have been tested and the results have been disappointing (64;79). In 2011, a Korean study tested 14 different biological markers in 205 breast cancer patients with sentinel node metastases but none of the markers were significantly associated with non-sentinel node metastases (80). In Denmark, we have tested Tissue Inhibitor of Metalloproteinase 1 (TIMP1) IHC, Ki67 and HER2 for the prediction of non-sentinel node metastases *(Paper VII).* All three factors are involved in cancer cell dissemination. TIMP1 is a protease inhibitor found to be associated with lymph node metastases and risk of recurrence in breast cancer patients (81;82). KI67 is a nuclear antigen used as a marker for cell proliferation and associated with poor prognosis (83). Finally, HER2 is a tyrosine kinase receptor involved in regulation of breast cell growth and associated with poor prognosis (84). The three markers were tested in a matched case-control study including 75 patients from Herlev Hospital operated between 2001 and 2007 with micrometastases in the sentinel node and a completion ALND. None of the three markers were significantly associated with non-sentinel node metastases (85).
At present, testing of additional markers is going on in the same patient material; TIMP1 FISH, Plasminogen activator inhibitor 1 (PAI1), BMI1 and Mel-18. Translational research has indicated that these markers may serve as prognostic markers in breast cancer (86-88). Testing of BMI-1 and Mel-

18 is performed at the Klinikk for kreft, kirurgi og transplantasjon in Oslo, Norway.
In 2000 Perou et al. described four different breast cancer subtypes based on gene expression patterns (89). The use of these genetic subtypes as prognostic markers in breast cancer is increasing (89). It is possible that the subtypes could be used in the selection of sentinel node positive patients for completion ALND. The genetic subtypes have a high concordance with subtypes based on traditional IHC analyses of estrogen receptor status, progesterone receptor status, HER2 status, and KI67 index. A recent nationwide Danish study based on more than 20,000 patients has shown that the risk of axillary lymph node metastases is associated with breast cancer subtypes based on receptor status (90). A similar association between subtypes and non-sentinel node metastases has not yet been found (91-93), and so far genetic subtypes have not been used to select sentinel node positive patients for ALND.

Table 5: Performance of models for predicting low and high risk of non-sentinel node metastases in original, internal validation and external validation cohorts. (From *Paper VI* (74))

Model	Cohort	No.	No. with NSN metastases (%)	Sensitivity (Low-risk)†	Specificity (Low-risk)†	Low-risk patients (%)†	Sensitivity (High-risk)‡	Specificity (High-risk)‡	High-risk patients (%)‡	AUC (95% CI)
Helsinki model	**Original**	484	36 (7.4)	0.36	0.86	407 (84.1)	0.03	0.998	2 (0.4)	0.68 (0.59-0.77)
	Internal validation	51	5 (9.8)	0.80	0.76	36 (70.6)			0	0.79 (0.64-0.95)
	External validation	1831	304 (16.6)	0.11	0.91	1667 (91.0)	0.00	0.998	3 (0.2)	0.58 (0.55-0.62)
DBCG model (MIC)	**Original**	1521	273 (17.9)	0.97	0.05	66 (4.3)	0.25	0.88	219 (14.4)	0.64 (0.60-0.67)
	Internal validation	720	121 (16.8)	1.00	0.05	31 (4.3)	0.36	0.81	155 (22.0)	0.63 (0.57-0.68)
	External validation	278	24 (8.6)	0.96	0.05	14 (5.0)	0.42	0.83	52 (18.7)	0.63 (0.49;0.76)
DBCG model (ITC)	**Original**	299	28 (9.4)	0.93	0.34	95 (32.0)	0.43	0.85	52 (17.0)	0.73 (0.64-0.82)
	Internal validation	180	23 (12.8)	0.61	0.39	71 (39.0)	0.48	0.88	30 (17.0)	0.60 (0.46-0.75)
	External validation	206	12 (5.8)	0.42	0.49	102 (49.5)	0.25	0.91	20 (9.7)	0.52 (0.32;0.71)

Abbreviations: DBCG: Danish Breast Cancer Cooperative Group, CI: Confidence Interval, AUC: Area Under the Curve, MIC: Micrometastases, ITC: Isolated Tumor Cells

†Cut point of 10% risk of non-sentinel node metastases or (for DBCG ITC model) no risk factors present

‡ Cut point of 30% predicted risk of non-sentinel node metastases or (for DBCG ITC model) more than one risk factor present

Method of detection as a risk factor

Another way to identify patients with sentinel node metastases that may be more likely to spread beyond the sentinel node is to look at the method of cancer detection. After introduction of mammographic screening programs in many countries in the western world a peak in the incidence of breast cancers was seen (94). This is thought to be due to lead time bias, where breast cancers are identified before they become clinically evident. It is well known, that these screen-detected cancers are smaller and have a lower rate of lymph node metastases compared to symptomatic cancers (95;96). It has also been stated, that these cancers represent a group of slow growing cancers with a lower malignancy grade, lower Ki67 index and higher proportion of positive hormone receptor status (96;97). These characteristics are related to a less aggressive disease and better prognosis and it could be hypothesized, that these patients have a lower risk of non-sentinel node metastases. If this is true, the method of detection could be included in the predictive models and patients with cancers detected by screening should be offered less extensive axillary surgery.
Only few studies have looked at the risk of non-sentinel node metastases in patients with screen-detected breast cancers. The studies are small and only limited adjustment for confounders have been made (98-100). The most rmarkable results are found in a Swedish study including 143 breast cancer patients with micrometastases or ITC in the sentinel node. The study found a five-fold increased risk of non-sentinel node metastases in symptomatic cancers compared to screen-detected cancers after adjustments for tumor size and malignancy grade (OR=5.1; 95% CI=1.4-19) (99). These results pointed in the direction of a modified treatment of the axilla in patients with screen-detected cancers. Patients with screen-detected breast cancers are however a

highly selected group of patients. The Swedish study included patients between the age of 30 and 88 in the group with symptomatic cancers. Studies have shown that tumor characteristics vary by age, and that young age is associated with poor prognosis (101). In the Swedish study no adjustment for age was performed, but only adjustments for malignancy grade and tumor size, and residual confounding cannot be excluded.

We tried to verify the Swedish results in a Danish dataset of 955 patients from the screening population between the age of 50 and 70, registered in the DBCG database with micrometastases or ITC in the sentinel node, after introduction of the national mammographic screening program *(Paper VIII)*. 481 of these patients were identified in the nationwide Danish Quality Database of Mammography Screening with screen-detected cancers. The remaining 474 patients were considered having symptomatic cancers. We found no difference in the risk of non-sentinel node metastases between the two groups (OR=1.07; 95% CI=0.77-1.49; P=0.69), neither in patients with micrometastases nor in patients with ITC (102). In contrast to the Swedish study, adjustments for tumor size, proportion of positive sentinel nodes, lymphovascular invasion, hormone receptor status, and location of tumor in the breast were made in the Danish study; all risk factors identified in the Danish models for predicting non-sentinel node metastases developed in 2012 (15). 181 patients with micrometastases and 756 patients with ITC were included in the multivariate analyses (Table 6). Still, no significant difference was found in the risk of non-sentinel node metastases between patients with screen-detected cancers and symptomatic cancers.

Based on the Danish results, the method of detection cannot be used in the prediction of non-sentinel node metastases and the data does not support a less aggressive treatment of the axilla in patients with screen-detected breast cancer.

Prediction of axillary recurrence

The largest concern for breast cancer patients is not the risk of occult non-sentinel node metastases but the risk of recurrence. Accordingly, the predictive models for non-sentinel node metastases ought to be tested for their ability to predict axillary recurrences. Only one study exists on such a testing (103). In this study, four different models developed for the prediction of non-sentinel node metastases in patients with macrometastases in the sentinel node was tested for the prediction of axillary recurrence in 486 Dutch patients with micrometastases in the sentinel node without completion ALND. Only one of the four models identified a group of patients with a risk of axillary recurrence just above 10%. It is possible that a model developed for the prediction of a high risk of non-sentinel node metastases in patients with micrometastases in the sentinel node could more accurately predict axillary recurrence in this patient series. Hence, testing of the Danish micrometastatic model (15) for prediction of axillary recurrences in the Dutch data material has been proposed and initiated in collaboration with researchers in Maastricht.

Prognostic significance of axillary lymph node dissection

Studies on the the impact of ALND on axillary recurrence and survival in patients with micrometastases or ITC's in the sentinel node are few and limited by short follow-up, small sample sizes, lack of multivariate analyses, and information on adjuvant treatment (104-108) and the results are conflicting. Some studies show that patients with ITC or micrometastases have a worse outcome if ALND is omitted (107;109) while others can not show any difference (106;110;111). Two large register studies exist, including 6,838 and 10,259 patients with micrometastases in the sentinel node, respectively, from the American SEER database and the American National Cancer Database (NCDB). No significant difference in overall survival or axillary recurrence was found between patients treated by SLND + ALND or SLND alone (112;113), indicating that ALND could safely be omitted in these patients. These studies were however limited by missing registration of recurrences, imprecise number of removed sentinel nodes, and missing information on adjuvant treatment. The results from two recent randomized trials have further questioned the benefit from ALND in patients with micrometastases in the sentinel node. In the European IBSCSG 23-01 study, where 934 breast cancer patients with micrometastases or ITC in the sentinel node were randomized to either ALND or no ALND, no difference was found in axillary recurrence or survival between groups (78). Similar results were found in the Spanish AATRN 048/13/2000 trial, including 233 patients (114). It should however be noted that both randomized trials closed down before they met the planned accrual and might be underpowered.

In contrast to these studies, a Dutch research group has published the results of a large register study, including 795 patients with ITC and 1028 patients with micrometastases in the sentinel node. They found a non-significant adjusted

Table 6: Risk factors for NSN metastases in a multivariate analysis of 756 Danish breast cancer patients with micrometastases and 181 patients with ITC in the sentinel node from the screening population operated between 2008 – 2010. (From *Paper VIII* (102))

Variable	OR	95 % CI	P-value
	Micrometastases		
Tumor size, cm, trend	1.36	1.12-1.64	0.002
Proportion of pos SN, 100% vs. <100%	1.46	1.01-2.10	0.04
Lymphovascular invasion	1.78	1.11-2.86	0.02
Hormone receptor status, neg vs. pos	1.25	0.70-2.24	0.46
Location of tumor in upper lateral quadrant	1.41	0.97-2.06	0.07
Symptomatic vs. screen-detected	1.12	0.77-1.62	0.55
	Isolated tumor cells		
Tumor size, >2 vs. ≤2 cm	2.54	0.93-6.89	0.07
Proportion of pos SN, 100% vs. <100%	0.78	0.29-2.14	0.63
Symptomatic vs. screen-detected	0.45	0.16-1.27	0.13

Abbreviations: SN: Sentinel Node, NSN: Non-Sentinel Node, ITC: Isolated Tumor Cells, neg: negative, pos; positive, OR: Odds Ratio, CI: Confidence Interval

hazard ratio of 2.4 (95% CI= 0.67 – 8.48) for regional recurrence if ALND was omitted in patients with ITC in the sentinel node, and a significantly increased hazard ratio on 4.4 (95% CI=1.46 – 13.24) for regional recurrence if ALND was omitted in patients with micrometastases in the sentinel node after 5 years follow-up (115). Due to these results, the authors recommended ALND in patients with micrometastases in the sentinel node and unfavorable tumor characteristics.

Table 7: Axillary recurrence and overall survival in 2074 Danish breast cancer patients with micrometastases or ITC in the sentinel node treated between 2002 and 2008. (From *Paper IX* (116))

5 years cumulated incidence	Micrometastases		Isolated tumor cells	
	ALND		ALND	
	Yes	No	Yes	No
Axillary recurrence	1.44	1.04	1.90	3.96
OS (95% CI)	91.8 (90.3-93.1)	79.4 (71.6-85.3)	93.3 (89.8-95.6)	87.3 (79.1-92.4)

Abbreviations: ITC: Isolated Tumor Cells, ALND: Axillary Lymph Node Dissection, OS: Overall Survival

To investigate the safety of omitting ALND in breast cancer patients with micrometastases or ITC in the sentinel node we initiated a retrospective study in Denmark based on national data from the DBCG database *(Paper IX).* Until 2012 the standard treatment of Danish breast cancer patients with micrometastses or ITC in the sentinel node was a completion ALND. Still, some patients did not undergo ALND. The reason for not choosing ALND in these patients is basically unknown, but is probably due to age, comorbidity and patient preference. Accordingly, patients without ALND is a highly selected group of patients. In total, 256 patients with ITC or micrometastases in the sentinel node but without completion ALND have been registered in the DBCG database from the start of the sentinel node era in 2002 and until 2008. The axillary recurrence rate in this group of patients was very low (Table 7); 1.6% after 6 years of follow-up (116), despite between 9 – 18 % of these patients were expected to have non-sentinel node metastases (15). After adjustment for age, no significant difference was seen in axillary recurrence between patients with and without ALND; neither for patients with micrometastases in the sentinel node (HR=1.79; 95% CI= 0.41 – 7.80; P=0.44) nor for patients with ITC (HR=2.21; 95% CI= 0.54 – 8.95; P=0.27) (Table 8)(116). Development of axillary recurrence from minimal metastatic disease left in the axilla might take longer than what is expected for macrometastatic disease. In a study including patients with haematoxylin-eosin negative sentinel node, where patients with metastases detected by IHC were not offered an ALND, the medium time to recur
rence was 4.8 years (117). Sufficiently long follow-up for at least 5 years, like in the Danish study, is important for patients with minimal metastatic disease, to give time for an axillary recurrence to develop. As expected, the group without ALND had a significantly worse overall survival compared to patients with ALND, because one of the main reasons for omitting ALND in these patients could be age or comorbidity (Table 7). When adjusting for known risk factors for non-sentinel node metastases, age, comorbidity, and adjuvant systemic treatment and radiotherapy, there was no signify cant difference in overall survival between patients with and without ALND (HR=1.13; 95% CI=0.84 – 1.52; P=0.41)((Table 8) (116). These results support the safety of omitting ALND in patients with only micrometastases or ITC in the sentinel node. The remarkable difference between these results and the result from the Dutch study could be explained by the differences in the adjuvant treatment guidelines. In contrast to Danish treatment guidelines, only half of the patients included in the Dutch study received any kind of adjuvant treatment (115). In addition, it is possible that differences in adjuvant radiotherapy between studies play an important role. In fact, in the recent European AMAROS trial, where 4823 sentinel node positive patients were randomized to either ALND or axillary radiotherapy, no difference was found in the risk of axillary recurrence between groups after 6.1 years of follow-up (118). It is possible that adjuvant systemic treatment and adjuvant radiotherapy can offset the impaired prognosis after omitting ALND in patients with micrometastases or ITC in the sentinel node.

In the AMAROS trial, 60 % of the included patients had macrometastases in the sentinel node. Still no significant difference in 5 years axillary recurrence, disease free survival or overall survival was seen between groups (118). These results are in line with the results from the American ACOSOG Z0011 trial, where 891 patients with clinically node negative primary breast cancer treated by breast conserving surgery and maximum 2 positive sentinel nodes were randomized to ALND or no ALND. No significant difference in axillary recurrence and survival was shown after 6.3 years of follow up (110;111). Like in the AMAROS trial 60% of patients had macrometastases in the sentinel node. This study had however several methodological weaknesses (119), and both the ACOSOG Z0011 trial and the AMAROS trial were underpowered due to a low number of events. Still these studies indicate that it could be safe to omit ALND even in patients with macrometastases in the sentinel node. Recently, a Swedish randomized trial, the SENOMAC trial, has been initiated where breast cancer patients with macrometastases in the sentinel node are randomized to ALND or no ALND. Differences in recurrence and survival will be estimated. Danish breast surgery centers are going to participate in the trial. It is possible that the results from this trial will further reduce the proportion of sentinel node positive patients offered a completion ALND.

Table 8: Adjusted Cox proportional hazard ratios for axillary recurrence and death if ALND is omitted compared to patients with ALND in 2074 Danish breast cancer patients with micrometastases or ITC in the sentinel node treated between 2002 and 2008. (From *Paper IX* (116))

	Axillary recurrence			Death		
	HR*	95% CI	P-value	HR**	95% CI	P-value
Micrometastases n=1673	1.79	0.41-7.80	0.44	1.21	0.86-1.69	0.27
ITC n=401	2.21	0.54-8.95	0.27	0.96	0.57-1.62	0.89
Micrometastases or ITC n=2074	1.99	0.72-5.50	0.18	1.13	0.84-1.52	0.41

Abbreviations: ITC: Isolated Tumor Cells, ALND: Axillary Lymph Node Dissection, HR: Hazard Ratio, CI: Confidence Interval
**** Adjustment for age***
***** Adjustment for age, tumor size, histology type, malignancy grade, lymphovascular invasion, hormone receptor status, nodal status, comorbidity, adjuvant systemic treatment and adjuvant radiotherapy***

Changing guidelines

Studies on the prognostic significance of ALND in patients with micrometastases or ITC in the sentinel node have resulted in a trend towards omission of ALND in these patients. In 2009 the St. Gallen International Expert Consensus Conference stated, based on the low risk of non-sentinel node metastases, that ALND can be avoided in selected breast cancer patients with micrometastases or ITC in the sentinel node (120) (Fig 4a).Two years later, in 2011, after the publication of the results from the ACOSOG Z0011 trial, the option of omitting ALND was extended to patients with macrometastases in the sentinel node. It was however underlined that this option was only accepted for patients fulfilling the criteria for the ACOSOG Z0011 trial, e.g. clinically node negative patients, with 1 – 2 positive sentinel nodes, undergoing breast conserving treatment with adjuvant radiotherapy (50). In line with these recommendations, Denmark changed the DBCG treatment guidelines for patients with micrometastases or ITC in the sentinel node. Since 2012 ALND is no longer recommended in patients with less than 3 micrometastatic sentinel nodes, regardless of type of surgery in the breast (Fig 4b)(11).

It is possible that the predictive models for non-sentinel node metastases, based on patients from the DBCG database with micrometastases in the sentinel node, could give a better prediction of a high risk of non-sentinel node metastases than the presence of metastases in 3 or more sentinel nodes (15). In contrast to the majority of models, the Danish micrometastatic model focused on patients with high risk of non-sentinel node metastases. In the data material for model development and validation only 32 patients had 3 or more positive sentinel nodes, and only 3 (9.4%) of these patients had non-sentinel node metastases. By using the developed model on the common development and validation cohort a group of patients was identified with a particularly high risk of non-sentinel node metastases on more than 35%. ALND might be more beneficial in this group of patients than in patients with 3 or more micrometastatic sentinel nodes (Table 9).

When looking at Danish patients with axillary recurrence after omitting ALND, only 5 patients without ALND, included in the study of prognostic significance of ALND in patients with micrometastases or ITC in the sentinel node (116), had axillary recurrence as first event; 2 with micrometastases and 3 with ITC in the sentinel node. One of the two patients with micrometastases had 4 or more risk factors present and was identified as high risk patient according to the predictive model developed for patients with micrometastases (Unpublished data). However the number of patients was too small to test if the predictive model for non-sentinel node metastases could predict axillary recurrence as well. Results from testing of the models to predict axillary recurrence in the Dutch data material might shed further light on this issue.

In Denmark ALND is still recommended to breast cancer patients with macrometastases in the sentinel node but will be offered participation in the SENOMAC trial.

Axillary staging in locally recurrent breast cancer

Use of SLND in locally recurrent breast cancer

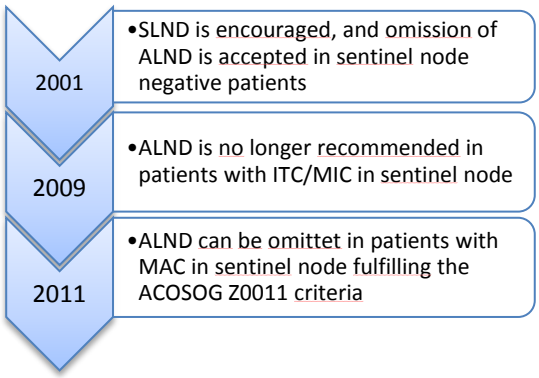
In Denmark, treatment guidelines for patients with locally recurrent breast cancer describes that patients with previous SLND should be offered an ALND and patients with previous ALND should not receive additional axillary surgery (11). However, less than 10% of patients with local recurrence has lymphatic spread at time of recurrence (121). This means that more than 90% of patients with local recurrence and a previous SLND could again be spared an ALND. In patients with previous ALND, a new lymphatic pathway to a de novo sentinel node might have been formed. If this new sentinel node contains metastases, identification by SLND might improve locoregional control.

In Denmark, there has been a growing interest to extend the potential benefits from the SLND to patients with local recurrence and some departments have started to use the procedure in selected patients. It is however questionable whether the previous axillary surgery and/or previous radiotherapy have changed the lymph drainage from the breast in such a way that SLND is hampered.

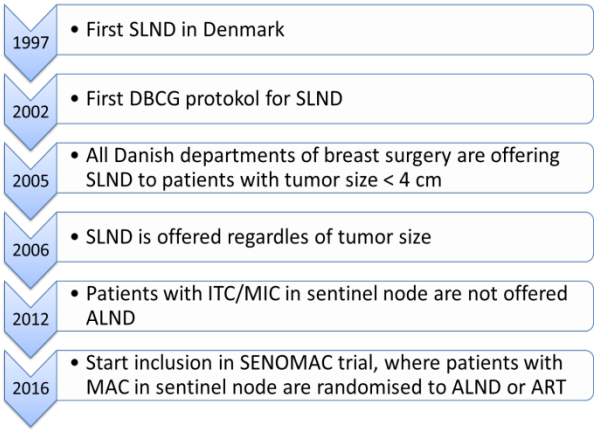
Only few and smaller studies exist on the subject (122-125). In 2011 and 2015 respectively, two studies were published with a reasonable number of patients; a Dutch study from 2011 including 150 patients and our Danish study from 2015 including 147

Figure 4:

a) Changes in the St. Gallen Guidelines for treatment of the axilla



b) Changes in the DBCG guidelines for treatment of the axilla



Abbreviations: SLND: sentinel lymph node dissection; ALND: axillary lymph node dissection, MAC: macrometastases; MIC: micrometastases; ITC: isolated tumor cells; DBCG: Danish Breast Cancer Group; ART: axillary radiotherapy

Table 9: Risk of NSN metastases in 2300 Danish breast cancer patients with micrometastases in the sentinel node according to risk groups defined by number of positive sentinel nodes or predictive model

	Low risk group		High risk group		Total
	Total	NSN metatastases (%)	Total	NSN metatastases (%)	
No. of positive SN; 1-2 vs. >2	2268	402 (17.7)	32	3 (9.4)	2300
Predictive model: No. of risk factors; 0-3 vs. 4-5	2142	359 (16.8)	99	35 (35.4)	2241*

59 patients had missing values for predictive model

Abbreviations: No.: Number, SN: Sentinel Node, NSN: Non-Sentinel Nodes

patients from 12 different departments of breast surgery in Denmark; 73 patients with a previous SLND and 74 patients with a previous ALND *(Paper X).* The results from both studies points in the same direction: The SLND seems to be feasible in patients with locally recurrent breast cancer. The Dutch study (the SNARB study) found a detection rate on 52%; higher in patients with previous SLND compared to previous ALND, and in patients with previous mastectomies compared to breast conserving surgery (126). In the Danish study we found a detection rate on 50%. Again the highest detection rate was found in patients with previous SLND (66%) compared to previous ALND (34%)(P=0.0001), and in patients with previous mastectomies (64%) compared to previous breast conserving surgery (48%)(121)(Table 10).

However, the difference in detection rate between previous mastectomy and previous breast conserving surgery was not significant.

It is possible that the trend towards an impaired detection rate in patients with previous breast conserving surgery is caused, not by the surgery itself but by the subsequent external radiation towards the residual breast. A recent Italian study included 212 patients with local recurrence after breast conserving surgery and a previously negative sentinel node. In this study they found a much higher detection rate on 92.5%. 36% of the patients had been treated with intraoperative radiotherapy or no radiotherapy at all (127).

In the published studies, only few sentinel node negative patients underwent completion ALND. No additional metastases were found in these patients corresponding to a false negative rate on zero. In the Danish study, no axillary recurrences were seen after more than 3 years of follow-up (121), whereas a cumulative incidence of axillary recurrence on 3.9% was seen in the Italian study (127). An increased risk of aberrant drainage was seen in patients with previous ALND. In patients with primary breast cancer aberrant lymphatic drainage is uncommon. Especially drainage to the contralateral axilla is rare (0 – 2 %) (128). However, if SLND is performed at locally recurrent breast cancer after previous ALND, aberrant drainage to contralateral axilla is seen in 11 - 71% of the patients at lymphoscintigraphy (121;126;128). In the Danish study, 25% of patients with previous ALND had complete or partly aberrant drainage outside the ipsilateral axilla (6 patients)(121). In half of these patients sentinel node was located in the contralateral axilla and in one patient (17%) the aberrant sentinel node contained metastases (Table 10). This is in line with international results where metastases are found in 20 % of the cases if a sentinel node in the contralateral axilla is removed (128). This emphasizes the need for lymphoscintigraphy in these patients, to be able to identify the location of additional metastatic lymph nodes. In the Danish study, 37 out of 73 patients (51%) with a previous SLND had a sentinel node identified without metastases. These patients could again be spared an ALND and the risk of arm morbidity. Another 11 patients did only have micrometastases or ITC in the sentinel node, and it could be argued if ALND could be omitted in these patients. In 6 out of 74 patients (8%) with a previous ALND, a positive sentinel node was identified and removed. This could potentially leading to a better local control(121).

Traditionally, patients with lymph node metastases in the contralateral axilla are considered as having disseminated disease and the treatment is regarded as palliative (128). However, due to the change of lymphatic drainage after previous axillary surgery and radiotherapy the prognostic significance of lymph node metastases outside the ipsilateral axilla might have changed. Patients with lymph node metastases in aberrantly draining lymph nodes at local recurrence might have the same prognosis as patients with metastases in the ipsilateral axilla (129).

The prognostic significance of lymph node metastases in the contralateral axilla at recurrence is difficult to investigate because this condition is extremely rare, and even by using national register it would be difficult to obtain a sufficient sample size. Studies built on multinational cooperation are therefore of great importance. A multinational collaboration on the prognostic significance of axillary recurrence in the contralateral axilla has recently been initiated by researchers in Maastricht. Denmark is participating in this study and we have delivered data from the DBCG database on patients with recurrence in contralateral axilla. If these patients have the same prognosis as patients with ipsilateral recurrence the results can change the treatment towards a more aggressive approach to obtain locoregional control.

Proposed changes in guidelines

The DBCG guidelines do not yet recommend SLND at recurrence (11). Based on the current evidence, it could be suggested that SLND is used at local recurrence, including preoperative lymphoscintigraphy. If a sentinel node can be identified, frozen sections should be performed. If no metastases are found, patients with a previous SLND should not undergo ALND. In case of metastases in the sentinel node, ALND should be performed after pre-

Table 10: Results of SLND at recurrence according to primary axillary operation in 147 Danish breast cancer patients. (From *Paper X* (121))

	SLND		ALND	
	No.	%	No.	%
Total	73	100	74	100
Previous operation in breast				
Mastectomy	4	5.5	10	13.5
BCS	69	94.5	64	86.5
Scintigraphy				
Not performed	17	23.3	9	12.2
Performed	56	76.7	65	87.8
Non-detection of SN	24	42.9	33	50.8
Detection of SN	32	57.1	32	49.2
Ipsilateral drainage	23	71.9	17	53.1
Aberrant drainage	9	28.1	15	46.9
SLNDAR				
Not performed	0	0	3	4.1
Performed	73	100	71	95.9
Non-detection of SN	25	34.2	47	66.2
Detection of SN	48	65.8	24	33.8
Ipsilateral drainage	42	87.5	18	75.0
Npos	9	21.4	5	27.8
Nneg	33	78.6	13	72.2
Aberrant drainage	6	12.5	6	25.0
Npos	2	33.3	1	16.7
Nneg	4	66.7	5	83.3

Abbreviations: SLNDAR: Sentinel Lymph Node Dissection After Recurrence, SLND: Sentinel Lymph Node Dissection, BCS: Breast Conserving Surgery, SN: Sentinel Node

vious SLND. If a new sentinel node is found in the ipsilateral axilla after previous ALND no further surgery should be performed unless previous ALND was incomplete. Attempts to remove sentinel nodes at extra-axillary sites should be considered, especially in the contralateral axilla. Depending on the results of the Dutch study on the prognostic significance of recurrence in contralateral axilla a contralateral ALND could be recommended. Removing internal mammary sentinel nodes is related to substantial morbidity and a prognostic advantage in primary breast cancer is still discussed (130;131). The significance of removing these sentinel nodes at recurrence is unknown. A change in radiation fields in patients with sentinel nodes at extra-axillary sites should be considered as well. In case of non-detection, treatment should follow the current guidelines.

Conclusion and perspectives

Conclusion

The introduction of the SLND in breast cancer treatment spares each year hundreds of thousands of women an ALND and the following risk of arm morbidity. The more extensive examination of the lymph nodes after introduction of the SLND has however resulted in identification of more micrometastases and ITC. Only a small proportion of patients with these metastases in the sentinel node have metastases in other axillary lymph nodes and some are caused by iatrogenic displacement. Removal of additional lymph nodes by ALND in these patients do not improve prognosis. As a result ALND is no longer recommended in the majority of these patients and the use of IHC on frozen sections has become redundant. Still a group might exist, with a higher risk of axillary recurrence where ALND should still be recommended. The developed predictive model can be used to identify this group. If final histology identifies such a high risk patient where ALND is still recommended, a two-stage procedure can be offered without impairment of surgical results or increased risk of side effects compared to a one-stage procedure.

This thesis adds to the increasing evidence of lack of prognostic advantage of ALND in sentinel node positive breast cancer patients. This evidence has resulted in dramatic changes in surgical treatment guidelines in Europe and Denmark, with a reduction in the proportion of patients offered ALND. It is expected that in the following years additional subgroups, where ALND can be omitted, will be identified.
In addition, the thesis suggests an extension of the use of SLND to patients with locally recurrent breast cancer, and a change in treatment guidelines for this group of patients is proposed.

Future reduction in axillary surgery

Further extension of the SLND to other patient groups could spare an additional proportion of patients an ALND. Especially, a change in the use of SLND in patients treated by neoadjuvant treatment could reduce the use of ALND.
The use of neoadjuvant treatment is increasing, to reduce tumor size and improve the possibilities of breast conserving surgery. In Denmark these patients are offered SLND before neoadjuvant treatment if they are clinically node negative. If metastases are found in the sentinel node, an ALND is offered at final surgery after neoadjuvant treatment. This does not take advantage of the neoadjuvant treatment that might reduce metastatic load in the axilla and convert patients from node positive to node negative and make further axillary surgery redundant. If the SLND is performed after neoadjuvant treatment, international studies have shown a detection rate between 72 -100%, and a false negative rate (FNR) between 0 and 33% (132-134). Two prospective studies have recently been published on the subject. The American ACOSOC Z1071 trial included 687 breast cancer patients with metastases in the axilla verified by biopsy before neoadjuvant treatment and a clinically node negative axilla after end of treatment. The detection rate at the subsequent SLND was 92.9% and the FNR was 12.6% at completion ALND (135). In the German SENTINA trial 592 patients with a positive sentinel node before neoadjuvant treatment was offered another SLND and ALND after end of treatment. Here the detection rate was 60% and the FNR was 52%. In the same study 360 patients with lymph node metastases at biopsy before neoadjuvant treatment and a subsequent

clinically negative axilla had a detection rate on 80% and a FNR on 14% at the following SLND and ALND (136). The FNR are in both studies higher than accepted by SLND at primary breast cancer. However a decreasing FNR was seen when using more than one tracer and by removing more sentinel nodes. In addition it is possible that marking of positive nodes before neoadjuvant treatment (Targeted axillary dissection) with a radioactive seed could further reduce the FNR (137), thereby making a SLND after neoadjuvant treatment acceptable.

Despite the advantages of introducing the SLND, the procedure is not without side effects. It has been shown that 25 - 50% of patients experience pain after the SLND and 31 – 56% experience sensory disturbances (3;4). Thus, caution should be taken to avoid overused of the procedure. Today, all patients with primary invasive breast cancer are offered axillary surgery, either SLND or ALND. Sometimes axillary surgery is even offered to patients with only DCIS, because in 13 – 40% of these patients an unsuspected small area with invasive carcinoma is found at histopathological examination after final surgery (138;139). International studies have shown that metastases are found in the sentinel node in only 1 - 2% of patients diagnosed with only DCIS or microinvasive disease <1 mm. The SLND is therefore redundant in the vast majority of these patients (38;140;141). It is possible that some of these sentinel node metastases are caused by iatrogenic displacement of tumor cells from the breast. The risk of metastases to other lymph nodes and the need for a completion ALND in these cases is basically unknown. The number of patients diagnosed with DCIS or microinvasive disease <1 mm has increased after introduction of the National Mammographic screening program, thereby increasing the risk of overtreatment by unnecessary SLND (94). Identification of risk factors for sentinel and non-sentinel node metastases in these patients could enable a tailor-made treatment of the axilla, and avoiding overtreatment by redundant SLND.

Traditionally, the advantage of axillary surgery has been based on the need for staging information for adjuvant treatment decisions and therapeutic removal of positive lymph nodes. Adjuvant treatment is now increasingly based on biological tumor characteristics and a survival advantage of removing axillary lymph nodes has never been shown. As early as in 1971 the NSABP B-04 trial was initiated where patients were randomized to radical mastectomy, total mastectomy with radiation, or total mastectomy with ALND only if nodes became positive (142). No difference in survival was shown between groups. With the increasingly precise axillary ultrasound (143) it is possible that axillary surgery with time will be reserved for patients with substantial tumor load in the axilla, whereas patients with fewer metastatic lymph nodes might be offered axillary radiotherapy which is connected to minor risk of morbidity than ALND, as shown in the AMAROS trial (118). The role for SLND will then be questioned.

Summary

This thesis is based on 10 original articles, of which 3 were previously included in the PhD thesis "Staging of women with breast cancer after introduction of sentinel node guided axillary dissection". In the PhD thesis is was shown that the introduction of sentinel lymph node dissection (SLND) in the treatment of breast cancer in Denmark has resulted in an increased identification of patients with micrometastases or isolated tumor cells (ITC) in the lymph nodes. Not all these small metastases are likely to disseminate to non-sentinel nodes. This thesis provides evidence that a previous surgical excision of a breast tumor is likely to lead to iatrogenic displacement of tumor cells resulting in a nearly four-fold increased risk of ITC in the sentinel node. These tumor cells are not associated with non-sentinel node metastases. Especially ITC, but also micrometastases and some macrometastases, are not identified on perioperative frozen sections, but found postoperatively at the conventional histopathological examination. These patients are offered an axillary lymph node dissection (ALND) as a second procedure. It has been suggested that this two-stage procedure reduces the number of lymph nodes removed, because of fibroses from previous surgery. In this thesis it was shown that a two-stage procedure does not result in a clinically relevant impairment of the number of lymph nodes removed by ALND.

Based on patient, tumor, and sentinel node characteristics from the Danish Breast Cancer Group database, two predictive models for non-sentinel node metastases, when only micrometastases or ITC are found in the sentinel node, were developed, as a part of the PhD thesis. These two models have now been internally validated, and a cross-validation in a Finnish patient material has been performed in cooperation with researchers from Helsinki. The model for patients with micrometastases proved to be robust under internal as well as external validation and could be used to identify patients with micrometastases that might still benefit from an ALND.

Efforts should continue to improve the model. As a part of the PhD thesis, new molecular markers were tested for prediction of non-sentinel node metastases. In addition, method of detection of the breast cancer could be a possible predictor of non-sentinel node metastases. It has been hypothesized that breast cancers detected by screening represent a clinical indolent group of cancers with lower risk of non-sentinel node metastases compared to symptomatic cancers. This hypothesis was tested in this thesis in a large Danish dataset. No significant difference in the risk of non-sentinel node metastases was found between patients with screen-detected and symptomatic breast cancers, and a less aggressive treatment of the axilla in patients with screen-detected breast cancers cannot be supported. Likewise, the method of detection is not expected to be able to improve the predictive models.

Until 2012, the standard treatment of Danish patients with micrometastases or ITC in the sentinel node was ALND. Still, in selected patients ALND was not performed. This thesis includes a comparison of the risk of axillary recurrence and survival between patients with and without ALND. The overall axillary recurrence rate was only 1.6% after 6 years of follow-up, despite between 9 – 18 % of these patients are expected to have non-sentinel node metastases. No significant difference was seen in axillary recurrence and overall survival between patients with and without ALND. These results support the safety of omitting ALND in patients with only micrometastases or ITC in the sentinel node and since 2012 Danish breast cancer patients with ITC or up to two micrometastatic sentinel nodes are no longer offered an ALND.

In Denmark the standard surgical treatment of the axilla in locally recurrent breast cancer is no further treatment of the axilla in case of previous ALND, and ALND in case of previous SLND. To investigate whether SLND can be extended to this patient group, a Danish multicenter study was performed. Despite a reduced detection rate, especially after previous ALND, SLND seemed to be a

feasible procedure in locally recurrent breast cancer. The procedure can spare a clinically significant number of patients an unnecessary ALND and improve staging and local control after previous ALND. The increased number of patients with aberrant drainage underlines the importance of preoperative lymphoscintigraphy at local recurrence.

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