# Refining prognostication in early breast cancer

More about the tumor, less about the nodes

**Julia van Steenhoven**

# REFINING PROGNOSTICATION IN EARLY BREAST CANCER:
## MORE ABOUT THE TUMOR, LESS ABOUT THE NODES

by
Julia Everdina Cato van Steenhoven

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# Refining prognostication in early breast cancer:
## more about the tumor, less about the nodes

# Prognose in vroeg stadium borstkanker patiënten:
## Het draait vooral om de primaire tumor en steeds minder om de lymfeklieren

(met een samenvatting in het Nederlands)

**Proefschrift**

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# Chapter 1

Introduction and outline of this thesis

## THE THEORY OF BREAST CANCER: FROM LOCAL TO SYSTEMIC DISEASE

It was long thought that breast cancer is a local disease that spreads through the body in an orderly manner: from the primary tumor in the breast to lymph nodes, and from there to distant sites. Based on this concept, Halsted's radical mastectomy offered the foundation of breast cancer treatment for nearly a century through the performance of extensive surgery consisting of removal of the entire breast, pectoral muscles and regional lymph nodes [1]. Still confronted with distant metastases, Lacour *et al.* attempted to expand local intervention by adding internal mammary chain dissection to Halsted's mastectomy but found no impact on outcome in a randomized controlled trial (RCT) and did show a harmful effect in terms of morbidity [2]. The recognition that ever more and aggressive surgery did not improve outcome was followed by a trend in the opposite direction. RCTs by Veronesi and Fisher starting in the 70's of the last century revealed equivalent outcomes for radical mastectomy and the less invasive quadrantectomy or lumpectomy (followed by local radiotherapy) supporting less extensive surgery of the primary breast tumor [3,4]. Similarly, the NSABP B-04 trial, that randomized patients to radical mastectomy, mastectomy with nodal irradiation, or mastectomy followed by delayed axillary lymph node dissection (ALND) showed that axillary management could be tapered as well with no detrimental effect on survival [5]. These large RCTs set the stage for less invasive breast cancer surgery and corroborated the hypothesis that breast cancer is a systemic disease and that prognosis is linked to the presence or absence of distant metastases, or at least the growth of those.

In line with this paradigm shift, landmark trials of Fisher, Bonadonna and Cole *et al.* in the early 1970s showed that the addition of chemotherapy and endocrine therapy to local treatment did improve outcome [6-8]. Chemotherapy and endocrine therapy became important pillars of breast cancer treatment in the 1990s. While adjuvant systemic therapy was initially promoted as treatment for patients with more advanced breast cancer subsequent trials also demonstrated a beneficial effect of systemic therapy in early breast cancer [9-11]. This led to a worldwide increase in the use of adjuvant systemic therapy, which was the main driver behind the improved breast cancer survival in subsequent years [12]. In the Netherlands, several guideline adaptations were implemented in the years between 2002 and 2008, each time including new categories of patients eligible for adjuvant chemo- and/or endocrine therapy [13]. With the expansion of adjuvant systemic therapy indication areas, physicians became aware that growing numbers of patients were potentially 'overtreated' systemically as the absolute risk reduction became less.

Focus shifted towards more personalized oncology in more recent years with the ultimate goal to select patients in whom the benefit of chemotherapy outweighs the side effects, and to select patients in whom chemotherapy can be forgone. Prognostic tools, such as Adjuvant!Online, were developed at the beginning of this century to serve this purpose.

## TOWARDS BETTER SELECTION OF PATIENTS IN NEED OF CHEMOTHERAPY

The selection of patients at high risk for disease recurrence – and therefore likely to benefit from adjuvant systemic therapy – was traditionally based on classic patient and tumor characteristics (e.g. age, tumor grade, size, nodal involvement). The first Dutch guideline for the treatment of breast cancer (CBO 2002) regarded patients <35 years, patients who had lymph node metastases, and patients with tumors of high malignancy grade candidates for adjuvant systemic treatment. Malignancy grade remains one of the most important factors in outcome prediction [14,15] and is commonly expressed according to the modified Bloom and Richardson grading system which combines pathologists' scores of tubule formation, nuclear pleomorphism, and tumor proliferation by mitotic index [16]. Despite efforts to optimize histopathological assessment standards, reproducibility of histological grading remains an issue [17-19]. More reliable and reproducible markers of tumor proliferation remains of interest to improve grading. Mitotic Activity Index assessment (MAI) and more recently staining for the proliferation related antigen Ki67 have been propagated as an additional marker for malignancy grade [20,21], but standardized methodologies for staining, measurement and uniform cut-off points are lacking [21]. More recently, phoshohistone H3 (PhH3) was proposed as a novel, more reproducible and reliable, proliferation marker.

Apart from refining classical grading, the identification of four intrinsic ("molecular") subtypes of human breast cancer tumors by Perou in the beginning of this millennium revolutionized the perception of the disease [22-27]. Four breast cancer subtypes were identified by expression of genes associated with estrogen receptor (ER) and HER2 status and the presence of proliferation associated genes. Luminal A (estrogen receptor positive (ER+), low proliferation), luminal B (ER+, high proliferation), HER2-driven (HER2+), and basal-like (ER-/HER2-/high proliferation) cancers are associated with different treatment response and outcome.

At the same time, gene-expression profiles (GEPs) were developed that either identify the intrinsic molecular tumor subtype [28] or discriminate between tumors that have a high or low risk of developing metastases [29-32]. These GEPs entered clinical practice with the ambition to guide adjuvant chemotherapy decisions in addition to the aforementioned classical clinicopathological factors and were soon incorporated in breast cancer guidelines to tailor adjuvant systemic therapy decisions since 2012 [33]. Since proliferation is uniformly higher among HER2+ and basal-like cancer, but variable among ER+ cancers, the clinical utility of genomic profiling lies mainly in aiding chemotherapy decision-making in ER+ cancers.

While information of the primary tumor has expanded, developments in axillary surgery influenced the availability of prognostic information as well. Until the mid 1990's, ALND was standard of care for each patient and provided information on the number of metastatically involved regional lymph nodes. Since the introduction of the sentinel lymph node biopsy (SLNB) as a less invasive staging procedure, ALND was no longer routinely performed in all breast cancer patients [34]. With the surgical procedure of the SLNB, pathologic work-up of the sentinel lymph nodes (SLN) intensified from gross pathological examination of all axillary nodes towards a meticulous approach of one or two lymph nodes by applying step-sectioning and immunohistochemical staining of SLNs. Consequently, small metastatic deposits such as micro-metastases and isolated tumor cells were observed more frequently [35-37]. Then, the Z0011 and AMAROS trials revealed that completion ALND (cALND) may be omitted in clinically node negative patients with one or two metastatic sentinel lymph nodes (SLNs) [38,39], and as a consequence information about the overall number of involved lymph nodes is no longer routine practice. This loss of detail on the extent of nodal involvement does not compromise outcome [38,39], but may affect the clinical decision-making process and certainly comes with a sense of uncertainty for many clinicians.

The increased use of neo-adjuvant chemotherapy (NAC) further challenged staging of the axilla, providing the clinician with less information on nodal involvement, which impedes locoregional treatment decision making. Since the 2000´s the use of NAC is emerging and has proven to be of great value in downstaging both breast and axillary disease, which decreases the need for extensive surgery for both breast and axilla. Meanwhile, SLNB after NAC is considered appropriate for staging the axilla in neo-adjuvant treated patients with a clinically negative axilla (cN0) [40]. However, staging the axilla in clinically node positive (cN+) patients downstaged after NAC with SLNB, remains controversial and is widely addressed in several trials. The most important concern is the potential false negative rate of SLNB after NAC. The NSABP-B27, SENTINA and ACOSOG Z1071 trial demonstrated false negative rates ranging from 10.7 – 14.2% after NAC in cN1-2 patients downstaged to cN0, with lower rates if at least 3 SLNs were removed [41-43]. Subsequent studies affirm that in axillary downstaged cN0 patients, cALND can be omitted if the SLNB is negative [44,45]. Attempts have been made to further reduce the false negative rate by marking the axillary lymph nodes with radioactive iodine seed (MARI) or targeted axillary dissection (a combination of SLNB and a MARI-like procedure) [46-49]. To date, patients who remain node positive after NAC are treated with ALND. However, the ALLIANCE A011202 trial is studying if omission of ALND is safe in neo-adjuvant treated patients with a clinically partial nodal response treated with axillary radiotherapy [50].

In summary, surgical treatment of the breast and axilla has become ever less aggressive over the last 30 years with less axillary surgery as the most recent ongoing trend. Over

the past decade, genomic classifiers have entered the mainstream of clinical practice and specially in ER+ breast cancer patients knowledge of tumor biology has contributed to a tendency among clinicians becoming more selective to administer adjuvant chemotherapy too. These trends appear independent developments within different medical disciplines, but in effect they do interfere. Local treatment historically provided the information to guide systemic treatment and with less and different timing of surgery, deliberations regarding systemic treatment change. More recently, evolutions in systemic treatment have allowed more reticent local treatment and will continue to do so.

## OUTLINE OF THIS THESIS

The first part of this thesis focuses on pathological and genomic classifiers for prognosis and molecular subtyping, and its impact on the administration of adjuvant chemotherapy in early stage breast cancer patients in The Netherlands. In **chapter 2** we compare the distinction between the four intrinsic tumor subtypes based on conventional pathology assessment and an 80-gene signature. In **chapter 3** we evaluate the reproducibility of the MAI, Ki67 and PhH3 in ER+ breast cancer patients. In **chapter 4** we evaluate the association between 70-GS use and the administration of adjuvant chemotherapy in Dutch early stage breast cancer patients treated between 2013 and 2016.

The second part of this thesis focuses on developments and trends in axillary staging, the clinical consequences of suboptimal staging and the effects of non-surgical treatment modalities on regional recurrence risk. In **chapter 5** we evaluate patterns of care in axillary treatment of Dutch patients diagnosed with primary invasive breast cancer, who underwent mastectomy and were staged as SLN+, in the years following the publication of the Z0011 and AMAROS trials. In **chapter 6** we assess the 'true' 5-year regional risk in SLNB-negative breast cancer patients who underwent mastectomy without radiotherapy or adjuvant systemic therapy. In **chapter 7** we aim to quantify the effects of non-surgical treatments on regional recurrence incidence among SLNB-negative breast cancer patients.

The last part of this thesis focuses on patients' perceptions regarding 70-GS testing and institutional factors that are associated with the tendency to adopt early or late to 'less is more' strategies. In **chapter 8** we evaluate the impact of 70-GS use on patients' decisions to undergo chemotherapy and the perceived decision conflict during decision-making. In **chapter 9** we analyze patient, treatment and hospital factors that are associated with the omission of cALND in SLNB+ breast cancer patients, with a particular interest of a hypothesized effect of GEP deployment within an institution as a means to de-escalate systemic treatment.

The results of this thesis and future perspectives are discussed in **chapter 10.**

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# PART I

Extending primary tumor examination by conventional pathology and applying gene-signatures

# Chapter 2

## Conventional pathology versus gene signatures for assessing Luminal A and B type breast cancers: results of a prospective cohort study

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## ABSTRACT

In this study, in estrogen receptor positive (ER+) early stage breast cancer patients who were considered candidates for 70-gene signature (70-GS, "MammaPrint") use, we compared molecular subtyping (MS) based on the previously validated 80-gene signature (80-GS, "BluePrint") versus surrogate pathological subtyping (PS). Between 1 January 2013 and 31 December 2015, 595 clinical intermediate risk ER+ early stage breast cancer patients were enrolled. Hormone receptor (HR) and HER2 receptor status were determined by conventional pathology using immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH). Ki67 was assessed in a subset of patients. The overall concordance between PS and MS for luminal type cancers (A and B together) was 98%. The concordance between PS and MS for luminal A and luminal B type cancers based on the Bloom Richardson histological grade (BR) ($n$ = 586) or Ki67 ($n$ = 185) was low: 64% (Kappa 0.20 [95% CI 0.11–0.28]) and 65% (Kappa 0.22 [95% CI 0.062–0.37]), respectively. In this prospective study (NCT02209857) of a selection of ER+ and predominantly HER2- early-stage breast cancer patients the additional value of the 80-GS to distinguish between Luminal, HER2-type and Basal like cancers was inherently very limited. The distinction of Luminal-type tumors into A and B according to Ki67 status or BR grade versus the 70-GS revealed poor concordance.

## INTRODUCTION

The identification of intrinsic ("molecular") subtypes of human breast cancer tumors by Perou et al. 15 years ago catalyzed the concept of individualized cancer therapy [1]. The improved understanding of molecular subtypes is of clinical importance, as different subtypes require specific treatment regimens and are associated with different outcomes. The majority of early stage breast cancer patients are diagnosed with an estrogen receptor (ER) positive (+) and HER2 receptor negative (HER2−) disease, associated with favorable outcomes, and patients benefit from endocrine therapy. HER2-driven and basal-like tumors are more aggressive breast cancer subtypes, and patients are sensitive to chemotherapy. The population diagnosed with ER+/HER2− (luminal type) disease is highly heterogeneous as patients with similar clinicopathological features can have strikingly different outcomes. In patients diagnosed with luminal A type disease, the additional value of chemotherapy over endocrine therapy is questionable, whereas chemotherapy seems to be of more benefit to patients with luminal B tumors [2–4].

In clinical practice, pathological determination of the ER, progesterone receptor (PR), HER2-status (HER2), Bloom Richardson histological grade (BR) and Ki67 are generally used to determine surrogate intrinsic cancer subtypes [5]. Gene expression profiles may be used in patients with ER+ breast cancer to make a distinction between groups with a low or high risk of developing distant metastases in order to optimize patient selection for chemotherapy [6–12]. Based on the expression of 80 genes, a molecular subtyping profile ("BluePrint") has been developed for the stratification of breast cancer tumors into the three main molecular subtypes: luminal, HER2 and basal [13]. By adding the prognostic risk profile of the 70-gene signature (70-GS), a substratification of luminal-type tumors into low risk (luminal A) and high risk (luminal B) type cancers can be made [14].

The St. Gallen International Expert Consensus panel defined a surrogate to distinguish Luminal A-type breast cancer from Luminal B-type, based on a combination of ER, PR, HER2, and the expression of the proliferation marker, Ki67 [15,16]. Furthermore, histological grade is also being used as an alternative to Ki67 in Luminal-type breast cancers. While hormone- and HER2 receptor status have been proven to be highly reproducible [17], a standardized methodology for Ki67 level assessment is lacking and the role of Ki67 in clinical decision making remains uncertain [18-22]. Reliable distinction of Luminal-type tumors into A and B is important, since therapeutic consequences are large. Misinterpretation of these surrogates may lead to the risk of patients being over- or undertreated.

As part of a prospective observational multicenter study in a selection of ER+ breast cancer patients who were considered candidates for 70-GS use [23], a conventional

pathology assessment was performed as well as gene-expression profiling. We assessed molecular subtypes (luminal/HER2/basal) using an 80-GS in ER+, mostly HER2- and partly HER2+ cancers determined by conventional pathology. In addition, concordance between Luminal A and B type cancers was evaluated using local pathology, stratified by Ki67 status or Bloom-Richardson (BR) grade versus gene signatures (80-GS/70-GS).

## MATERIALS AND METHODS

### PATIENTS

As part of a prospective observational multicenter study regarding the influence of the 70-GS on adjuvant chemotherapy decision-making in patients with surgically-treated ER+ breast cancer, conventional (local) pathology data and gene expression read-outs were obtained between January 1, 2013 and 31 December 2015. The study was approved by the medical ethics committee of the University Medical Center Utrecht (12-450) and by institutional review boards of participating centers. The study protocol (protocol number 12-450) was registered in the clinicaltrial.gov database (NCT02209857). Within the study, patients diagnosed with early stage ER+ invasive ductal breast cancer with an uncertain benefit of adjuvant chemotherapy based on traditional prognostic factors were eligible for inclusion. Twenty- three out of thirty-three participating hospitals offered patients the opportunity for their tumor samples to be additionally evaluated by the 80-GS (BluePrint ©). In total, 595 patients treated in these 23 hospitals had both tests performed, and these patients were included in the present study.

### ROUTINE PATHOLOGY ASSESSMENT

The determination of hormone receptor status (ER and PR) and HER2-receptor status was done routinely and locally in the pathology labs in accordance with national pathology guidelines. ER and PR status were routinely determined by immunohistochemistry (IHC) and positive identification was defined as the presence of nuclear staining in ≥10% of breast cancer cells, in accordance with the Dutch Breast Cancer Guidelines [24]. The results were identical if we applied a cut-off of ≥1%, as all cases included in this study with any ER or PR positive staining of the nuclei, showed at least 10% positive nuclear staining [25]. HER2-expression was scored by IHC according to international guideline recommendations [26]: 0 if no staining was observed or membrane staining was incomplete and faint/barely perceptible and within less than 10% of the tumor cells, 1+ if staining was incomplete and faint/barely perceptible, but within more than 10% of the tumor cells, 2+ if more than 10% of the tumor cells displayed circumferential staining of moderate intensity or complete and circumferential strong staining within less than 10% of the tumor cells, and 3+ for strong circumferential membrane staining within ≥10% of the tumor cells. A tumor was considered HER2-negative when a score of 0 or 1+ was found and positive when a score of 3+ was observed. Tumors with 2+ HER2 expression were additionally evaluated by

HER2 fluorescent in situ hybridization (FISH). In accordance with the Dutch guidelines, the cut-offs for HER2 low level and high-level amplification were defined as >6 and >10 copies of the HER2 gene or clusters, respectively [23]. Ki67 assessment was routinely performed in 11 of the participating hospitals and the tests were done in five different pathology laboratories. When Ki67 had been determined (n = 185), the average scoring method was performed and a Ki67 cut-off value of 20% [25] was used for the designation of Ki67 into luminal A or luminal B type tumors.

### PATHOLOGICAL SUBTYPING (PS)



In accordance with the 2013 recommendations from the St. Gallen guidelines, surrogate molecular subtypes were determined as follows: luminal A-like (ER+ and PR≥20%, HER2− and Ki67<20%) and luminal B-like (ER+/HER2−/PR<20%, or ER+/HER2−/Ki67≥20%, or ER+/HER2+) [22]. Using surrogate molecular subtyping based on grade, Bloom Richardson (BR) histological I and II were combined into the low proliferative group and BR grade III represented the high proliferative tumors, and surrogate subtypes were determined as follows: luminal A- like (ER+ and PR≥20%, HER2− and BR I/II) and luminal B-like (ER+/HER2−/PR<20%, or ER+/HER2−/BR III or ER+/HER2+).

### MOLECULAR SUBTYPING (MS)

All tumor samples were routinely evaluated by the 80-GS and 70-GS methods at the Agendia Laboratory in Amsterdam, The Netherlands; the individuals who conducted the analysis were blinded to clinical and pathological data. The 80-GS (“BluePrint”) stratified breast cancers into the molecular subtypes: luminal, HER2, and basal-like [13]. Combining the 80-GS with the 70-GS method enabled further stratification of luminal tumors into luminal A (70-GS low risk) and luminal B (70- GS high risk) [14].

### STATISTICAL ANALYSIS

A comparison of molecular subtyping (MS) and pathological subtyping (PS) was done with two by three (Table 2) and two by four (Table 3 and 4) contingency tables, calculating overall concordance. In addition, comparison of pathological subtyping (PS) and molecular subtyping (MS) for Luminal A and Luminal B tumors based on BR grade and Ki67 was done with the Kappa statistic to evaluate the agreement between these two classifications on a nominal scale and accompanying 95% confidence intervals (CI) were calculated [27].

## RESULTS

### PATIENTS

There were 595 patients with a median age of 58 years. The majority of the patients had intermediate grade tumors (74%), with no or micro-metastatic axillary lymph node involvement (pN0 or pN1mi ≥93%, Table 1). A local pathology assessment determined

that all 595 patients had ER+ tumors, 87% had PR+ and 2% had HER2+ tumors. The 70-GS classified 59% of the patients as having a 'low risk' form of breast cancer. In the subset of 185 patients in whom Ki67 levels were assessed, 83% had Ki67 levels <20%, reflecting a low risk. This subset of patients had comparable proportions of PR+ and HER2+ tumors (88% and 1%, respectively) (Table 1).

**Table 1.** Baseline characteristics in patients assessed by local pathology (*n* = 595) and in patients assessed by local pathology enhanced by Ki67 level determination (*n* = 185).

| Characteristics | Total *n* = 595 (%, valid) | Subset Ki67 *n* = 185 (%, valid) |
|---|---|---|
| **Age, years, median** | 58 | 57 |
| **(*range)*** | (35–80) | (35–74) |
| **Pathological T-stage** | | |
| T1 | 480 (80.6) | 153 (82.7) |
| T2 | 114 (19.2) | 31 (16.8) |
| T3 | 1 (0.2) | 1 (0.5) |
| **Pathological N-stage** | | |
| N0(i+) | 496 (84.5) | 164 (89.6) |
| N1mi | 54 (9.2) | 11 (6) |
| N1(a-c) | 37 (6.3) | 8 (4.4) |
| Nx | 8 | 2 |
| **Tumor grade** | | |
| 1 | 86 (14.5) | 30 (16.3) |
| 2 | 438 (73.7) | 125 (67.9) |
| 3 | 70 (11.8) | 29 (15.8) |
| Unknown | 1 | 1 |
| **ER status** | | |
| ER+ | 595 (100) | 185 (100) |
| **PR status** | | |
| PR+ | 518 (87.2) | 163 (88.6) |
| PR- | 76 (12.8) | 21 (11.4) |
| Unknown | 1 | 1 |
| **HER2 status** | | |
| HER2+ | 12 (2) | 2 (1.1) |
| HER2− | 576 (98) | 182 (98.9) |
| Unknown | 7 | 1 |
| **Ki67 Level** | | |
| <20%, low | 153 (83) | 153 (83) |
| [3]20%, high | 32 (17) | 32 (17) |
| Not assessed | 410 | - |
| **70-GS** | | |
| Low risk | 349 (59) | 109 (59) |
| High risk | 246 (41) | 76 (41) |

ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth receptor 2; Ki-67: proliferation marker; N0/N0(i+): no axillary lymph node involvement/isolated tumor cells; Nmi: micro-metastasis; N1a-c: metastasis in movable ipsilateral Level I, II axillary lymph node(s), Nx axillary lymph node status not assessed.

## PATHOLOGICAL SUBTYPING VERSUS MOLECULAR SUBTYPING USING THE 80-GS ONLY

Using local pathology, 98% of patients (*n* = 576) were regarded as [HR+/HER2−, luminal A] and 2% (*n* = 12) of patients as [HR+/HER2+, luminal B]. In seven patients (1%), the HER2 receptor status was not conclusive and these individuals were therefore excluded from the analysis. The 80-GS classified 98% (*n* = 583) of all patients as luminal-type, 1% (*n* = 7) as HER2-type and 1% (*n* = 5) as basal-type. The comparison of MS and PS for luminal A and B together resulted in an overall concordance of 98% (Table 2). The ER expression percentages of patients reclassified as HER2-type were 50% (*n* = 1), 70% (*n* = 1), 90% (*n* = 1) or 100% (*n* = 4). The ER expression percentages of patients reclassified as basal-type were 10% (*n*= 1), 50% (*n* = 2), 70% (*n* = 1) or 100% (*n* = 1).



**Table 2.** Pathological subtyping using hormone and HER2 receptor status versus molecular subtyping using the 80-GS (*n* = 588).

| | Molecular subtypes | | | |
|---|---|---|---|---|
| **Clinical subtypes** | **80-GS Luminal (%)** | **80-GS HER2 (%)** | **80-GS Basal (%)** | **Total** |
| ER+/PR+, HER2− | 567(98) | 4(1) | 5(1) | 576 |
| ER+/PR+, HER2+ | 9(75) | 3(25) | 0(0) | 12 |
| Total | 576 | 7 | 5 | 588 |

80-GS: 80-gene signature; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2. The overall concordance between PS and MS was 98%.

## COMPARISON OF LUMINAL A AND LUMINAL B TUMORS BY MOLECULAR OR PATHOLOGICAL SUBTYPING

Based on the BR grade, stratification of luminal-type tumors into A and B was performed. Using PS, 74% of patients (*n* = 448) were classified as luminal A, and 26% of patients (*n* = 138) were classified as luminal B. Using MS, 58% of patients (*n* = 342) were classified as luminal A, 40% of patients (*n* = 232) as luminal B, 1% of patients (*n* = 7) as HER2-type and 1% of patients (*n* = 5) as basal-type. Thirty-four percent of patients (*n* = 154) considered to be PS luminal A were reclassified as MS luminal B. Thirty-eight percent of patients (*n* = 52) regarded as PS luminal B were reclassified as MS luminal A. The overall concordance between PS and MS was 63%. The concordance between PS and MS within the luminal group was 64% (Kappa 0.20 [95% CI 0.11–0.28], Table 3).

**Table 3.** Comparison of pathological subtyping using Bloom Richardson histological grade versus molecular subtyping (*n* = 586).

| | Molecular subtypes | | | | |
|---|---|---|---|---|---|
| **Clinical subtypes** | **Luminal A (%)** | **Luminal B (%)** | **HER2 (%)** | **Basal (%)** | **Total** |
| ER+, PR ≥20%, HER2-, BR I/II | 290(65) | 154(34) | 4(1) | - | 448 |
| ER+ & (PR<20%, or HER2+ or BR III) | 52(38) | 78(57) | 3(2) | 5(3) | 138 |
| Total | 342 | 232 | 7 | 5 | 586 |

*ER* estrogen receptor, *PR* progesterone receptor, *HER*2 human epidermal growth factor receptor 2. The overall concordance between PS and MS was 63%. Concordance between PS and MS within the Luminal group was 64% (Kappa 0.20 [95% CI 0.11– 0.28])

## COMPARISON OF LUMINAL A AND LUMINAL B TUMORS BY MOLECULAR AND PATHOLOGICAL SUBTYPING

Based on hormone and HER2 receptors and Ki67 status, stratification of molecular subtypes into luminal A or luminal B tumors could be performed. Based on local pathology, 82% (n = 151) were classified as PS luminal A and 18% (n = 34) as PS luminal B. Of the patients classified as PS Luminal A, 64% (n = 96) were also classified as MS luminal A. Thirty-four percent of patients (n = 52) and 2% (n = 3) of patients were reclassified as MS luminal B and MS HER2-type, respectively. Of the patients classified as PS luminal B type, 65% of patients (n=22) were also classified as MS luminal B. Eleven percent of patients (n = 32) and 1% of patients (n = 3) were reclassified as MS luminal A type and MS basal-type, respectively. The overall concordance between PS and MS in this subset of patients was 64%. The concordance between PS and MS within the luminal group was 65% (Kappa 0.22 [95% CI 0.062–0.37]) (Table 4).

**Table 4.** Comparison of pathological subtyping based on Ki67 status versus molecular subtyping (*n* = 185).

| | Molecular subtypes | | | | |
|---|---|---|---|---|---|
| **Clinical subtypes** | **Luminal A (%)** | **Luminal B (%)** | **HER2 (%)** | **Basal (%)** | **Total** |
| ER+, PR ≥20%, HER2-, Ki67<20% | 96(64) | 52(34) | 3(2) | 0 | 151 |
| ER+ & (PR<20%, or HER2+ or Ki67 ≥20%,) | 11(32) | 22(65) | 0 | 1(3) | 34 |
| Total | 107 | 74 | 3 | 1 | 185 |

ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; Ki67: proliferation marker protein. The overall concordance between PS and MS was 64%. The concordance between PS and MS within the luminal group was 65% (Kappa 0.22 [95% CI 0.062–0.37]).

## DISCUSSION

In this prospective multicenter ER+ breast cancer study, we observed high concordance between conventional pathology assessment and gene-expression profiling for luminal-type cancers (A and B together). When Ki67 expression or BR grade was used in addition to routine pathology and compared to molecular subtyping to differentiate between luminal A/B type cancers, concordance was low.

The current study, therefore, shows that molecular subtyping using local pathology or 80-GS results in classification of similar proportions of luminal-type tumors. The observed concordance is in line with a previous study conducted by Nguyen et al. (*n* = 135) in which concordance between luminal-type tumors by IHC/FISH and 80-GS was 96% [28]. Similarly, results of a study evaluating the effect of locally and centrally assessed hormone and HER2 receptor statuses revealed comparable proportions of tumors classified as ER+ and HER2-, 97% and 98%, respectively. In the latter study, central reclassification rates were higher in tumors originally assessed as ER− (14%) and HER2+ (21%). These results suggest that 80-GS based molecular subtyping has little additional value in patients that are classified as HR+/HER2− by conventional pathology.



In the present study, stratification of luminal-type tumors into types A and B by conventional pathology based on the expression of the proliferation marker, Ki67, compared to 70-GS was associated with lower concordance (64%). This apparent discordance within the luminal group is in line with results reported in the MINDACT trial where 54% of patients with a luminal B subtype according to conventional pathology were reclassified as luminal A by the 70/80-GS [29]. As a consequence, using conventional or microarray analysis (70/80-GS) for breast cancer subtyping may lead to discordant chemotherapy decision-making with the risk of patients being potentially over or undertreated.

The pathological distinction between ER+ tumors into low risk (luminal A) and high risk (luminal B) for developing metastases is mostly based on the assessment of proliferative activity. Histologic grade, in which the mitotic activity index as measure of proliferation plays a major role, is generally used to this end, but the St. Gallen breast cancer consensus panel also recommends Ki67 as a means of determining proliferative activity and therefore, selecting patients for chemotherapy. However, a standardized methodology for Ki67 assessment is lacking [18] and revisions within the St. Gallen expert panel for the most appropriate cut-off for high proliferative tumors are still pending [30–33]. In accordance with the 2013 St Gallen recommendations, we set the Ki67 cut-off for high proliferation at 20%. If a 14% Ki67 cut-off value had been applied, the comparison between low-risk and high-risk tumors would have resulted in even higher discordance (37%) of tumors reclassified by the 80- and 70-GS methods (Appendix A). Intra-tumoral heterogeneity of Ki67 expression levels, inter-laboratory and inter-observer variability of

Ki67 staining and differences in Ki67 Labelling Index values have been observed by others and hamper the utility of this biomarker as a reproducible prognostic tool [18,22,34,35]. In our study, 11 hospitals and five different pathology labs were used to determine Ki67. Among these pathology labs, different staining methods may exist and could have caused the discordance between molecular and pathological subtyping.

In this prospective multicenter trial, molecular subtyping by local pathology versus gene signatures was evaluated for a large group of patients. Furthermore, this selection of ER+ patients is very relevant as this is the subset of patients in whom gene signatures are most commonly deployed to guide chemotherapy decisions and, as such, best reflects current clinical practice. Unfortunately, the composition of our study population precluded the performance of 80-GS in HER2 driven and basal-like cancers and so, to be able to study these groups, future studies need to be performed.

It is noteworthy that discordance between conventional pathology and the 80-GS for HER2-driven tumor types reported in trials in the neo-adjuvant setting, was high [4,36,37]. In the Neoadjuvant Breast Register Symphony Trial (NBRST), approximately half of patients (48%) regarded as HER2+/ER+ by conventional pathology were classified as BluePrint luminal-type. In addition, a comparison of molecular subtyping in clinical HER2+ patients in the MINDACT trial revealed 38% and 5% of patients reassigned by the BluePrint as luminal-type and basal-type, respectively. These results confirm the presence of different underlying dominant pathways indicating that expression of the luminal pathway is often dominant compared to the HER2-driven tumor profile.

In conclusion, in this prospective study of a selection of ER+ and predominantly HER2– early-stage breast cancer patients, the additional value of the 80-GS to distinguish between luminal, HER2-type and basal like cancers was inherently very limited. However, agreement between luminal A and luminal B type tumors based on local pathology enhanced by Ki-67 or BR grade versus the 70-GS, was poor. The main implication of our study is the existence of disparity between the two classification methods and the concomitant risk of inadequate treatment allocation. In that regard, there may be a role for gene expression profiling as a consistent tool to discriminate between luminal A and B to guide adjuvant chemotherapy decision-making.

**Supplementary table 1** Comparison of Luminal A and Luminal B type tumors with Ki67 versus gene- signatures (70-GS/80-GS) (n=179).

| | Molecular subtypes | | |
|---|---|---|---|
| **Clinical subtypes** | **Luminal A (%)** | **Luminal B (%)** | **Total** |
| ER+, PR ≥20%, HER2-, Ki67 <14% | 86(64) | 49(36) | 135 |
| ER+ & (PR<20%, or HER2+, or Ki67 ≥14%) | 17(39) | 27(61) | 44 |
| Total | 103 | 76 | 179 |

*70-GS* 70-gene signature, *80-GS* 80-gene signature *Ki67* proliferation marker protein. The overall concordance between Ki67 and the 70-GS was 65%. Kappa 0.20 95% CI 0.048 – 0.35. Using a 14% Ki67 cut off value, 6 patients could not be stratified into Ki67 'low' or 'high' based on their Ki67 percentage and were excluded from the current analysis.



**Supplementary Materials:** The following are available online at www.mdpi.com/xxx/s1, Table S1: Comparison of Luminal A and Luminal B type tumors with Ki67 versus gene-signatures. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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# Chapter 3

## Assessment of tumour proliferation by use of the mitotic activity index, and Ki67 and phosphohistone H3 expression, in early-stage luminal breast cancer

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## ABSTRACT

**Aim:** Phosphohistone H3 (PhH3) has been proposed as a novel proliferation marker in breast cancer. This study compares the interobserver agreement for assessment of the mitotic activity index (MAI), Ki67 expression, and PhH3 in a cohort of oestrogen receptor (ER)-positive breast cancer patients.

**Methods and results:** Tumour samples of 159 luminal breast cancer patients were collected. MAI and PhH3 scores were assessed by three breast cancer pathologists. Ki67 scores were assessed separately by two of the three pathologists. PhH3-positive cells were counted in an area of 2 $mm^2$, with a threshold of ≥13 positive cells being used to discriminate between low-proliferative and high-proliferative tumours. Ki67 expression was assessed with the global scoring method. Ki67 percentages of <20% were considered to be low. The intraclass correlation coefficient (ICC) and Cohen's $\kappa$ statistics were used to evaluate interobserver agreement. The impact on histological grading of replacing the MAI with PhH3 was assessed. Counting PhH3-positive cells was highly reproducible among all three observers (ICC of 0.86). The $\kappa$ scores for the categorical PhH3 count ($\kappa$ = 0.78, $\kappa$ = 0.68, and $\kappa$ = 0.80) reflected substantial agreement among all observers, whereas agreement for the MAI ($\kappa$ = 0.38, $\kappa$ = 0.52, and $\kappa$ = 0.26) and Ki67 ($\kappa$ = 0.55) was fair to moderate. When PhH3 was used to determine the histological grade, agreement in grading increased (PhH3, $\kappa$ = 0.52, $\kappa$ = 0.48, and $\kappa$ = 0.52; MAI, $\kappa$ = 0.43, $\kappa$ = 0.35, and $\kappa$ = 0.32), and the proportion of grade III tumours increased (14%, 18%, and 27%).

**Conclusion:** PhH3 seems to outperform Ki67 and the MAI as a reproducible means to measure tumour proliferation in luminal-type breast cancer. Variation in the assessment of histological grade might be reduced by using PhH3, but would result in an increase in the proportion of high-grade cancers.

## INTRODUCTION

Histological tumour grade is one of the most robust prognostic factors in breast cancer [1-5]. The modified Bloom and Richardson (BR) Nottingham grading system, which has been globally incorporated in breast cancer guidelines [6], reflects three features, i.e. nuclear polymorphism, tubular formation, and mitotic count, the last of which reflects tumour proliferation. By the assignment of a score to each of these features, tumours are divided into three categories. Category 1 contains the well-differentiated tumours with an inherently good prognosis, and category 3 contains the poorly differentiated tumours [1-5].

Assessment of histological grade is applied worldwide, and adds important prognostic information to other clinicopathological features in order to guide systemic treatment decisions. Patients with grade 3 tumours are often candidates for treatment with adjuvant chemotherapy, whereas those with grade I tumours are candidates for less toxic hormonal therapy [6]. A substantial proportion (30–60%) of patients are diagnosed with grade 2 tumours, and in these patients the indication for adjuvant systemic treatment is less clear. Especially in this category of patients, high interobserver grading variability and institutional inconsistencies have been reported [7-9].



Over time, determination of the roles of individual genes in breast cancer dissemination have increased our knowledge. Although studies have revealed an important role for tumour proliferation-related genes [10-12], the functional end result remains cell division. The latter is detectable for the examining pathologist as mitotic figures showing a typical appearance of chromosome sets. Assessment of mitotic figures, expressed as the mitotic activity index (MAI), is the oldest method of evaluating tumour proliferation and an important component of histological grade. The MAI has shown to be an important independent prognostic factor [13,14], but its reproducibility remains limited [15-17].

Tumour proliferation can also be determined immunohistochemically by staining for the proliferation-related antigen Ki67. Several studies have demonstrated prognostic significance of assessing Ki67 in invasive breast cancer [18,19], but variation in the methodology of this assay has limited its adoption in clinical practice [20-24].

Phosphohistone H3 (PhH3) has been proposed as a novel proliferation marker. This protein is involved in chromatin condensation and decondensation, and is present in the active phases of the cell cycle ($G_2$ to M transition). Unlike Ki67 assessment, PhH3 assessment is performed according to a standardised protocol, similar to that used for traditional mitosis counting. The contrast-rich PhH3 staining enhances the recognition of mitotic figures, and the scoring resembles assessment of the MAI. PhH3 has been shown to have prognostic value in lymph node-negative breast cancer patients [25], but studies regarding the reproducibility of PhH3 assessment in breast cancer are scarce. In the present study,

we aimed to compare the interobserver agreement for assessment of the MAI, Ki67 and PhH3 in a cohort of oestrogen receptor (ER)-positive breast cancer patients. Furthermore, the impact of replacing the MAI with PhH3 to determine histological grade was assessed.

## MATERIALS AND METHODS

### PATIENTS

As part of a prospective observational multicentre study regarding the influence of the 70-gene signature on adjuvant chemotherapy decision-making in patients treated for ER+ early-stage (i.e. absence of distant metastasis) invasive ductal breast cancer, tumour samples were obtained between 1 January 2013 and 31 December 2015. The study was approved by the medical ethics committee of the University Medical Centre Utrecht (12-450) and by the institutional review boards of participating centres. Patients enrolled in this study were asked for their consent to use their tumour samples for future research. The current side-study was conducted according to the principles of Human Tissue and Medical Research: Code of conduct for responsible use (2011). For the present study, tissue samples of 159 patients were randomly retrieved from seven of the 31 participating centres.

### CLINICOPATHOLOGICAL INFORMATION

Clinicopathological data were obtained from the study database: patient age, tumour size, grade (based on nuclear polymorphism, tubular formation, and mitotic count), histological subtype, lymph node involvement, and ER, progesterone receptor (PR) and HER2 status.

### PATHOLOGICAL EXAMINATION

Pathological ER, PR and HER2 assessments had been routinely performed on all tumour samples ($n$ = 159). Immunohistochemistry and fluorescence *in-situ* hybridisation (FISH) were performed according to local standards at each institution. According to the Dutch guideline [26], positive ER or PR identification was defined as the presence of nuclear staining in ≥10% of breast cancer cells. Immunohistochemical expression of HER2 was scored as follows: 0 as <10% of tumour cells staining positively; 1+ as >10% of tumour cells staining positively, but no circumferential staining being present; 2+ as >10% of tumour cells showing weak or moderate circumferential staining; and 3+ as >10% of tumour cells showing strong circumferential staining. Scores of 0 and 1+ were considered to indicate a negative result, 2+ an equivocal result, and 3+ a positive result. HER2 2+ scores were re-evaluated with FISH.

Tissue samples were assessed for the MAI by three dedicated breast cancer pathologists, employed in different institutions, who were blinded to the clinicopathological data, according to the protocol guidelines of Van Diest and Baak [27]. One pathologist (observer 2) assessed the MAI in 106 of 159 included patients, whereas the other two pathologists (observers 1 and

3) assessed the MAI in all 159 patients. MAI was categorised on the basis of the total number of mitotic figures in an area of 2 $mm^2$, as follows: 0–7 = 1, 8–12 = 2, and ≥13 = 3. Whole tumour tissue sections of the 159 patients were immunohistochemically stained for PhH3 (clone BC37, 1:250; Biocare, CA, USA). The PhH3-based mitotic count was scored by the same observers. As for traditional mitosis counting, the area of highest proliferation, preferably at the periphery of the tumour, was identified to assess the PhH3 mitotic count. PhH3-positive objects, usually with mitosis morphology, were counted in an area of 2 $mm^2$, whereas intact nuclei with fine granular PhH3 staining were not counted, as these cells were regarded as not being in the $G_2$/M phase (Figure 1) [28]. The previously reported PhH3 threshold of 13 positive cells was used to discriminate between patients with a high or a low number of PhH3-positive cells, as this cut-off value was associated with 20-year recurrence-free survival rates for patients with distant metastases of 58% and 96%, respectively [25].



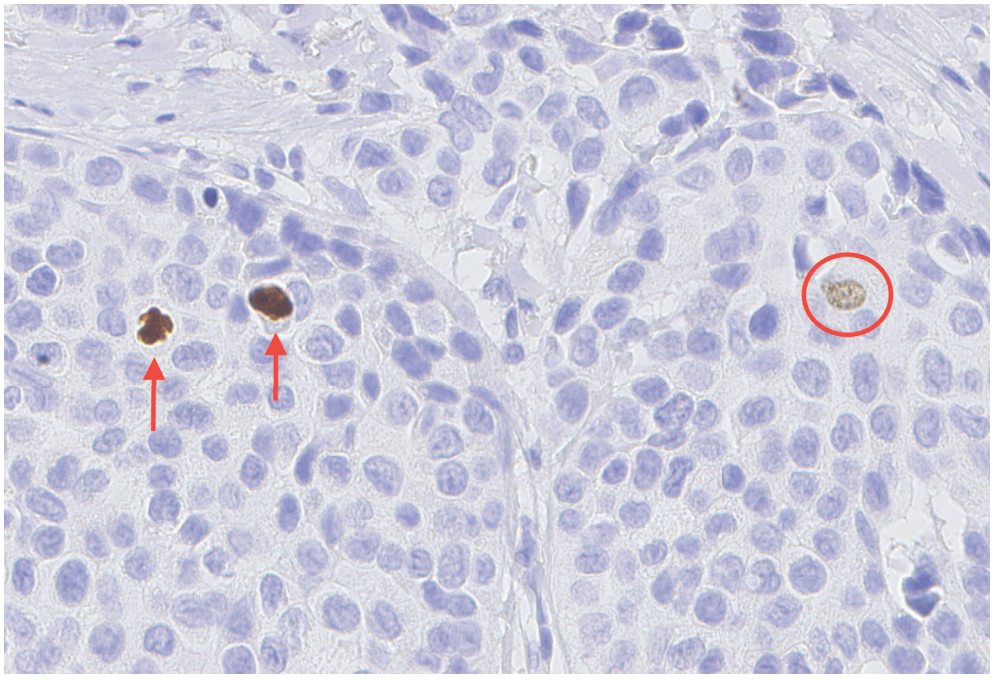

**Figure 1.** Microscopic image of phosphohistone H3 (PhH3) staining. True mitoses (arrows) are highlighted by the PhH3 immunostain. Intact nuclei with fine granular PhH3 staining (circle) were not counted, as these cells were regarded as not being in $G_2$/M phase.

In a non-selected subset of 105 patients, tumour tissue was additionally stained for Ki67 in one laboratory (Mib-1 antibody, ready-to-use; Dako, Denmark). Ki67 expression was assessed in 105 patients by observers 2 and 3, using the global scoring method. A cut-off value of 20% of nuclei positively stained for Ki67 was used to discriminate between high-proliferative and low-proliferative tumours, as previously established [29].

### STATISTICAL ANALYSES

Data were analysed with R, Version 3.2.2. The intraclass correlation coefficient (ICC), determined with the two-way random effects model for multiple raters [ICC with 95% confidence interval (CI)], was used to assess inter-rater agreement for numerical variables (PhH3, Ki67 and MAI score on a continuous scale), and Cohen's $\kappa$ was used to assess inter-rater reliability for categorical variables (PhH3, Ki67 and the MAI categorised on the basis of the aforementioned thresholds). Furthermore, we created an alternative histological grade by replacing the MAI-based mitotic count with the PhH3-based mitotic count as follows: 1 point for a PhH3 mitotic number of ≤7 per 2 $mm^2$; 2 points for a PhH3 mitotic number of 8–12 per 2 $mm^2$; and 3 points for a PhH3 mitotic number of ≥13 per 2 $mm^2$. This PhH3-based histological grade of PhH3 was compared with the traditional MAI-based grade by use of the chi-square test. Two reasonable scales for the interpretation of the ICC and Cohen's $\kappa$ are shown in Table S1 [30].

## RESULTS

### PATIENTS

In total, 159 early breast cancer patients with a median age of 57 years were included in this study. All patients had ER+ disease, 88% of patients had PR+ disease, and 98% of patients were HER2–. The majority of the patients had no axillary lymph node involvement (87%) (Table 1).

On the basis of the original pathology assessment, 16% of patients had low-grade (I) cancers and 67% of patients had intermediate-grade (II) tumours. For traditional mitosis counting, the median total number of mitotic figures were 2 [interquartile range (IQR) of 3], 3 (IQR of 6) and 5 (IQR of 8) for observers 1, 2 and 3, respectively, resulting in a MAI score of 1 in 84%, 70% and 60% of patients.

The median total PhH3 scores were 10 (IQR of 18), 8 (IQR of 12) and 9 (IQR of 16) for observers 1, 2, and 3, respectively. The percentages of low-proliferative tumours based on the PhH3 mitotic count (<13 points per 2 $mm^2$) were 60% (observer 1), 62% (observer 2), and 63% (observer 3) (Table 2). Median numbers of total nuclei positively stained for Ki67 were 5 (IQR of 5) and 2 (IQR of 3) for observers 2 and 3, respectively. The percentages of low-proliferative tumours based on the Ki67 score (<20% of positively stained nuclei) were 81% and 89%, respectively (Table 2).

**Table 1.** Clinical and pathological characteristics of oestrogen receptor-positive breast cancer patients included in the study (*n* = 159)

| Characteristic | Value |
|---|---|
| **Age (years), median (minimum–maximum)** | 57 (33–70) |
| **Progesterone receptor status, *n* (%)** | |
| Negative | 19 (12) |
| Positive | 140 (88) |
| **HER2 status, *n* (%)** | |
| Negative | 156 (98) |
| Positive | 2 (2) |
| **Grade, *n* (%)** | |
| 1 | 26 (16) |
| 2 | 106 (67) |
| 3 | 27 (17) |
| **Histological tumour type, *n* (%)** | |
| Invasive ductal breast cancer | 159 (100) |
| **Unifocal tumour, *n* (%)** | |
| No | 9 (6) |
| Yes | 150 (94) |
| **Tumour diameter (mm), median (minimum–maximum)** | 15 (5–35) |
| **T stage, *n* (%)** | |
| T1 | 132 (83) |
| T2 | 27 (17) |
| **N stage, *n* (%)** | |
| N0 | 138 (87) |
| Nmi | 13 (8) |
| N1a | 6 (4) |
| Unknown | 2 (1) |
| **Type of surgery, *n* (%)** | |
| Lumpectomy | 134 (84) |
| Mastectomy | 25 (16) |

**Table 2.** Mitotic activity index, phosphohistone H3 (PhH3) scores and Ki67 percentages assessed by three different breast cancer pathologists

| | Observer 1 (*n* = 159) | Observer 2 (*n* = 106) | Observer 3 (*n* = 159) |
|---|---|---|---|
| **Mitotic activity index** | | | |
| 1 (0–7 mitotic figures) per 2 $mm^2$ | 134 | 74 | 94 |
| 2 (8–12 mitotic figures) per 2 $mm^2$ | 13 | 19 | 28 |
| 3 (≥13 mitotic figures) per 2 $mm^2$ | 12 | 13 | 37 |
| **PhH3 score** | | | |
| <13 positively stained cells per 2 $mm^2$ | 95 | 66 | 100 |
| ≥13 positively stained cells per 2 $mm^2$ | 64 | 40 | 59 |
| **Ki67 percentage** | | | |
| <20% positively stained cells | – | 85 | 93 |
| ≥20% positively stained cells | – | 20 | 12 |
| Not assessed | 159 | 1 | 54 |

PhH3, phosphohistone H3.

## AGREEMENT OF CONTINUOUS PHH3, MAI AND KI67 SCORES

The ICCs of the PhH3 mitotic count on a continuous scale for observer 1 versus 2, observer 1 versus 3 and observer 2 versus 3 were 0.79 (95% CI 0.67–0.86), 0.97 (95% CI 0.96–0.98) and 0.76 (95% CI 0.72–0.86), respectively (Figure 2A–C). Interobserver agreement for PhH3 among all three pathologists reflected almost perfect agreement (ICC of 0.86, 95% CI 0.80–0.89). The ICCs for the total mitotic figure count were lower than those for PhH3: 0.62, 95% CI 0.48–0.72 (observer 1 versus observer 2), 0.41, 95% CI 0.16–0.60 (observer 1 versus observer 3), and 0.61, 95% CI 0.39–0.75 (observer 2 versus observer 3) (Figure 2D–F). The ICC for the total mitotic figure count among all three pathologists was 0.57 (95% CI 0.41–0.69). The ICC for Ki67 for the two pathologists who assessed Ki67 was 0.64 (95% CI 0.39–0.78) (observer 1 versus observer 2).

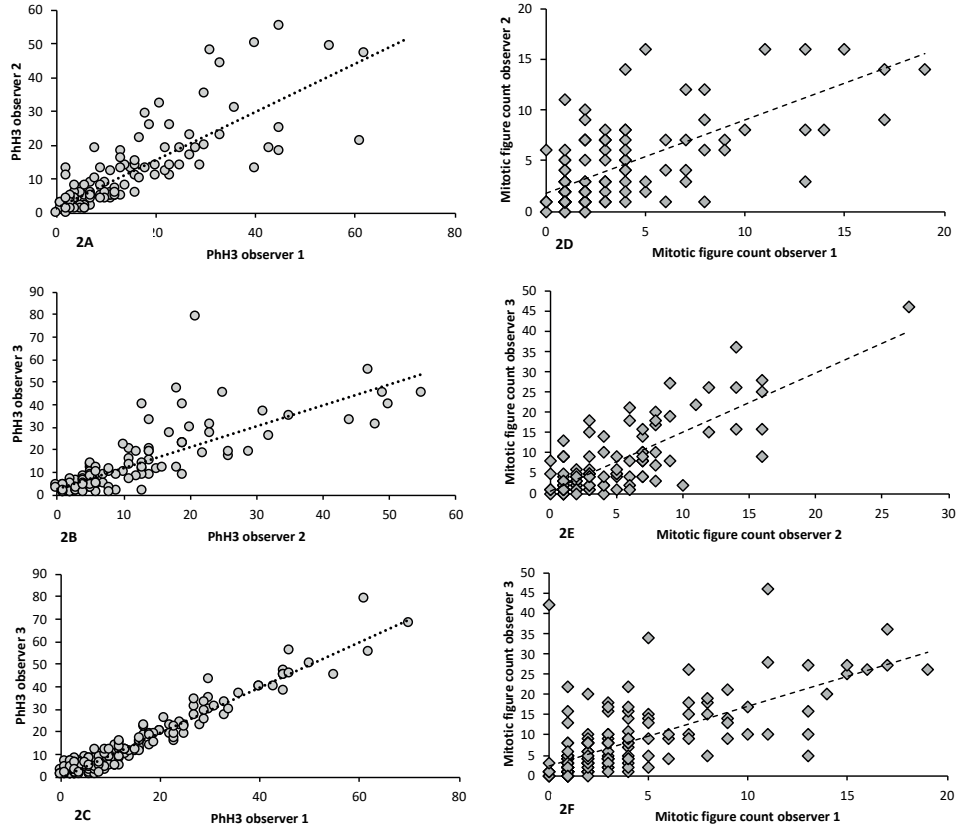


**Figure 2. A–C,** Interobserver agreement of the total phosphohistone H3 (PhH3) score for observers 1 and 2 (**A**), observers 2 and 3 (**B**), and observers 1 and 3 (**C**). **D–F,** Total mitotic figure count assessed in an area of 2 $mm^2$ by observers 1 and 2 (**D**), observers 2 and 3 (**E**), and observers 1 and 3 (**F**). **A,** PhH3 intraclass correlation coefficient (ICC) score of 0.79 [95% confidence interval (CI) 0.67–0.86]. **B,** PhH3 ICC score of 0.76 (95% CI 0.72–0.86). **C,** PhH3 ICC score of 0.97 (95% CI 0.96–0.98). **D,** Mitotic count ICC of 0.62 (95% CI 0.48–0.72). **E,** Mitotic count ICC of 0.61 (95% CI 0.39–0.75).**F,** Mitotic count ICC of 0.42 (95% CI 0.16–0.60).

## AGREEMENT OF CATEGORICAL PHH3, MAI AND KI67 SCORES

The $\kappa$ scores for the categorical PhH3 score ($\kappa$ = 0.78 for observer 1 versus observer 2, $\kappa$ = 0.80 for observer 1 versus observer 3, $\kappa$ = 0.68 for observer 2 versus observer 3; Table S2) reflected substantial agreement between the three observers. Interobserver agreement for Ki67 and the MAI was only fair to moderate: Ki6, $\kappa$ = 0.55 (observer 1 versus observer 2); and MAI, $\kappa$ = 0.38 (observer 1 versus observer 2), $\kappa$ = 0.26 (observer 1 versus observer 3), and $\kappa$ = 0.52 (observer 2 versus observer 3), respectively (Tables S3 and S4).

## ASSESSMENT OF HISTOLOGICAL GRADE BASED ON THE MAI VERSUS GRADE BASED ON PHH3

When PhH3 was used in the modified BR Nottingham grading score instead of the MAI, interobserver agreement in determining histological grade improved (MAI, $\kappa$ = 0.43, $\kappa$ = 0.35, and $\kappa$ = 0.32; PhH3, $\kappa$ = 0.52, $\kappa$ = 0.48, and $\kappa$ = 0.52). At the same time, when the grading score was re-evaluated on the basis of PhH3 assessment, it shifted from grade I to grade II in 8% (observer 1), 12% (observer 2) and 4% (observer 3) of the patients, and from grade II to III in 27% (observer 1), 18% (observer 2) and 14% (observer 3) of the patients ($P$ < 0.001) (Table 3). Among all three observers, there were a few patients who were downgraded from grade II to grade I ($n$ = 1, $n$ = 2 and $n$ = 3 for observers 1, 2 and 3, respectively) or downgraded from grade III to grade II ($n$ = 1, $n$ = 2 and $n$ = 6 for observers 1, 2, and 3, respectively) (Table 3). The majority of the patients who were upgraded from grade II to grade III had a PhH3 score of ≥13 (86%, 95% and 69% for observers 1, 2 and 3, respectively), whereas a substantial proportion in whom the histological grade was shifted from grade I to II had a PhH3 score of <13 (31%, 43% and 83% for observers 1, 2 and 3, respectively).

**Table 3**. Impact of replacing the mitotic activity index with phosphohistone H3 on the modified Bloom–Richardson Nottingham grade score

| Change in histological grading score | Observer 1 (%) | Observer 2 (%) | Observer 3 (%) |
|---|---|---|---|
| Upgraded from grade I to grade II | 8 | 12 | 4 |
| Upgraded from grade I to grade III | – | 1 | – |
| Upgraded from grade II to grade III | 27 | 18 | 14 |
| Downgraded from grade II to grade I | 1 | 2 | 2 |
| Downgraded from grade III to grade II | 1 | 2 | 4 |

## DISCUSSION

In this study, the reproducibility of three different proliferation-related variables that contribute to the assessment of tumour grade was compared in patients with luminal-type breast cancer. Our results demonstrate that PhH3-based mitotic counting provides a more reproducible means for observing tumour proliferation in ER+ early breast cancers than MAI or Ki67 assessment. Incorporating PhH3 as an alternative to the traditional MAI in the BR Nottingham grading system would decrease the variation in histological grading, but would increase the proportion of cancers that would be considered to be high-grade tumours.

Assessment of mitotic activity is routinely performed as part of determining histological tumour grade, and has been established as an independent prognostic factor [31-34]. The reproducibility of the MAI is limited [15-17]. This may in part be attributable to a lack of strict protocols, and to difficulties in selecting the mitotically most active area [16-17], but it may also result from the coexistence of cells that mimic mitosis, such as apoptotic and necrotic cells, especially in cases of poor fixation [35]. Optimal assessment of mitotic activity requires the experience of trained pathologists and dedication, as this may take ~10 min [36].

PhH3 showed better interobserver agreement in the present study than did the MAI, supported by higher ICC and Cohen's $\kappa$ scores. PhH3 is a proliferation marker that is specific for mitosis, as it is expressed from the late $G_2$ phase to M transition, and rapidly degrades on entry into the $G_1$ phase [37]. Therefore, PhH3 labelling has been reported to closely correlate with mitotic figure detection on standard haematoxylin and eosin (H&E)-stained sections [38,39]. As compared with the MAI, PhH3 is relatively easy to assess, as its bright staining offers easy visualisation of mitotic figures by morphology, resulting in a high accuracy of detection. The results of our study showed that PhH3 revealed higher numbers of mitotic cells than did H&E staining, which is in line with previous literature [28,40]. This difference in sensitivity may be explained by the fact that prophase figures are not well recognised with regular H&E stains, but can be easily identified in PhH3-stained specimens [28]. Because of the sharp contrast with non-stained elements, PhH3 allows rapid detection of the mitotically most active area [40]. A previous study demonstrated that PhH3 staining was particularly useful in detecting mitotic cells in high-grade cancers with dense cellularity and with numerous apoptotic and necrotic cells [28]. In addition, PhH3 assessment may serve as a better means to assess proliferative activity in core needle biopsies, as PhH3 labelling was found to be more accurate at identifying mitotic figures than routine H&E staining [41]. In the light of these advantages, it is conceivable that PhH3 staining results in a higher accuracy of mitotic figure detection, even in specimens with poor fixation, or specimens that contain dense, distorted tumour infiltrate or crush artefacts. Then again, others have shown that antigenicity for PhH3 can be lost if tissue is

not immediately fixed after sampling [42]. Hence, fixation delay should be kept as short as possible.

In addition to the conventional factors, immunohistochemical assessment of the proportion of cells staining for the nuclear antigen Ki67 is used for determination of tumour proliferation. Many studies have demonstrated the prognostic value of Ki67 [43]. However, the clinical utility of this marker has been disputed because of poor reproducibility, which is also reflected by the results of the present study. Flaws in Ki67 assessment are attributed to a lack of scoring consensus among experts and an undefined cut-off point for clinical decision-making. In an effort to harmonise the analytical methodology of Ki67, the International Ki67 Breast Cancer Working Group proposed a set of guidelines for the analysis and reporting of Ki67 [44]. However, even after standardisation, the assessment of Ki67 among some of the world's most experienced laboratories turned out to be poor [45]. Although interlaboratory variability in staining methods contributed to differences in Ki67 scoring, the working group also observed substantial discrepancies in Ki67 interpretation when the staining was performed centrally. These results are in line with those of another study reporting high interobserver variability in Ki67 assessment among 15 pathologists [46]. The Ki67 working group stated that 'unless an individual pathology laboratory has demonstrated that its staining and scoring methodology, including cut-off determination, meet the highest level of evidence for clinical utility, clinicals should use Ki67 results with caution [45].



As PhH3 assessment is also based on immunohistochemistry, one may wonder to what extent PhH3 assessment suffers from similar limitations. In contrast to the variability in Ki67 scoring methods, PhH3 assessment is performed according to a standardised protocol similar to that used for traditional mitosis counting. Furthermore, PhH3-positive cells can be unambiguously identified, even at low-power magnification and by inexperienced observers [40]. Finally, there is less debate regarding cut-off values for PhH3 assessment.

In the present study, the use of PhH3 instead of the MAI to determine the modified BR histological grade resulted in the histological grade being upgraded in 14–27% of cases. This increase in the proportion of patients with high-grade tumours is in line with other studies [25,28,38,48]. PhH3 was shown to have independent prognostic value, which exceeded the prognostic value of the MAI [hazard ratio (HR) of 9.6 versus HR of 3.6] [49]. These findings support the concept of replacing the MAI with PhH3 in order to improve the prognostic value of histological grading through better identification of mitotic figures. At the same time, PhH3-based mitotic indices should be evaluated in larger studies before their use in clinical practice can be recommended.

To our knowledge, this study has provided a unique comparison between the reproducibility of traditional proliferation markers and that of the novel proliferation marker PhH3. Interobserver agreement was reliable, as the pathology examination was performed by three dedicated breast cancer pathologists, working in different institutions. It is important to note that we performed this study in a selection of ER+ cancers, and this should be taken into consideration when the results are interpreted. However, optimisation of the assessment of tumour proliferation is especially needed in this subset of patients, as the patient group was a selected group in whom genomic profiling was undertaken to decide on adjuvant chemotherapy. It is important to note that the prognostic value of the different proliferation markers was not addressed in the present study, as follow-up data were not available, and the follow-up period would have been too short. In due course, outcome data will become available, and these will enable us to also further evaluate PhH3 assessment in terms of prognostication. We also aim to explore deep-learning algorithms to automatically identify PhH3-positive objects, as has successfully been performed before for mitoses in H&E-stained and PhH3-stained sections [50,51].

In conclusion, our results demonstrate that PhH3 is a more reproducible proliferation marker in breast cancer than are the MAI and Ki67. The association between PhH3 and outcome, and the potential increase in the proportion of high-grade cancers when PhH3 is used, need to be further addressed.

## SUPPORTING INFORMATION

**Table S1.** Interpretation of intraclass correlation coefficient (ICC) and Cohen's $\kappa$ score.

| ICC | Interpretation of ICC |
|---|---|
| <0.5 | Poor agreement |
| 0.5-0.74 | Moderate agreement |
| 0.75-0.9 | Good agreement |
| >0.9 | Excellent agreement |

| Kappa | Interpretation of Cohen's Kappa score |
|---|---|
| < 0 | Poor agreement |
| 0.01-0.20 | Slight agreement |
| 0.21-0.40 | Fair agreement |
| 0.41-0.60 | Moderate agreement |
| 0.61-0.80 | Substantial agreement |
| 0.81-1.00 | Almost perfect agreement |

**Table S2.** Concordance of PhH3 scored by three different breast cancer pathologists

| | Obs. 2 | | Obs. 3 | | | Obs. 3 | |
|---|---|---|---|---|---|---|---|
| **Obs. 1** | **PhH3<13** | **PhH3 ≥13** | **PhH3<13** | **PhH3≥13** | **Obs. 2** | **PhH3<13** | **PhH3≥13** |
| **PhH3<13** | 58 | 3 | 90 | 5 | **PhH3<13** | 59 | 7 |
| **PhH3 ≥13** | 8 | 37 | 10 | 54 | **PhH3 ≥13** | 9 | 31 |

The overall concordance of PhH3 for observer 1 vs. 2, 1 vs. 3 and 2 vs. 3 were 90% (κ 0.78), 91% (κ 0.80) and 85% (κ 0.68), respectively. Abbreviations: obs, observer, PhH3, phosphohistone H3

**Table S3.** Concordance of MAI classes scored by three different breast cancer pathologists.

| | Obs. 2 | | | Obs. 3 | | | | Obs. 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **MAI** | **MAI** | **MAI** | **MAI** | **MAI** | | | **MAI** | **MAI** | **MAI** |
| **Obs. 1** | **0-7** | **8-12** | **≥13** | **0-7** | **8-12** | **MAI ≥13** | **Obs. 2** | **0-7** | **8-12** | **≥13** |
| **MAI** | | | | | | | **MAI** | | | |
| **0-7** | 71 | 11 | 6 | 92 | 23 | 19 | **0-7** | 62 | 8 | 4 |
| **MAI** | | | | | | | **MAI** | | | |
| **8-12** | 1 | 5 | 3 | 1 | 3 | 9 | **8-12** | 2 | 8 | 9 |
| **MAI** | | | | | | | **MAI** | | | |
| **≥13** | 2 | 3 | 4 | 1 | 2 | 9 | **≥13** | 2 | 1 | 10 |

The overall concordance of MAI for observer 1 vs. 2, observer 1 vs. 3 and observer 2 vs. 3 were 75% κ 0.38 95% CI 0.10-0.42, 65% κ 0.26 95% CI 0.10-0.42 and 75% k 0.52 95% CI 0.36-0.68 respectively. Abbreviations: obs, observer, MAI, Mitotic Activity Index

**Table S4.** Concordance of Ki67 scored by two different breast cancer pathologists.

| | Obs. 3 | |
|---|---|---|
| **Obs. 2** | **Ki67 <20%** | **Ki67 ≥20%** |
| **Ki67 <20%** | 83 | 2 |
| **Ki67 ≥20%** | 10 | 10 |

The overall concordance of Ki67 for observer 1 vs. 2 was 88% k 0.52 95% CI 0.30-0.74

## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest. No funding was received for this study.

## AUTHOR CONTRIBUTIONS

All persons listed as authors were actively involved in one or more key aspects of the reported study. J. E. C. van Steenhoven: conception and design, analysis and interpretation of data, drafting of the article, and final approval. A. Kuijer: conception and design, acquisition of data, analysis and interpretation of data, critical revision, and final approval. R. Kornegoor: interpretation of data, critical revision, and final approval. A. M. van Leeuwen: acquisition of data, critical revision, and final approval. J. van Gorp: acquisition of data, interpretation of data, critical revision, and final approval. T. van Dalen: conception and design, interpretation of data, drafting of the article, and final approval. P. J. van Diest: acquisition of data, interpretation of data, critical revision, and final approval.

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# Chapter 4

## The changing role of gene-expression profiling in the era of de-escalating adjuvant chemotherapy in early stage breast cancer

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## ABSTRACT

**Purpose:** We assessed recent trends in the administration of adjuvant chemotherapy thereby evaluating the role of the 70-gene signature (70-GS) testing in decision-making in the systemic treatment of lymph node negative (N0) and lymph node positive (N+) breast cancer patients.

**Methods:** Patients with a national guideline directed indication for 70-GS use treated between 2013-2016 were selected from the Netherlands Cancer Registry. Time trends in the administration of adjuvant chemotherapy were evaluated within guideline- and age delineated subgroups. The influence of the 70-GS on chemotherapy use was assessed with logistic regression.

**Results:** During the study period, the overall administration of adjuvant chemotherapy decreased from 49% to 23% and 70-GS use increased from 24% to 51%. The 70-GS was not associated with a decreased likelihood for N0 patients to receive chemotherapy (OR 1.0 95%CI 0.86-1.17), as the proportion of N0 patients who received chemotherapy in the absence of 70-GS use decreased during the study period. In patients with N1a disease, 70-GS testing was associated with a decreased likelihood to receive chemotherapy (OR 0.21 95% CI: 0.15-0.29). In patients <50 years and 50-59 years of age, 70-GS use was associated with a consistent lower proportion of patients receiving chemotherapy throughout the study period (OR 0.17, 95% CI: 0.13-0.23 and OR 0.53 95% CI: 0.43-0.65, respectively).

**Conclusion:** In this population-based study, the administration of adjuvant chemotherapy in ER+ breast cancer strongly declined. For node-positive and younger patients, 70-GS use was associated with a decreased probability for patients to receive adjuvant chemotherapy.

## INTRODUCTION

Over the past two decades, the importance of tumor biology in relation to breast cancer outcome and the varying beneficial effect of adjuvant chemotherapy for the molecular cancer subtypes are increasingly recognized [1,2]. Particularly in patients with estrogen receptor (ER)-positive (+) breast cancer, the routine use of adjuvant chemotherapy has been questioned in recent years [3,4]. Gene-expression profiles (GEPs) were developed and validated for outcome prediction in ER+ early-stage breast cancer patients, and its use has been incorporated in both national and international breast cancer guidelines [5-12].

The Dutch guideline of 2012 recommended to administer adjuvant chemotherapy in all lymph node-positive patients and in lymph node-negative patients with unfavorable clinicopathological characteristics (i.e., T2 grade I or T1c grade II tumors, and all grade III tumors) [13]. However, the same national guideline also suggested to consider the use of a validated GEP in patients with ER+/Her2− tumors of low or intermediate malignancy grade with no or limited metastatic lymph node involvement. In 2015, the St. Gallen expert panel was the first to reconsider the routine administration of chemotherapy in "Luminal A-like" breast cancer (i.e., HR+, Her2−, Ki-67 low or gene signature low risk), thereby questioning mere tumor size and involvement of one to three lymph nodes as criteria to warrant chemotherapy administration [3].



In a previous nationwide study, we demonstrated that the use of the 70-GS was associated with a significant reduction of chemotherapy administration in the subset of ER+/Her2− disease without overt lymph-node metastasis (≤ Nmi) treated between 2011 and 2013 [14]. Recent randomized trials studying the contribution of GEPs to the decision to administer adjuvant chemotherapy also suggest a role for GEPs in lymph node-positive patients [8,15].

In the present study, we describe the time trends in chemotherapy use in a large population-based cohort of ER+/Her2− breast cancer patients considered eligible for GEP use according to national guidelines, encompassing the period of time since the Dutch breast cancer guideline first suggested a role for GEP use until the period that the results of the GEP trials were available. Furthermore, the use and impact of the 70-GS on chemotherapy administration was evaluated in different subgroups delineated by lymph node status, grade, tumor size, and age.

## PATIENTS AND METHODS

Data on patient, tumor, and treatment characteristics were derived from the Netherlands Cancer Registry (NCR). All Dutch female patients (> 17 years) surgically treated for primary unilateral invasive ductal breast cancer between January 2013 and December 2016 were identified in the NCR database. Patients with a prior history of malignancy or those who received neoadjuvant chemotherapy, were excluded from the analysis.

During the study period, the national guideline of 2012 was effective. According to this guideline, adjuvant chemotherapy should be administered to all patients with lymph node positive disease (≥ N1a) and to patients without lymph node involvement but with unfavorable clinicopathological features (all grade III tumors, grade II tumors > 1 cm, any tumor > 2 cm, or Her2+ tumors), as well as in patients of young age (< 35 years). This guideline also suggested the use of validated GEP in ER+ breast cancer, when there is doubt about the indication for adjuvant chemotherapy based on traditional clinicopathological risk factors [13]. Patients with grade III tumors were not included, because these patients were not considered candidates for GEP use. Although the 70-GS and OncotypeDx are both commercially available in the Netherlands, OncotypeDx was rarely used during the study period [16]. We therefore focused on the use and impact of 70-GS only.

We delineated four groups of patients < 70 years of age, suffering from ER+/Her2− invasive ductal breast cancer, who were considered eligible for GEP use based on the aforementioned guideline criteria. In addition, we included patients with macro-metastatic lymph involvement based on the more recently suggested role of GEP use in this subset of patients. The following four groups were composed: group A (pN0; grade I; > 2 cm), group B (pN0; grade II; > 1 cm), group C (pNmi, grade I/II, any size), and group D (pN1a, grade I/II, any size).

### STATISTICAL ANALYSIS

Frequencies of patient and tumor characteristics of patients eligible for GEP use (i.e., clinical intermediate risk) were compared between patients who received the 70-GS versus patients who did not receive the test, using a $\chi^2$ test for differences in categorical data. For normally distributed continuous variables (age and size), means were calculated and a *t* test was performed. For the whole group, the proportions of patients who received adjuvant chemotherapy, irrespective of GEP use, were calculated for the years 2013–2016. For the defined subgroups A–D, the proportions of patients in whom the 70-GS was applied were calculated and observed over time. Adherence to the test result in terms of the administration of chemotherapy in the overall study population and the aforementioned subgroups (A–D) was calculated by dividing the sum of patients with a low-risk test result in whom adjuvant chemotherapy was omitted and patients with a high-risk test result who received adjuvant chemotherapy by all patients with a known test

result. The differences in chemotherapy administration between patients who received the 70-GS versus patients who did not receive the test were evaluated using a $\chi^2$ test or Fisher's exact test when the proportions of patients within this category were small.

In addition, we investigated the association between 70-GS use on the administration of adjuvant chemotherapy within three different age categories (< 50 years, 50–59 years, and 60–69 years) using a $\chi^2$ test. Subsequently, logistic regression analysis was performed within the different guideline and age delineated subgroups to assess whether GEP use was independently associated with the administration of adjuvant chemotherapy after correction for clinicopathological confounders (age, grade, tumor size, N-status, PR status) and incidence year. Results are presented as odds ratios (OR) and accompanying 95% confidence intervals (95% CI). All tests were two-sided, and *P* value < 0.05 was considered to be statistically significant. All statistical analyses were performed in R (Version 3.2.1).



## RESULTS

### STUDY POPULATION

A total of 6780 breast cancer patients treated between 2013 and 2016 who were eligible for GEP could be identified in the NCR, of whom 281 patients (4%) were assigned to group A (BR I, > 2 cm, N0), 3571 patients (53%) to group B (BR II, > 1 cm, N0), 1040 patients (15%) to group C (BR I/II, any size, Nmi), and 1888 of patients (28%) to group D (BR I/II, any size, N1a). Chemotherapy was administered in 40% of all patients and decreased during the study period: in 2013, 49% of patients within the delineated indication area for GEP use received adjuvant chemotherapy versus 23% of patients in 2016 (Fig. 1).

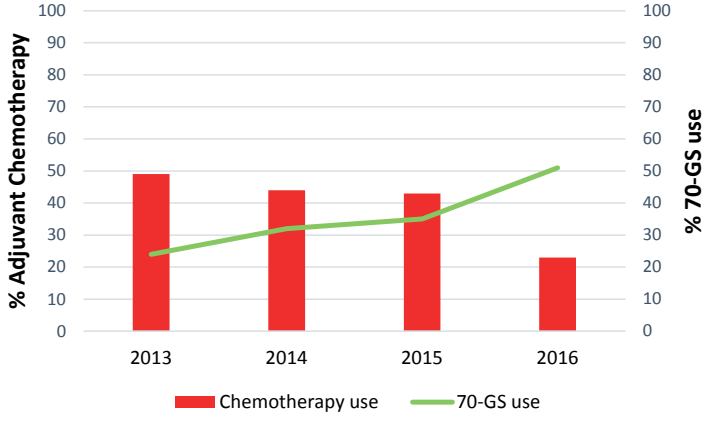



**FIG 1** Time trend in the administration of adjuvant chemotherapy in the total study population eligible for gene-expression profiling and the use of the 70-gene signature

The 2399 patients (35%) who received a 70-GS, were slightly younger, had smaller tumors, and tumors of intermediate grade and less often positive lymph nodes compared with their counterparts in whom no 70-GS was used (Table 1). The use of the 70-GS increased from 24% of the eligible patients in 2013 to 51% of patients eligible for GEP use in 2016. This rising trend in 70-GS use was observed within all subgroups (A-D), but the clinically most significant increase in 70-GS use was seen in subgroup D (N1a patients): from 6% in 2013 to 50% of patients in 2016 (Fig. 2). The majority of patients who received the 70-GS were assigned to the 70-GS low-risk category: 68% in the whole group of patients and 85, 65, 73, and 73% in subgroups A, B, C, and D, respectively. The test result was adhered to in 91% of the overall study population: only in subgroup D compliance to the test was lower (85%; Supplementary Table 1).

**Table 1** Patient and tumor characteristics according to 70-gene signature use in 6780 patients within the indicated area for 70-GS use (all younger than 70 years of age with ER+/HER2- invasive ductal carcinoma)

| | **70-GS not used** ***n* = 4381 *n* (%)** | **70-GS used** ***n* =2399 *n* (%)** | ***P* value*** |
|---|---|---|---|
| *Patient characteristics* | | | |
| Age at diagnosis (year), mean | 57 | 56 | <0.001** |
| Age Categories | | | |
| <50 | 875 (20) | 558 (23) | <0.001 |
| 50-59 | 1453 (33) | 975 (41) | |
| 60-69 | 2053 (47) | 866 (36) | |
| Incidence year | | | |
| 2013 | 1365 (31) | 434 (18) | <0.001 |
| 2014 | 1151 (26) | 551 (23) | |
| 2015 | 1032 (24) | 545 (23) | |
| 2016 | 833 (19) | 869 (36) | |
| *Tumor characteristics* | | | |
| Pathological axillary status (pN) | | | |
| pN0 (i-,i+) | 2076 (47) | 1776 (74) | <0.001 |
| pNmi | 739 (17) | 301 (13) | |
| pN1a | 1566 (36) | 322 (13) | |
| Pathological tumor size (mm), mean | 18 | 17 | <0.001** |
| Tumor size categories | 2980 (68) | 1820 (76) | <0.001 |
| ≤2 cm | 1387 (32) | 573 (24) | |
| >2cm | 14 (0.3) | 6 (0.2) | |
| *NA* | | | |
| Invasive tumor grade | | | |
| Grade I | 987 (23) | 333 (14) | <0.001 |
| Grade II | 3394 (77) | 2066 (86) | |

70-GS; 70 gene-signature, CT; chemotherapy, BR; Bloom-Richardson grade, N0; no axillary lymph node involvement, Nmi; micro-metastasis, N1a: 1-3 ipsilateral positive axillary lymph nodes (at least one >2mm)
*$\chi^2$ test was used to compare frequencies in clinicopathological characteristics between patient who received the 70-GS (n=2399) versus patients who did not receive the test (n=4381)
** t-test to assess the difference in mean age and tumor size (continuous variables)

## CHEMOTHERAPY ADMINISTRATION AND 70-GS USE WITHIN GUIDELINE DELINEATED SUBGROUPS

For the whole study population, the proportion of patients who received adjuvant chemotherapy was lower when the 70-GS was used (30% vs. 46% of patients when the 70-GS was not used; $P < 0.001$). In a multivariable logistic regression analyses, 70-GS use remained associated with a decreased probability of administering adjuvant chemotherapy (odds ratio [OR] 0.65; 95% CI 0.57–0.73). For the whole study period, use of the 70-GS was associated with a nonsignificant decreased probability of administering chemotherapy in group A (OR 0.53; 95% CI 0.24–1.14, data not shown). In group B, the 70-GS was associated with a nonsignificant increased probability of receiving chemotherapy (OR 1.03; 95% CI 0.88–1.21, data not shown). For group C (Nmi) and group D (N1a), use of the 70-GS resulted in a significant decreased probability to receive chemotherapy (group C: 0.37, 95% CI 0.27–0.51, and group D: OR 0.21, 95% CI 0.15–0.29).



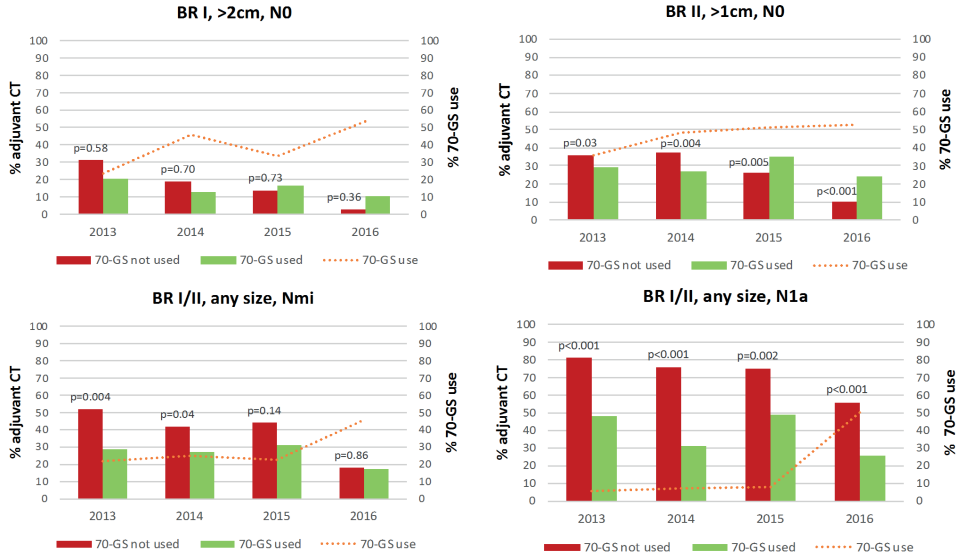



**FIG 2** Adjuvant chemotherapy use in patients who received the 70-gene signature versus patients who did not receive the 70-gene signature in different guideline delineated subgroups: group A (BR I, >2cm, N0) (n=218), group B (BR II, >1cm, N0) (n=3571), group C (BR I/II, any size, Nmi) (n=1040) and group D (BR I/II, any size, N1a) (n=1888). *P*-values were calculated using a $\chi^2$ test for differences in categorical data.

Different time trends for the interplay between 70-GS use and chemotherapy administration were observed for the four subgroups (Fig. 2a–d). In groups A and B, chemotherapy administration in the selection of patients in whom the 70-GS was deployed fluctuated during the study period within a limited range. Without using the 70-GS, the use of chemotherapy decreased over time and was rarely administered to N0 patients in 2016.

Hence, while the use of the 70-GS in N0 patients (group A and B together) was associated with the administration of less chemotherapy in 2013 and 2014, more chemotherapy was administered in the recent years. In group C (Nmi), 70-GS use was associated with less chemotherapy administration in 2013 and 2014, while in more recent years this association was no longer observed. In N1a patients, the lower proportion of patients receiving chemotherapy when the 70-GS was deployed was consistent throughout the study period.

## ADJUVANT CHEMOTHERAPY ADMINISTRATION AND 70-GS USE BY AGE CATEGORIES

Subgroup analyses in patients delineated by age demonstrated a significant decrease in the administration of adjuvant chemotherapy in patients < 50 years and 50–59 years of age who received the 70-GS versus patients who did not receive the 70-GS (Supplementary Fig. 1a–c). Without the use of the 70-GS the likelihood of administering chemotherapy decreased with age: 79, 55, and 26% of patients were treated with adjuvant chemotherapy in the < 50, 50–59, and 60–69 years group, respectively. In multivariable logistic regression analysis, 70-GS use in patients < 50 years and in the 50–59 years group was independently associated with a decreased chance of chemotherapy administration (OR 0.17, 95% CI 0.13–0.23 and OR 0.53, 95% CI 0.43–0.65). In the older age group (60–69 years), a reverse association was observed; the 70-GS was independently associated with an increased chance of chemotherapy administration (OR 1.76, 95% CI 1.41–2.19). Age was not associated with a higher proportion of patients being assigned to a risk category based on the 70-GS test result: 68, 70, and 65% of patients were classified as low risk in the < 50, 50–59, and 60–69 years age groups, respectively.

## DISCUSSION

In the present population-based study, in early-stage breast cancer patients who are considered candidates for GEP use, an increased use of the 70-GS was observed over time as well as a decrease in the administration of chemotherapy. For patients with lymph node positive disease and in younger patients, 70-GS use was associated with a consistent lower proportion of patients receiving adjuvant chemotherapy. In lymph node-negative patients, we observed a decrease in the use of chemotherapy over time, irrespective of 70-GS use.

The increased use of the 70-GS and the decrease in chemotherapy administration (from 49% of patients in 2013 to 23% in 2016) both demonstrate the growing restraint of Dutch clinicians to administer chemotherapy in the selection of patients identified as having luminal A-type breast cancers. The decline in chemotherapy administration coincides with recent international guideline recommendations [3]. In 2015, the St. Gallen international consensus meeting stated that for patients with ER+/Her2− disease, a spectrum exists in degree of risk and responsiveness to chemotherapy and noted the increasing evidence for the use of multiparameter molecular test (e.g., the 70-GS and the 21-RS) to discriminate between “Luminal A-like” and “Luminal B-like” disease in order to better guide chemotherapy decisions. Interestingly, the results of our study indicate that this decline in chemotherapy use is not only explained by the use of GEPs, since also in patients in whom no 70-GS was deployed (i.e., in whom no difference between Luminal A or B disease was made) less chemotherapy was administered over time. Clinicians move away from administering chemotherapy in HR+/Her2−/N0 disease and apparently do not consider a multiparameter molecular test necessary to do so. These results are in line with a study conducted in the United States that examined trends in OncotypeDx deployment and chemotherapy use over the years 2013–2015. In the latter study, chemotherapy use in node-negative and micro-metastatic patients declined from 26.6 to 14.1%, and the reported decrease was independent of OncotypeDx use [17].

In an earlier population-based study conducted in The Netherlands between 2011 and 2013, a period in which chemotherapy was commonly administered in patients with lymph-node negative disease, the 70-GS was independently associated with a decreased likelihood to receive chemotherapy [14]. In the current study, which was conducted in more recent years, no independent association between 70-GS use and chemotherapy administration was observed in N0 patients as the administration of chemotherapy mostly decreased without 70-GS deployment. This more reluctant attitude among clinicians in administering chemotherapy in this patient category is supported by recent international chemotherapy recommendations as well as by recent studies that support omission of chemotherapy in clinical low-risk luminal type breast cancer patients [3,8,10]. According to the MINDACT trial, there was no difference in 5-year DMFS in clinical low-risk patients

assigned to the 70-GS high-risk category who did or did not receive chemotherapy, illustrating that there is no role for the 70-GS in clinical low-risk patients. This is different for lymph-node positive patients. In this category, a strong association between 70-GS use and less chemotherapy administration was observed and a lower proportion of patients in this category received a 70-GS in the current study. Because international guidelines are more cautious concerning 70-GS use in lymph-node positive patients, this is not surprising. However, in those lymph-node positive patients who did receive a 70-GS, less chemotherapy was administered. This finding also was reported by others and indicates a potential important benefit of 70-GS use in lymph-node positive patients, supported by the results of the MINDACT trial in which omission of chemotherapy in lymph-node positive patients (pN1a) with a 70-GS low-risk result appeared to be safe [8,18-21].

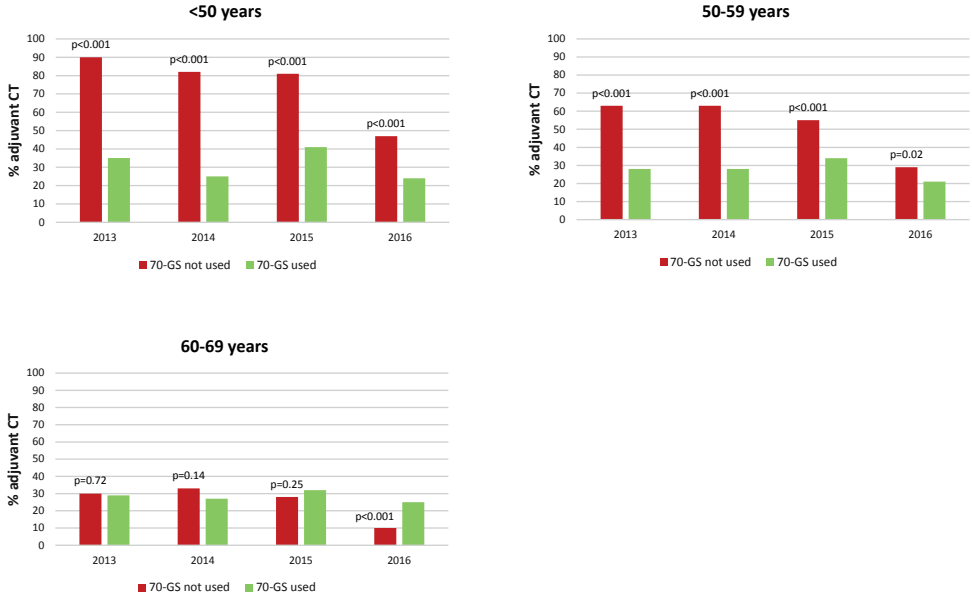
Another important finding of our study was the age dependent effect of the 70-GS use. The reduction in the proportion of patients who received chemotherapy in association with 70-GS use was observed in the younger age categories (< 50 years and 50–59 years). Younger women more often present with more aggressive types of breast cancer compared with the older age category, and it is becoming clearer that tumor biology largely explains the impact of young age on breast cancer outcomes [22-24]. In the present study, however, the aggressive molecular subtypes were not included, and the proportion of eligible patients who underwent genomic profiling and were assigned to the genomic high risk category was similar for all age groups. Notwithstanding the similar intrinsic molecular composition of the tumors in the age groups, chemotherapy was still substantially more often administered to young patients when the 70-GS was not used. A reversed relationship was seen in the older age category (60–69 years) as the use 70-GS was associated with an increased risk of receiving chemotherapy in the context of a limited tendency to administer chemotherapy without the 70-GS. The use of GEPs among young women with breast cancer apparently helps to reduce the tendency to “overtreat” young women.

A strength of this study is the nationwide character and the large cohort of breast cancer patients in whom the association of a GEP on the administration of adjuvant chemotherapy could be assessed. The retrospective design of this study is an important limitation of the study and prevents us from formulating statements that imply causality. During the study period, the national guideline of 2012 was effective suggesting the use of a GEP in ER+/HER2− breast cancer when there is doubt about the adjuvant chemotherapy benefit. Then again, international guidelines were formulated in the meantime providing different recommendations regarding the indications for gene expression profiling [25,26].

## CONCLUSIONS

At a nationwide level in ER+/Her2− breast cancer patients, this study demonstrates a strong decrease in the administration of adjuvant chemotherapy over time without an adjustment in the national breast cancer guideline but in line with contemporary international consensus statements. For lymph node-negative patients, this decline in chemotherapy administration was independent of the 70-GS use, whereas in lymph node-positive disease and in younger patients, the 70-GS was associated with a significantly decreased likelihood that patients received adjuvant chemotherapy.

## SUPPORTING INFORMATION




**Supplementary Fig 1** Adjuvant chemotherapy use in patients who received the 70-gene signature versus patients who did not receive the 70-gene signature in relation to different age categories: <50 years (n=1433) (**A**), 50-59 years (n=2428) (**B**) and 60-69 years of age (n=2919) (**C**). *P*-values were calculated using a $\chi^2$ test for differences in categorical data.

**Availability of data and material:** The data is available on request.

**Conflict of interest**: We declare that we have no conflicts of interest.

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## AUTHOR CONTRIBUTIONS:

Conception and design: J.E.C van Steenhoven, A. Kuijer, T. van Dalen, S. Siesling
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Manuscript writing: all authors
Final approval of manuscript: all authors.
Accountable for all aspects of the work: all authors

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# PART II

Decreasing significance of removing regional lymph nodes

# Chapter 5

## Trends of axillary treatment in sentinel-node positive breast cancer patients undergoing mastectomy

*Accepted - Annals of Surgical Oncology*

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## ABSTRACT

**Background:** The ACOSOG-Z0011- and the AMAROS-trial obviated the need for axillary surgery in most sentinel node positive (SLN+) breast cancer patients undergoing breast conserving surgery (BCS). Data for patients who undergo mastectomy is scarce. The aim of this study is to investigate patterns of axillary treatment in SLN+ patients treated by mastectomy in the years following the publication of landmark studies regarding axillary treatment in SLN+ breast cancer patients undergoing BCS.

**Methods:** This was a population-based study in cT1-3N0M0 breast cancer patients treated by mastectomy and staged as SLN+ between 2009 and 2018. The performance of an axillary lymph node dissection (ALND) and/or administration of postmastectomy radiotherapy (PMRT) were primary outcomes and studied over time.

**Results:** The study included 10,633 patients. The frequency of ALND performance decreased from 78% in 2009 to 10% in 2018, while PMRT increased from 4% to 49% ($P < 0.001$). In ≥N1a patients, ALND performance decreased from 93% to 20%, while PMRT increased to 70% ($P < 0.001$). In N1mi and N0itc patients, ALND was abandoned during the study period, while PMRT increased to 38% and 13% respectively ($P < 0.001$), respectively. Age, tumor subtype, N-stage and hospital type affected the likelihood that patients underwent ALND.

**Conclusion:** In this study in SLN+ breast cancer patients undergoing mastectomy, use of ALND decreased drastically over time. By the end of 2018 most ≥N1a patients received PMRT as the only adjuvant axillary treatment, while the majority of N1mi and N0itc patients received no additional treatment.

## INTRODUCTION

During the last decade, several randomized trials have cast doubt on the need to perform axillary lymph node dissection (ALND) in patients with sentinel lymph node metastases (SLN+). The Z0011 trial of The American College of Surgeons Oncology Group (ACOSOG), published in 2011, demonstrated that ALND in cT1-2 patients undergoing breast conserving surgery (BCS) who were found to have one or two positive sentinel lymph nodes (SLNs) showed no lower regional recurrence risk or better survival, compared to those undergoing sentinel lymph node biopsy (SLNB) only [1,2]. The International Breast Cancer Study Group trial (IBCSG 23-01) showed similar results for patients with micro-metastases in the SLN [3]. The results of the 'After Mapping of the Axilla: Radiotherapy or Surgery?' (AMAROS) trial, published in 2014, demonstrated that axillary radiotherapy (RT) could serve as a safe alternative to ALND resulting in equivalent regional control [4].

Results of these trials led to a broad discussion about the need of performing ALND in SLN+ patients and about the use of RT as an alternative to ALND in SLN+ patients who would previously had been candidates for ALND. International guidelines suggest to consider foregoing axillary surgery in patients meeting the Z0011 criteria, i.e. patients who were treated by breast conserving surgery (BCS) followed by routine external beam RT of the breast [5-7]. Other guidelines advocate the use of regional RT as an alternative for ALND in SLN+ patients [6], applying the AMAROS results both to patients who undergo BCS as well as to patients treated by mastectomy.



Some years ago, a substantial decrease has been reported in ALND frequency among SLN+ patients both in those undergoing BCS and mastectomy [8,9]. In a previous Dutch population-based study, describing patients treated from 2011 to 2015 the proportion of SLN+ patients receiving ALND alongside BCS versus mastectomy was 31% versus 52% at the start, but had decreased to 11% and 26%, respectively, by the end of the study period [8]. These trend lines show a stronger reduction of ALND in the context of BCS versus mastectomy, which may reflect an altered protocol with regard to the anticipated effectivity of ALND in conjunction with BCS. Since for mastectomy patients the Z0011 criteria do not apply, one might expect that postmastectomy radiotherapy (PMRT) would have been applied as a substitute for ALND.

Therefore, the aim of this study was to investigate patterns of care in axillary treatment for Dutch cT1-3N0 SLN+ breast cancer patients undergoing mastectomy. Furthermore, patient-, tumor-, treatment-, and hospital related factors that are associated with ALND performance were evaluated.

## METHODS

Data were obtained from the nationwide population-based Netherlands Cancer Registry (NCR), which is hosted by the Netherlands Comprehensive Cancer Organisation (IKNL). Based on notification through the national pathology database (PALGA) specially trained IKNL data managers register patient-, tumor- and treatment-related characteristics directly from the patient's files.

### PATIENTS AND HOSPITALS

For the present study, all Dutch adult female patients diagnosed with cT1-3N0M0 invasive breast cancer who underwent mastectomy including SLNB between January 2009 and December 2018 were selected from the NCR. Patients who had SLNs containing metastases were included. Those who received neo-adjuvant systemic therapy, underwent mastectomy without SLN biopsy, as well as patients in whom the SLN could not be identified intraoperatively were excluded.

### CONSTRUCTION OF VARIABLES

Patients were subdivided in groups according to axillary treatment following SLNB: ALND, PMRT, a combination of the two (ALND + PMRT) or no subsequent axillary treatment. Detailed information regarding radiation fields was not available. In the Netherlands, the indication for RT of the chest wall in the primary setting is dependent on the estimated risk of recurrence and the absence or presence of risk factors. In case regional RT is indicated in postmastectomy patients (dependent on the extent of nodal disease and the absence or presence of risk factors), the chest wall is generally included in the radiotherapy field. Metastatic lymph node involvement was categorized into isolated tumor cells (N0itc), micro-metastases (N1mi) or macro-metastases (≥N1a) based on the pathology examination of the retrieved SLNs. Hospitals were categorized based on surgical hospital volume. They were divided into low volume (<150 breast cancer operations for primary breast cancer), middle volume (150-300 operations), and high volume (>300 operations) on average per year. Cut-off points were based on those reported by EUSOMA, the European Society of Breast Cancer Specialists [10], and those reported in an article from Greenup et al. [11] Hospitals were also categorized by their teaching status as general nonteaching, teaching, or academic centers.

### STATISTICAL ANALYSIS

Patient-, tumor-, treatment- and hospital-related characteristics are presented as baseline characteristics according to the different treatment groups and compared using chi-squared tests. Descriptive analyses were used to report on the annual proportions of axillary treatments. Univariable and multivariable logistic regression analyses were used to identify patient-, tumor-, treatment- and hospital related factors that are associated

with ALND performance. A P-value of <0.05 was considered statistically significant. Data analyses were performed using Stata version 17.0 (StataCorp, TX, USA).

## RESULTS

### PATIENTS

In total 10,633 patients were included in the analysis. Most of the SLN+ patients were diagnosed with a cT1-2 tumor (93%, n=9,864). The remaining 7% of the patients were diagnosed with a cT3 tumor (n=769) (Table 1). In most of the patients receiving SLNB alone and no ALND (n=6,457), 1 to 3 lymph nodes were removed and examined (83%, n=5,355; median 2; IQR 1-3).

**Table 1** Baseline characteristics of all SLN+ patients treated with ALND, ALND + RT, RT or no adjuvant axillary treatment (N = 10.633).

| Characteristics | ALND | | ALND + RT | | RT | | No adjuvant axillary treatment | | P-value |
|---|---|---|---|---|---|---|---|---|---|
| **Year of diagnosis** | *Number* | *%* | *Number* | *%* | *Number* | *%* | *Number* | *%* | |
| 2009 | 462 | 54.1% | 206 | 24.1% | 33 | 3.9% | 153 | 17.9% | *<0.001* |
| 2010 | 561 | 53.9% | 198 | 19.0% | 52 | 5.0% | 230 | 22.1% | |
| 2011 | 537 | 44.6% | 216 | 17.9% | 121 | 10.0% | 331 | 27.5% | |
| 2012 | 431 | 36.0% | 187 | 15.6% | 180 | 15.0% | 399 | 33.3% | |
| 2013 | 342 | 29.6% | 134 | 11.6% | 258 | 22.3% | 423 | 36.6% | |
| 2014 | 205 | 18.0% | 132 | 11.6% | 362 | 31.7% | 443 | 38.8% | |
| 2015 | 137 | 12.5% | 75 | 6.8% | 477 | 43.4% | 409 | 37.3% | |
| 2016 | 86 | 8.5% | 56 | 5.6% | 458 | 45.4% | 408 | 40.5% | |
| 2017 | 67 | 6.8% | 49 | 5.0% | 451 | 45.9% | 415 | 42.33% | |
| 2018 | 64 | 6.7% | 31 | 3.3% | 463 | 48.8% | 391 | 41.2% | |
| **Age group** | | | | | | | | | |
| <40 years | 165 | 29.7% | 111 | 20.0% | 150 | 27.0% | 130 | 23.4% | *<0.001* |
| 40-49 years | 624 | 31.5% | 317 | 16.0% | 492 | 24.9% | 546 | 27.6% | |
| 50-59 years | 774 | 30.7% | 328 | 13.0% | 681 | 27.0% | 738 | 29.3% | |
| 60-69 years | 675 | 27.0% | 311 | 12.5% | 681 | 27.3% | 831 | 33.3% | |
| 70-79 years | 426 | 24.3% | 150 | 8.6% | 547 | 31.2% | 631 | 36.0% | |
| >79 years | 228 | 17.2% | 67 | 5.1% | 304 | 22.9% | 726 | 54.8% | |
| **Histological tumour type** | | | | | | | | | |
| Ductal | 2,193 | 29.8% | 836 | 11.3% | 1,850 | 25.1% | 2,493 | 33.8% | *<0.001* |
| Lobular | 493 | 20.4% | 361 | 14.9% | 750 | 31.0% | 815 | 33.7% | |
| Mixed | 152 | 24.8% | 65 | 10.6% | 208 | 33.9% | 189 | 30.8% | |
| Other | 54 | 23.7% | 22 | 9.7% | 47 | 20.6% | 105 | 46.1% | |
| **Differentiation grade** | | | | | | | | | |
| Grade I | 544 | 30.1% | 159 | 8.8% | 419 | 23.2% | 684 | 37.9% | *<0.001* |
| Grade II | 1,502 | 25.9% | 684 | 11.8% | 1,557 | 26.8% | 2,065 | 35.6% | |
| Grade III | 755 | 27.4% | 413 | 15.0% | 833 | 30.2% | 758 | 27.5% | |
| Unknown | 91 | 35.0% | 28 | 10.8% | 46 | 17.7% | 95 | 36.5% | |

**Table 1** Continued

| Characteristics | ALND | | ALND + RT | | RT | | No adjuvant axillary treatment | | P-value |
|---|---|---|---|---|---|---|---|---|---|
| **Clinical tumour stage** | | | | | | | | | |
| cT1 | 1,466 | 31.0% | 444 | 9.4% | 1,054 | 22.3% | 1,766 | 37.3% | *<0.001* |
| cT2 | 1,330 | 25.9% | 694 | 13.5% | 1,453 | 28.3% | 1,657 | 32.3% | |
| cT3 | 96 | 12.5% | 146 | 19.0% | 348 | 45.3% | 179 | 23.3% | |
| **Multifocality** | | | | | | | | | |
| No | 2,002 | 27.9% | 867 | 12.1% | 1,739 | 24.3% | 2,557 | 35.7% | *<0.001* |
| Yes | 877 | 25.6% | 408 | 11.9% | 1,107 | 32.3% | 1,032 | 30.1% | |
| Unknown | 7 | 20.0% | 9 | 25.7% | 7 | 20.0% | 12 | 34.3% | |
| **Breast cancer subtype** | | | | | | | | | |
| HR+/HER2- | 2,290 | 26.8% | 999 | 11.7% | 2,345 | 27.5% | 2,901 | 34.0% | *<0.001* |
| HR+/HER2+ | 260 | 28.9% | 108 | 12.0% | 218 | 24.3% | 313 | 34.8% | |
| HR-/HER2+ | 108 | 30.1% | 57 | 15.9% | 82 | 22.8% | 112 | 31.2% | |
| HR-/HER2- | 193 | 30.9% | 104 | 16.6% | 156 | 25.0% | 172 | 27.5% | |
| Other/unknown | 41 | 19.1% | 16 | 7.4% | 54 | 25.1% | 104 | 48.4% | |
| **SLNB result** | | | | | | | | | |
| Isolated tumour cells | 70 | 3.6% | 17 | 0.8% | 224 | 10.3% | 1,850 | 85.3% | *<0.001* |
| Micrometastasis | 743 | 26.1% | 104 | 3.7% | 739 | 26.0% | 1,261 | 44.3% | |
| Macrometastasis | 2,070 | 36.9% | 1,163 | 20.7% | 1,892 | 33.7% | 491 | 8.7% | |
| **Hormonal therapy** | | | | | | | | | |
| No | 509 | 24.2% | 234 | 11.1% | 461 | 21.9% | 902 | 42.8% | *<0.001* |
| Yes | 2,383 | 28.0% | 1,050 | 12.3% | 2,394 | 28.1% | 2,700 | 31.7% | |
| **Chemotherapy** | | | | | | | | | |
| No | 1,101 | 21.2% | 243 | 4.7% | 1,360 | 26.2% | 2,495 | 48.0% | *<0.001* |
| Yes | 1,791 | 33.0% | 1,041 | 19.2% | 1,495 | 27.5% | 1,107 | 20.4% | |
| **Hospital volume** | | | | | | | | | |
| <150 resections per year | 1,277 | 29.9% | 512 | 12.0% | 1,021 | 23.9% | 1,467 | 34.3% | *<0.001* |
| 150-300 resections per year | 1,515 | 25.5% | 733 | 12.3% | 1,685 | 28.3% | 2,018 | 33.9% | |
| >300 resections per year | 99 | 24.8% | 37 | 9.3% | 148 | 37.0% | 116 | 29.0% | |
| **Hospital type** | | | | | | | | | |
| General non-teaching | 1,288 | 22.5% | 498 | 10.8% | 1,247 | 27.1% | 1,571 | 34.1% | *<0.001* |
| Teaching hospital | 1,402 | 27.3% | 707 | 13.8% | 1,334 | 26/0% | 1,687 | 32.9% | |
| Academic hospital | 201 | 22.5% | 77 | 8.6% | 273 | 30.5% | 343 | 38.4% | |

*ALND* axillary lymph node dissection, *RT* radiotherapy, *HR* hormone receptor, *HER2+* human epidermal growth factor receptor 2, *SNLB* sentinel lymph node biopsy

## TRENDS IN AXILLARY TREATMENT IN CT1-3 SLN+ BREAST CANCER PATIENTS UNDERGOING MASTECTOMY

The proportion of SLN+ patients who underwent ALND following mastectomy (n=10,633) decreased from 78% in 2009 to 10% in 2018 (Figure 1). The frequency of ALND decreased from 93% to 20% in ≥N1a patients, from 85% to 0.4% in N1mi patients, and from 21% to 0% in N0itc patients, respectively (Figure 1).

Figure 2 shows the trend of adjuvant axillary treatment. Both ALND and ALND combined with PMRT decreased, from 54% in 2009 to 7% in 2018 and from 24% to 3% respectively. The use of PMRT as the only type of adjuvant treatment increased from 4% to 49% (P<0.001 for all). For patients with a cT3 tumor ALND (ALND alone or combined with PMRT) decreased from 72% to 13%. Excluding patients with T3 tumors had no significant impact on the results for the whole group: in the selection of patients with cT1-2 tumors the proportion of ALND decreased, from 55% to 7% and treatment with PMRT increased from 4% to 48%.



The trends of adjuvant axillary treatment varied for the different N+ categories groups. In ≥N1a patients, the increase of PMRT from 2% in 2009 to 70% in 2018 was accompanied by a decrease in ALND from 57% to 13% (P<0.001 for all) (Figure 3a). In the N1mi group the decrease of ALND appeared most prominent, from 75% to 0.4% (P<0.001) (Figure 3b). This decrease in ALND performance was only in part accompanied by an increase of PMRT, from 4% to 38% (P<0.001). In the latter years, a substantial number of patients did not receive axillary treatment at all. In N0itc patients ALND was abandoned rapidly from 17% to about 0% since 2012 (P<0.001) (Figure 3c), the use of PMRT being approximately 10% throughout the study period.

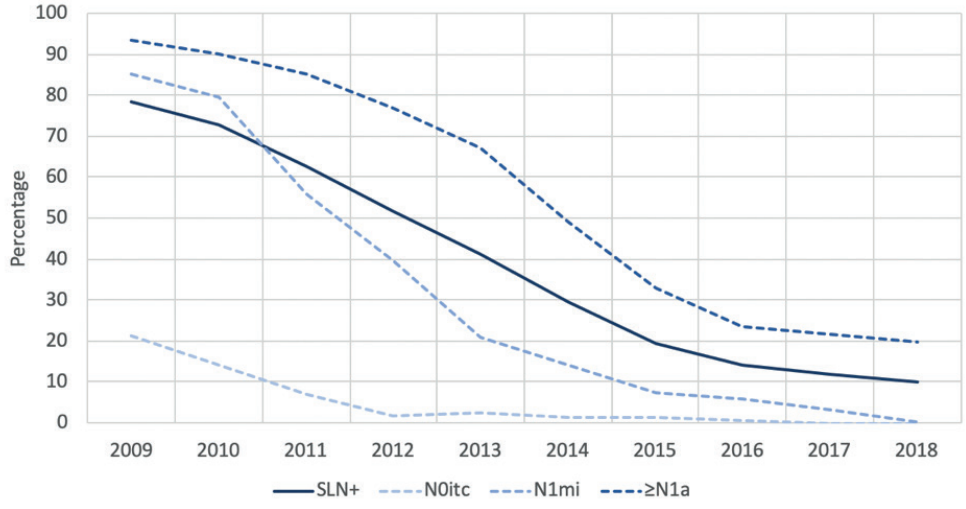


**Figure 1:** Frequency of ALND in SLN+ patients undergoing mastectomy according to N-stage during the study period (2009-2018)

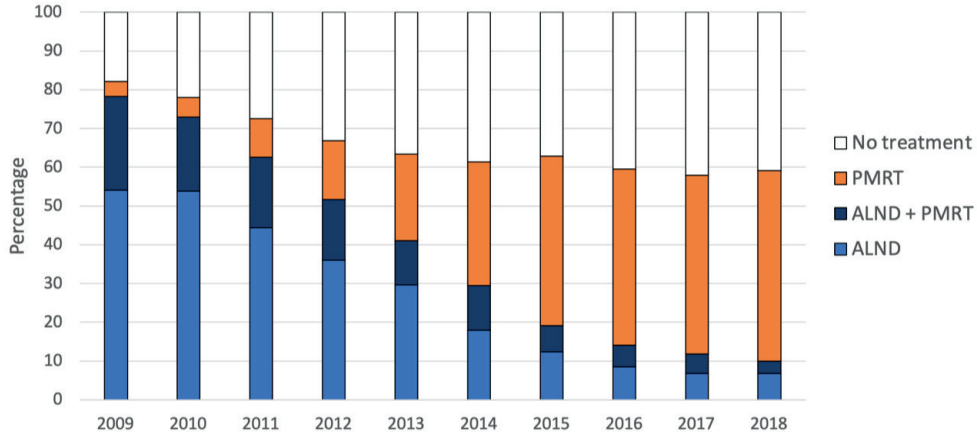


**Figure 2:** Frequency of adjuvant axillary treatment strategies in all SLN+ patients over the study period (2009-2018).

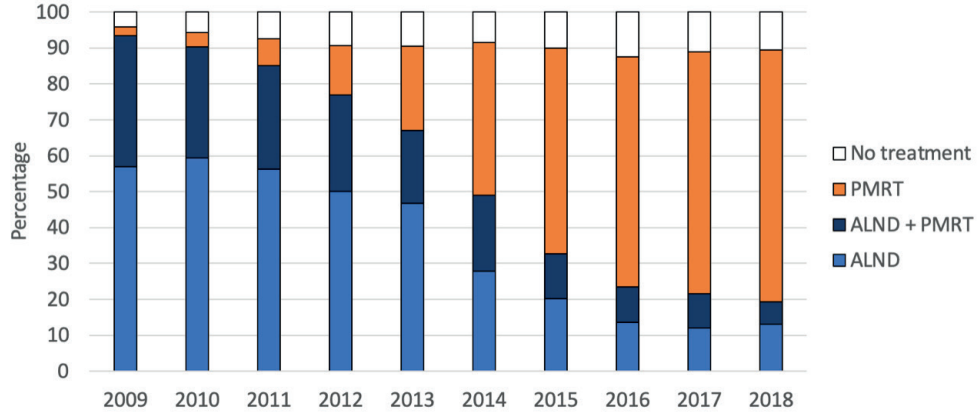


**Figure 3a:** Frequency of adjuvant axillary treatment strategies in ≥N1a patients over the study period (2009-2018).

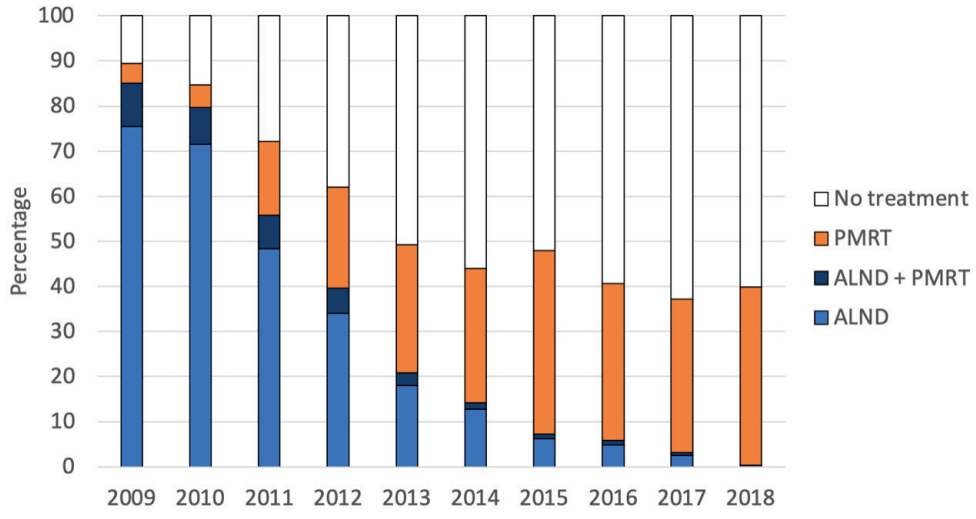


**Figure 3b:** Frequency of adjuvant axillary treatment strategies in N1mi patients over the study period (2009-2018).



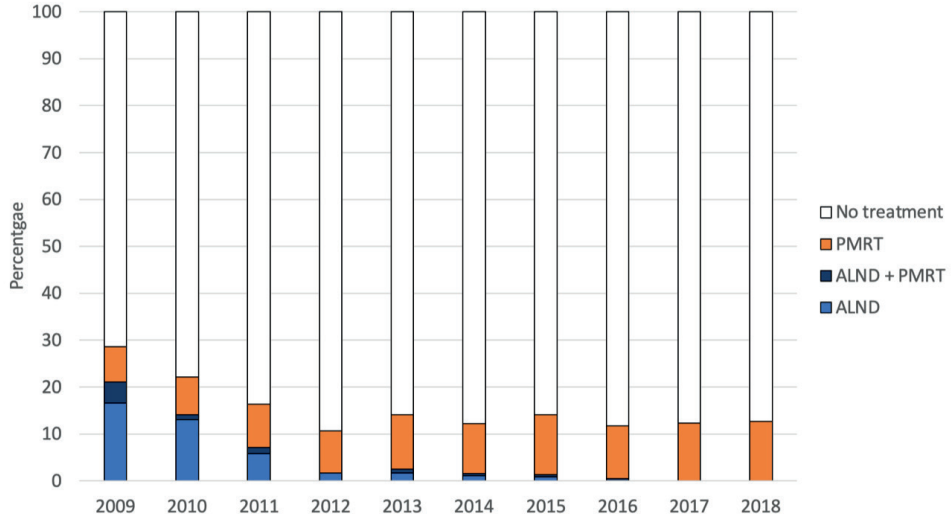


**Figure 3c:** Frequency of adjuvant axillary treatment strategies in N0itc patients over the study period (2009-218).

## PATIENTS-, TUMOR- AND HOSPITAL CHARACTERISTICS WHICH INFLUENCE THE CHOICE OF OMITTING ALND

In addition to the effect of time, factors that were associated with a decreased chance of undergoing ALND were patients >79 years (OR 0.27; 95%CI 0.21-0.35) compared to age 50-59 years, treatment with PMRT (OR 0.14; 95%CI 0.12-0.17), patients with tumor's differentiation grade II (OR 0.83; 95%CI 0.70-0.98) compared to grade I, and patients with sentinel nodes containing isolated tumor cells (OR 0.00; 95%CI 0.00-0.01) or micro-metastases (OR 0.10; 95%CI 0.08-0.11) compared to macro-metastases.

Factors that were associated with a higher chance of ALND performance were age < 40 years (OR 1.28; 95%CI 0.96-1.70) compared to age 50-59 years, lobular (OR 1.23; 95%CI 1.05-1.43) compared to ductal tumor type, basal-like (OR 1.83; 95%CI 1.33-2.53) compared to hormone receptor positive (HR+)/HER2 receptor negative tumor subtype, receiving chemotherapy (OR 2.34; 95%CI 1.98-2.77) compared to not receiving adjuvant chemotherapy, as well as treatment outside an academic institution (teaching hospital: OR 2.19; 95%CI 1.71-2.81, general hospital: OR 1.58; 95%CI 1.25-2.00) (Table 2).

**Table 2:** Univariable and multivariable analysis patient-, tumor- and hospital characteristics associated with the performance of ALND.

| | Univariable | | | | Multivariable | |
|---|---|---|---|---|---|---|
| | **N** | **% ALND** | **Odds ratio** | **95% CI** | **Odds ratio** | **95% CI** |
| **Year of incidence** | | | | | | |
| 2009 | 854 | 78 | 1.334 | 1.079-1.650 | 1.65 | 1.20-2.27 |
| 2010 | 1,041 | 73 | Ref | | Ref | |
| 2011 | 1,205 | 62 | 0.619 | 0.517-0.741 | 0.45 | 0.34-0.59 |
| 2012 | 1,197 | 52 | 0.397 | 0.332-0.474 | 0.21 | 0.17-0.28 |
| 2013 | 1,157 | 41 | 0.260 | 0.217-0.311 | 0.12 | 0.09-0.16 |
| 2014 | 1,142 | 30 | 0.156 | 0.129-0.187 | 0.08 | 0.06-0.10 |
| 2015 | 1,098 | 19 | 0.089 | 0.073-0.109 | 0.04 | 0.03-0.05 |
| 2016 | 1,008 | 14 | 0.061 | 0.049-0.076 | 0.03 | 0.02-0.04 |
| 2017 | 982 | 12 | 0.050 | 0.039-0.063 | 0.02 | 0.02-0.03 |
| 2018 | 949 | 10 | 0.041 | 0.032-0.053 | 0.02 | 0.01-0.03 |
| **Age (years)** | | | | | | |
| <40 | 556 | 50 | 1.27 | 1.06-1.53 | 1.32 | 1.00-1.76 |
| 40-49 | 1.979 | 48 | 1.17 | 1.04-1.31 | 1.08 | 0.86-1.25 |
| 50-59 | 2,521 | 44 | Ref | | Ref | |
| 60-69 | 2,498 | 39 | 0.84 | 0.75-0.94 | 1.04 | 0.88-1.24 |
| 70-79 | 1,754 | 33 | 0.63 | 0.55-0.71 | 0.93 | 0.74-1.16 |
| >79 | 1,325 | 22 | 0.37 | 0.32-0.43 | 0.27 | 0.21-0.35 |
| **Histological tumor type** | | | | | | |
| Ductal | 7,372 | 41 | Ref | | Ref | |
| Lobular | 2,419 | 35 | 0.78 | 0.71-0.86 | 1.23 | 1.05-1.43 |
| Mixed | 614 | 35 | 0.78 | 0.66-0.93 | 0.86 | 0.66-1.12 |

**Table 2:** Continued

| | Univariable | | | | Multivariable | |
|---|---|---|---|---|---|---|
| | **N** | **% ALND** | **Odds ratio** | **95% CI** | **Odds ratio** | **95% CI** |
| Other | 228 | 33 | 0.72 | 0.54-0.95 | 0.91 | 0.59-1.42 |
| **Differentiation grade** | | | | | | |
| Grade I | 1,806 | 39 | Ref | | Ref | |
| Grade II | 5,808 | 38 | 0.95 | 0.85-1.06 | 0.83 | 0.70-0.98 |
| Grade III | 2,759 | 42 | 1.15 | 1.02-1.30 | 1.00 | 0.82-1.22 |
| Unknown | 260 | 46 | - | - | | |
| **Clinical tumour stage** | | | | | | |
| cT1 | 4,730 | 40 | Ref | | Ref | |
| cT2 | 5,134 | 39 | 0.96 | 0.89-1.04 | 1.08 | 0.95-1.23 |
| cT3 | 769 | 31 | 0.68 | 0.58-0.80 | 1.11 | 0.87-1.41 |
| **Multifocality** | | | | | | |
| No | 7,165 | 40 | Ref | | Ref | |
| Yes | 3,424 | 38 | 0.90 | 0.83-0.98 | 0.98 | 0.86-1.11 |
| Unknown | 35 | 46 | - | - | | |
| **Pathological N-stage** | | | | | | |
| Isolated tumour cells | 2,170 | 4 | 0.034 | 0.028-0.042 | 0.00 | 0.00-0.01 |
| Micrometastasis | 2,847 | 30 | 0.312 | 0.284-0.344 | 0.10 | 0.08-0.11 |
| Macrometastasis | 5,616 | 58 | Ref | | Ref | |
| **Breast cancer subtype** | | | | | | |
| HR+/HER2- | 8,535 | 39 | Ref | | Ref | |
| HR+/HER2+ | 899 | 41 | 1.11 | 0.96-1.27 | 0.75 | 0.60-0.93 |
| HR- /HER2+ | 359 | 46 | 1.36 | 1.10-1.68 | 1.15 | 0.78-1.69 |
| HR-/HER2- (basal-like) | 625 | 48 | 1.44 | 1.23-1.70 | 1.83 | 1.33-2.53 |
| Unknown | 215 | 27 | 0.58 | 0.42-0.78 | 0.72 | 0.45-1.16 |
| **Adjuvant hormonal therapy** | | | | | | |
| No | 2,106 | 35 | Ref | | Ref | |
| Yes | 8,527 | 40 | 1.24 | 1.12.-1.37 | 1.26 | 1.02-1.55 |
| **Adjuvant chemotherapy** | | | | | | |
| No | 5,199 | 26 | Ref | | Ref | |
| Yes | 5,434 | 52 | 3.12 | 2.88-3.39 | 2.34 | 1.98-2.77 |
| **Radiotherapy** | | | | | | |
| No | 6,494 | 45 | Ref | | Ref | |
| Yes | 4,139 | 31 | 0.56 | 0.52-0.61 | 0.14 | 0.12-0.17 |
| **Hospital volume** | | | | | | |
| Low (<150) | 4,277 | 42 | Ref | | Ref | |
| Medium (150-300) | 5,951 | 38 | 0.84 | 0.78-0.91 | 0.75 | 0.65-0.86 |
| High (>300) | 400 | 34 | 0.72 | 0.58-0.89 | 0.91 | 0.65-1.26 |
| **Hospital type** | | | | | | |
| Academic | 894 | 31 | Ref | | Ref | |
| Teaching | 5,130 | 41 | 1.55 | 1.33-1.80 | 2.19 | 1.71-2.81 |
| General | 4,604 | 39 | 1.40 | 1.20-1.64 | 1.58 | 1.25-2.00 |

*ALND* subsequent axillary lymph node dissection, *HR* hormone receptor, *HER2+* human epidermal growth factor receptor 2, *SNLB* sentinel lymph node biopsy

## DISCUSSION

In this population-based study in Dutch cT1-3N0M0 breast cancer patients who underwent mastectomy and were SLN+ a substantial decrease in the proportion of patients undergoing ALND was observed. In patients diagnosed as having ≥N1a disease, ALND performance decreased and PMRT increased substantially over the years while in patients with isolated tumor cells and micro-metastasis a substantial proportion had no adjuvant regional treatment at the end of the study period.

Ten years after the publication of the Z0011 and AMAROS trials the proportion of Dutch patients undergoing mastectomy who were SLN+ and underwent ALND decreased to 10%. This seems to reflect the clinicians' confidence in a restrained surgical policy in this category of patients, albeit that the aforementioned trials included patients undergoing BCS exclusively (Z0011) or mostly (82% in the AMAROS trial) [2, 4, 12]. A recent population-based study from the USA in a similar cohort of 12,190 patients also showed a decrease in the proportion patients undergoing ALND from 58% in 2005 to 36% in 2014 [13], while another large population-based study in Germany showed a decrease from 90% in 2008 to 56% in 2015 [14].

The present study shows replacement of ALND with PMRT as axillary treatment after mastectomy in patients staged as ≥N1a. While only 20% of ≥N1a patients underwent ALND at the end of the study period, 70% received PMRT. This trend to omit ALND and increasingly use PMRT has been reported by others too [13, 15], arguing in favor of this treatment switch citing the evidence from the AMAROS trial results. In addition, a remarkable decrease in both performing ALND and administering PMRT as adjuvant axillary treatment is observed. Others reported this decrease too [16]. Proceeding with PMRT instead of ALND in SLN+ patients precludes the identification of patients with N2 or N3 patients. Long term outcome remains to be awaited, but the short term advantage in terms of less arm morbidity when fewer patients undergo both local treatment modalities goes without saying.

In N0itc and N1mi patients, the decreasing trend in axillary surgery was observed earlier during the study period and the decrease was to a lesser extent accompanied by an increase in PMRT compared to ≥N1a patients. This may partly be clarified by the Dutch breast cancer treatment guideline from 2012 [17], which recommended that adjuvant axillary treatment was unnecessary in N0itc patients and questioned the need of axillary treatment in a selection of N1mi patients, e.g. depending on the number of lymph nodes that contained micrometastasis or the presence of other risk factors, such as young age (<40 years), grade 3 disease, lymphovascular invasion or triple negative disease. The conceivable association between the degree of metastatic lymph node involvement and the proportion of patients who undergo axillary surgery was observed by others too [13-15,

18]. Apart from the observed decreased performance of ALND, the association between the extent of metastatic involvement of the SLN and the subsequent administration of PMRT suggests that in SLN+ patients who undergo mastectomy and are diagnosed with ≥N1a disease, the AMAROS trials results are adhered to, while in patients with N1mi and N0itc, adjuvant treatment is considered unnecessary by many clinicians in the majority of patients [3, 16, 19].

In addition to N-stage and histologic subtype of the tumor, several other factors were associated with the decision whether or not to perform ALND. Women above the age of 79 had a lower chance of undergoing ALND, while women under the age of 40 and women with basal-like tumor subtype had a higher chance of undergoing ALND [14, 15]. It seems that surgeons are more reserved in omitting ALND in young patients with an aggressive tumor subtype, albeit that a recent study suggests that clinicians may forego ALND in young patients when PMRT will be administered [20]. Furthermore, the results of our study showed that patients who undergo adjuvant chemotherapy were also more likely to receive ALND. The higher likelihood of macrometastatic disease or high grade disease in patients undergoing adjuvant chemotherapy probably contributes to this correlation, albeit that hospital type and the innovative characteric within a hospital also influences the use of systemic therapies and axillary treatment.



Albeit that patients with a cT3 tumor were not included in the Z0011 and AMAROS trial, we decided on including these patients in our dataset to evaluate patterns of care for this particular subgroup too. Despite the lack of evidence to de-escalate axillary treatment within this category of patients, the results of our study illustrated a similar decreasing trend in the performance of ALND in patients with cT3 tumors compared to those with T1-2 tumors.

The finding that patients treated outside an academic hospital were more likely to undergo axillary surgery is in line with the findings of another study from the Netherlands [8], but contrasts with the opposite finding of three cohort studies from the US and Germany [13-15]. In the German study, patients who were treated in community cancer centers, in comparison to academic cancer centers, were more likely to undergo treatment with SLN dissection without ALND or PMRT (37.4% and 32.1%, respectively) [14]. Weiss et al showed similar results: 37-38% of the patients treated in community centers only underwent SLN biopsy versus 32% in academic centers. The latter authors also observed that patients with public insurance were more likely to receive SLN biopsy only [13]. Then again, in another American study it was observed that patients undergoing an upfront ALND were more likely to be treated in a community center than those undergoing SLN biopsy alone [15]. All in all this implies that opinions regarding axillary treatment differ between institutions, clinicians and surgical societies.

The main strengths of the present study are the size of the study population, the quality of the items that were uniformly registered by personnel of the NCR and the study period of 10 years. As a result, robust data regarding treatment trends are presented. Some limitations of the study are the absence of the number of removed and examined sentinel nodes in patients who underwent ALND following SLN biopsy, the timing of axillary surgery (SLN biopsy with the ALND versus delayed ALND) and the absence of information regarding the radiation fields. In the Netherlands, the indication for RT of the chest wall in the primary setting is dependent on the estimated risk of recurrence and the absence or presence of risk factors. In case regional RT is indicated in postmastectomy patients (dependent on the extent of nodal disease and the absence or presence of risk factors), the chest wall is generally included in the radiotherapy field. Another important limitation of the study design is the absence of follow-up information, as this is not routinely collected for all patients in the NCR. While evidence from clinical trials support the interchangeability of ALND and regional RT in patients treated with BCS, we are still awaiting the results of several clinical trials exploring the impact of omitting adjuvant local treatment in SLN+ patients who undergo mastectomy [21- 23]. These trials mostly included patients treated with BCS, while data specifically for patients undergoing mastectomy is scarce. The Dutch BOOG 2013-07 registry study assesses the oncologic safety of different extents of additional axillary treatment following a positive SLN, specifically in patients who underwent mastectomy [24]. Follow-up of this trial was recently completed. While awaiting the results of these trials to determine optimal axillary treatment strategies in postmastectomy patients with a positive SLN, it seems sensible to avoid treating patients with both ALND and regional RT, since this combination is associated with the worst patient-reported outcomes, compared to less invasive axillary treatments (SLNB or regional RT only) [25]. Based on the results of our study, specialists seem to already actively avoid this combination in daily practice, as these rates decreased further each year.

In conclusion, this study shows a descending trend in the execution of ALND in SLN+ Dutch cT1-3N0M0 breast cancer patients undergoing mastectomy within the 10 years following the AMAROS and Z0011 trial results. ALND was omitted in the vast majority of SLN+ patients. In ≥N1a patients PMRT increased drastically, while less than half of N1mi and only a tenth of N0itc patients received PMRT as the only adjuvant axillary treatment by the end of 2018.

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# Chapter 6

## Regional recurrence risk following a negative sentinel node procedure does not approximate the false-negative rate of the sentinel node procedure in breast cancer patients not receiving radiotherapy or systemic treatment

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## ABSTRACT

**Background:** Although the false-negative rate of the sentinel lymph node biopsy (SLNB) in breast cancer patients is 5-7%, reported regional recurrence (RR) rates after negative SLNB are much lower. Adjuvant treatment modalities probably contribute to this discrepancy. This study assessed the 5-year RR risk after a negative SLNB in the subset of patients who underwent breast amputation without radiotherapy or any adjuvant treatment.

**Methods:** All patients operated for primary unilateral invasive breast cancer between 2005 and 2008 were identified in the Netherlands Cancer Registry. Patients with a negative SLNB who underwent breast amputation and who were not treated with axillary lymph node dissection, radiotherapy, or any adjuvant systemic treatment were selected. The cumulative 5-year RR rate was estimated by Kaplan-Meier analysis.

**Results:** A total of 13,452 patients were surgically treated for primary breast cancer and had a negative SLNB, and 2012 patients fulfilled the selection criteria. Thirty-eight RRs occurred during follow-up. Multifocal disease was associated with a higher risk of developing RR (P = 0.04). The median time to RR was 27 months and was significantly shorter in patients with estrogen receptor-negative (ER-) breast cancer (9.5 months; P = 0.003). The 5-year RR rate was 2.4% in the study population compared with 1.1% in the remainder of 11,440 SLNB-negative patients (P = 0.0002).

**Conclusions:** Excluding the effect of radiotherapy and systemic treatment resulted in a twofold higher 5-year RR risk in breast cancer patients with a tumor-free SLNB. This 5-year RR rate was still much lower than the reported false-negative rate of the SLNB procedure.

## INTRODUCTION

Sentinel lymph node biopsy (SLNB) has replaced axillary lymph node dissection (ALND) as a minimally invasive staging procedure for patients with invasive breast cancer. While meta-analyses documented a false-negative rate (FNR) of the SLNB of 5–7%, the incidence of regional (axillary) recurrence after a negative SLNB in literature is much lower; recently conducted systematic reviews reported an incidence of axillary recurrence after a negative SLNB of 0.3–0.6% [1-5].

Additional treatment modalities contribute to the discrepancy between the FNR of the SLNB and the reported regional recurrence (RR) rates. Randomized, controlled trials have shown a favorable effect of adjuvant systemic therapy on the risk of locoregional recurrence [6-9]. In a previous population-based study, a lower risk of RR was observed following breast conserving surgery (BCS) than after amputation of the breast, suggesting a positive effect of radiotherapy to the breast on the RR rate [10].

It is conceivable that patients with negative SLNB outcomes who are not treated with adjuvant systemic therapy and who do not undergo radiotherapy of the breast or thoracic wall will develop clinically manifest regional disease more often. For this group, the risk of developing a RR might shift towards the reported FNR of the SLN procedure over time.



To eliminate the contribution of the additional nonsurgical treatments, we evaluated in a population-based cohort the 5-year risk of developing a RR after a negative SLNB in the subset of breast cancer patients who were surgically treated with breast amputation and who did not undergo axillary lymph node dissection (ALND), were not treated with radiotherapy, and did not receive adjuvant systemic therapy.

## METHODS

### STUDY DESIGN AND PATIENTS

All patients operated for primary unilateral invasive breast cancer between January 1, 2005 and December 31, 2008 were identified in the Netherlands Cancer Registry (NCR). The NCR is a nationwide, population-based cancer registry containing information on patient, tumor, and treatment characteristics. The registration of data from patients' medical records is performed by registration employees. For the primary endpoint of the present study, we selected patients who were treated with breast amputation and who had a tumor-negative SLNB (pN0). In addition, patients did not have an additional ALND, no treatment with radiotherapy, and no adjuvant or neoadjuvant systemic therapy (chemotherapy, endocrine therapy, or trastuzumab). Exclusion criteria yielded a history of previous breast cancer, a synchronous contralateral breast cancer, and incomplete follow-up data (e.g., no information, missing event date). For comparison of the endpoint

of interest, the aforementioned study group was compared to the remaining group of surgically treated breast cancer patients with a tumor-free SLNB. The latter group consisted of patients who received radiotherapy as part of breast-conserving therapy, patients who received radiotherapy following amputation, and the patients who received adjuvant systemic chemotherapy or endocrine therapy.

The following items were extracted from the NCR: age, gender, histologic type (ductal, lobular, mixed ductal/lobular or other), pathologic tumor size (pT), histologic grade (Bloom–Richardson), multifocality (yes/no), hormone receptor status (ER/PR), HER2 status, intrinsic subtype (HR+/HER2-, HR+/HER2+, HR-/HER2+, HR-/HER2-), operative treatment (breast amputation/BCS), resection margin status (positive/negative), ALND (yes/ no), radiotherapy (yes/no), chemotherapy (yes/no), endocrine therapy (yes/no), and trastuzumab (yes/no). Standard assessment of HER2-status was implemented in the Netherlands mid-2005.

## OUTCOMES

Frequencies of clinicopathological characteristics were compared between patients who developed RR versus patients who remained free of RR during 5-years of follow-up. In patients who developed RR, the time to RR with respect to the baseline characteristics was evaluated. Five-year follow-up data on RR (and local recurrence) were collected for all patients treated during the study period through active surveillance by NCR registrars (in addition to routine annual surveillance to detect any disease recurrence) [11]. Incomplete data on follow up were mainly applicable for the years 2007 and 2008 in which 47% (n = 43) of the hospitals provided follow-up data since data collection for those years was only performed on request. The 5-year RR rate for patients with a negative SLNB treated with breast amputation without ALND, radiotherapy, or adjuvant systemic therapy was extracted and compared with the 5-year RR rate of the remainder of all patients with a negative SLNB, irrespective of their adjuvant treatment. In patients who developed a RR, simultaneous occurrence of a local recurrence (LR) was assessed and defined as the establishment of a LR within 3 months of the occurrence of a RR.

## DEFINITIONS OF ENDPOINTS

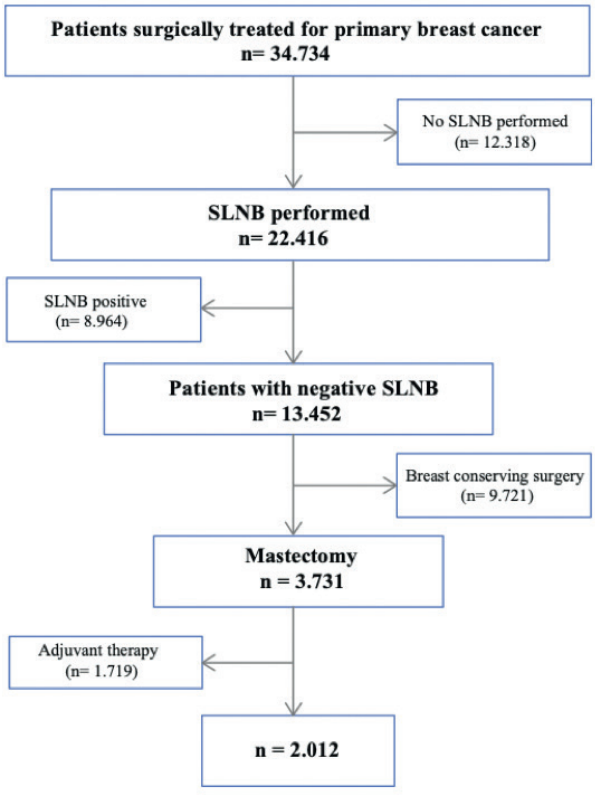
Regional recurrence (RR) was defined as recurrence of breast cancer in ipsilateral regional lymph nodes (e.g., axillary, infra/supraclavicular, or in the internal mammary chain). Local recurrence (LR) was defined as the occurrence of breast cancer or ductal carcinoma in situ in the ipsilateral breast or in the skin or subcutaneous tissue of the ipsilateral chest wall. Follow-up commenced at the date of final surgery and ended with any type of recurrence (event), death (censored), or the date of last follow-up (censored).

### STATISTICAL ANALYSIS

The distribution of clinicopathologic characteristics is presented in percentages. Frequencies of baseline characteristics were compared between patients who developed RR versus patients who remained free of RR during 5 years of follow up, using a $\chi^2$ test for differences in categorical data. For the normally distributed continuous variable (age), means were calculated, and a t-test was performed. In patients who had developed a regional recurrence, the difference in median time to recurrence in relation to the baseline characteristics was evaluated performing a Kruskal–Wallis test. Cumulative 5-year RR rates were calculated through Kaplan–Meier estimates. The RR rate in the study group was compared to the rate in the remainder of SLNB-negative patients by means of a log-rank test. Statistical analysis was performed using STATA (version 13.1 2013, Texas).

## RESULTS

During the study period, 34,734 patients underwent surgery for breast cancer, of whom 22,416 underwent the SLNB procedure. In total, 13,452 of these patients had a tumor-negative SLNB (pN0). Of the latter group, 3731 patients had undergone breast amputation (Fig. 1).



The study population consisted of 2012 SLNB-negative patients who underwent breast amputation without ALND and who did not receive radiotherapy to the breast or adjuvant systemic treatment (Table 1). The mean age of the study population was 64 years [standard deviation (SD) 12.5 years]. Pathologic tumor size was classified as T1-2 in 98.0% of patients.

**Table 1** Baseline characteristics of all patients who underwent breast amputation and sentinel lymph node biopsy (pN0) who did not undergo ALND, and did not receive radiotherapy or adjuvant systemic therapy (n=2012) and of the patients who developed a regional recurrence during 5-yr follow-up (n=38).

| **Characteristics** | **All patients n=2012(%)** | **Patients developing regional recurrence n=38(%)** | ***P*-values*** |
|---|---|---|---|
| Age in years (mean, SD) | 64 (12.5) | 59 (14.2) | 0.008** |
| Age (categories) | | | |
| <35 | 3 (0.10%) | 0 (0%) | 0.20 |
| 35-50 | 292 (14.5%) | 10 (26.3%) | |
| 50-70 | 953 (47.4%) | 17 (44.7%) | |
| >70 | 764 (38%) | 11 (29%) | |
| Histological type | | | |
| Ductal | 1570 (78%) | 33 (86.9%) | 0.39 |
| Lobular | 230 (11.4%) | 4 (10.5%) | |
| Mixed | 100 (5%) | 1 (2.6%) | |
| Other | 112 (5.6%) | - | |
| **Tumor size (T-stage)** | | | |
| **T1a/1M** | 223 (11.1%) | 2 (5.2%) | 0.80 |
| **T1b** | 463 (23%) | 9 (23.7%) | |
| T1c | 1010 (50.2%) | 21 (55.3%) | |
| T2 | 277 (13.8%) | 6 (15.8%) | |
| T3 | 9 (0.5%) | - | |
| T4 | 0 | - | |
| X | 30 (1.5%) | - | |
| Bloom-Richardon Histologic grade | | | |
| I | 707 (35.1%) | 10 (26.3%) | |
| II | 899 (44.7%) | 22 (57.9%) | 0.32 |
| III | 253 (12.6%) | 6 (15.8%) | |
| Unknown | 153 (7.6%) | - | |
| Multifocality | | | |
| No | 1594 (79.2%) | 25 (68%) | 0.04 |
| Yes | 377 (18.8%) | 12 (32%) | |
| Unknown | 41 (2%) | 1 | |
| Estrogen receptor status | | | |
| Negative | 367 (18.2%) | 9 (24%) | 0.22 |
| Positive | 1623 (80.7%) | 29 (76%) | |
| Unknown | 22 (1.1%) | - | |
| Progesterone receptor status | | | |
| Negative | 733 (36.4%) | 13 (37%) | 0.54 |
| Positive | 1244 (61.8%) | 22 (63%) | |
| Unknown | 35 (1.8%) | 3 | |

**Table 1** Continued

| Characteristics | All patients n=2012(%) | Patients developing regional recurrence n=38(%) | P-values* |
|---|---|---|---|
| HER2 receptor status | | | |
| Negative | 154 (76.6%) | 26 (87%) | 0.84 |
| Positive | 202 (10%) | 4 (13%) | |
| Unknown | 270 (13.4%) | 8 | |
| Intrinsic subtype | | | |
| HR+/HER2- | 1297 (64.5%) | 22 (73.3%) | 0.67 |
| HR+/HER2+ | 99 (4.9%) | 1 (3.3%) | |
| HR-/HER2+ | 102 (5%) | 3 (10%) | |
| HR-/HER2- | 163 (8.1%) | 4 (13.3%) | |
| Unknown | 351 (17%) | 8 | |

Categorical variables are displayed as n (%), ALND: axillary lymph node dissection, HR: hormone receptor status, RR: regional recurrence SD: standard deviation

* $\chi^2$ test was used to compare frequencies in clinicopathological characteristics between patient who developed RR (n=38) versus patients who remained free of RR during 5 years of follow up (n=1974)

** refers to the t-test to assess the difference in mean age (continuous variable)

In the 2012 SLNB-negative patients who underwent breast amputation without ALND and who did not receive radiotherapy to the breast or adjuvant systemic treatment, a total of 38 RRs occurred during the follow-up period. In 10 of these patients (26.3%), a LR was detected simultaneously. Patients who developed RR were younger (P = 0.008) and suffered more often from multifocal disease (P = 0.04) compared with the group of patients who remained free of RR (Table 1). In the 38 patients who developed a RR, the median time to recurrence was 27 (interquartile range [IQR] 13–44) months. ER-negative breast cancers were not associated with a higher risk of developing RR, but the median time to recurrence was significantly shorter in patients with a RR of an ER-negative breast cancer (9.5 months; Table 2).

The cumulative 5-year risk of developing a RR in the study population was 2.4% (95% confidence interval [CI] 1.7–3.4%; Fig. 2). A total of 38 RRs occurred with a median time to recurrence of 27 (IQR 13–44) months. No flattening of the slope of the curve was observed throughout the 5-year follow-up. The cumulative 5-year RR of the SLNB-negative patients who did receive radiotherapy or systemic therapy as part of their treatment (n = 11,440) was 1.1% (117 events; 95% CI 1.0–1.4%; P = 0.0002) with a median time to recurrence of 29 months (IQR 16–44 months).

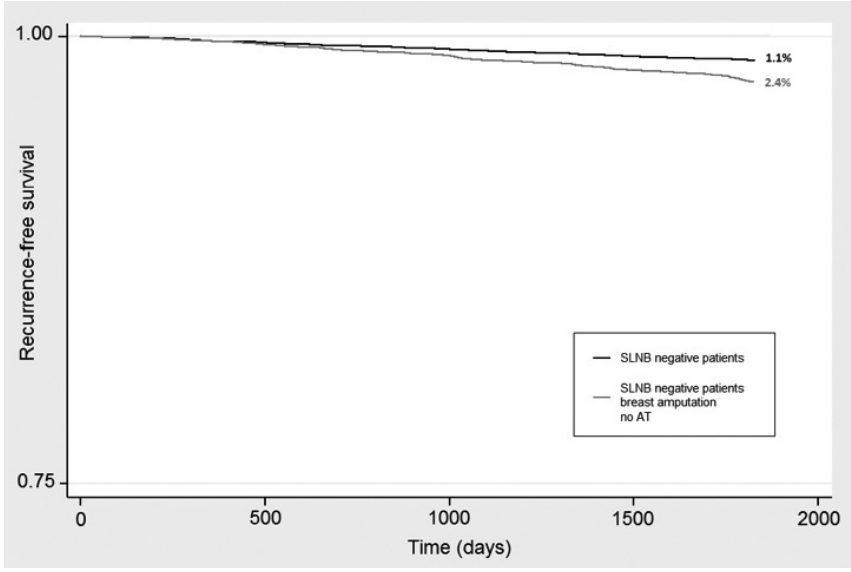



**Figure 2.** Five-year regional recurrence-free survival in patients with a negative SLNB treated with breast amputation without radiotherapy or adjuvant therapy (n=2012) compared to the remainder of all SLNB negative patients (n=11.440)

**Legend** SLNB: sentinel lymph node biopsy, AT: adjuvant treatment

**Table 2** The cumulative regional recurrence rate in patients who underwent breast amputation and sentinel lymph node biopsy (pN0) and did not undergo ALND, radiotherapy or adjuvant systemic therapy (n=2012) and the time to recurrence for patients developing regional recurrence during 5-yr follow-up (n=38)

| **Characteristics** | **5 years regional recurrence rate (%)*** | **Median time to regional recurrence (months)** | ***P***** |
|---|---|---|---|
| Age (categories) | | | |
| <35 | - | - | 0.90 |
| 35-50 | 3.7 | 26.7 | |
| 50-70 | 2.4 | 26 | |
| >70 | 1.8 | 36.9 | |
| Histological type | | | |
| Ductal | 2.7 | 22.1 | 0.62 |
| Lobular | 2.0 | 37.6 | |
| Mixed | 1.1 | 36.9 | |
| Other | - | - | |
| **Tumor size (T-stage)** | | | |
| **T1a/1M** | 1.0 | 20 | 0.10 |
| **T1b** | 2.5 | 39.4 | |
| T1c | 2.7 | 32 | |
| T2 | 2.4 | 10.7 | |
| T3 | - | - | |
| T4 | - | - | |
| X | - | - | |

**Table 2** Continued

| Characteristics | 5 years regional recurrence rate (%)* | Median time to regional recurrence (months) | *P*** |
|---|---|---|---|
| Bloom Richardon Histologic | | | |
| grade | 1.6 | 29.5 | 0.11 |
| I | 3.3 | 31.6 | |
| II | 2.6 | 10.7 | |
| III | - | - | |
| Unknown | | | |
| Multifocality | | | |
| No | 2.1 | 28.9 | 0.95 |
| Yes | 3.6 | 26.7 | |
| Unknown | 2.6 | - | |
| Estrogen receptor status | | | |
| Negative | 2.6 | 9.5 | 0.003 |
| Positive | 2.3 | 35 | |
| Unknown | - | - | |
| Progesterone receptor status | | | |
| Negative | 2.4 | 13.4 | 0.001 |
| Positive | 2.4 | 39.8 | |
| Unknown | - | - | |
| HER2 receptor status | | | |
| Negative | 2.4 | 22 | 0.67 |
| Positive | 3.0 | 18.9 | |
| Unknown | 1.8 | - | |
| Intrinsic subtype | | | |
| HR+/HER2- | 2.2 | 30.1 | 0.05 |
| HR+/HER2+ | 2.7 | 60 | |
| HR-/HER2+ | 3.2 | 11.8 | |
| HR-/HER2- | 2.7 | 9.1 | |
| Unknown | 2.7 | - | |

ALND: axillary lymph node dissection, HR: hormone receptor status RR: regional recurrence

*the 5-year cumulative regional recurrence rate using Kaplan-Meier estimates

**P values refer to the Kruskal-Wallis test to assess differences in time to recurrence

## DISCUSSION

In this study, a significantly higher risk of developing a RR was observed in SLNB-negative patients who had been treated with breast amputation and who did not receive radiotherapy or systemic therapy as part of their routine treatment, implying that nonsurgical treatments contribute significantly to the risk of developing a RR. Recurrence events were evenly distributed over the study period of 5 years. Tumor characteristics other than multifocality were not associated with a risk of developing RR.

The 5-year RR rate in the selected group of patients was 2.4% and was higher than the RR rate of SLNB-negative patients who did receive additional nonsurgical therapies as part of routine treatment (1.1%). A multicenter analysis on axillary recurrences after a negative SLNB in 929 patients with cT1-3N0 breast cancer reported a 5-year estimated axillary recurrence rate of 1.6% [12]. The observed difference between the RR rates of our study population and the remainder of all SLNB-negative patients confirms the beneficial effect of nonsurgical treatment modalities. A risk-reducing effect of adjuvant treatment on RR has been reported previously. Van Wely et al. [13] reported an axillary recurrence rate of 2.8% in a prospective institutional study. RRs were detected in 11 patients after a median of 27 months. Ten of these patients had been primarily treated with mastectomy, and only three of these patients had received adjuvant systemic treatment. The patients had been surgically treated between 1998 and 2004, in a period when adjuvant systemic treatment guidelines were more lenient than after 2004. The same author also conducted a meta-analysis demonstrating a significant risk reducing effect on RR of external beam radiotherapy to the breast [5]. Bulte et al. [14] retrospectively analyzed 54 patients who had developed axillary metastases after a tumor-negative SLNB procedure: 36 patients had been treated with mastectomy, and 37% had received adjuvant systemic treatment.

While the absence of nonsurgical therapy contributed to a higher risk of RR, the observed RR rate of the study population was still two to three times lower than the previously reported FNR of the SLNB procedure, i.e., 5–7% in literature. The duration of the follow-up period of the present study may be one explanation for the discrepancy between the RR rate and the FNR of the SLNB, because RR rates are likely to be higher after a longer follow-up. Primary tumor characteristics, such as tumor size, malignancy grade, and molecular subtype, did not affect the risk of developing RR, albeit that the time to recurrence was significantly shorter in patients with ER- breast cancer.

Because our study population mainly comprised ER+ breast cancer patients, a follow-up period beyond 5 years may reveal more RRs. The latter theory is supported by the slope of the RR curves in the present study: RR occurred evenly throughout the study period. In a study by Matsen et al. [15], the incidence of late axillary recurrence in 1529 SLNB-negative patients increased from 0.6% after 5 years to 0.9% after 10 years of follow-up. The 10-year follow-up results of the Z0011 also support the theory that RRs will develop

after the 5-year follow-up period, because the 10-year RR increased from 0.9 to 1.5% in the SLNB-arm of the study [16]. On the other hand, it also may be possible that not all positive axillary lymph nodes will eventually evolve into a clinically detectable RR.

Support for the latter is paradoxically found in the Z0011 trial as well [17]. In the study arm that received completion ALND, 27% additional tumor-containing lymph nodes were found while the reported rate of patients who developed overt metastases in the SLNB alone arm (0.9%) is in sharp contrast with this. Even though radiotherapy and adjuvant systemic treatments will have played a substantial role in lowering the recurrence rate in the study, it is unlikely that these treatments can account for the whole difference.

A strength of the present study is the nationwide study design in a large population with complete 5-year follow-up, although one may argue that the 5-year follow-up period is too short for a definitive answer. Another limitation of this study is that we do not have additional data on how the SLNB procedure was conducted in the different centers and cannot assess whether RRs possibly occurred more when a particular approach was applied. Also, in the 38 patients who developed a RR in 5 years of follow-up, approximately a quarter of patients developed a simultaneous local recurrence. In these patients, it remains unclear whether the SLNB procedure may have been false negative or if the detected RR developed from their local recurrence with a true negative initial SLNB.



In the present study, we explored the discrepancy between the FNR of the SLNB procedure and the rare occurrence risk of regional recurrence by studying SLNB-negative patients who received no additional nonsurgical treatment. In this particular subset of patients, the clinical implications in terms of additional treatment adjustment are limited. Then again, the unselected group of all SLNB-negative patients also may serve as a model to evaluate the effects of whole breast radiotherapy and systemic therapies on the risk that additional metastases become overt. We will study these effects as part of a future project. Together with the results of the present study, the latter data may be extrapolated to patients with tumor-positive SLNBs to better grasp the even larger discrepancy between the rate of additional non-sentinel lymph node metastases (25–30%) and the observed regional recurrence rate when axillary clearance is omitted (1%) [17,18].

In this study, a higher risk of developing a RR was observed in SLNB-negative patients who had been treated with breast amputation without radiotherapy or systemic therapy. The risk of developing a RR remains lower than what would be expected based on the known FNR of the SLNB procedure, and even after a longer period of follow-up, it remains questionable whether the 5–7% of all patients who undergo breast cancer surgery and who have a false-negative SLNB procedure will eventually develop overt lymph node metastases.

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# Chapter 7

## Quantifying the mitigating effects of whole breast radiotherapy and systemic treatments on regional recurrence incidence in breast cancer patients

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## ABSTRACT

**Purpose:** Despite the potential for residual lymph node metastases after a negative or positive sentinel lymph node biopsy (SLNB), breast cancer patients rarely develop regional recurrences (RR). In this study, we aimed to quantify the effects of non-surgical treatments on RR incidence in sentinel lymph node biopsy negative (SLNB N0) breast cancer patients.
**Methods:** All primary SLNB N0-staged breast cancer patients diagnosed between 2005 and 2008, with 5-year follow-up data on recurrences were selected from the Netherlands Cancer Registry. The cumulative incidence function (CIF) for RR was calculated as the first event at 5 years, taking into account any other first event (local or distant recurrence, contralateral breast cancer or death) as competing risks. Cox regression analysis was used to model the cause-specific hazard of developing RR as first event to quantify the effect of adjuvant systemic therapy and whole-breast radiotherapy (RT) on RR incidence at 5-years.
**Results:** The study included 13,512 patients. Of these patients, 162 experienced an RR. The CIF of RR at 5 years was 1.3% [95% confidence interval [CI] 1.1-1.5], whereas the CIF for death and any other event were 4.4% and 9.5%, respectively. Cox regression analysis showed hazard ratios (HR) of 0.46 [95%CI 0.33-0.64], 0.31 [95%CI 0.18-0.55] and 0.40 [95%CI 0.24-0.67] for patients treated by RT as routine part of breast conserving therapy (BCT), chemotherapy and hormonal therapy.
**Conclusion:** RT as routine part of BCT, chemotherapy and hormonal therapy independently exerted a mitigating effect on the risk of developing RR. The three modalities at least halved the risk.

## INTRODUCTION

Sentinel lymph node biopsy (SLNB) has replaced axillary lymph node dissection (ALND) as a minimally invasive staging procedure for patients with invasive breast cancer. While meta-analyses documented a false negative rate (FNR) of the SLNB procedure of 5 to 7% as proven in patients undergoing SLNB followed by ALND, only 0.3 to 0.6% of the patients who are staged as N0 by SLNB will develop axillary recurrence [1-5]. Likewise, patients who have tumor positive sentinel lymph nodes (SLN N+) but do not undergo completion ALND will also rarely develop axillary recurrence. The Z0011 study and the AMAROS study showed that a 27 to 33% chance of additional lymph node metastases translates into only 1.5% of patients developing regional metastases without further axillary surgery [6-8]. The discrepancy between the frequent presence of additional lymph node metastases in both SLN N0 and SLN N+ patients and the rare occurrence of regional recurrence (RR) is intriguing and commonly attributed to the effects of additional nonsurgical treatments like radiotherapy (RT), chemotherapy (CT) and hormonal therapy (HT).

In a previous study we evaluated the 'natural course' of developing RR in patients who underwent ablative surgery, had a tumor free SLNB and did not receive additional non-surgical treatments [9]**.** The observed risk of developing a RR (2.4%) was less than half of the false-negative rate (5-7%) of the procedure, implying that residual lymph node metastases do not automatically develop into RR when left untreated. As such this observation closely resembled the historical findings of the National Surgical Adjuvant Breast and Bowel Project (NSABP)-04 trial, in which less than half of the patients with nodal metastases (based on the incidence of metastases in the ALND arm) developed clinically apparent RR, while none of these patients had received adjuvant systemic or RT [10].

The current study aimed to address the contribution of nonsurgical treatments that were not primarily given to reduce the risk of developing RR. For this purpose, we quantified the effects of RT on the breast and systemic treatments in a large population-based cohort of SLN N0 breast cancer patients.

## METHODS

### STUDY DESIGN AND PATIENTS

A nationwide cohort study was conducted using data of the Netherlands Cancer Registry (NCR). The NCR is a national, population-based cancer registry containing information on patient, tumor and treatment characteristics. Event data (e.g. local recurrences (LR), RR and distant metastases (DM)) within the first 5 years following primary breast cancer treatment had been collected directly from the patients' files by NCR registrars [11]. Data on vital status and date of death or last observation were derived through linkage with

the Municipal Personal Records database. The Committee of Privacy of the NCR approved the use of data for this study.

All patients who had primary unilateral invasive breast cancer without DM diagnosed between 1 January 2005 and 31 December 2008 who underwent surgery, including SLNB, at the time of diagnosis were selected from the NCR. Patients not undergoing SLNB and patients having a positive SLNB were excluded. We used this cohort of SLNB N0 patients to quantify the hypothesized mitigating effects of RT and systemic treatments on the regional recurrence risk. By using this subset of node negative patients, confounding by additional axillary RT or axillary surgery could be excluded.

To address the impact of routine RT on the breast, patients who received RT after mastectomy and patients who did not receive RT after breast conserving therapy (BCT) were excluded, because these treatment strategies were not routine practice during the study period. Hence, all patients in the study cohort undergoing BCT were treated by RT on the breast and all patients undergoing mastectomy did not receive RT. Other exclusion criteria were macro- or microscopic tumor residue after the final surgery of the primary tumor, patient who received neoadjuvant systemic therapy and patients who underwent ALND.

The following patient and tumor characteristics were collected: age, histologic type (ductal, lobular, mixed ductal/lobular or other), pathologic tumor size (pT), histologic grade (Bloom Richardson I/II/III), multifocality (yes/no), hormone receptor (HR) status (ER/PR), HER2 status, intrinsic subtype (HR+/HER2-, HR+/HER2+, HR-/HER2+, HR-/HER2-), operative treatment (mastectomy/BCT), RT (yes/no), CT (yes/no), HT (yes/no) and trastuzumab (yes/no). During the study period, the national breast cancer clinical guideline of 2005 was effective. According to this guideline, systemic therapy (CT and/or HT in ER+ patients) was advised for N0 patients with unfavorable clinicopathological features (tumors >3cm, grade 3 tumors (unless <1cm), or grade 2 tumors >2cm) as well as for young patients (<35 years). HER2 positive patients were advised to receive trastuzumab in addition to adjuvant CT. Standard assessment of HER2-status was implemented in the Netherlands mid-2005 while treatment with trastuzumab was advised and reimbursed from 2006 onward.

## DEFINITIONS OF ENDPOINTS

The primary endpoint of the current study was RR as first event, defined as recurrence of breast cancer in ipsilateral regional lymph nodes (e.g. axillary, infra/supraclavicular or in the internal mammary chain) [12]. Local recurrence (LR) was defined as the occurrence of breast cancer or ductal carcinoma *in situ* in the ipsilateral breast or in the skin or subcutaneous tissue of the ipsilateral chest wall and contra-lateral breast cancer (CLC) was the occurrence of invasive breast cancer in the contralateral breast. Follow-up

commenced at the date of diagnoses plus 91 days and ended with any type of recurrence (event), death (censored), or the date of last follow-up (censored). The first event and any additional events occurring within 91 days of the first event were included for analyses (e.g. in patients who presented with DM after which RR was diagnosed during further examination within 91d, both events were included in the analyses).

### STATISTICAL ANALYSIS

Distribution of baseline characteristics is presented in percentages. The cumulative incidence function (CIF) of RR as first event at 5-years was calculated with death, and any other type of event (LR, CLC, DM) as competing events. As a reference, the CIFs of death and CIF of any other type of event (LR, CLC, DM) were calculated as well. Cumulative 5-year RR rates in relation to clinicopathological and treatment characteristics were assessed through Kaplan–Meier estimates and accompanying 95% CI were calculated. Univariable analyses was performed by using the log-rank test.

Multivariable Cox proportional hazards regression analyses was used to model the cause-specific hazard of developing RR as first event within 5 years in order to quantify the effects of nonsurgical treatments on RR risk. From a clinical perspective, we included all clinicopathological characteristics (i.e. age, grade, hormone receptor status, multifocality, histologic subtype) and non-surgical treatments (RT, CT, HT, Trastuzumab) in the analysis because all variables potentially influence RR risk. To deal with competing events, patients were censored at date of death or another event (LR, CLC, DM). Multivariable analyses were repeated calculating the cause-specific hazards for death, LR, CLC and DM as well. Besides the variable regarding HER2 status and trastuzumab, we performed complete case analyses. Statistical analysis was performed using STATA version 14.2 (StataCorp, Texas). A *P*-value of less than 0.05 was considered statistically significant.



## RESULTS

### BASELINE CHARACTERISTICS

From the NCR, 34,734 patients with a diagnosis of primary unilateral invasive breast cancer treated surgically between 2005 and 2008 and complete 5-year follow-up assessment of recurrences were identified. The exclusion criteria ruled out patients who did not undergo SLNB (n=12,318), those with a positive SLNB (n=6791), those who had a mastectomy and received local RT (n=228), those who had BCT but did not receive RT of the breast (n=235), patients who underwent ALND (n=1091), patients with a pT4 tumor (n=94) and patients with macro- or microscopic tumor residue after final surgery (n=465) (Fig 1. S1). This resulted in a study population of 13,512 patients.

The mean age of the study population was 59 ±12 years (Table 1). The pathological tumor size was classified as T1a-c in 76% of the patients. Of all patients, 72% (n=9674) underwent BCT and received routine RT on the breast, whereas 28% patients (n=3828) underwent mastectomy. Adjuvant CT was administered to 22% (n=3010), adjuvant HT to 25% (n=3424) and CT in addition to HT to 12% (n=1617) of the patients. In the patient categories with a guideline-directed indication for systemic treatment in the absence of lymph node metastases, (i.e. patients <70 years and having large tumors [>3cm], grade 3 tumors or grade 2 tumors [>2cm], respectively 71%, 70% and 61% of patients received adjuvant CT and 82%, 80% and 86% of patients received HT, respectively. Furthermore, of patients with information regarding HER2 status and who were <70 years of age, 46% of patients classified as HER2+ received trastuzumab.

**Table 1** Patient, tumor and treatment characteristics of the 13,512 primary breast cancer patients operated between 2005 and 2008 and who were staged as N0 according to sentinel lymph node biopsy

| | N | % |
|---|---|---|
| **Mean age (SD)** | 59 (12) | - |
| **Age (categories)** | | |
| <35 | 209 | 2 |
| 35-49 | 2724 | 20 |
| 50-59 | 3856 | 29 |
| 60-69 | 3706 | 27 |
| ≥ 70 | 3017 | 22 |
| **Histological type** | | |
| Ductal | 11,016 | 81 |
| Lobular | 1221 | 9 |
| Mixed | 496 | 4 |
| Other[a] | 779 | 6 |
| **Tumor size (T-stage)** | | |
| T1a/1M | 790 | 6 |
| T1b | 2847 | 21 |
| T1c | 6565 | 49 |
| T2 | 3273 | 24 |
| T3 | 37 | 0 |
| **Grade** | | |
| I | 3785 | 28 |
| II | 5606 | 42 |
| III | 3566 | 26 |
| Unknown | 555 | 4 |
| **Multifocality** | | |
| No | 12,005 | 89 |
| Yes | 1335 | 10 |
| Unknown | 172 | 1 |

**Table 1** Continued

| | N | % |
|---|---|---|
| **Intrinsic subtype** | | |
| HR+/HER2- | 9025 | 67 |
| HR+/HER2+ | 957 | 7 |
| HR-/HER2+ | 549 | 4 |
| HR-/HER2- | 1461 | 11 |
| Unknown | 1520 | 11 |
| **RT on the breast**[b] | | |
| No | 3838 | 28 |
| Yes | 9674 | 72 |
| **Chemotherapy** | | |
| No | 10,502 | 78 |
| Yes | 3010 | 22 |
| **Hormonal therapy** | | |
| No | 10,088 | 75 |
| Yes | 3424 | 25 |
| **HER2 and trastuzumab** | | |
| HER2-, no trastuzumab | 10,956 | 81 |
| HER2+, no trastuzumab | 923 | 7 |
| HER2+, trastuzumab | 598 | 4 |
| Unknown[c] | 1035 | 8 |

[a]Percentages may not add up to 100% due to rounding.

[b]Histological tumor subtype 'other' (e.g mucinous, medullary, metaplastic carcinoma)

[c] All patients who received radiotherapy (RT) of the breast were treated with breast-conserving therapy, and patients not receiving RT on the breast were treated with mastectomy

[d]The majority of patients in the 'unknown' category were diagnosed in earlier years since standard HER2 testing and treatment with trastuzumab were routinely implemented starting September 2005



## REGIONAL RECURRENCE INCIDENCE AS FIRST EVENT WITHIN 5-YEARS

The total number of patients who experienced an RR was 162. The CIF of RR as first event within 5 years was 1.3% [95%CI 1.1-1.5]. The CIF for death within 5 years was 4.4% [95%CI 4.0-4.8] and for LR, CLC or DM as first event, the CIF was 9.5% [95%CI 9.0-10.0].Of the 162 patients, 82 (50%) experienced an isolated RR as first event. For 24 patients (15%), RR was diagnosed simultaneously with LR, whereas for 42 patients (26%), RR occurred simultaneously with DM and for 14 patients (9%), RR was diagnosed simultaneously with LR and DM (Table 2).

**Table 2** Site of first event of the 13.512 breast cancer patients operated between 2005 and 2008 and who were staged as N0 according to sentinel lymph node biopsy

| Site of first event | Total | % of all events |
|---|---|---|
| **Number of events** | 1338 | |
| **Isolated events** | | |
| · **RR** | 82 | 6% |
| · **LR** | 203 | 15% |
| · **CLC** | 414 | 31% |
| · DM | 516 | 39% |
| **Two simultaneous events** | | |
| · **RR, LR** | 24 | 2% |
| · **RR, DM** | 42 | 3% |
| · **LR, CLC** | 9 | 1% |
| · **LR, DM** | 30 | 2% |
| · **CLC, DM** | 2 | 0.1% |
| **Three simultaneous events** | | |
| · **RR, LR, DM** | 14 | 1% |
| · **LR, CLC, DM** | 2 | 0.1% |

RR, regional recurrence, LR, local recurrence, CLC, contralateral breast cancer, DM, distant metastasis

## TREATMENT EFFECTS - UNIVARIABLE ANALYSIS

The cumulative incidence of an RR as the first event in patients who underwent BCT and received RT as part of their routine treatment was 1.0%, whereas it was 2.3% for the patients treated with mastectomy (*p<0.001*). The cumulative incidence of RR as first event for all the patients who had received CT was 1.3% versus 1.4% for those not treated with CT (*p=0.93*). The cumulative incidence of RR as first event for patients who received HT versus patients who did not was 1.1% versus 1.5%, respectively (*p=0.20*). For HER2+ patients who received trastuzumab, the cumulative RR as first event was 1.2% compared to 2.3% in HER2+ patients who did not receive trastuzumab (*p=0.10)* (Table 3).

**Table 3** The regional recurrence incidence as first event within 5-years according to clinicopathological and treatment factors of the 13,512 breast cancer patients operated between 2005 and 2008 and who were staged as N0 according to sentinel lymph node biopsy.

| | N | Absolute number of RRs | RR%[a] | 95% CI |
|---|---|---|---|---|
| **Total** | 13,512 | 162 | 1.4 | |
| **Age (categories)** | | | | |
| <35 | 209 | 4 | 2.1 | 0.8-5.5 |
| 35-49 | 2724 | 49 | 2.0 | 1.5-2.6 |
| 50-59 | 3856 | 48 | 1.5 | 1.8-2.0 |
| 60-69 | 3706 | 33 | 1.0 | 0.7-1.4 |
| >70 | 3017 | 28 | 1.1 | 0.7-1.5 |

**Table 3** Continued

| | N | Absolute number of RRs | RR%[a] | 95% CI |
|---|---|---|---|---|
| **Histological type** | | | | |
| Ductal | 11,016 | 148 | 1.5 | 1.3-1.8 |
| Lobular | 1221 | 9 | 0.9 | 0.5-1.8 |
| Mixed | 496 | 4 | 0.9 | 0.33-2.3 |
| Other[b] | 779 | 1 | 0.15 | 0.02-1.1 |
| **Tumor size (T-stage)** | | | | |
| **T1a/1M** | 790 | 5 | 0.7 | 0.3-1.7 |
| **T1b** | 2847 | 19 | 0.90 | 0.5-1.4 |
| **T1c** | 6565 | 86 | 1.5 | 1.2-1.9 |
| T2 | 3273 | 52 | 1.7 | 1.3-2.3 |
| T3 | 37 | - | - | - |
| **Grade** | | | | |
| I | 3785 | 19 | 0.6 | 0.4-1.0 |
| II | 5606 | 77 | 1.6 | 1.3-2.0 |
| III | 3566 | 61 | 1.9 | 1.5-2.4 |
| Unknown | 550 | 5 | - | - |
| **Multifocality** | | | | |
| No | 12,005 | 134 | 1.3 | 1.1-1.5 |
| Yes | 1335 | 26 | 2.1 | 1.5-3.1 |
| Unknown | 172 | 2 | 1.2 | 0.3-4.8 |
| **Hormone receptor status** | | | | |
| Negative | 2295 | 42 | 2.0 | 1.5-2.7 |
| Positive | 11,140 | 119 | 1.2 | 0.9-1.4 |
| Unknown | 77 | 1 | 1.4 | 1.2-2.2 |
| **RT on the breast** | | | | |
| No | 3838 | 76 | 2.3 | 1.8-2.9 |
| Yes | 9674 | 86 | 1.0 | 0.8-1.3 |
| **Chemotherapy** | | | | |
| No | 10,502 | 125 | 1.4 | 1.2-1.7 |
| Yes | 3010 | 37 | 1.3 | 1.0-1.8 |
| **Hormonal therapy** | | | | |
| No | 10,088 | 128 | 1.5 | 1.2-1.8 |
| Yes | 3424 | 34 | 1.1 | 0.8-1.5 |
| **HER2 and trastuzumab** | | | | |
| HER2-, no trastuzumab | 10,956 | 129 | 1.4 | 1.1-1.6 |
| HER2+, no trastuzumab | 923 | 18 | 2.3 | 1.4-3.6 |
| HER2+, trastuzumab | 598 | 7 | 1.2 | 0.6-2.6 |
| Unknown[c] | 1035 | 8 | 0.9 | 0.5-1.8 |

RR regional recurrence; CI confidence interval; RT radiotherapy; HER2, human epidermal growth factor receptor 2

[a] Represent Kaplan-Meier estimates

[b] Histological tumor subtype 'other' (e.g mucinous, medullary, metaplastic carcinoma)

[c] The majority of patients in the 'unknown' category were diagnosed in earlier years since standard HER2 testing and treatment with trastuzumab were routinely implemented starting September 2005

## TREATMENT EFFECTS - MULTIVARIABLE ANALYSIS

Multivariable Cox regression analyses showed a significant impact of nonsurgical treatment methods on RR as first event within 5 years. The patients treated with RT as part of BCT had a lower risk for development of RR as the first event than the patients who underwent mastectomy [HR 0.46 95% CI 0.33-0.64]. The administration of adjuvant CT and HT was significantly associated with a lower risk for development of RR as the first event (HR 0.31 95%CI 0.18-0.55 and HR 0.40 95%CI 0.24-0.67, respectively) (Fig 1). The effect of the combination CT/HT and all treatments combined (RT, CT and HT) resulted in HRs 0.12 (95% CI 0.06-0.30) and HR 0.05 (95% 0.015-0.15), respectively (Table 4). Treatment with trastuzumab had no significant impact on RR as the first event (HR 0.78 95%CI 0.29-2.08) (Table 4).

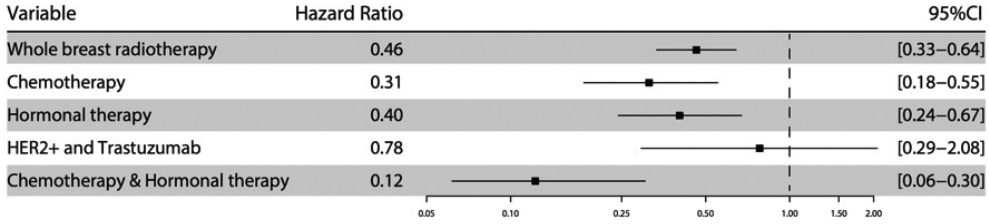



**Figure 1** The quantitative effects of nonsurgical treatments on the regional recurrence (RR) incidence as the first event within 5 years after the 13,512 breast cancer patients had surgery between 2005 and 2008 and were staged as N0 according to sentinel lymph node biopsy. Hazard Ratios were assessed using multivariable Cox Proportional hazards regression analyses adjusted for all clinicopathological (age, grade, tumor size, histologic subtype, multifocality, hormone receptor status) and treatment characteristics (e.g radiotherapy on the breast, endocrine therapy, adjuvant chemotherapy and human epidermal growth factor receptor 2 [HER2] receptor status and trastuzumab)

**Table 4** The quantitative effects of non-surgical treatments and clinicopathological factors on the RR incidence as first event within 5-years of the 13,512 breast cancer patients operated between 2005 and 2008 who were staged as N0 according to sentinel lymph node biopsy.

| | | Multivariable | |
|---|---|---|---|
| | **N** | **HR[a]** | **95% CI** |
| **RT on the breast** | | | |
| No | 3838 | Ref. | - |
| Yes | 9674 | 0.46 | 0.33-0.64 |
| **Chemotherapy** | | | |
| No | 10,502 | Ref. | - |
| Yes | 3010 | 0.31 | 0.18-0.55 |
| **Hormonal therapy** | | | |
| No | 10,088 | Ref. | - |
| Yes | 3424 | 0.40 | 0.24-0.67 |

**Table 4** Continued

| | | Multivariable | |
|---|---|---|---|
| **HER2 and trastuzumab** | | | |
| HER2-, no trastuzumab | 10,956 | 1.0 | 0.58-1.76 |
| HER2+, no trastuzumab | 923 | Ref. | - |
| HER2+, trastuzumab | 598 | 0.78 | 0.29-2.08 |
| Unknown[b] | 1035 | 0.71 | 0.30-1.68 |
| **Age (categories)** | | | |
| <35 | 209 | 2.14 | 0.73-6.21 |
| 35-49 | 2724 | 1.49 | 0.99-2.47 |
| 50-59 | 3856 | Ref. | - |
| 60-69 | 3706 | 0.62 | 0.39-0.99 |
| >70 | 3017 | 0.50 | 0.30-0.84 |
| **Histological type** | | | |
| Ductal | 11,016 | Ref. | - |
| Lobular | 1221 | 0.52 | 0.25-1.08 |
| Mixed | 496 | 0.59 | 0.22-1.62 |
| Other[c] | 779 | 0.12 | 0.02-0.88 |
| **Tumor size (T-stage)** | | | |
| T1a/1M | 745 | Ref. | - |
| T1b | 2851 | 1.26 | 0.42-3.75 |
| T1c | 6575 | 2.84 | 1.03-7.86 |
| T2 | 3273 | 4.74 | 1.64-13.68 |
| **Grade** | | | |
| I | 3785 | Ref. | - |
| II | 5606 | 2.96 | 1.78-4.95 |
| III | 3566 | 4.96 | 2.62-9.39 |
| **Multifocality** | | | |
| No | 12,005 | Ref. | - |
| Yes | 1335 | 1.51 | 0.96-2.36 |
| **Hormone receptor status** | | | |
| Negative | 2295 | 0.99 | 0.57-1.67 |
| Positive | 11,140 | Ref. | - |

Subjects n=12,702, number of missings n=810, events n=154.

[a] Hazard Ratios were assessed using multivariable Cox Proportional hazards regression analyses adjusted for all clinicopathological and treatment characteristics (e.g radiotherapy of the breast, hormonal therapy, adjuvant chemotherapy and trastuzumab)

[b] The majority of patients in the 'unknown' category were diagnosed in earlier years since standard HER2 testing and treatment with trastuzumab were routinely implemented starting September 2005

[c] Histological tumor subtype 'other' (e.g mucinous, medullary, metaplastic carcinoma)

## MULTIVARIABLE ANALYSIS - COMPETING EVENTS

Table 5 lists the HRs of the nonsurgical treatments according to the 5-year probability of LR, CLC, DM or death as a first event, adjusted for all clinicopathological characteristics. Treatment with whole-breast RT was associated with a lower LR risk as a first event compared to patients treated with mastectomy, but it was shown to have no significant impact on the risk for development of DM or CLC as the first event. Treatment with

adjuvant CT was associated with a lower LR risk as the first event and a lower risk for the development of DM compared with no CT treatment. Treatment with HT was associated with a lower risk of LR, CLC or DM as the first event than no HT treatment, with the strongest association seen on the risk of developing CLC as the first event.

**Table 5** The quantitative effects of non-surgical treatments on LR, CL, DM and death within 5-years of the 13,512 breast cancer patients operated between 2005 and 2008 who were staged as N0 according to sentinel lymph node biopsy

| | | Multivariable | | | |
|---|---|---|---|---|---|
| | | **LR** | **CLC** | **DM** | **Death** |
| | **N** | **HR[a] [95% CI]** | **HR[a] [95% CI]** | **HR[a] [95% CI]** | **HR[a] [95%CI]** |
| **RT on the breast** | | | | | |
| No | 3838 | Ref. | Ref. | Ref. | Ref. |
| Yes | 9674 | 0.73[0.56-0.95] | 0.82[0.65-1.03] | 0.91[0.75-1.09] | 0.62[0.52-0.74] |
| **CT** | | | | | |
| No | 10,502 | Ref. | Ref. | Ref. | Ref. |
| Yes | 3010 | 0.46[0.28-0.74] | 0.70[0.44-1.13] | 0.56[0.43-0.74] | 0.62[0.42-0.94] |
| **HT** | | | | | |
| No | 10,088 | Ref. | Ref. | Ref. | Ref. |
| Yes | 3424 | 0.44[0.29-0.67] | 0.38[0.26-0.56] | 0.74[0.58-0.95] | 0.79[0.61-1.02] |
| **HER2 and TT** | | | | | |
| HER2-, no TT | 10,956 | 0.94 [0.61-1.45] | 1.13[0.73-1.74] | 0.64[0.49-0.84] | 1.33[0.93-1.88] |
| HER2+, no TT | 923 | Ref. | Ref. | Ref. | Ref. |
| HER2+, TT | 598 | 0.94 [0.42-2.10] | 0.64[0.25-1.68] | 0.58[0.37-0.90] | 0.84[0.35-1.99] |
| Unknown[b] | 1035 | 0.56 [0.28-1.10] | 1.18[0.69-2.01] | 0.70[0.47-1.04] | 1.19[[0.75-1.86] |

LR, local recurrence; CLC, contralateral breast cancer; DM, distant metastasis; HR, hazard ratio; CI, confidence interval; RT, radiotherapy, CT, chemotherapy; HT, hormonal therapy; HER2, human epidermal growth factor receptor 2; TT, trastuzumab

[a]Hazard Ratios were assessed using multivariable Cox Proportional hazards regression analyses adjusted for all clinicopathological (e.g grade, size, age, histologic subtype, multifocality, hormone receptor status) and treatment characteristics

[b]The majority of patients in the 'unknown' category were diagnosed in earlier years since standard HER2 testing and treatment with trastuzumab were routinely implemented starting September 2005

## CLINICOPATHOLOGICAL FACTORS ASSOCIATED WITH REGIONAL RECURRENCE RISK

Besides the effects of various treatment modalities, larger primary tumor size and higher grade were strongly associated with an increased 5 year RR risk (Table 4), whereas histology other than ductal or lobular carcinoma was associated with lower RR risk. Age was inversely related with the risk of developing a RR. Patients older than 70 years of age had a decreased risk for the development of an RR (HR 0.50 95%CI 0.30-0.84).

## DISCUSSION

In this population-based study of early breast cancer patients staged N0, the use of whole breast RT as routine part of BCT, HT and CT were associated with a lower risk of RR development as first event within 5 years from diagnosis. Besides the nonsurgical treatment modalities, younger age, larger tumor size and higher grade were associated with higher RR incidence. To our knowledge, this is the first study which reports the magnitude of these effects in a large population-based cohort.

The patients who received RT as part of BCT had a significantly lower risk [HR 0.46 95% CI 0.33-0.64] for the development of RR. The mitigating effect of local RT on the risk for the development of RR has been described before [5,13-15]. A prospective study by Van Wely et al. [14] showed a disproportionately high number of axillary recurrences after negative SLNB in patients who underwent ablative surgery and attributed this observation to the absence of external beam radiation therapy (EBRT) in patients who undergo a mastectomy. The hypothesis that EBRT reduces RR risk was supported by a meta-analysis performed by the same author [5] and is line with other studies [13-15].

The presence of an effect of RT on the breast on the risk for the development of RR may be explained by the incidental irradiation of the lower axilla by local RT. Studies have demonstrated that the SLN site is radiated in 79 to 94% of patients using conventional two-dimensional (2D) breast irradiation of the breast [16,17]. Radiation techniques have evolved, and 3D CT planning usually was applied in the Netherlands during the period that the patients in this study were treated. Even with 3D CT-planning techniques, the 95% isodose line still has been found to encompass 55% of the axillary level 1 and 2 lymph node anatomic volume [18], and it has been hypothesized that in 76% of patients the site of the SLN received an elective radiation dose [19]. However, in the current era, radiation techniques have improved further, with such procedures as Intensity Modulated Radiotherapy (IMRT), Volumetric Modulated Arc Therapy (VMAT) [20] and protontherapy, resulting in even more conformal dose distributions around strictly defined target volumes [21]. These techniques reduce accidental dose to the axillary nodes considerably and are expected to reduce side-effects due to lower doses to healthy tissues. Because we have shown a significant effect of accidental axillary dose, axillary recurrences may increase in the current era due to these new radiation techniques.

The current study, was able to address the effects of adjuvant systemic therapies on the RR risk for a substantial proportion of patients. The beneficial effects of adjuvant CT and HT were strong [HR 0.31 95%CI 0.18-0.55 and HR 0.40 95%CI 0.24-0.67, respectively] and the combined effect was even stronger [HR 0.12 95% CI 0.06-0.30].

During the last decades, systemic treatment modalities have evolved extensively, leading to improved survival [22,23] and better locoregional control [22-26]. The overview of the Early Breast Cancer Trialists' Collaborative Group **(**EBCTCG) illustrated that treatment with tamoxifen diminished the LR rates by nearly 50% versus placebo [23] and the use of aromatase inhibitors decreased the LR rates even further [27]. Treatment with cytotoxic CT and targeted therapy improved locoregional control to an even greater extent [28-31].

An effect of trastuzumab on the RR risk could not be demonstrated in this study. The absence of this effect may be explained by the fact that HER2 receptor status testing was not routinely applied, and only a small proportion of HER2+ patients received this type of treatment at that time. Besides the effect of local RT and systemic therapies, our results demonstrate that tumor malignancy grade, tumor size, histologic subtype and age are associated with RR risk.

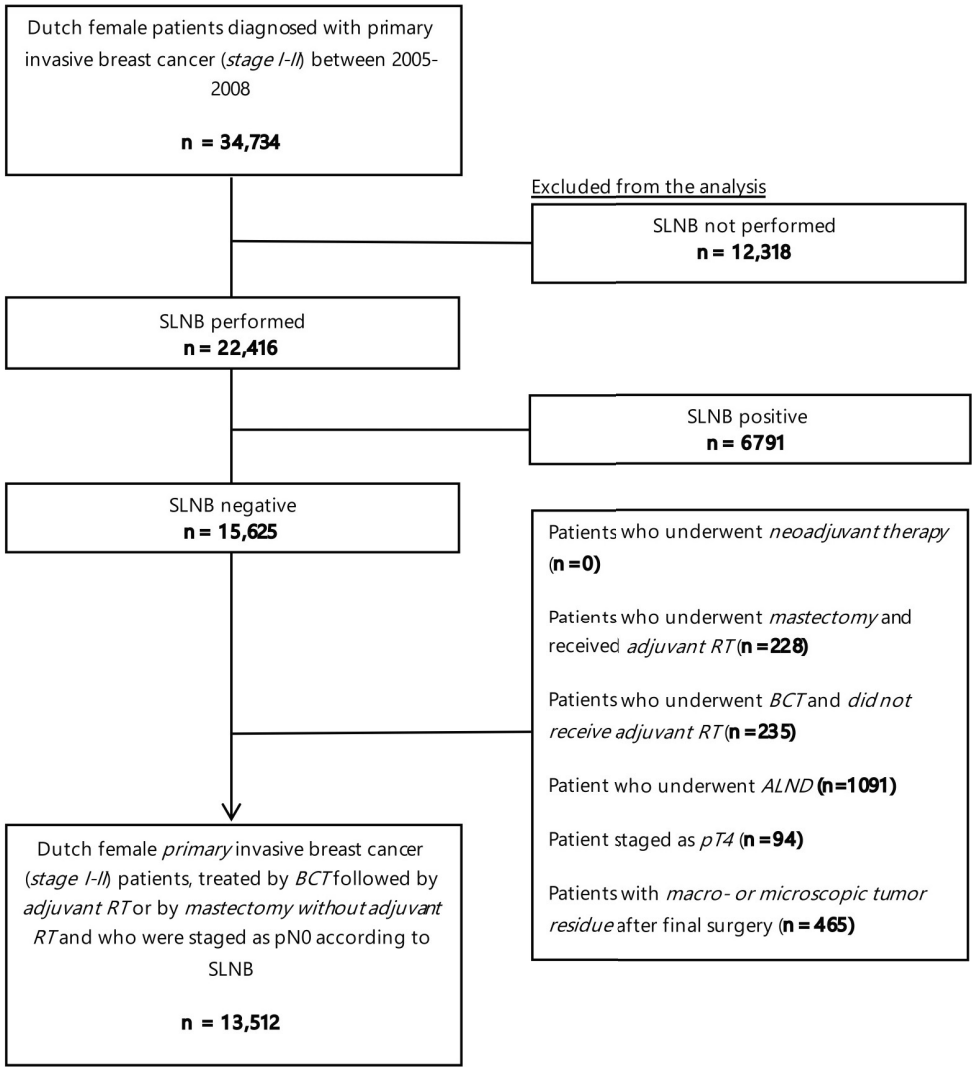
Although nonsurgical therapies contribute to a lower RR risk, the observed 1.4% RR rate of the study population was still much lower than the previously reported FNR of the SLNB procedure (i.e., 5–7% in literature).

In a previous study, we concluded that not all residual lymph node metastases will develop into clinically overt RR when left untreated [9]. As such, this finding coincides with randomized trials in the past comparing ALND to no-ALND and reporting much lower RR rates based on the incidence of nodal metastases in the ALND arm [10]. On the one hand, this may be explained by the natural course of nodal metastases, but on the other hand it may also be due to the fact that DMs occur before the nodal metastases become clinically overt. Once DMs have occurred, no or little attention will be paid to RR, and in addition, usually systemic treatment is started, which also influences subclinical RR. However, in this study, the absolute percentage of patients experiencing DM as first event was only 4%, suggesting that the impact of the aforementioned problem is small. Furthermore, the CIF for RR as first event was calculated with DM as the competing event taken into account.

Some strengths of this study were its population-based design and its large number of analyzed patients with a complete data on first events. Using a SLNB-negative cohort may be considered a weakness of the study. Ideally, a subset of SLNB positive patients would have been used as well since these patients have a higher baseline risk of developing RR. Then again, before 2010 the latter patients would have undergone routine ALND, and later on these patients were considered candidates for RT of the axilla in line with the results of the AMAROS-trial. Another limitation is that we have no detailed information on how RT treatment planning was done at the time that these patients were treated.

Many trials have provided evidence that breast cancer management is often too extensive, and a focus towards de-escalating treatment in a selection of patients has been proposed [32-35]. In this study, we quantified the mitigating side effects of whole-breast RT, CT and HT on RR incidence in a large cohort SLNB N0 breast cancer patients. We demonstrated that the three described methods at least halved the risk. When we also take into account the historical finding of the NSABP-04 trial that residual metastatic lymph nodes will not automatically develop into a clinically detectable RR, even in the absence of the aforementioned therapies [9,10], the findings of the present study may help to explain the observed discrepancy between the false-negative rate of SLNB and regional recurrence in N0-patients. If we extrapolate the effect size of the nonsurgical treatments to SLNB N+ patients, the findings may even help the clinician to better grasp the discrepancy between the rate of additional non-SLN (27%) and the observed RR (1.5%) rate when axillary clearance is omitted.

## SUPPORTING INFORMATION




**Supplementary figure 1.** Flowchart of the analysed study-population

SLNB; sentinel lymph node biopsy, RT; radiotherapy, BCT; breast conserving therapy, ALND; axillary lymph node dissection

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# PART III

Patient and doctors in the era of new developments

# Chapter 8

## Patients' perceptions of 70-gene signature testing: commonly changing the initial inclination to undergo or forego chemotherapy and reducing decisional conflict

*Breast Cancer Research and Treatment 2020; 182;107-115.*

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## ABSTRACT

**Purpose:** Little is known about the impact of 70-gene signature (70-GS) use on patients' chemotherapy decision-making. The primary aim of this study was to evaluate the impact of 70-GS use on patients' decisions to undergo chemotherapy. The perceived decision conflict during decision-making was a secondary objective of the study.

**Methods**: Patients operated for estrogen receptor-positive early breast cancer were asked to fill out a questionnaire probing their inclination to undergo chemotherapy before deployment of the 70-GS test. After disclosure of the 70-GS result patients were asked about their decision regarding chemotherapy. Patients' decisional conflict was measured using the 16-item decisional conflict scale (DCS); scores <25 are associated with a persuaded decision while a score >37.5 implies that one feels unsure about a choice.

**Results:** Between January 1th 2017 and December 31th 2018, 106 patients completed both questionnaires. Before deployment of the 70-GS, 58% of patients (n=62) formulated a clear treatment preference, of whom 21 patients (34%) changed their opinion on treatment with chemotherapy following the 70-GS. The final decision regarding chemotherapy was in line with the 70-GS result in 90% of patients. The percentage of patients who felt unsure about their preference to be treated with chemotherapy decreased from 42% to 5% after disclosure of the 70-GS. The mean total DCS significantly decreased from pre-test to post-test from 35 to 23, irrespective of the risk estimate ($p<0.001$).

**Conclusion:** Deployment of the 70-GS changed patients' inclination to undergo adjuvant chemotherapy in one third of patients and decreased patients' decisional conflict.

## INTRODUCTION

In patients with early-stage breast cancer, adjuvant systemic therapy is administered to reduce the risk of cancer recurrence and to improve overall survival [1]. The advice to administer adjuvant chemotherapy (CT) is based on patients' estimated risk of recurrence. Prognostic tools such as 'Adjuvant!Online' and 'UK.Predict' incorporate clinical and pathological risk factors to determine the recurrence risk and to guide clinical decision-making [2,3]. Even with the aid of these algorithms individual risk assessment remains challenging as patients with comparable tumors may have different outcomes.

In general, patients with estrogen-receptor positive (ER+), Her2 receptor negative (HER2-) breast cancer, have good prognosis and the incremental benefit of adding adjuvant CT to endocrine therapy (ET) is limited. However, some ER+/HER2- patients have more aggressive tumor types who could benefit from CT. Over the past decades, focus has shifted towards optimal patient selection to determine in which patients the benefits of treatment with CT outweigh the negative effects. The use of adjuvant CT in ER+/HER2- patients with no or limited axillary lymph node involvement has been decreasing during recent years [4-5].

Several gene-expression profiles (GEP), such as the 70-gene signature (70-GS; MammaPrint) have been developed to provide more accurate risk assessment by classifying patients into two subgroups (low risk vs. high risk) on the basis of the risk of distant recurrence at 5 years and at 10 years. [6-11]. Current breast cancer guidelines suggest the use of a validated GEP when there is doubt about the indication to administer CT in patients with ER+ invasive ductal carcinoma based on traditional prognostic factors [12,13].



In a previous study, we assessed the impact of the 70-GS on CT-decisions in ER+ early breast cancer by asking physicians to formulate their advice before and after use of the 70-GS [14]. The results of that study showed that the 70-GS changed the physicians intended recommendation to administer CT in about half of the patients in line with the GEP result. Whereas the body of literature on the impact of GEP use on CT-decision making from a physicians' perspective is growing [14-18], reports on patients' perceptions on GEP use are scarce.

The primary aim of this prospective study was to evaluate the impact of 70-GS use on patients' decisions to undergo adjuvant chemotherapy or not. Furthermore, we aimed to explore the perceived decisional conflict during decision-making and gain insight in patients' understanding of 70-GS testing.

## MATERIAL AND METHODS

### STUDY DESIGN AND PATIENTS

This observational, prospective, questionnaire study was designed to assess the impact of the 70-GS test on patients' decision-making to undergo adjuvant CT or not. Patients for whom 70-GS test deployment was deemed indicated based on the prevailing national guideline [12] were eligible for participation. Exclusion criteria were a history of malignancy, the presence of distant metastasis, previous neo-adjuvant systemic treatment and inability to read or write Dutch. The study was approved by the medical ethics committee of the University Medical Center Utrecht and by institutional review boards of participating centers.

Between January 1 2017 and December 31 2018, patients were enrolled in nine participating centers in the Netherlands. The centers comprised both general non-teaching and teaching hospitals, located in the northern part and middle part of the country.

Figure 1 details the study flowchart. Eligible patients were identified during postoperative multidisciplinary team meetings based on the indication for 70-GS use to support the decision to administer adjuvant CT. Patients were informed about the study by their surgical oncologists or the medical oncologists following referral. Before deployment of the 70-GS test, informed consent was obtained from all participating patients. After enrollment, the tumor sample was sent for 70-GS analysis, and the result was disclosed to the oncologist within 10 working days. 70-GS analysis was carried out centrally by Agendia N.V. (Amsterdam, the Netherlands). A minimum tumor percentage of 30% in the tissue sample was required to obtain a valid result. After the tissue was sent for 70-GS analysis, the treating physician completed the first clinical report form, in which information on clinicopathological characteristics and the preliminary CT recommendation to administer adjuvant CT, withhold adjuvant CT, or state uncertainty (i.e depends on 70-GS result) —were registered. This CT recommendation was not disclosed to the patient. Simultaneously, an electronic questionnaire was sent to the patient. In this first questionnaire, information was obtained about the patients' CT preference (to undergo CT-or-not, or 'unsure' when uncertain) without knowledge of the 70-GS. After the 70-GS test result was disclosed, the treating physician reported the post-test CT recommendation and whether CT was actually administered in a second clinical report form. Patients received a second questionnaire regarding their final decision to undergo CT after receiving the 70-GS test, including survey items addressing the influence of the 70-GS test result on patients' CT preference (Figure 1).

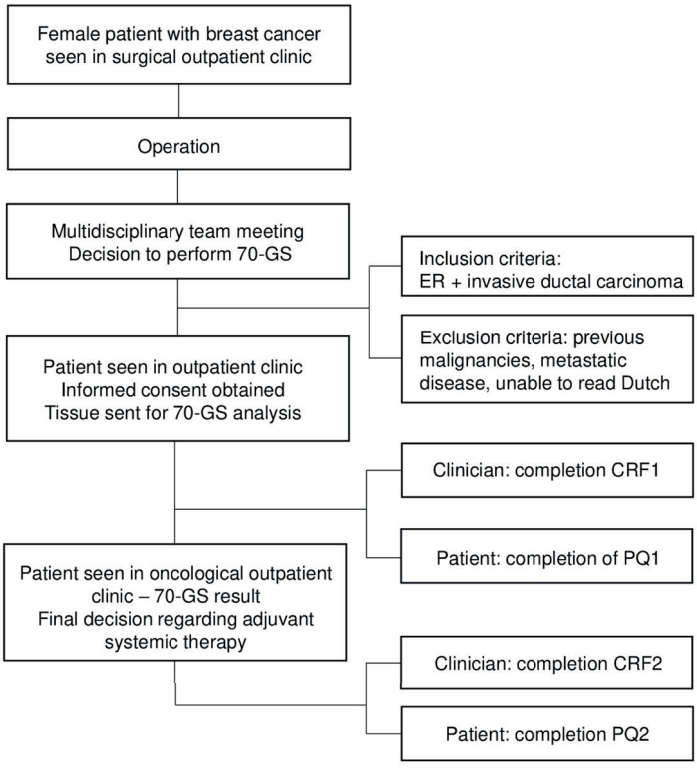


**Figure 1** Flowchart of study inclusion between January 2017 and December 2019.

*Legend:* Abbreviations: 70-GS, 70-gene signature, CRF1, clinical report form, PQ, patient questionnaire, ER, estrogen receptor



## DECISIONAL CONFLICT

Before and after disclosure of the 70-GS test result, patients were asked to fill out a decisional conflict scale (DCS). The DSC is a questionnaire widely used in health care studies of decision-making processes which measures the level of decisional conflict that patients experience while making treatment decisions and it has been validated in a breast cancer sample [19]. The DCS measures modifiable factors contributing to uncertainty in choosing options (e.g. support, information, clarity about personal values) and measures the eventual quality of the decision (figure 2). DCS scores range from 0 to 100, with 0 representing no decisional conflict and 100 reflecting the highest decisional conflict possible. According to this instrument, scores lower than 25 are associated with implementing decision, whereas scores exceeding 37.5 are associated with decision delay or feeling unsure about implementation [20]. Decisional conflict especially exists when a choice has to be made that involves uncertain risks or outcomes, which is the case in adjuvant therapy decision-making in cancer patients [19] and particularly in patients who receive systemic therapy in the adjuvant setting.

The DCS encompasses 16 items, each using a five-point response format (completely agree, agree, neither agree nor disagree, disagree, completely disagree). These items were categorized into five subscales measuring: being informed (extent to which one is informed about options, risks and benefit), values clarity (extent to which one feels clear about personal values and value trade-offs in the decision), support (extent to which one feels supported in making a choice), experiencing uncertainty (level of uncertainty in decision-making), and effective decision (extent to which one agrees their decision was informed, consistent with personal values and is likely to be implemented).

## UNDERSTANDING OF THE 70-GS TEST RESULT

In the second questionnaire, patients were queried about their understanding of the genomic test result using six knowledge questions. Patients received +50 points when one of the following questions were answered with yes: 'the 70-GS provides me information about the risk of distant metastases' or 'the 70-GS aids decisions about undergoing adjuvant CT'. Patient received -50 points when one of the following questions were answered with yes: 'the 70-GS gives me information about the presence of hereditary breast cancer', 'the 70-GS gives me information about the success of the operation', 'the 70-GS gives me information about my chance that adjuvant chemotherapy will be a success' or 'the 70-GS gives me information about my life expectancy. Scores of +50 points or higher were associated with good understanding of the 70-GS test. Furthermore, patients were asked to report, to their personal opinion, their chance of breast cancer recurrence within 5-years. In order to identify characteristics associated with a patient's understanding of the 70-GS, we obtained patient demographics including education level, employment status, family composition, county of birth and household income.

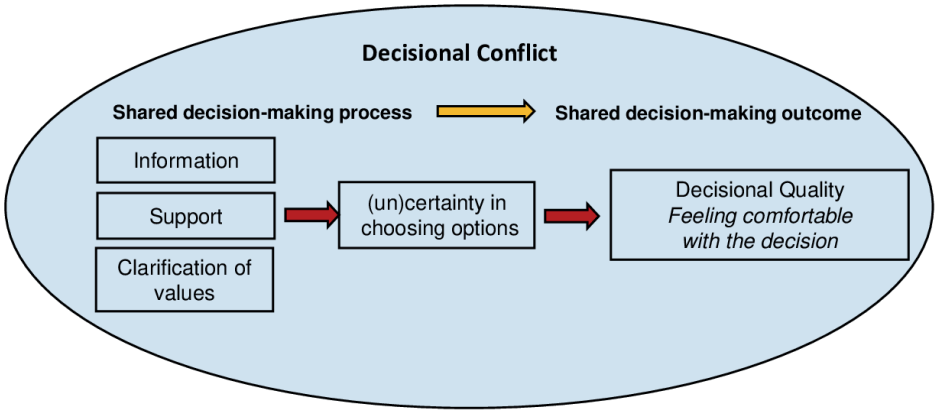


**Figure 2** Decisional Conflict Model.

### END POINTS

The primary end point of this study was defined as the percentage of patients for whom 70-GS use led to an altered adjuvant CT treatment preference (no CT, CT or CT unsure). Secondary endpoints included the change in mean DCS scores prior to and after deployment of the 70-GS, evaluation of patients' understanding regarding 70-GS use, agreement on CT treatment preference between patients' and oncologists' recommendation and the adherence to the 70-GS test result. Patient characteristics associated with patients' understanding of 70-GS testing were explored.

### STATISTICAL ANALYSIS

The frequency of patients' preferences to undergo CT was evaluated before and after use of the 70-GS. The change in mean total DCS scores before and after the 70-GS result and for the subscales were compared by a Wilcoxon signed-rank test. P-values ≤ 0.05 were considered statistically significant.

We calculated standardized effect sizes (*d)* by dividing the mean difference in DCS scores before and after use of the 70-GS by the pooled standard deviation (SD). Effect sizes around 0.2, 0.5 and 0.8 are considered small, medium, and large, respectively. Patients' adherence to the 70-GS result was calculated by the sum of patients who adhered to the 70-GS result (i.e. prefer no CT in case of a low-risk profile and prefer administration of CT in case of a high-risk result) divided by the total number of patients. Agreement between patients' preference and oncologists' recommendation on CT treatment was evaluated. Logistic regression analysis was used to identify patient characteristics associated with a poor understanding of the 70-GS test.



## RESULTS

### PATIENTS

A total of 106 ER+/HER2- negative breast cancer patients were enrolled in the study (median age 55 years). The majority of patients was surgically treated for unifocal, intermediate grade and T1c tumors. Fifty-nine percent of patients were diagnosed with pN0(i-/i+) disease, the remaining patients had (limited) axillary lymph node involvement (pNmi-pN1a). Eighty-seven percent of patients had been treated by breast conserving surgery (table 1). The 70-GS stratified 77% of patients into the 70-GS genomic low risk category.

**Table 1** Clinical characteristics and demographics of estrogen receptor positive breast cancer patients (n=106).

| | % |
|---|---|
| **Age (median, min-max)** | 55 years (34-70) |
| **Type of surgery** | |
| Breast conserving | 87 |
| Mastectomy | 13 |
| **Progesterone receptor status** | |
| Negative | 3 |
| Positive | 97 |
| **Grade** | |
| 1 | 17 |
| 2 | 77 |
| 3 | 5 |
| **Unifocal tumor** | |
| No | 9 |
| Yes | 91 |
| **Tumor diameter in mm** | |
| (median, min-max) | 18 (8-48) |
| **T-stage** | |
| T1 | 62 |
| T2 | 38 |
| **N-Stage** | |
| N0 | 59 |
| Nmi | 15 |
| N1a | 25 |
| Unknown | 1 |
| **70-Gene Signature test result** | |
| High risk | 23 |
| Low risk | 77 |
| **Education** | |
| Primary school | 4 |
| High school diploma | 27 |
| Secondary vocational education | 31 |
| Higher professional education | 23 |
| University | 15 |
| **Household income** | |
| < €20.000 | 9 |
| €20.000-€40.000 | 12 |
| €40.000-€60.000 | 16 |
| >€60.000 | 22 |
| Prefer not to answer | 41 |

## PATIENTS' ADJUVANT CT PREFERENCE

Before deployment of the 70-GS, 58% of patients formulated a clear preference to undergo CT (n=9) or not (n=53), whereas 42% of patients felt unsure regarding this decision (figure 3). After disclosure of the 70-GS, 95% of patients formulated a clear decision and the percentage of patients who remained in doubt regarding their treatment decreased to 5% (figure 3). Of the 62 patients who formulated a clear preliminary decision before 70-GS deployment, 21 patients (34%) subsequently changed their opinion (from CT to no CT or vice versa). The overall agreement between the patients' post-test CT preference and the 70-GS result was 90%: five patients eventually decided to have adjuvant CT despite having a low risk test result and five patients preferred not to receive CT despite the presence of a 70-GS high risk test result. Eighty percent of patients (n=85) considered 70-GS a decisive factor regarding their final treatment plan.

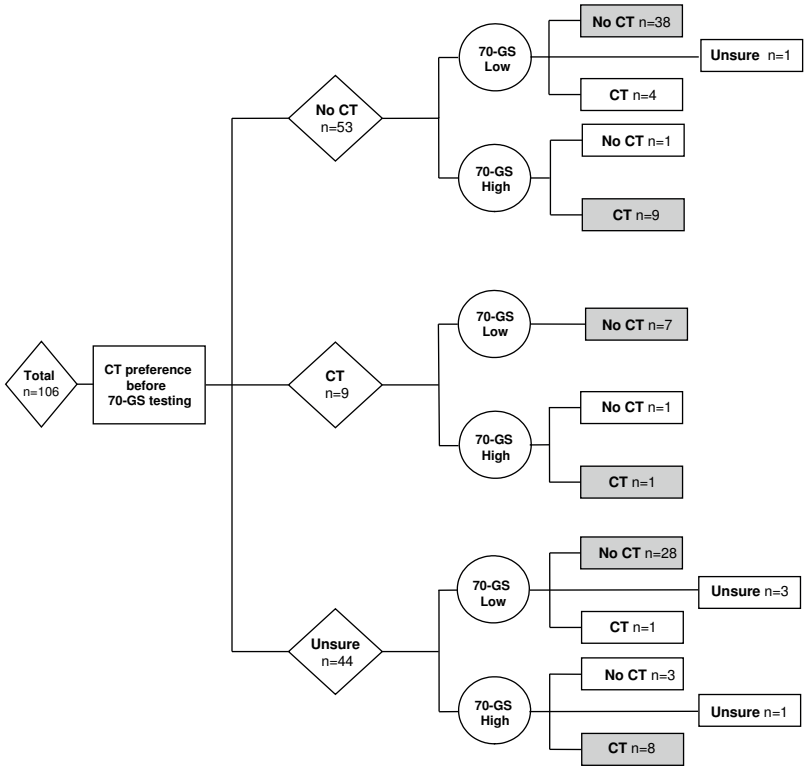


**Figure 3** Flowchart describing patients' inclination to undergo adjuvant CT before use of the 70-GS and the final decision to undergo adjuvant CT after the 70-GS result was disclosed to the patient.

*Legend:* Patients in whom the final CT decision was in line with the 70-GS result are represented in gray. Abbreviations: CT, chemotherapy, ER, estrogen receptor, 70-GS, 70-gene signature

## DECISIONAL CONFLICT

The mean total DCS-score before deployment of the 70-GS was 35 out of 100 and the mean total score decreased to 23 after disclosure of the 70-GS test result (p<0.001, table 2). We determined an effect size of 0.8 for the mean change in DCS following the 70-GS, which is considered large. The initial decisional conflict was highest in patients who preferred not to undergo CT (table 3 and 4). However, this subgroup of patients also showed the largest decrease in DCS when the final decision not to undergo CT was in line with the preliminary decision (mean change total DCS 14.0 points, Table 4). In the small subset of patients who remained unsure about CT, the mean total DCS-score increased (+ 8.0 points, DCS post 70-GS 41 out of 100, Table 4). In ten patients, the decision to undergo or forego adjuvant CT was not in line with the 70-GS result. In the five patients who eventually decided to undergo adjuvant CT despite having a low risk test result, the post-test DCS was higher compared to the total group of patients (mean post-test DCS 32 vs. 23). In the five patients who decided to refrain from CT despite a high risk test result, the mean total DCS decreased from 31 to 25 (data not shown).

Table 2 shows the difference in DCS for each subscore. Four out of five subscores significantly decreased after disclosure of the 70-GS test result. Only the 'support' score did not significantly decrease, albeit that the initial score was already low (25 at baseline), implying that patients felt supported regarding their decision making throughout the decision-making process.

**Table 2** Changes in total decisional conflict scores and sub-scores with regard to the decision to undergo adjuvant chemotherapy for the total cohort of estrogen receptor positive breast cancer patients before and after being informed on the results of the 70-gene signature test (n=106).

| | Decisional Conflict Scores | | |
|---|---|---|---|
| | **Pre-test score** | **Post-test score** | **P-value*** |
| **Total** | 35 | 23 | <0.001 |
| **Subscores** | | | |
| Informed score | 28 | 19 | <0.001 |
| Clarity score | 35 | 22 | <0.001 |
| Support score | 25 | 22 | 0.26 |
| Uncertainty score | 47 | 28 | <0.001 |
| Effective decision score | 37 | 25 | <0.001 |

*P-values represent Wilcoxon signed rank test.

**Table 3** Patients' chemotherapy (CT) inclination prior to the 70-gene signature test (70-GS), baseline decisional conflict scale (DCS) scores and DCS scores after being informed on the 70-GS test results.

| Pre-70-GS CT inclination | Pre 70-GS DCS-score | 70-GS result | Post 70-GS DCS-score |
|---|---|---|---|
| **No CT (N=53)** | 38 | **Low risk** | 25 |
| | | **High risk** | 24 |
| **CT (N=9)** | 29 | **Low risk** | 19 |
| | | **High risk** | 25 |
| **CT Unsure (N=44)** | 33 | **Low risk** | 21 |
| | | **High risk** | 25 |
| **Total (N=106)** | 35 | **Low risk** | 23 |
| | | **High risk** | 24 |

**Table 4** Patients' chemotherapy (CT) inclination prior to the 70-gene signature (70-GS), baseline decisional conflict scale (DCS) scores and DCS scores after being informed on the 70-GS test results stratified by the patients' final CT decision.

| Pre-70-GS CT inclination | Pre 70-GS DCS-score | Post 70-GS CT decision | Post 70-GS DCS-score |
|---|---|---|---|
| **No CT (N=53)** | 38 | **No CT** | 24 |
| | | **CT** | 26 |
| | | **Unsure** | 25 |
| **CT (N=9)** | 29 | **No CT** | 19 |
| | | **CT** | 25 |
| | | **Unsure** | - |
| **CT Unsure (N=44)** | 33 | **No CT** | 19 |
| | | **CT** | 23 |
| | | **Unsure** | 41 |
| **Total (N=106)** | 35 | **No CT** | 22 |
| | | **CT** | 25 |
| | | **Unsure** | 38 |



## PHYSICIANS' ADJUVANT CT RECOMMENDATION

Before deployment of the 70-GS, physicians refrained from recommending CT-or-not in 94% (n=100) of patients. Physicians apparently preferred to await the 70-GS test result. In the remaining six patients, physicians did advise CT. The physician's final treatment recommendation was in line with the 70-GS test result in 96% of patients (four patients were advised to receive adjuvant CT despite a low risk test result). Agreement between patients' final decision and the oncologists' recommendation for treatment with CT was 92%.

## PATIENTS' UNDERSTANDING OF THE 70-GS

After disclosure of the 70-GS test result, 68% percent of patients understood that the 70-GS had provided information regarding their adjuvant CT benefit and 59% of patients understood that the test provided information regarding the risk for metastatic disease.

See supplementary Table 1. Thirteen percent answered that the test gave them information about their life expectancy and small proportions of patients thought that the 70-GS had provided information regarding the success of the operation and that the test provided information about the presence of hereditary breast cancer. Furthermore, we observed a large variation in the patients' self-reported risk of locoregional or distant recurrence at 5 years. For example, some 70-GS low risk patients who were aware of this test result reported to have a 98% chance that their cancer would return within 5 years and some high risk patients reported to have a 1% chance (data not shown).

In relation to patient characteristics, low education level of the patient (high school or less vs. at least some college) and older age (>65 years vs. <55 years) were negatively associated with a correct understanding of the 70-GS (OR 0.19 95%CI 0.03-0.84 and OR 0.25 95%CI 0.07-0.86, respectively). Other patient demographics (household income, employment status, country of birth, family composition) failed to identify any significant correlations (data not shown). Understanding of the 70-GS did not differ between patients with a low or high-risk 70-GS test result or between patients with a high or low DCS (data not shown).

## DISCUSSION

In this prospective study in breast cancer patients in whom the 70-GS was deployed, one third of patients changed their intended decision to undergo adjuvant CT following disclosure of the test result. Deployment of the 70-GS into the decision-making regarding CT was associated with a significant decrease in decisional conflict and a significant increase in the proportion of patients that felt sure about their decision. Low education level and older age were negatively associated with a correct understanding of the 70-GS test.

Thirty-four percent of the patients changed their mind after disclosure of the 70-GS test result. While most tests expressed a genomic low risk, twenty five percent of the patients who initially felt they should not undergo CT eventually decided that they would receive CT and eight of the nine patients refrained from chemotherapy despite an initial preference for it. In addition, we found that the percentage of patients who were initially unsure about treatment with adjuvant CT decreased from 42% to 5% after use of the 70-GS. These results complement previous findings evaluating the impact of GEP use on the shared decision-making process regarding adjuvant CT [21-24]. In a large prospective study conducted by Levine et al., the impact of Oncotype Dx on the patient's CT preference was assessed in the same category of ER+/HER2- breast cancer patients and they reported a comparable 31% change in the patient's CT treatment choice following Oncotype Dx. Their study also reported a similar proportion of patients (42%) feeling

initially unsure about their CT choice [21]. Most patients downgraded their choice from CT to no CT following Oncotype Dx. Comparable results regarding the impact of genomic testing in the clinical decision-making process of the patient following EndoPredict have been reported as well [25]. The use of Endopredict led to an altered CT preference in 37% of patients, of which half of the patients upgraded their choice to CT and half of the patients downgraded their choice to endocrine therapy. We observed high adherence rates of patients and clinicians to the 70-GS test result which is in line with other studies [26,27]. This finding supports a previous study evaluating how patients valued GEP testing in their treatment decision. Many of these patients described the test as an element that empowered them, allowed them to feel confident in their decision, and in many cases, rescued them from unnecessary CT [28].

Another important finding of this study was the reduction in decisional conflict following use of the 70-GS. A mean post-test DCS of 23 implies that patients were convinced of their choice. The magnitude of the reduction measured by the effect size (*d=0.8)* outpaced the effect size what is considered a clinically important and meaningful difference (*d=0.40)* for this tool [19,20]. Our findings are in line with previous studies who also found a significant reduction in DCS and a substantial decrease in patient anxiety too [18, 21, 25-28]. In the present study, the decrease in DCS was influenced by differences in the patients' pre- and post-test CT treatment preferences. Before the test was deployed, patients who intended not to undergo CT felt most uncertain about their decision, while the post-test score was the lowest in those in whom treatment was downgraded. Decisional conflict was the highest in the small group of patients in whom uncertainty remained despite the use of this test and in patients who chose to undergo CT despite a low risk test result.



Exploring the patients' understanding of the 70-GS, we observed that most patients (68%) were aware of the purpose of the test, i.e. they knew that the test provided information regarding the benefit of CT. At the same time, a substantial proportion of patients (41%) did not understand that this information also implied a higher or lower risk of developing distant recurrence. The lack of knowledge of GEP testing is also illustrated by another study in which patients tended to overestimate the truth-value of the test based on misperception on its validity [29]. Given the increased use of multigene assays to guide systemic treatment decisions [4], it is of importance to identify knowledge gaps in patients' understanding regarding the clinical implication of a GEP-test. Despite the large confidence intervals as a result of limited sample size, our findings suggested that low education level and older age were associated with poor understanding of the 70-GS test. These findings should stimulate clinicians to optimize their communication strategies in order to explain the purpose of the test, adjusted to the education level and age of the patient. A previous study reported that oncologists considered explaining GEP testing to patients simple, but, paradoxically, they remained uncertain about patients' understanding of genomic testing [30].

There are some limitations of this study. First, the number of patients within the study cohort is limited and information regarding the total number of eligible patients within the institutions is lacking. The limited number of patients precludes firm conclusions, particularly regarding the DCS variation and the identification of factors associated with a poor understanding of 70-GS testing. Furthermore, while we observed an important decrease in the proportion of patients who felt unsure about whether or not to undergo CT following the 70-GS test, this could well be the result of the fact that these patients were aware that the 70-GS would provide additional information regarding the effect of the therapy. In addition, in this study we cannot correct for the effect of time on the decrease in decisional conflict, since contemplation during a cooling-off of 10-14 days may well have an effect on the perceived decisional conflict. Ideally, we would have used a control group of breast cancer patients in whom the 70-GS was not applied to compare the difference in decisional conflict within these two groups. On the other hand, our study examined the 70-GS associated treatment preference together with the effect on patients' decision conflict. The study design and population best mimics routine practice.

In conclusion, use of the 70-GS changed the patient-intended preference to undergo adjuvant CT in one third of patients and helped patients to feel more confident about their adjuvant CT choice. Deployment of the 70-GS was associated with a significant and clinically relevant decrease in patients' decisional conflict.

**Supplementary Table 1**. Responses to questions assessing patients' understanding of the 70-GS test.

| **Questions** | **Response Total N=106** | |
|---|---|---|
| | Yes N (%) | No N (%) |
| The 70-GS gives me information about the presence of hereditary breast cancer | 5 (5%) | **101 (95%)** |
| The 70-GS gives me information about the success of the operation | 9 (8%) | **97 (92%)** |
| The 70-GS gives me information about the risk of distant metastases | **63 (59%)** | 43 (41%) |
| The 70-GS gives me information about the benefit of adjuvant chemotherapy | **72 (68%)** | 34 (32%) |
| The 70-GS gives me information about my chance that adjuvant chemotherapy will be a success | 10 (9%) | **96 (91%)** |
| The 70-GS gives me information about my life expectancy | 14 (13%) | **92 (87%)** |
| Abbreviations: 70-GS, 70-gene signature. Numbers in bold represent a correct response. | | |

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# Chapter 9

## Inequalities in the omission of axillary dissection in sentinel lymph node positive patients in the Netherlands: innovative hospitals are early adopters of a de-escalating approach

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## ABSTRACT

During the last decade completion axillary lymph node dissection (cALND) was gradually omitted in sentinel lymph node positive (SLN+) breast cancer patients. However, adoption varies among hospitals. We analyzed factors associated with the omission of cALND in all Dutch SLN+ patients. As one of the focus hospital related factors we defined “innovative’ as the percentage of gene-expression profile (GEP) deployment within the indicated group of patients per hospital as a proxy for early adoption of innovations. cT1-2N0M0 SLN+ patients treated between 2011-2018 were selected from the Netherlands Cancer Registry. Hospitals were defined to be innovative based on their GEP use. Multivariable logistic regression (MLR) was performed to assess the relationship between innovative capacity, patient-, treatment- and hospital-related characteristics and cALND performance. 14 317 patients were included. Treatment in a hospital with high innovative capacity was associated with a lower probability of receiving cALND (OR 0.69, OR 0.46 and OR 0.35 in modestly, fairly and very innovative, respectively). Other factors associated with a lower probability of receiving a cALND were age 70-79y and ≥79y (ORs 0.59 (95%CI 0.50-0.68) and 0.21 (95%CI 0.17-0.26)) and treatment in an academic hospital (OR 0.41 (95%CI 0.33-0.51)). Factors associated with an increased probability of undergoing cALND were HR-/HER2- tumors (OR 1.46 (95%CI 1.19-1.80)), macro-metastatic lymph node involvement (OR 6.37 (95%CI 5.70-7.13) and mastectomy (OR 4.57 (95%CI 4.09-5.10)). Patients treated in a hospital that early adopted innovations were less likely to receive cALND. Our findings endorse the need for studies on barriers and facilitators of implementing innovations.

## INTRODUCTION

During the last decade, the treatment spectrum for breast cancer has changed radically. While outcome has improved, attention progressively focused on individualization of treatment and minimization of morbidity [1-5].

In terms of local treatment, a number of randomized controlled trials (RCTs) catalyzed the shift towards less aggressive axillary surgery in patients with lymph node positive disease. Between 2011 and 2014, the Z0011, IBCSG 23-01 and the AMAROS trial demonstrated that completion axillary lymph-node dissection (cALND) was no longer necessary for all patients with tumor positive sentinel lymph nodes (SLNs) [2-5]. Since 2012, both national and international guidelines suggested to consider no further axillary surgery in these patients [6-8]. A decrease in cALND rates among patients with positive SLNs has been observed [9], however the adoption of the implications of the Z0011 and AMAROS results appeared to vary among hospitals and countries [9,10].

At the same time gene-expression profiles (GEPs), that had been developed and validated for better outcome prediction [11-15], were incorporated into clinical practice to contribute to chemotherapy decision-making in hormonal receptor positive (HR+) / HER2-receptor negative (HER2-) disease. RCTs [13,14] commonly led to less chemotherapy use in patients with genomic low risk breast cancers [13-17]. Since 2012, the Dutch national guideline suggests the use of a GEP in a selection of HR+/HER2-patients [7]. In previous nationwide studies we demonstrated an increased use of GEPs and an overall decrease of chemotherapy use in categories of patients [16,17]. Nevertheless, only a modest proportion of Dutch breast cancer patients who are eligible for GEP use, actually received a GEP [16].



The common denominator in the implementation of less extensive local therapy and the decreasing use of chemotherapy through the use of GEP is an attitude of surgeons, medical oncologists and multidisciplinary teams to adhere to novel treatment insights and adjusted guidelines that propagate a de-escalating treatment approach. Little is known about factors that are associated with the tendency to adopt early or late to 'less is more' strategies.

In the present study, patient-, treatment- and hospital-related factors were analyzed that are associated with the omission of cALND in SLN+ breast cancer patients. Of particular interested was a potential effect of early adoption of innovations within hospitals. In this study we hypothesized that GEP deployment to guide chemotherapy administration in patients who are considered eligible for GEP use was used as a proxy for being innovative, thus early adopt innovations within a multidisciplinary breast care team within a hospital.

## METHODS

### STUDY DESIGN

In this population-based historic cohort study, we used the Netherlands Cancer Registry, which is hosted by the Netherlands Comprehensive Cancer Organisation (IKNL). Trained and dedicated data managers register data on patient-, tumor-, hospital-, and treatment-related characteristics of all newly diagnosed malignancies following notification by the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA). This study has been approved by the privacy committee of the Netherlands Cancer Registry (request number K18.149).

### STUDY POPULATION

All female cT1-2N0M0 staged breast cancer patients were included if diagnosed between 2011-2018. Patients who underwent neoadjuvant systemic treatment, patients with a history of breast cancer and patients treated in a foreign hospital were excluded. In addition, patients with an unknown or negative SLN, patients with isolated tumor cells, and patients in whom the SLN could not be identified during surgery were also excluded.

### OUTCOMES AND DEFINITIONS

The primary outcome of this study was cALND performance in SLN+ patients. In addition to previously described patient and hospital characteristics [9], the use of the 70-gene signature (GS) to guide adjuvant systemic treatment administration in patients who are considered eligible for GEP use was used as a proxy for early adaption to innovations within hospitals. GEP use was defined as a hospital factor reflected by the percentage of GEPs that were performed in a hospital between 2011 and 2013 in ER+/HER2- Bloom-Richardson grade 1 and >2cm or grade 2 and >1cm patients with no or isolated lymph node metastases.

During these years, GEPs were recommended, and therefore mainly deployed, in node negative patients. We used 2011-2013 as these were the first years that GEPs entered clinical practice. In this time period, the use of a GEP was associated with a reduction in the administration of adjuvant chemotherapy in node negative patients [16], whereas in later years (2013-2016) there were other factors contributing to the decrease in the administration of adjuvant chemotherapy in this category of patients [17].

The percentage of GEP use in these years was considered to be a proxy indicator of ‘innovation’ of hospitals being early adopters in the multidisciplinary field of medical oncology.

Based on the proportional use of the 70-GS use in this study, hospitals were categorized into four groups: 0-5%, 6-10%, 11-15% and more than 15% use, reflecting not, modest, fair and very innovative, hospitals respectively. Hospital volume, which was used as a covariable in the analysis, was defined as the number of new breast cancer incidences per hospital.

### STATISTICAL ANALYSIS

Patient-, tumor-, treatment- and hospital-related characteristics were summarized using descriptive statistics. Missing data were considered to be missing at random. To increase accuracy of the estimates, missing data were corrected by applying multiple imputation statistics using the multiple impute chained equation command in Stata. The imputation was performed 20 times. Estimates and standard errors of all imputed datasets were combined using Rubin's rule [18]. Multivariable logistic regression analysis was performed to assess the relationship between innovative capacity and chance of cALND performance, and to evaluate the influence of patient-, tumor-, treatment- and hospital-related characteristics on this association. Variables included in the multivariable analysis were selected based on clinical foreknowledge and literature. The data was hierarchically structured, therefore a multilevel logistic regression analysis was performed in addition to logistic regression analyses. Hospital of first excision was used as hierarchical level to account for the dependency of patients within hospitals, thereby providing more accurate estimates than traditional logistic regression analysis [19]. Individual variable effects were expressed as odds ratios (ORs) with corresponding 95% confidence intervals (CIs). Complete case analysis was performed to assess whether the estimates obtained using imputed datasets were similar to those derived from the original dataset. Statistical tests were two-sided and a p-value<0.05 was considered to be statistically significant. All statistical analyses were performed in Stata version 17.0 (StataCorp LLC, College Station, TX, USA).



## RESULTS

### STUDY POPULATION

Between 2011 and 2018, 84 765 female patients underwent surgery for cT1-2N0 invasive breast cancer in the Netherlands. Patients who underwent neo-adjuvant systemic therapy (n = 7400), had a prior history of breast cancer (n = 8008), did not receive SLN biopsy (SLNB) (n = 1978) or were treated in a foreign hospital (n=43) were excluded for the current study, leaving 67 336 patients in whom a SLNB was performed. Of these patients, 47 555 had a negative SLN result, 746 had an unknown SLNB result, in 968 patients the SLN could not be identified during surgery, and 3750 patients had only isolated tumor cells and were therefore excluded in the current study (79% of all patients in whom SLNB was

performed). Our study population comprised the remaining 21% (n = 14 317) of patients who were diagnosed with nodal micro- or macrometastasis according to SLN (Figure 1).

## BASELINE CHARACTERISTICS

Overall, 28.0% (n = 4015) of the patients in this study cohort received a cALND and we observed a clear downward trend in the use of cALND over time: 71% versus 7% of patients underwent cALND in 2011 versus 2018, respectively (Table 1). Patients in whom cALND was omitted were treated in more recent years and suffered of less aggressive (HR+/HER2-) tumors of smaller size and grade, as compared to patients who underwent cALND. Furthermore, patients who did not receive cALND more frequently underwent breast-conserving surgery (67% vs 41%) and radiation therapy (86% vs 58%), as compared to patients in whom cALND was performed. Adjuvant chemotherapy was administered in 69% vs 45% of patients who did, and did not, receive cALND respectively (Table 1).

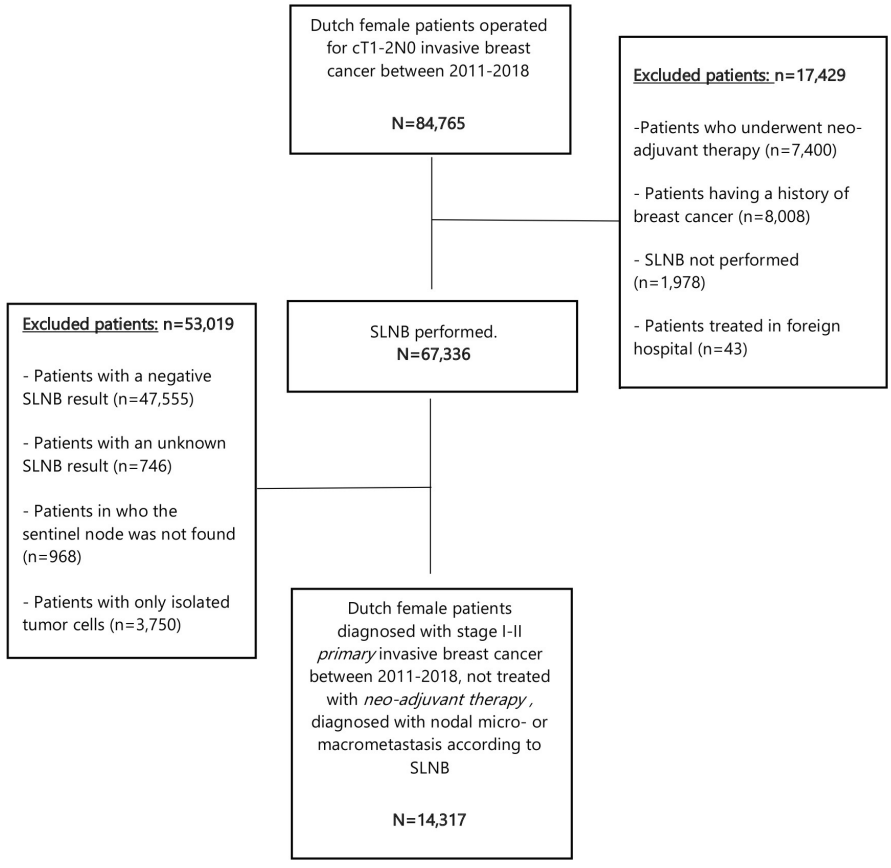


**Figure 1.** Flowchart of included patients

Patients were treated in 79 individual hospitals and the number of treated patients ranged from 2 to 281 per hospital for this cohort. Sixty-nine hospitals (87%) deployed a GEP during the study period. The percentage of patients within the indicated area who received a GEP ranged from 0% - 43.8% within hospitals. Twenty-one percent of patients in whom cALND was omitted were treated in a hospital in which GEPs were frequently used (>15%) as compared to 14% of patients who did receive a cALND (Table 1).

**Table 1.** Baseline characteristics of all included patients diagnosed with nodal micro- or macro-metastasis according to a sentinel lymph node biopsy (n=14,317).

| Characteristics | No cALND (n=10,302) | | cALND (n=4,015) | |
|---|---|---|---|---|
| | **N** | **%** | **N** | **%** |
| **Year of diagnosis** | | | | |
| 2011 | 588 | 5.7% | 1,415 | 35.2% |
| 2012 | 1,042 | 10.1% | 931 | 23.2% |
| 2013 | 1,253 | 12.2% | 604 | 15.0% |
| 2014 | 1,411 | 13.7% | 396 | 9.9% |
| 2015 | 1,449 | 14.1% | 237 | 5.9% |
| 2016 | 1,556 | 15.1% | 178 | 4.4% |
| 2017 | 1,555 | 15.1% | 146 | 3.6% |
| 2018 | 1,448 | 14.1% | 108 | 2.7% |
| **Age group** | | | | |
| <40 years | 348 | 3.4% | 227 | 5.7% |
| 40-49 years | 1,664 | 16.2% | 879 | 21.9% |
| 50-59 years | 2,859 | 27.8% | 1,154 | 28.7% |
| 60-69 years | 2,809 | 27.3% | 1,049 | 26.1% |
| 70-79 years | 1,838 | 17.8% | 542 | 13.5% |
| >79 years | 784 | 7.6% | 164 | 4.1% |
| **Socioeconomic status** | | | | |
| Low | 3,372 | 32.7% | 1,506 | 37.5% |
| Medium | 2,434 | 23.6% | 956 | 23.8% |
| High | 3,471 | 33.7% | 1,190 | 29.6% |
| Unknown | 1,025 | 9.9% | 363 | 9.0% |
| **Histological tumour type** | | | | |
| Ductal | 8,465 | 82.2% | 3,212 | 80.0% |
| Lobular | 1,248 | 12.1% | 588 | 14.6% |
| Mixed | 390 | 3.8% | 137 | 3.4% |
| Other | 199 | 1.9% | 78 | 1.9% |
| **Differentiation grade** | | | | |
| Grade I | 2,460 | 23.9% | 793 | 19.8% |
| Grade II | 5,456 | 53.0% | 2,005 | 49.9% |
| Grade III | 2,251 | 21.9% | 1,132 | 28.2% |
| Unknown | 135 | 1.3% | 85 | 2.1% |

**Table 1.** Continued

| Characteristics | No cALND (n=10,302) | | cALND (n=4,015) | |
|---|---|---|---|---|
| **Clinical tumour size** | **N** | **%** | **N** | **%** |
| cT1 not further specified | 54 | 0.5% | 25 | 0.6% |
| cT1a | 114 | 1.1% | 38 | 1.0% |
| cT1b | 1,476 | 14.3% | 447 | 11.1% |
| cT1c | 5,098 | 49.5% | 1,726 | 43.0% |
| cT2 | 3,560 | 34.6% | 1,779 | 44.3% |
| **Multifocality** | | | | |
| No | 8,499 | 82.5% | 3,126 | 77.9% |
| Yes | 1,780 | 17.3% | 880 | 21.9% |
| Unknown | 23 | 0.2% | 9 | 0.2% |
| **Breast cancer subtype** | | | | |
| HR+/HER2- | 8,766 | 85.1% | 3,206 | 79.9% |
| HR+/HER2+ | 682 | 6.6% | 344 | 8.6% |
| HR-/HER2+ | 208 | 2.0% | 125 | 3.1% |
| HR-/HER2- | 465 | 4.5% | 300 | 7.5% |
| Unknown | 181 | 1.8% | 40 | 1.0% |
| **Treatment characteristics** | | | | |
| **Type of surgery** | | | | |
| Breast-conserving surgery | 6,926 | 67.2% | 1,663 | 41.4% |
| Mastectomy | 3,376 | 32.8% | 2,352 | 58.6% |
| **Result of SLNB** | | | | |
| Micrometastasis | 4,710 | 45.7% | 678 | 16.9% |
| Macrometastasis | 5,592 | 54.3% | 3,337 | 83.1% |
| **Radiation therapy** | | | | |
| No | 1,475 | 14.3% | 1,673 | 41.7% |
| Yes | 8,827 | 85.7% | 2,342 | 58.3% |
| **Hormonal therapy** | | | | |
| No | 1,700 | 16.5% | 656 | 16.3% |
| Yes | 8,602 | 83.5% | 3,359 | 83.7% |
| **Chemotherapy** | | | | |
| No | 5,691 | 55.2% | 1,227 | 30.6% |
| Yes | 4,611 | 44.8% | 2,788 | 69.4% |
| **Hospital characteristics** | | | | |
| **Hospital volume** | | | | |
| <150 resections per year | 3,484 | 33.8% | 1,526 | 38.0% |
| 150-300 resections per year | 6,144 | 59.6% | 2,314 | 57.6% |
| >300 resections per year | 674 | 6.5% | 175 | 4.4% |
| **Hospital type** | | | | |
| General non-teaching | 2,443 | 23.7% | 1,072 | 26.7% |
| Teaching hospital | 7,172 | 69.6% | 2,745 | 68.4% |
| Academic hospital | 687 | 6.7% | 198 | 4.9% |
| **Hospital innovative capacity** | | | | |
| Not innovative (0-5% GEP use) | 2,638 | 25.6% | 1,395 | 34.7% |
| Moderately innovative (6-10% GEP use) | 3,384 | 32.8% | 1,440 | 35.9% |
| Fairly innovative (11-15% GEP use) | 2,092 | 20.3% | 626 | 15.6% |
| Very innovative (>15% GEP use) | 2,188 | 21.2% | 554 | 13.8% |

Abbreviations: cALND = completion axillary lymph node dissection HR = hormonal receptor, HER2 = human epidermal growth factor receptor 2, c = clinical, p = pathological, SLNB = sentinel lymph node biopsy, NOS = not otherwise specified, mi = micrometastasis, GEP = gene expression profile

## MULTIVARIABLE (MULTILEVEL) LOGISTIC REGRESSION ANALYSIS

Multivariable logistic regression analyses revealed that treatment in a hospital with high innovative capacity (i.e. in which a GEP was often deployed) was associated with a significant lower chance of receiving a cALND (OR 0.69, OR 0.46 and OR 0.35 in hospitals with 6-10%, 11-15% and more than 15% GEP use, respectively, compared to hospitals with <5% GEP use). Other factors that were significantly associated with a lower chance of cALND were year of diagnosis (OR 0.52 (95%CI 0.51-0.54), age 70-79 and ≥79 years (ORs 0.59 (95%CI 0.50-0.68) and 0.21 (95%CI 0.17-0.26), compared to age 50-59 years) medium and high socio-economic status (SES) (OR 0.88 (95%CI 0.77-0.99) and 0.68 (95%CI 0.68-0.86), compared to low SES), mixed tumor histology (OR 0.73 (95%CI 0.57-0.95), compared to ductal histology) and treatment in an academic hospital (OR 0.41 (95%CI 0.33-0.51), compared to general non-teaching hospitals) (Table 2). Factors associated with an increased chance of undergoing a cALND were HR-/HER2- tumors (OR 1.46 95%CI 1.19-1.80, compared to HR+/HER2-), macrometastasis according to SLN (OR 6.37 (95%CI 5.70-7.13), compared to micrometastasis), and treatment with mastectomy (OR 4.57 95%CI 4.09-5.10, compared to breast-conserving surgery). Hospital volume was not associated with the risk of cALND (Table 2). Multilevel logistic regression analyses, in which the dependency of patients within hospitals was accounted for, showed similar results except for SES. The latter was not significantly associated with a lower risk of receiving cALND anymore. In addition, grade was not significant in the conventional logistic regression model, but it was significant in the multilevel model. However, the OR estimates were similar for the two models (Table 3). Multilevel and logistic regression analyses performed on complete cases only yielded similar results (Supplementary Table 1 and 2).

**Table 2.** Multivariable logistic regression analyses to assess factors associated with receiving completion axillary lymph node dissection (cALND).

| | Odds ratio | Lower 95% limit | Upper 95% limit | p-value |
|---|---|---|---|---|
| **Hospital innovative capacity** | | | | |
| Not innovative | *Reference* | | | |
| Moderately innovative | 0.69 | 0.62 | 0.78 | **<0.001** |
| Fairly innovative | 0.46 | 0.39 | 0.53 | **<0.001** |
| Very innovative | 0.35 | 0.30 | 0.40 | **<0.001** |
| | | | | |
| **Year of diagnosis**[1] | 0.52 | 0.51 | 0.54 | **<0.001** |
| | | | | |
| **Age group** | | | | |
| <40 years | 1.21 | 0.95 | 1.55 | 0.115 |
| 40-49 years | 1.10 | 0.96 | 1.26 | 0.176 |
| 50-59 years | *Reference* | | | |
| 60-69 years | 0.90 | 0.80 | 1.02 | 0.114 |
| 70-79 years | 0.59 | 0.50 | 0.68 | **<0.001** |
| >79 years | 0.21 | 0.17 | 0.26 | **<0.001** |
| **Socioeconomic status** | | | | |
| Low | *Reference* | | | |
| Medium | 0.88 | 0.77 | 0.99 | **0.039** |
| High | 0.76 | 0.68 | 0.86 | **<0.001** |
| **Histological tumour type** | | | | |
| Ductal | *Reference* | | | |
| Lobular | 0.99 | 0.86 | 1.15 | 0.942 |
| Mixed | 0.73 | 0.57 | 0.95 | **0.018** |
| Other | 0.96 | 0.68 | 1.36 | 0.822 |
| **Differentiation grade** | | | | |
| Grade I | *Reference* | | | |
| Grade II | 1.04 | 0.92 | 1.17 | 0.576 |
| Grade III | 1.14 | 0.99 | 1.33 | 0.076 |
| **Clinical tumour size** | | | | |
| T1 | *Reference* | | | |
| T2 | 1.06 | 0.96 | 1.18 | 0.238 |
| **Multifocality** | | | | |
| No | *Reference* | | | |
| Yes | 0.99 | 0.87 | 1.12 | 0.875 |
| **Breast cancer subtype** | | | | |
| HR+/HER2- | *Reference* | | | |
| HR+/HER2+ | 0.93 | 0.77 | 1.11 | 0.397 |
| HR-/HER2+ | 0.92 | 0.68 | 1.23 | 0.558 |
| HR-/HER2- | 1.46 | 1.19 | 1.80 | **<0.001** |
| **Type of surgery** | | | | |
| Breast-conserving surgery | *Reference* | | | |
| Mastectomy | 4.57 | 4.09 | 5.10 | **<0.001** |
| **SLNB result** | | | | |
| Micrometastasis | | | | |
| Macrometastasis | 6.37 | 5.70 | 7.13 | **<0.001** |

**Table 2.** Continued

| | Odds ratio | Lower 95% limit | Upper 95% limit | p-value |
|---|---|---|---|---|
| **Hospital volume** | | | | |
| <150 resections per year | *Reference* | | | |
| 150-300 resections per year | 0.94 | 0.83 | 1.07 | 0.335 |
| >300 resections per year | 0.90 | 0.70 | 1.15 | 0.391 |
| **Hospital type** | | | | |
| General non-teaching | *Reference* | | | |
| Teaching hospital | 1.05 | 0.92 | 1.20 | 0.442 |
| Academic hospital | 0.41 | 0.33 | 0.51 | **<0.001** |

[1.] 2011 served as reference year, the OR represents the OR per year increase.

**Table 3.** Multivariable multilevel logistic regression to assess factors associated with receiving completion axillary lymph node dissection (cALND). Hospital of first excision was used as hierarchical level to account for the dependency of patients within hospitals.

| | Odds ratio | Lower 95% limit | Upper 95% limit | p-value |
|---|---|---|---|---|
| **Hospital innovative capacity** | | | | |
| Not innovative | *Reference* | | | |
| Moderately innovative | 0.49 | 0.26 | 0.92 | **0.025** |
| Fairly innovative | 0.43 | 0.21 | 0.88 | **0.020** |
| Very innovative | 0.27 | 0.12 | 0.60 | **0.001** |
| | | | | |
| **Year of diagnosis**[1] | 0.47 | 0.46 | 0.49 | **<0.001** |
| | | | | |
| **Age group** | | | | |
| <40 years | 1.29 | 0.99 | 1.67 | 0.055 |
| 40-49 years | 1.11 | 0.95 | 1.29 | 0.179 |
| 50-59 years | *Reference* | | | |
| 60-69 years | 0.88 | 0.77 | 1.01 | 0.071 |
| 70-79 years | 0.57 | 0.48 | 0.67 | **<0.001** |
| >79 years | 0.16 | 0.13 | 0.21 | **<0.001** |
| **Socioeconomic status** | | | | |
| Low | *Reference* | | | |
| Medium | 0.95 | 0.83 | 1.09 | 0.448 |
| High | 0.99 | 0.78 | 1.01 | 0.069 |
| **Histological tumour type** | | | | |
| Ductal | *Reference* | | | |
| Lobular | 1.03 | 0.88 | 1.20 | 0.759 |
| Mixed | 0.88 | 0.66 | 1.16 | 0.367 |
| Other | 0.90 | 0.62 | 1.31 | 0.575 |
| **Differentiation grade** | | | | |
| Grade I | *Reference* | | | |
| Grade II | 1.00 | 0.88 | 1.15 | 0.958 |
| Grade III | 1.22 | 1.04 | 1.44 | **0.016** |
| **Clinical tumour size** | | | | |
| cT1 | *Reference* | | | |
| cT2 | 1.09 | 0.98 | 1.22 | 0.133 |

**Table 3.** Continued

| | Odds ratio | Lower 95% limit | Upper 95% limit | p-value |
|---|---|---|---|---|
| **Multifocality** | | | | |
| No | *Reference* | | | |
| Yes | 1.09 | 0.95 | 1.25 | 0.215 |
| **Breast cancer subtype** | | | | |
| HR+/HER2- | *Reference* | | | |
| HR+/HER2+ | 0.93 | 0.77 | 1.13 | 0.455 |
| HR-/HER2+ | 0.87 | 0.63 | 1.19 | 0.376 |
| HR-/HER2- | 1.41 | 1.12 | 1.76 | **0.003** |
| **Type of surgery** | | | | |
| Breast-conserving surgery | *Reference* | | | |
| Mastectomy | 4.80 | 4.26 | 5.43 | **<0.001** |
| **SLNB result** | | | | |
| Micrometastasis | *Reference* | | | |
| Macrometastasis | 8.52 | 7.52 | 9.65 | **<0.001** |
| **Hospital volume** | | | | |
| <150 resections per year | *Reference* | | | |
| 150-300 resections per year | 0.77 | 0.59 | 1.02 | 0.070 |
| >300 resections per year | 0.68 | 0.45 | 1.04 | 0.072 |
| **Hospital type** | | | | |
| General non-teaching | *Reference* | | | |
| Teaching hospital | 1.00 | 0.57 | 1.76 | 0.993 |
| Academic hospital | 0.34 | 0.14 | 0.83 | **0.017** |

[1.] 2011 served as reference year, the OR represents the OR per year increase.

Random-effects parameters: SD 1.08 (95%CI 0.91-1.29).

## DISCUSSION

Between 2011-2018, 28% of the Dutch breast cancer patients who suffered from cT1-2 breast cancer received an cALND, after SLN revealed nodal metastases. Besides known patient- and tumor characteristics, undergoing a cALND was associated with several hospital factors in the current study. Patients who were treated in a hospital with high innovative capacity, based on frequent GEP use in routine breast cancer care, or in an academic hospital, had a lower probability of receiving a cALND. Our findings suggest that early adoption to new innovations within a multidisciplinary breast cancer team results in a more reticent attitude towards axillary treatment.

As a result of the Z0011 and the AMAROS study, the Dutch national guidelines of 2012 first suggested to consider no further axillary surgery or to propose axillary radiotherapy as an alternative in some cases, in cT1-2 patients with a positive SLN [20]. The effect of this guideline change was clear: as reported in this study, in 2011 71% of cT1-2N1 patients received a cALND compared to 44% of patients in 2012. Over time this percentage further decreased to only 7% of patients receiving a cALND in 2018, illustrating a slow but almost full adaption of this de-escalating approach in Dutch clinical practice.

In the current study, several factors were associated with the risk of receiving cALND. Older age was associated with a lower risk of receiving a cALND, whereas a more aggressive tumor subtype (HR-/HER2-) increased the risk of receiving cALND. Ong et al. reported similar results in a nation-wide cohort of patients eligible for the Z0011 (cT1-2N0) criteria in the United States treated between 2009-2014 [21]. These results suggest that physician-driven risk stratification may drive the extent of axillary surgery, resulting in higher rates of cALND in younger patients or patients with aggressive tumor biology. This is despite the fact that several studies have shown that more extensive axillary therapy may not always be warranted in these patients and is associated with higher morbidity rates as compared to radiotherapy [22, 23].



Patients treated in a hospital with high innovative capacity (i.e. early adopters of GEP use), had a lower risk of receiving cALND. This finding suggests that in hospitals in which multidisciplinary teams tend to individualize systemic treatment by using GEPs, those teams are also more inclined to de-escalate axillary surgery. In line with this finding is a study conducted by Morrow et al. that showed that surgeons with less acceptance of the ‘no ink on tumor’ as a definition of a negative margin, where also less likely to implement the results of the Z0011 trial [24]. While the latter data indicates that variation exists in the acceptance of a more limited surgical approach among breast cancer surgeons, the present study suggests that innovative propensity is an asset of a multidisciplinary team or hospital and underscores the need for ongoing education of surgeons and multidisciplinary teams in order to improve acceptance.

Our findings also show that patients treated in an academic hospital had a lower risk of receiving cALND. Ong et al. also reported a lower incidence of cALND in patients treated in academic centers as compared to patients treated in community cancer centers. Prior literature already suggested that although individual doctors may adapt novel clinical trial insights, as a whole, academic centers preceded community hospitals in evidence-based practice change [25, 26]. This could partially be explained by higher participation rates of academic hospitals in clinical trials in which innovations are implemented in clinical practice. The fact that hospitals in which GEPs were applied more often – and thus de-escalate systemic treatment – were also more inclined to limit axillary treatment, further endorse the finding that hospitals with an innovative propensity are more likely to make evidence-based practice change in other parts of breast cancer treatment.

In the current study, with robust nation-wide data, factors were revealed which are associated with the probability of receiving cALND in SLN+ breast cancer patients. We are the first to assess the association between omission of cALND (which can be interpreted as a proxy for de-escalating local breast cancer treatment) and a hospitals innovative capacity (defined as GEP use). It should be noted that both GEP use as omission of cALND could be mediated by a high innovative capacity of multidisciplinary teams. Furthermore, it is important to note that the time period for early adoption of GEP use is arbitrarily chosen and based on previous data on GEP use and chemotherapy administration in the Netherlands [16,17]. Following closure of patient accrual for the MINDACT study in 2011, GEPs first entered clinical practice. In a previous nationwide study conducted between 2011-2013 evaluating the impact of GEP use on the administration of adjuvant chemotherapy, we observed that the use of a GEP was accompanied by a decrease in chemotherapy administration mainly in node negative patients [16]. However, in the years thereafter (2013-2016), this association was no longer observed. In the latter years, a further decline in chemotherapy administration was observed in all node negative patients and this trend was irrespective of GEP use [17]. We therefore decided to focus only on the years 2011-2013, since in this period the use of GEPs had the major clinical implication of withholding chemotherapy. However, a possibility of bias resulting from this approach cannot be ruled out. Therefore, our study should mainly be interpreted as hypothesis-generating material on adaption of innovation, rather than assessing causality between cALND and the use of GEPs.

In conclusion, a downward trend was observed in the use of cALND in Dutch SLN+ breast cancer patients between 2011-2018. Patients treated in hospitals with innovative capacity, based on the use of GEPs in clinical practice, had a lower probability of receiving cALND. This suggests that hospitals that early adopt innovations to de-escalate systemic treatment are also more likely to de-escalate axillary treatment. The latter observation is not only of importance for involved patients, as de-escalation is often accompanied by

less morbidity, but also affects our health care system since costs are rising and adapting to innovation to de-escalate treatment plays a key role in keeping our health care system viable. Therefore, our findings endorse the need for studies on barriers and facilitators of implementing innovations to increase the nation-wide uptake of innovation, ultimately to reduce inter-institutional inequality in breast cancer care.

**Supplementary Table 1.** Multivariable logistic regression analyses on complete cases only, to assess factors associated with receiving completion axillary lymph node dissection (cALND).

| | Odds ratio | Lower 95% limit | Upper 95% limit | p-value |
|---|---|---|---|---|
| **Hospital innovative capacity** | | | | |
| Not innovative | *Reference* | | | |
| Moderately innovative | 0.75 | 0.66 | 0.85 | **<0.001** |
| Fairly innovative | 0.45 | 0.39 | 0.53 | **<0.001** |
| Very innovative | 0.35 | 0.30 | 0.41 | **<0.001** |
| | | | | |
| **Year of diagnosis**[1] | 0.52 | 0.51 | 0.535823 | **<0.001** |
| | | | | |
| **Age group** | | | | |
| <40 years | 1.18 | 0.92 | 1.52 | 0.202 |
| 40-49 years | 1.09 | 0.94 | 1.26 | 0.245 |
| 50-59 years | *Reference* | | | |
| 60-69 years | 0.91 | 0.79 | 1.04 | 0.153 |
| 70-79 years | 0.61 | 0.52 | 0.72 | **<0.001** |
| >79 years | 0.21 | 0.16 | 0.27 | **<0.001** |
| **Socioeconomic status** | | | | |
| Low | *Reference* | | | |
| Medium | 0.87 | 0.77 | 0.99 | **0.036** |
| High | 0.75 | 0.67 | 0.85 | **<0.001** |
| **Histological tumour type** | | | | |
| Ductal | *Reference* | | | |
| Lobular | 0.99 | 0.85 | 1.16 | 0.939 |
| Mixed | 0.71 | 0.54 | 0.94 | **0.017** |
| Other | 0.95 | 0.65 | 1.38 | 0.782 |
| **Differentiation grade** | | | | |
| Grade I | *Reference* | | | |
| Grade II | 1.05 | 0.92 | 1.20 | 0.468 |
| Grade III | 1.14 | 0.98 | 1.34 | 0.098 |
| **Clinical tumour stage** | | | | |
| cT1 | *Reference* | | | |
| cT2 | 1.06 | 0.95 | 1.18 | 0.335 |
| **Multifocality** | | | | |
| No | *Reference* | | | |
| Yes | 1.01 | 0.88 | 1.15 | 0.907 |
| **Breast cancer subtype** | | | | |
| HR+/HER2- | *Reference* | | | |
| HR+/HER2+ | 0.99 | 0.82 | 1.20 | 0.951 |
| HR-/HER2+ | 0.88 | 0.64 | 1.19 | 0.403 |
| HR-/HER2- | 1.36 | 1.09 | 1.69 | **0.007** |
| **Type of surgery** | | | | |
| Breast-conserving surgery | *Reference* | | | |
| Mastectomy | 4.51 | 4.00 | 5.08 | **<0.001** |
| **SLNB result** | | | | |
| Micrometastasis | *Reference* | | | |
| Macrometastasis | 6.55 | 5.81 | 7.39 | **<0.001** |

**Supplementary Table 1.** Continued

| | Odds ratio | Lower 95% limit | Upper 95% limit | p-value |
|---|---|---|---|---|
| **Hospital volume** | | | | |
| <150 resections per year | *Reference* | | | |
| 150-300 resections per year | 0.96 | 0.84 | 1.10 | 0.601 |
| >300 resections per year | 0.90 | 0.68 | 1.17 | 0.421 |
| **Hospital type** | | | | |
| General non-teaching | *Reference* | | | |
| Teaching hospital | 1.04 | 0.90 | 1.20 | 0.565 |
| Academic hospital | 0.43 | 0.34 | 0.55 | **<0.001** |

p-values in bold indicate statistical significance (p<0.05). Abbreviations: GEP = gene expression profile, c = clinical, HR = hormonal receptor, HER2 = human epidermal growth factor receptor 2, SLNB = sentinel lymph node biopsy

1. 2011 served as reference year, the OR represents the OR per year increase.

**Supplementary Table 2.** Multivariable multilevel logistic regression on complete cases only to assess factors associated with receiving completion axillary lymph node dissection (cALND). Hospital of first excision was used as hierarchical level to account for the dependency of patients within hospitals.

| | Odds ratio | Lower 95% limit | Upper 95% limit | p-value |
|---|---|---|---|---|
| **Hospital innovative capacity** | | | | |
| Not innovative | *Reference* | | | |
| Moderately innovative | 0.53 | 0.29 | 0.98 | **0.044** |
| Fairly innovative | 0.43 | 0.21 | 0.87 | **0.019** |
| Very innovative | 0.27 | 0.12 | 0.60 | **0.001** |
| | | | | |
| **Year of diagnosis**[1] | 0.47 | 0.45 | 0.49 | **<0.001** |
| | | | | |
| **Age group** | | | | |
| <40 years | 1.28 | 0.97 | 1.68 | 0.082 |
| 40-49 years | 1.09 | 0.93 | 1.28 | 0.275 |
| 50-59 years | *Reference* | | | |
| 60-69 years | 0.88 | 0.76 | 1.01 | 0.078 |
| 70-79 years | 0.59 | 0.50 | 0.70 | **<0.001** |
| >79 years | 0.16 | 0.12 | 0.21 | **<0.001** |
| **Socioeconomic status** | | | | |
| Low | *Reference* | | | |
| Medium | 0.95 | 0.83 | 1.09 | 0.493 |
| High | 0.89 | 0.78 | 1.02 | 0.089 |
| **Histological tumour type** | | | | |
| Ductal | *Reference* | | | |
| Lobular | 1.02 | 0.87 | 1.21 | 0.778 |
| Mixed | 0.83 | 0.62 | 1.13 | 0.237 |
| Other | 0.90 | 0.60 | 1.34 | 0.603 |
| **Differentiation grade** | | | | |
| Grade I | *Reference* | | | |
| Grade II | 1.00 | 0.87 | 1.16 | 0.957 |
| Grade III | 1.21 | 1.02 | 1.44 | **0.032** |

**Supplementary Table 2.** Continued

| | Odds ratio | Lower 95% limit | Upper 95% limit | p-value |
|---|---|---|---|---|
| **Clinical tumour stage** | | | | |
| cT1 | *Reference* | | | |
| cT2 | 1.07 | 0.95 | 1.21 | 0.241 |
| **Multifocality** | | | | |
| No | *Reference* | | | |
| Yes | 1.11 | 0.96 | 1.28 | 0.170 |
| **Breast cancer subtype** | | | | |
| HR+/HER2- | *Reference* | | | |
| HR+/HER2+ | 0.98 | 0.80 | 1.21 | 0.884 |
| HR-/HER2+ | 0.85 | 0.61 | 1.19 | 0.341 |
| HR-/HER2- | 1.30 | 1.03 | 1.66 | **0.030** |
| **Type of surgery** | | | | |
| Breast-conserving surgery | *Reference* | | | |
| Mastectomy | 4.77 | 4.19 | 5.43 | **<0.001** |
| **SLNB result** | | | | |
| Micrometastasis | *Reference* | | | |
| Macrometastasis | 8.58 | 7.51 | 9.79 | **<0.001** |
| **Hospital volume** | | | | |
| <150 resections per year | *Reference* | | | |
| 150-300 resections per year | 0.78 | 0.58 | 1.05 | 0.100 |
| >300 resections per year | 0.69 | 0.44 | 1.07 | 0.095 |
| **Hospital type** | | | | |
| General non-teaching | *Reference* | | | |
| Teaching hospital | 0.98 | 0.56 | 1.71 | 0.937 |
| Academic hospital | 0.35 | 0.15 | 0.84 | **0.019** |

[1.] 2011 served as reference year, the OR represents the OR per year increase.

Random-effects parameter of multilevel model: SD 1.06 (95%CI 0.89-1.27). A Likelihood Ratio test (LRT) was performed to compare the multilevel (random effects) model with the fixed effects model (logistic regression without hospital as hierarchical level), LRT vs. logistic regression: P-value <0.001. The residual intraclass correlation coefficient was 0.25 (95%CI 0.19-0.33), indicating that 25% of the variance in the outcome can be explained by clustering of patients within hospitals.

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# Chapter 10

Concluding remarks and future perspectives

## SUMMARY AND GENERAL DISCUSSION

Accurate assessment of histopathological variables of the tumor, including histologic grade, is important for individual risk assessment and adjuvant therapy decisions in patients with breast cancer. The most widely used modified Bloom and Richardson grading system combines tubular formation, nuclear pleomorphism and mitotic count, the last of which reflects tumor proliferation. Apart from counting mitosis, tumor proliferation can also be assessed by proliferation-associated biomarkers including nuclear protein Ki67 or phosphohistone H3 (PhH3). In the first part of this thesis the use of histopathological proliferation markers and genomic classifiers to optimize grading and enhance risk-assessment in (ER) positive (+) / HER2- breast cancer is studied.

In **chapter 2** the distinction of molecular breast cancer subtypes based on conventional pathology assessment was compared to gene-signatures (70-gene signature and 80-gene signature) in a selection of ER+ breast cancer patients. The additional value of a gene expression profile test (80-GS) over conventional pathology to discriminate between 'luminal' and 'HER2+' breast cancers and to identify missed 'basal' like cancers was very limited. However, the distinction between Luminal A and B like cancers, based on assessing tumor grade through routine pathology or by evaluation of Ki67 expression, was often discordant when compared to the use of the 70 gene signature (70-GS). Since outcomes of both methods are used in clinical practice to guide adjuvant chemotherapy decision making, and have documented prognostic value [1-6], follow-up data will have to be awaited to address their prognostic interdependency.

Although gene-expression profiles are appealing tools in clinical practice for their reproducible analysis, their deployment remains costly. Therefore, the quest continues for alternative, reliable predictors of breast cancer outcome that can be determined preferably by conventional pathology. In **chapter 3** we compared interobserver variation for routine assessment of the mitotic activity index (MAI), Ki67 expression and PhH3. We found that PhH3 was associated with a more reproducible assessment of tumor proliferation compared to the use of conventional MAI and Ki67. The use of PhH3 also reduced variation in determining malignancy grade through better identification of mitotic figures. Uniform assessment of malignancy grade is important as it remains an important parameter when adjuvant chemotherapy is considered for the individual patient. In due course, follow-up data will become available for these patients in this study, and will provide meaningful information about the prognostic value of Ki67 and PhH3 versus genetic profiling by the 70-GS.

In **chapter 4** we studied the deployment of gene-expression profile use and the administration of adjuvant chemotherapy in Dutch ER+ breast cancer patients who were treated between 2013 and 2016. Although national breast cancer guideline

recommendations regarding the use of gene-expression profiles for tailoring individual risk assessment and chemotherapy administration have not changed until 2017, we observed remarkable differences in the deployment of the 70-GS and its association with chemotherapy administration over the years 2013-2016 compared to earlier years (2011-2013). Until 2017, the Dutch national breast cancer guideline recommended to administer adjuvant chemotherapy to all node positive patients (≥N1a) and to patients without lymph node involvement but with unfavorable clinicopathological tumor features (grade III tumors >1cm, any tumor >2cm) as well as to patients <35 years of age [7]. In the current Dutch guideline, the definition of 'high risk' node negative patients shifted to patients with grade I tumors >3cm, grade II tumors >2cm, and grade III tumors >1cm [8]. In a previous nationwide study conducted between 2011 and 2013, we observed that the 70-GS was accompanied by a decrease in chemotherapy administration in node negative patients or patients with limited (Nmi) lymph node involvement [9]. However, in the years thereafter (2013-2016), this association was no longer observed. In the latter years, a decline in chemotherapy administration in all node negative patients was observed and this trend was irrespective of gene-expression profile use. For lymph node positive and younger patients (<60 years), the 70-GS did show a significant association with a decreased likelihood of chemotherapy administration compared to node negative patients or older patients (60-69 years). Since the decline in chemotherapy administration was seen in the absence of breast cancer guideline recommendations, clinicians apparently interpret the available and unchanged clinical information differently in more recent years. Our study results did show a potential impact of the 70-GS on chemotherapy administration in younger patients (<50 years), but the indication area for 70-GS use in younger patients is lately being discussed and, in many institutions, currently discommended. The long-term follow-up results of the MINDACT and TailorX trials, that do demonstrate a chemotherapy benefit in pre-menopausal women stratified to the 70-GS low risk group, corroborate to this recommendation [10,11].

In the second part of this thesis various aspects of lymph node staging in the time frame characterized by the results of the landmark Z0011 and AMAROS trials are studied.

The results of the Z0011 and AMAROS trials contributed to a paradigm shift in axillary surgery. The necessity to perform completion axillary lymph node dissection (cALND) in patients with 1-2 positive (+) sentinel lymph nodes (SLNs) treated by breast conserving surgery followed by whole breast irradiation and systemic therapy was soon abandoned. For SLN+ patients treated with mastectomy, the results of the AMAROS trial demonstrated that axillary radiotherapy was found to be effective as an alternative to cALND. Yet, evidence regarding the oncologic safety of 'observing the nodes' in SLN+ patients undergoing mastectomy is lacking. In **chapter 5** we evaluated patterns of care of Dutch breast cancer patients who underwent mastectomy and had a SLNB+. A marked decrease

in cALND performance in this category of patients was observed between 2009 and 2018. In the majority of pN1a patients, radiotherapy replaced cALND, i.e. in line with the AMAROS study, whereas half of pN1mi patients underwent no further axillary treatment by the end of the study period (2018). This apparently reflects the clinicians' confidence in a restrained surgical policy in this category of patients as well.

In **chapter 6** we explored the risk of developing a regional recurrence in a population-based study in patients who had a negative SLNB, given a reported false negative rate of the SLNB of approximately 5-7%. In the absence of any risk mitigating treatment option, i.e. patients treated by mastectomy without receiving adjuvant radiotherapy or systemic therapy, the 5-year regional recurrence risk was 2.4% and two-fold lower than one might expect based on the risk of residual disease. In line with the historical NSABP-04 study, these results demonstrate that residual metastatic lymph nodes do not automatically develop into a regional recurrence in the SLNB era. In **chapter 7** we subsequently aimed to quantify the 'unintended' side effects of the non-surgical treatments on the regional recurrence risk in a subset of SLNB negative breast cancer patients selected from the Netherlands Cancer Registry. We observed that radiotherapy as routine part of breast conserving therapy, chemotherapy, and hormonal therapy independently exerted a mitigating effect on the risk for the development of a regional recurrence. The three different treatment modalities separately at least halved the risk. These findings help to understand the observed discrepancy between the false-negative rate of SLNB and the low regional recurrence rate in N0 patients, and help the clinician to better grasp the discrepancy between the rate of additional positive non-SLN (27-33%) and the observed regional recurrence rate (<2%) in SLNB+ positive patients when axillary clearance is omitted.

In the last part of this thesis we focused on patients and doctors' perceptions of risk assessment in the era of more extensive grading and less invasive staging.



In **chapter 8** we evaluated the impact of 70-GS testing on patients' chemotherapy decisions. We found that use of the 70-GS commonly changed the initial inclination to undergo or forego adjuvant chemotherapy and reduced decisional conflict. The results illustrate that use of the 70-GS helped patients to come to a more 'informed' decision and confident attitude regarding adjuvant chemotherapy, leading to a higher number of patients that felt sure about their decision. We also observed that whether these patients did, or did not, understand the clinical implications of the test, was of little importance. The number of patients was limited, but we observed that low education level and older age were factors associated with poor understanding of the 70-GS test.

In **chapter 9** we aimed to explore patient-, treatment- and hospital-related factors associated with de-escalation in axillary surgery and focused on hospital factors that could reflect an innovative attitude. Besides various conceivable clinicopathological characteristics associated with cALND performance, we found that cALND performance indeed varied among institutions. Our results showed that patients who were treated in an academic hospital had a lower chance of undergoing cALND compared to patients treated outside the academic. We also found that patients treated in an institution in which gene-expression profiles were frequently applied in routine breast cancer care, were less likely to receive a cALND. These findings suggest that early adoption to new innovations (by using the 70-GS as proxy variable) by breast team members other than surgeons was associated with the swiftness to adopt to a more reticent attitude towards axillary treatment by their surgical counterparts in a breast team.

## FUTURE PERSPECTIVES

### MORE ABOUT THE TUMOR

Biology of the primary tumor has become ever more important for breast cancer treatment. The identification of the main molecular breast cancer subtypes, each with their different biological behavior, prognosis, and response to treatment, has shifted breast cancer management from a 'one-size-fits all' principle to individualized cancer therapy. The molecular subtype already dictates systemic therapy regimens, but increasingly influences the sequence of local and systemic therapy too and may dominate the extent of local therapy in the near future. Hence, accurate assessment of primary tumor characteristics reflecting tumor biology should be optimal.

Subtyping of luminal type breast cancer into low- or high risk ER+/HER2 negative (-) cancer is commonly done by assessment of the modified Bloom and Richardson malignancy grade and the immunohistochemical assessment of Ki67 expression or by the use of a gene expression profile. High Ki67 expression in luminal type breast cancers is used to advise in favor of adjuvant chemotherapy [12]. More recently, Ki67 expression is also used in the neo-adjuvant setting to come to a biomarker-driven treatment strategy in terms of the duration of eventual endocrine treatment [13-16]. The clinical utility of Ki67 expression, albeit available over the past 30 years, remains controversial. Various studies and meta-analyses have demonstrated an independent prognostic value of Ki67 in ER+ early stage breast cancer [1-4,17]. Despite incorporation of Ki67 in the St. Gallen Consensus guideline [12], its use is still hampered by the lack of a uniform method for assessing and scoring of Ki67 and unclear thresholds. In particular among patients with grade II cancers in whom the benefit of adjuvant chemotherapy is unsure, Ki67 levels are highly variable and show poor reproducibility [18]. Genomic signatures have their own strengths and weaknesses. In the

last ten years they contributed to risk stratification in ER+ early breast cancer, and catalyzed a tendency of clinicians to administer less adjuvant chemotherapy in patients with ER+/HER2-breast cancer [9,19]. These genomic tests are used in the neo-adjuvant setting as well. The costs and turnaround time of these multigene signatures remain limiting factors. As we demonstrated in this thesis and in line with other studies, the outcome of the 70-GS poorly relates to the result of Ki67 enhanced grading [20,21]. Therefore, the quest for less costly and easily accessible as well as reproducible alternatives continues.

PhH3 holds promise as a novel biomarker to assess tumor proliferation. Unlike mitotic figure detection on standard haematoxylin and eosin (H&E) stained sections, PhH3 is not expressed in cells that mimic mitosis, such as apoptotic or necrotic cells. Due to its bright staining, PhH3 enhances the recognition of mitotic figures, but also allows the identification of mitotic figures during prophase. As we demonstrate in this thesis, this does lead to an increased sensitivity of mitotic figure detection and therefore, PhH3-based mitotic counts are usually higher. The prognostic value of PhH3 in breast cancer patients has been demonstrated in several studies [22-27]. Yet, unrefined integration of the PhH3-based mitotic counting into the modified Bloom and Richardson grading systemic will result in a substantial proportion of patients being upgraded, especially from grade II to grade III cancers. Future studies should address the dependency between the prognostic impact of Ki67, PhH3 or other proliferation markers on the one hand and gene expression profiles on the other hand to further improve risk stratification. Furthermore, combining the histopathological analysis and results of genomic testing into a prognostic prediction model seems a sensible option too.

Another way to improve risk stratification might be to decrease interobserver variation and increase reproducibility through standardizing biomarker assessment methods by use of artificial intelligence (AI). Computerized algorithms for Ki67 have shown potential for that purpose [28]. A recent study conducted by *Ström et al.* demonstrated that AI models can be trained to detect and grade prostate cancer with international experts-level performance [29]. Similar developments are also ongoing for breast cancer with deep learning algorithms being applied for breast cancer grading and even prediction of the intrinsic molecular breast cancer subtype [30,31]. In particular in ER+/HER2- breast cancers, future prediction models should incorporate existing patient and histopathological and genomic tumor characteristics, but may also be bolstered by AI enhanced assessment of mitotic count, Phh3 or Ki67 expression.



## LESS ABOUT THE NODES

Concurrent with the increasing knowledge of tumor biology, the extent of axillary surgery has gradually decreased in the last decades. Originally, ALND was routinely performed in all breast cancer patients for the purpose of staging and locoregional control. In the mid

90's, ALND was replaced by SLNB in patients with clinically node negative breast cancer, providing accurate and even more detailed staging information with less morbidity and without affecting locoregional control [32-34]. Subsequently, landmark trials such as IBCSG 23-01, Z0011 and the AMAROS trials demonstrated that ALND can be safely omitted in terms of overall survival and locoregional control in patients with 1-2 positive SLNs [35-37]. These studies showed that a substantial risk (30%) of residual positive lymph does not impair survival nor locoregional control in patients receiving adjuvant radiotherapy or systemic therapy. The aforementioned trials merely included patients undergoing breast conserving surgery and results of randomized trials comparing ALND to axillary radiotherapy or observation in SLNB positive patients treated by mastectomy remain to be awaited [38-40]. While oncological safety is warranted in the context of ever less invasive axillary surgery, from a staging perspective, clinicians struggle with the increasing lack of information about the extent of lymph node involvement. In line with this trend, several trials explore whether SLNB can be omitted entirely in the 'surgery first setting', such as the BOOG 2013-08, SOUND and INSEMA trials [41-43]

Today, many breast cancer patients are treated with chemotherapy in the neo-adjuvant setting. Also in these patients it is challenging to find a balance between accurate staging of the axilla and oncological safety. In patients initially categorized as cN0, SLNB is accepted to stage the axilla before and after neo-adjuvant chemotherapy (NAC) [44]. In patients with clinically node positive disease (cN+) who receive NAC, optimal axilla surgery after NAC remains controversial. There are various minimal invasive strategies to evaluate residual nodal involvement, such as performing SLNB post-NAC, marking the axillary positive lymph node prior to NAC with an iodine seed (MARI) or another marker and remove it after NAC, or a combination of these two means (RISAS procedure) [45-48]. Accuracy of only performing SLNB following NAC in cN+ patients is hampered by a relatively high false negative rate (>10%) [49] and the same, although to a lesser extent, holds true for solely removing a pre-NAC clipped or iodine marked (MARI) tumor positive lymph node post NAC. Hence, from a 'staging' perspective, this would be in favor to combine the two techniques, as the documented false negative (FN) rate of this RISAS technique is significantly lower. Then again, from an oncological safety perspective, very low regional recurrence rates have been reported in cN+ patients who were assessed as ypN0 after NAC based on SLNB or MARI procedure and did not undergo subsequent ALND [48, 50-53].

## TOWARDS MOLECULAR SUBTYPE DRIVEN NODAL SURGERY

So, if axillary surgery does not impact survival and is not necessary for regional control, then what is the role and timing of nodal surgery? First and foremost, staging should not be a goal in itself. Any nodal intervention should be used to guide subsequent treatment, and this may differ for different tumor subtypes.

In luminal type breast cancer patients, systemic treatment is still commonly administered in the adjuvant setting and frequently based on an accurate assessment of the metastatic potential of the primary tumor. The need for axillary staging should be determined accordingly against the background of the limited clinical consequences of a false negative result of examination of the axilla. Several trials explore whether SLNB can be omitted entirely in the 'surgery first setting' in patients who are classified as cN0 based on axillary ultrasound (US), such as the BOOG 2013-08, SOUND and INSEMA trials [41-43]. Preliminary results of the INSEMA trial show that in the patients randomized to undergo axillary staging, only 0.4% of patients had pN2 disease, indicating that the chance of missing pN2 disease after a negative axillary US is very low. But even the implications of 'missing' pN1 disease are limited for certain categories of patients. The Rx PONDER trial showed no benefit of chemotherapy in postmenopausal patients with low risk 21-RS luminal type breast cancer with 1-3 positive SLNs, hence in these patients omitting SLNB seems a sensible approach [54].

For patients with luminal type breast cancers, the role of NAC is to enhance the chance of performing breast conserving surgery and/or limiting axillary surgery in cN+ patients. In the latter subgroup, the relevance of a potential false negative rate of the post NAC axillary staging is limited because ypN status does not guide subsequent systemic therapy. For some, this is reason to selectively remove the positive lymph nodes in cN1 luminal type patients as part of a surgery first treatment and then continue local treatment in line with the Z0011 or AMAROS results and give systemic therapy in the adjuvant setting.

In case of the other molecular (TN and HER2+) breast cancer subtypes, the role of axillary staging differs. Albeit that these subtypes are in itself usually an indication for chemotherapy, lymph node status as part of a 'surgery first' strategy may determine whether a more or less toxic chemotherapy is advised, e.g. a 'Tolaney-regimen' in HER2+ pT1N0 patients. Furthermore, in many of these patients, chemotherapy is often given in the neoadjuvant setting. The ypT as well as ypN status are important to distinguish complete (pCR) from non-complete responders, since the latter (non-pCR) patients nowadays have an indication for subsequent chemotherapy following NAC. In these patients, procedural false negative rate should be as low as possible and therefore benefit from the deployment of a procedure with the highest diagnostic accuracy.



Axillary management encompasses a variety of options ranging from still offering complete ALND to the omission of any form of axillary surgery. This reflects the interdependence of all treatment modalities, the increasing role played by the molecular breast cancer subtype, and very rarely the management of residual disease. As a consequence of ever less invasive axillary surgery, clinicians nowadays have to deal with having less pathological information available than they used to have and are comfortable with.

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# Chapter 11

Summary in Dutch

## BORSTKANKER IN NEDERLAND

Borstkanker is, zowel wereldwijd als in Nederland, de meest voorkomende vorm van kanker onder vrouwen. Ongeveer 1 op de 7 Nederlandse vrouwen ontwikkelt gedurende haar leven borstkanker. Dit betekent dat jaarlijks meer dan 15.000 vrouwen de diagnose borstkanker krijgen. Ondanks dat het aantal nieuwe borstkankergevallen de afgelopen jaren is toegenomen, is de kans om hieraan te overlijden juist afgenomen. Gemiddeld is 89% van de patiënten met borstkanker na vijf jaar nog in leven, en na 10 jaar ligt dit percentage op 80%. De daling in borstkankersterfte wordt onder andere toegeschreven aan vroege opsporing door screening en door verbetering en toename in het gebruik van adjuvante systeem therapie. Chemotherapie, hormoontherapie en doelgerichte therapie zijn vormen van systeem therapie. Met de uitbreiding van het indicatiegebied voor adjuvante systemische therapie, en een verbetering in de prognose van borstkanker, groeide de afgelopen jaren ook het besef dat niet iedere patiënt baat heeft bij deze aanvullende behandeling. Vooral bij patiënten met een hormoongevoelige borstkanker lijkt de overlevingswinst van systemische behandeling beperkt. De afgelopen jaren richt het onderzoek van borstkanker zich daarom onder andere op het identificeren van patiënten bij wie de te behalen overlevingswinst van systemische behandeling opweegt tegen de nadelen hiervan, en nog belangrijker, bij welke patiënten systemische therapie veilig achterwege gelaten kan worden. Daarmee vindt er een verschuiving plaats van het 'one size fits all' principe naar behandeling op maat.

## INZICHTEN IN DE BIOLOGIE VAN BORSTKANKER

In de afgelopen decennia is steeds duidelijker geworden dat tumor biologie een belangrijke rol speelt in borstkanker uitkomst. Aan het begin van de 21$^{e}$ eeuw konden vier verschillende moleculaire subtypes van borstkanker worden geïdentificeerd, ieder met een eigen biologisch gedrag en prognose. Tegelijkertijd zijn er genexpressieprofielen ontwikkeld die een onderscheid kunnen maken tussen patiënten met een hoog of laag risico op terugkeer van ziekte of uitzaaiingen. Deze inzichten zijn een belangrijke stap gebleken op het gebied van 'precision medicine', waarbij de systemische en lokale behandeling in toenemende mate worden afgestemd op de biologische kenmerken van de tumor. Er bestaan verschillende genexpressieprofielen en vrijwel allemaal worden die testen centraal uitgevoerd in laboratoria van de producenten van de respectievelijke genexpressieprofielen. In Nederland werd in de afgelopen jaren hoofdzakelijk het 70-genen profiel (70-GS; Mammaprint) toegepast om een onderscheid te kunnen maken tussen hoog- en laag risico hormoongevoelige (luminaal type) borstkanker. Inmiddels is deze test in de praktijk vervangen door OncotypeDx en wordt vergoed vanuit het basispakket.

Daarnaast worden klassieke pathologische technieken zoals de hormoonreceptor- en HER2neu receptor status bepaling nog altijd als variabelen gebruikt om het 'surrogaat'

moleculaire subtype vast te stellen. Daarmee zijn er grofweg drie hoofdgroepen te onderscheiden: het hormoon-gedreven 'luminale' subtype (ER+ of PR+), het HER2 gedreven 'HER2+' subtype en het 'basale subtype' (ER-, PR-, HER2-). Bij het luminale subtype wordt een nader onderscheid gemaakt tussen het luminale A en het luminale B subtype, waarbij de prognose van de laatste groep minder gunstig is. In veel pathologische laboratoria wordt naast de bepaling van de maligniteitsgraad, aanvullend een Ki67 kleuring gedaan om dit onderscheid tussen het luminale A en luminale B borstkanker subtype te maken.

Gelijktijdig met de toename in kennis en inzicht over het biologisch gedrag van de primaire tumor en de daarmee gepaard gaande terughoudendheid in het geven van systeemtherapie aan patiënten met gunstige tumor kenmerken, zien we ook een verschuiving ontstaan richting een steeds minder agressieve behandeling van de oksel. Historisch gezien bepaalde de betrokkenheid van de lymfeklieren (m.n. in de oksel) in sterke mate de keuze voor systemische therapie. Met de huidige de-escalatie in de stadiëring van de oksel, worden clinici geacht om met steeds minder informatie over de okselklieren toch een keuze te maken voor de aanvullende systemische therapie indicatie. Dit maakt dat er steeds meer focus komt te liggen op de kenmerken en biologisch gedrag van de primaire tumor, en steeds minder informatie beschikbaar is over eventuele uitzaaiingen in de okselklieren. Ondanks dat deze ontwikkelingen onafhankelijk van elkaar zijn opgetreden, beïnvloeden ze elkaar in sterke mate.

Het eerste deel van dit proefschrift richt zich voornamelijk op het gebruik van pathologische proliferatiemarkers en genexpressieprofielen om een onderscheid te maken in het biologische moleculaire borstkanker subtype. Daarnaast kijken we naar de impact van de inzet van een genexpressieprofiel op chemotherapie gebruik in Nederland.

Het tweede deel van dit proefschrift richt zich op de ontwikkelingen en trends in het stadiëren van de oksel en de klinische risico's daarvan.

In het derde deel van dit proefschrift kijken we naar patiënt ervaringen met het gebruik van genexpressieprofielen. Tot slot kijken we in hoeverre de implementatie van een meer terughoudende behandeling van de oksel varieert tussen Nederlandse ziekenhuizen.

## DEEL 1: STEEDS MEER INFORMATIE UIT DE PRIMAIRE TUMOR

In **hoofdstuk 2** vergelijken we de moleculaire subtypering van borstkanker met behulp van een genexpressieprofiel met een surrogaat subtypering op basis van conventionele diagnostiek van hormoon- en HER2 receptoren. Daarnaast vergelijken we de onderverdeling van het luminale A en luminale B subtype op basis van de uitkomst van een genexpressieprofiel (het 70-genen profiel; "Mammaprint") met de onderverdeling

op basis van de bovengenoemde pathologie bepalingen (tumor gradering aangevuld met een Ki67 bepaling) in een cohort van 595 patiënten met een oestrogeenreceptor (ER) positieve tumor. We zagen vergelijkbare proporties patiënten die geclassificeerd werden als zijnde 'luminal' op basis van een genexpressieprofiel of op basis van lokale pathologische bepalingen. Echter in het maken van het onderscheid tussen het luminale A en luminale B subtype, bestond een discrepantie tussen de subtypering op basis van een genexpressieprofiel en de routinematige pathologische bepalingen aangevuld met tumor graad en Ki67.

In **hoofdstuk 3** onderzoeken we de reproduceerbaarheid van drie verschillende tumor proliferatie markers die een rol spelen bij de het vaststellen van de maligniteitsgraad: de mitotische activiteitsindex, Ki67 bepaling en een telling van mitosen na kleuring met fosfohiston H3. In een cohort van 159 patiënten met een ER-positieve borstkanker, zien we dat het gebruik van fosfohiston H3 leidt tot een betere reproduceerbaarheid tussen pathologen in de beoordeling van tumorproliferatie dan het gebruik van de mitotische-activiteitindex en Ki67. Wanneer fosfohiston H3 wordt gebruikt als parameter om het aantal cellen met mitotische activiteit te bepalen en daarmee de tumor gradering volgens Bloom Richardson vast te stellen, zien we een afname in de variatie in tumorgradering. Tegelijkertijd zien we ook dat het gebruik van fosfohiston H3 leidt tot een toename van tumoren die als hooggradig wordt geclassificeerd (graad III). De verbetering in reproduceerbaarheid door het gebruik van fosfohiston H3 is relevant, omdat de beslissing om patiënten te behandelen met aanvullende systeemtherapie grotendeels afhangt van de vastgestelde maligniteitsgraad.

In **hoofdstuk 4** laten we zien dat de inzet van genexpressieprofielen in ER+/HER2- patiënten over de jaren 2013-2016 is toegenomen in Nederland en dat het gebruik van chemotherapie in dezelfde categorie patiënten aanzienlijk is gedaald. Terwijl in een eerdere landelijke studie duidelijk werd dat de inzet van een genexpressieprofiel leidde tot minder gebruik van chemotherapie valt het in de huidige studie op dat de inzet van een genexpressieprofiel bij patiënten met een negatieve lymfeklierstatus (pN0) niet is geassocieerd met minder chemotherapie gebruik. Het blijkt dat onafhankelijk van de inzet van een genexpressieprofiel in deze categorie van N0 patiënten een sterke daling in het gebruik van chemotherapie heeft plaatsgevonden in die periode. Bij patiënten met een positieve lymfeklierstatus (pN1) en bij jonge vrouwen zien we wel dat de inzet van een genexpressieprofiel is geassocieerd met minder chemotherapiegebruik. Het is opvallend dat deze trends in het gebruik van chemotherapie optraden in een tijdsperiode waarin geen wijzigingen hebben plaatsgevonden in de Nederlandse richtlijn voor de behandeling van borstkanker.

## DEEL 2: STEEDS MINDER INFORMATIE OVER DE LYMFEKLIEREN

Het afgelopen decennium is de okselbehandeling voor patiënten met een positieve schildwachtklier (SWK) behoorlijk veranderd. De IBCSG 23-01, ACOSOG Z0011 en de AMAROS-trials hebben hier een grote rol in gespeeld. Deze trials lieten zien dat een okselklierdissectie veilig achterwege gelaten kan worden voor patiënten met beperkte lymfekliermetastasen, ofwel vervangen kan worden door radiotherapie van de oksel (AMAROS). De studiepopulatie van de Z0011-trial (waarin het achterwege laten van een okselbehandeling werd vergeleken met een observatie van de oksel) betrof patiënten die een borstsparende behandeling ondergingen en dus wel routinematig radiotherapie van de borst kregen als onderdeel van hun behandeling. Voor de IBSCG 23-01 en de AMAROS-trial lag het percentage patiënten dat borstsparend werd behandeld op respectievelijk 91% en 83%. Dat maakt dat de bewijslast voor het achterwege laten van een okselklierdissectie voor patiënten met een positieve oksel die worden behandeld middels mastectomie, minder robuust is.

In **hoofdstuk 5** van dit proefschrift laten we zien dat het percentage aanvullende okselklierdissecties in Nederland bij patiënten die een mastectomie hebben ondergaan en een positieve SWK hadden (pN+) daalt van 78% in 2009 naar 10% in 2018. Dit laat zien dat chirurgen zich ook in patiënten behandeld middels mastectomie, comfortabel voelen om een okselklierdissectie achterwege te laten. We zien ook, in lijn met de resultaten van de AMAROS-studie, dat voor patiënten met macrometastasen (≥N1a), de okselklierdissectie vrijwel volledig is vervangen door aanvullende radiotherapie. Voor patiënten met micrometastasen of geïsoleerde tumorcellen zien we dat in het merendeel van de patiënten regionale behandeling achterwege gelaten wordt (in 60% en 92% van de patiënten, respectievelijk). Tumor karakteristieken zoals het moleculaire subtype of de leeftijd van de patiënt, spelen een rol bij de besluitvorming om wel of niet te kiezen voor een okselklierdissectie.

Halverwege de jaren negentig is de SWK-procedure geïntroduceerd als minimaal invasief stadiëringsonderzoek voor borstkanker patiënten met een klinisch negatieve lymfeklierstatus. Aan de techniek van de SWK-procedure kleeft een risico van 5-7% op achtergebleven metastasen bij een negatieve SWK-biopsie (vals negatieve test) en dit wordt als zodanig geaccepteerd in de kliniek. De categorie patiënten die een mastectomie ondergaat en een negatieve SWK had is op basis van die 5-7% kans op achtergebleven lymfekliermetastasen in de volgende studie als model gehanteerd om het risico op het ontwikkelen van een regionaal recidief (recidief in de oksel) nader te analyseren. Daarnaast hebben we in een vervolgstudie de effecten van radiotherapie en systemische therapie op de regionaal recidiefkans verder in kaart gebracht.

In **hoofdstuk 6** laten we met behulp van gegevens van de Nederlandse Kankerregistratie (NKR) zien dat de 5-jaars regionaal recidief kans bij SWK negatieve patiënten die worden behandeld middels mastectomie zonder aanvullende therapie (radiotherapie/ systeemtherapie) 2.4% bedraagt. Dit percentage ligt daarmee hoger dan voor pN0 patiënten die borstsparend zijn behandeld (ca 1.1%), maar is nog altijd veel lager dan het percentage patiënten dat naar verwachting additionele metastasen in de oksel had op grond van het percentage vals negativiteit van de SWK volgens de literatuur (5-7%). Blijkbaar ontwikkelen occulte lymfekliermetastasen zich lang niet altijd tot een regionaal recidief, wat in lijn is met de uitkomsten van de NSABP-04 studie uit de vorige eeuw.

In **hoofdstuk 7** gebruiken we opnieuw gegevens van de NKR om de 'onbewuste' effecten van niet-chirurgische therapieën op het risico op het ontwikkelen van een regionaal recidief te evalueren. We zien hierbij dat in een populatie van SWK-negatieve patiënten, radiotherapie als onderdeel van een standaard borstsparende behandeling, chemotherapie en anti-hormonale therapie elk het risico op een regionaal recidief met ca. de helft reduceren. Deze bevindingen gecombineerd met het fenomeen zoals beschreven in hoofdstuk 6 bieden een verklaring voor de discrepantie tussen het aandeel vals-negatieve biopsieën en de kans op een regionaal recidief bij SWK-negatieve patiënten. Wanneer we deze data extrapoleren naar patiënten een positieve SWK, helpen deze uitkomsten ons ook om de grote discrepantie tussen de kans op aanvullende lymfekliermetastasen (25-30%) en het lage regionaal recidief percentage (1%) bij patiënten die geen okselklierdissectie ondergaan, beter te begrijpen.

## DEEL 3: ARTSEN EN PATIËNTEN IN EEN TIJDPERK VAN NIEUWE ONTWIKKELINGEN OP HET GEBIED VAN GRADERING EN STADIËRING

In **hoofdstuk 8** worden de resultaten gepresenteerd van een prospectieve observationele studie naar de invloed van een genexpressieprofiel (het 70-genen profiel, Mammaprint) op de besluitvorming van de patient rondom chemotherapie. Borstkankerpatiënten bij wie, op basis van traditionele prognostische factoren, twijfel bestond over de indicatie voor chemotherapie, kwamen in aanmerking voor deze studie. In totaal werd aan 106 patiënten met hormoongevoelig borstkanker vóór de inzet van het genexpressieprofiel gevraagd naar hun voorkeur voor behandeling met aanvullende chemotherapie. Nadat de uitslag van de test was besproken tussen de arts en patiënt, werden patiënten opnieuw gevraagd om hun definitieve keuze voor behandeling met chemotherapie te formuleren. Op beide momenten werd ook naar de verwachtingen van, en ervaringen met de Mammaprint gevraagd. Inzet van een genexpressieprofiel leidde in meer dan een derde van patiënten tot een verandering in het besluit omtrent behandeling met chemotherapie. Daarbij zagen we dat patiënten zich na de test vaker zekerder voelden over hun keuze en gaf het een daling van de mate waarin zij 'decisional conflict' ervaarden. Tot slot zagen

we ook dat een aanzienlijk deel van de patiënten de klinische implicatie van de test niet helemaal begreep. Oudere leeftijd en een laag opleidingsniveau waren factoren die bij dit laatste een rol speelden.

In **hoofdstuk 9** is onderzocht in hoeverre de implementatie van een meer terughoudende behandeling van de oksel bij lymfeklierpositieve patiënten varieert tussen Nederlandse ziekenhuizen. We zien dat academische ziekenhuizen eerder geneigd zijn om een okselklierdissectie achterwege te laten dan perifere ziekenhuizen. Daarnaast zien we dat in ziekenhuizen waar medisch oncologen vooropliepen in de de-escalatie van systeemtherapie in combinatie met het inzetten van een genexpressieprofiel, chirurgen ook eerder geneigd zijn om de oksel terughoudender te behandelen. De dynamiek binnen een ziekenhuis of een multidisciplinair team lijkt dus van invloed te zijn op de mate waarmee nieuwe klinische inzichten worden geïmplementeerd in de praktijk. Dit vormt een belangrijk aangrijpingspunt voor vervolgonderzoek, maar geeft ook stof tot nadenken over hoe innovaties en richtlijnveranderingen optimaal geïmplementeerd kunnen worden in de praktijk.

# Chapter 12

Review committee of this thesis
List of publications
Acknowledgements (dankwoord)
About the author

## REVIEW COMMITTEE OF THIS THESIS

- Prof. dr. E. van der Wall
- Prof. dr. J. Wesseling
- Prof. dr. I.H.M. Borel Rinkes
- Prof. dr. P.W.B. Derksen
- Prof. dr. C.H. van Gils

## LIST OF PUBLICATIONS

Gooijer SA, Folkersma C, **van Steenhoven JEC**, de Kort J, Siesling S, Volders J, van Dalen T. Long-Term Outcome of Sustained Endocrine Monotherapy for Elderly Breast Cancer Patients. Ann Surg Oncol. 2023 30:1671-1677.

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## ABOUT THE AUTHOR

Julia van Steenhoven was born on the 11th of August 1992 in Utrecht, The Netherlands. She grew up in Zeist and graduated from the Herman Jordan Lyceum in Zeist in 2010. She then started her medical school at the University of Groningen.

Besides her study, she worked as a tutor medicine, she joined a medical team that assisted with collecting data and blood samples of level one trauma patients in the course of a research project of het UMCG Department of Trauma Surgery, and she joined the student association Vindicat Atque Polit where she participated multiple committees. After obtaining her bachelor degree, she went to Australia where she followed a Cambridge course and afterwards she went to Vietnam where she worked as an intern in the VietDuc University Hospital in Hanoi. She graduated from medical school in 2017 after completing her senior internship at General Surgery at the Diakonessen Hospital in Utrecht.

During her last year of medical school, Julia started medical research on breast cancer at the department of surgery under the supervision of dr. T. van Dalen at the Diakonessen Hospital. After graduating from medical school, she combined research with clinical work as a surgical resident (not in training) at the Diakonessen Hospital. From 2018, she started collaborating with Prof. P.J. van Diest at the department of pathology at the University Medical Center of Utrecht. This resulted in the current PhD thesis on refining prognostication in early stage breast cancer patients.

Unfortunately, Julia had to put her PhD on hold to recover from mild traumatic brain injury sustained in a cycling accident. During her recovery, she became increasingly aware of the importance of holistic medicine. In 2021, she started a foundation ''GETaHEAD Brain Recovery Support'', which provides a peer-support mentoring program to help patients with brain injury find footing, while navigating the challenges of recovery. Julia also works in a neurorehabilitation program called “Zelfzorg aan Zee” that utilizes surfing as therapeutic modality for patients with brain injury or other chronic symptoms. Having personally experienced the physical and mental benefits of this program during her own recovery, she is now passionate about helping other patients achieve their own personal recovery goals. She combines this work with a 2-year program on ‘Integrative and Lifestyle Medicine’ to further deepen her knowledge in this field.