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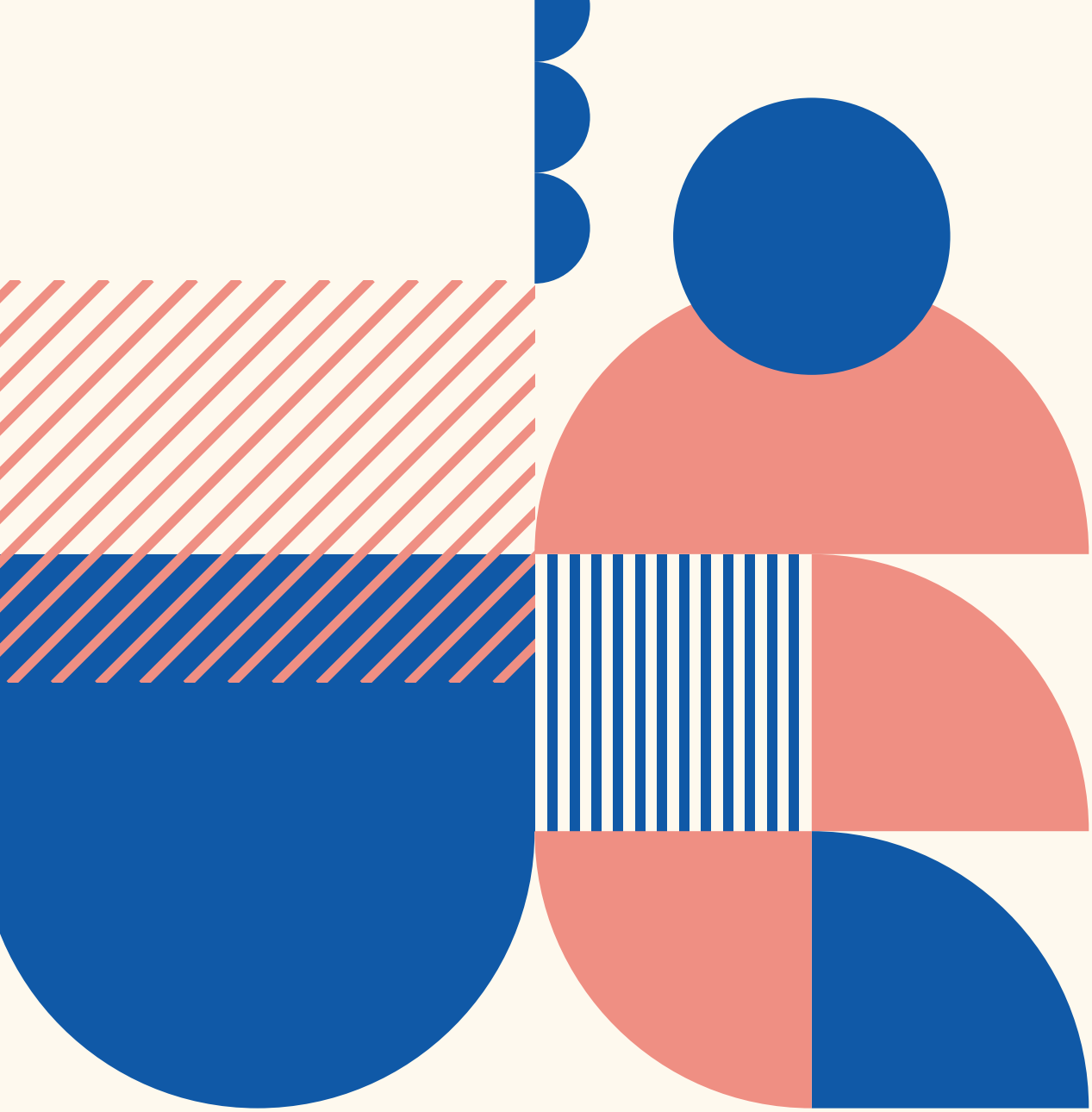

DE-ESCALATING LOCOREGIONAL TREATMENT AFTER NEOADJUVANT SYSTEMIC THERAPY IN BREAST CANCER

Marieke van der Noordaa

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DE-ESCALATING LOCOREGIONAL TREATMENT AFTER NEOADJUVANT SYSTEMIC THERAPY IN BREAST CANCER

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Chapter 1

Introduction and outline

Breast cancer is the most common diagnosed cancer among women worldwide.[1] Over the past three decades, the incidence rates of invasive breast cancer in the Netherlands have doubled, with 7.738 women being diagnosed in 1989, to 15.792 women in 2021.[2] The incidence of breast cancer is increasing in all continents of the world, but the highest incidence rates are reported in industrialized countries.[3] This trend can partially be explained by the westernization of lifestyles such as delayed and fewer pregnancies, reduced breastfeeding, lack of physical exercise and poor diet.[4,5] Other important causes for the increasing incidence are improved screening programs and imaging techniques, allowing earlier detection of breast cancer[1].

Whereas more women are being diagnosed with breast cancer, breast cancer mortality and breast cancer recurrence rates have decreased in high-income countries such as the Netherlands.[6] This decrease may be explained by earlier detection due to implementation of screening programs. However, improvement and more extensive use of systemic treatment are mainly accountable for the decrease of breast cancer mortality and recurrence.[7] Breast cancer is a heterogeneous disease with a wide variety in biological and morphological features, clinical behaviour and treatment response.[8] Four subtypes can be distinguished by determining oestrogen receptor and progesterone receptor (hormone receptors) status and human epidermal growth factor receptor 2 (HER2)-status, that each differ in treatment response and prognosis.[8,9]

Introduction of neoadjuvant systemic therapy for breast cancer patients

Neoadjuvant systemic therapy (NST; ie systemic therapy administered prior to surgery) was introduced in the 1970's, aiming to reduce locally advanced, inoperable breast cancer and make it operable.[10] Currently, NST is widely used, also in early-stage breast cancer. In the Netherlands, use of NST has increased from 9% in 2005 to 44% in 2020.[2] Although no survival advantage of NST over adjuvant systemic therapy (AST; ie systemic therapy administered after surgery) has been demonstrated,[11] the neoadjuvant treatment approach has several advantages. Most importantly, NST enables down-staging of the primary tumour and metastatic lymph nodes, permitting less extensive surgery in selected patients with good response to NST.[12,13] Furthermore, NST might be more likely to eradicate micrometastatic disease than AST.[11] In addition, it allows response monitoring, facilitating adjustments in the systemic therapy regimen or duration in case of either exceptional or non-responders.[14,15] NST also enables research by identifying predictors for response, and enables evaluation of new systemic treatment strategies by using pathologic complete response (pCR) as an early surrogate endpoint that correlates with

survival.[16-18] Systemic treatments are adapted to patient and tumour characteristics, resulting in pCR rates as high as 60% for triple-negative tumours and up to 90% for hormone-receptor (HR) negative, HER2-positive tumours.[14,19,20]

Pathologic complete response and residual cancer burden

Pathologic complete response is strongly associated with improved long-term survival outcomes. The meta-analysis of the Collaborative Trials in Neoadjuvant Breast Cancer (CTNeoBC) showed that patients with pCR have improved event-free and overall survival, with the greatest prognostic value in patients with aggressive tumour subtypes.[18] However, the binary outcome of pCR versus residual disease considers little information, without distinction among patients with varied amounts of residual disease. Another method, the residual cancer burden (RCB), was developed to address the shortcomings of pCR.[21] RCB provides a standardized pathologic method to evaluate and quantify the extent of residual invasive disease in the breast or regional lymph nodes after NST. The RCB method includes the diameter of residual disease, percentage of vital tumor cells, and diameter of the largest tumor-positive lymph node.[21,22] It provides a continuous measurement, with cutpoints at 0, 1.36 and 3.28 to define 4 RCB classes of increasing residual disease ranging from RCB-0 (corresponding to pCR) to RCB-III.[21] The prognostic value of RCB was demonstrated in the I-SPY1 (Investigation of Serial Studies to Predict Your Therapeutic Response with Imaging and Molecular Analysis) and I-SPY2 trials, and other single-institution and multicenter trials.[23-27] Briefly, I-SPY1 was a multi-center trial for women with locally advanced breast cancer treated with neoadjuvant anthracycline-based chemotherapy.[28,29] The I-SPY2 trial is a multicenter, adaptive randomized trial that compares, by subtype, investigational agents with a common controle of taxane-anthracycline-based chemotherapy in women with stage 2/3 breast cancer.[30,31]

In this thesis, we validated the prognostic value of RCB by performing a pooled participant-level analysis of multiple clinical trials and cohorts to evaluate the overall association between RCB and long-term outcomes, with emphasis on the breast cancer subtypes defined by hormone receptor and HER2 receptor status.

Ductal carcinoma in situ and neoadjuvant systemic therapy

Whereas increasing rates of pathologic complete response of invasive breast cancer are being observed, ductal carcinoma in situ (DCIS) is considered insensitive to systemic treatment.[32,33] Therefore, presence of DCIS adjacent to IBC, observed in 43-66% of patients with invasive breast cancer,[34,35] may impede de-escalation of surgery. Presence of a large area of calcifications on mammography or non-mass enhancement on MRI, both of which may be associated with DCIS, or DCIS adjacent to IBC in pre-NST biopsies are often considered contra-indications for breast conserving surgery (BCS), even in those with radiological complete response of the tumour on magnetic resonance imaging (MRI).

To facilitate potential de-escalation of surgery in the future in patients with adjacent DCIS, in this thesis, we aimed to estimate the response of adjacent DCIS to NST containing HER2-blockade in a large series of HER2-positive breast cancer patients. Furthermore we aimed to identify clinicopathological and radiological factors that predict response of DCIS.

De-escalating local treatment

While mastectomy used to be standard of care in patients with breast cancer, BCS is nowadays recommended for most patients with early-stage breast cancer. In the Netherlands, the use of BCS has increased from 36% in 1989 to 55% in 2020[2] (Figure 1). However, in patients with large breast tumours, BCS after NST remains controversial. An explanation for the reserved attitude towards BCS could be a concern about the safety of not removing the entire original tumour area after NST. Since tumour-positive margins after BCS are associated with a higher risk of local recurrence,[36,37] the selection of patients for BCS should be based on whether tumour-free margins can be achieved. Therefore, reliable assessment of residual disease is essential when considering de-escalating surgery. Magnetic resonance imaging (MRI) has been demonstrated to be the most adequate imaging modality to evaluate the presence or extent of residual disease after NST.[38] In this thesis, we discuss the safety of breast conserving therapy (BCS + radiation treatment) in cT3 breast cancer patients in whom MRI was used to assess the presence of residual tumour during and after NST.

With the increasing pCR rates after NST, breast cancer survival has greatly improved over the past decades. Therefore, locoregional treatments should be de-escalated whenever oncologically safe, to prevent unnecessary long-term side effects of these treatments. Although morbidity

occurs more frequently after mastectomy, in BCS, moderate to severe long-term morbidity such as pain, fibrosis, loss of flexibility, asymmetry and decreased psychosocial function is present in up to 45% of patients.[39-42] Therefore, we designed the MICRA trial (Minimally Invasive Complete Response Assessment) with the ultimate aim to eliminate surgery of the breast in patients who achieve pCR, consequently improving quality of life of these patients. To this end, we evaluated the value of ultrasound-guided biopsy of the breast in identifying pCR after NST in patients with radiologic complete response (rCR) on MRI. In this thesis, we present the study protocol, the feasibility and the interim analysis of the MICRA trial.

De-escalating regional treatment

Axillary lymph node status is an important prognostic factor in patients with breast cancer.[18] As with breast surgery, axillary surgery has undergone multiple changes. For decades, axillary lymph node dissection (ALND) was standard of care in all women with in invasive breast cancer.[43] However, ALND is associated with significant morbidity including lymphedema, chronic pain, numbness and limitation of shoulder movements.[44,45] In the adjuvant setting, studies have showed that leaving low-volume axillary metastasis in situ does not compromise oncologic safety in patients who are treated with radiation therapy.[46-48] In the neoadjuvant setting, there has been an on-going debate on axillary staging before and after NST. Generally, clinicians differentiate patients with node negative disease before and after NST (cN0, ypN0), and patients with node positive disease prior to NST (cN+) who remain node positive after NST (ypN+) or who convert to node negative disease after NST (ypN0).

In patients with clinically node-negative (cN0) breast cancer, sentinel lymph node biopsy (SLNB) has replaced axillary lymph node dissection (ALND).[49,50] Although the risk of co-morbidity associated with SLNB is lower than that of ALND, co-morbidities such as paraesthesia, numbness and pain are reported in 5-34% of patients after SLNB. Lymphedema occurs significantly less frequently after SLNB compared with ALND, but is still noted in up to 5% of patients.[51] After NST, the rate of nodal positivity is low in patients with cN0 disease. In those with triple negative (TN) breast cancer or HER2+ disease and a pCR in the breast, ypN+ rates lower than 2 percent have been demonstrated.[52] In these patients, the value of surgical axillary staging after NST may be limited. In this thesis, we identified factors that predict tumour-negative sentinel nodes after NST in patients with cN0 breast cancer. By identifying such characteristics, it would be possible to select patients in whom axillary staging by SLNB could safely be omitted after NACT.

Although a decline has been observed in performance of ALND in patients with cN+ disease, ALND is still frequently performed in these patients.[53] False-negative rates (FNR) of SLNB after NST range from 5-30% and therefore SLNB is only useful in select patients: the FNR can be reduced to <10% in cN1-2 patients, when ultrasound after NST shows no suspect axillary lymph nodes (ALNs), when both technetium-99m-nanocolloid and blue dye are used, and when ≥3 SLNs can be retrieved and examined.[54-57] At the Netherlands Cancer Institute (NKI), we introduced an alternative technique for axillary staging after NST: the MARI-procedure (Marking Axillary lymph nodes with Radioactive Iodine seeds).[58] With this technique, a tumor-positive ALN is marked with an iodine seed before NST and selectively removed after NST with a FNR of 7% in predicting pCR in the additional ALNs.[59] In the last part of this thesis, we first present a feasibility study in which we demonstrate that the combination of 18F-FDG positron emission tomography/ computed tomography (PET/CT) before NST and the MARI-procedure after NST can reliably select patients in whom ALND can be replaced by axillary radiotherapy or even omission of all axillary treatment. PET/CT is an optimal method for nodal staging prior to NST with a positive predictive value (PPV) of 77-98% for detecting ALNs metastases.[60,61] In addition, the number of FDG-avid ALNs can reliably be determined.[62,63]

Next, we demonstrate the results of the implementation of the axillary treatment protocol in cN+ patients, in which results of the PET/CT pre-NST and MARI-procedure post-NST are combined. Finally, we present the three-year axillary recurrence-free interval in cN+ patients that were treated according to the axillary treatment protocol at the Netherlands Cancer Institute.

Figure 1. Trends in various types of local treatment in patients with invasive breast cancer *2021 concerns the first quarter of 2021.

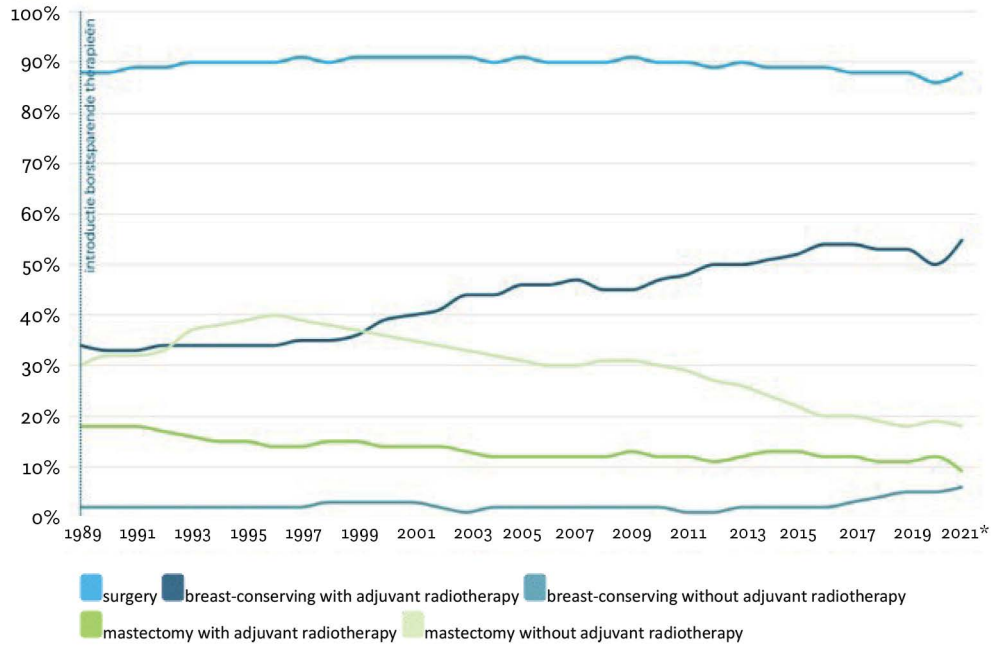

Figure adapted from: https://iknl.nl/borstkankercijfers

Rationale and outline of this thesis

The ultimate aim of this thesis is to de-escalate or eliminate surgical treatment in breast cancer patients that have exceptional response to NST, consequently preventing overtreatment and improving quality of life in these patients. To this end, several minimal and non-invasive methods for response prediction of the breast and axilla are investigated.

This thesis is divided into three sections. Section I consists of research that aims to predict response and prognosis in breast cancer patients that are treated with NST. In **chapter 2**, the prognostic value of the residual cancer burden is validated in a large multi-center pooled analysis. In **chapter 3**, the response of ductal carcinoma in situ after NST in patients with HER-2 positive breast cancer is assessed and predictors for response of DCIS are identified.

Section II focuses on de-escalating local treatment of the breast after NST. In **chapter 4** we investigated the safety of breast conserving therapy (BCS + radiation treatment) in cT3 breast cancer patients in whom MRI was used to assess the presence of residual tumour during and after NST. We present the study design and feasibility of the MICRA trial (Minimally Invasive Complete Response Assessment) in **chapter 5**. In this multi-center observational cohort study, we investigated the value of ultrasound-guided biopsy of the breast in identifying pCR after NST, with the ultimate aim to eliminate surgery of the breast in those who achieve pCR. In **chapter 6** the interim analysis of the MICRA trial is presented.

In section III, de-escalation of axillary treatment after NST in cNo and cN+ patients is assessed. In **chapter 7**, we identified pre-surgery factors that predict tumour-negative sentinel nodes after NST in patients with cNo breast cancer. The ultimate aim of this study is to identify patients groups in whom axillary staging could safely be omitted after NST. For cN+ patients, the MARI procedure was developed. We present a feasibility study in **chapter 8** that investigates if the combination of PET/CT before NST and the MARI-procedure after NST can reliably select patients in whom ALND can be replaced by axillary radiotherapy or even omission of all axillary treatment. In **chapter 9** the results of the implementation of this axillary treatment protocol for cN+ patients are presented. The three-year axillary recurrence-free interval in cN+ patients that were treated according to the axillary treatment protocol is investigated in **chapter 10**.

This thesis concludes with a general discussion including future perspectives in **chapter 11**. Additional research directions that require further exploration into de-escalating locoregional treatment and preventing overtreatment in breast cancer patients after NST are reviewed.

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Section I

Predicting response and prognosis in breast cancer patients treated with neoadjuvant systemic therapy

Chapter 2

Residual Cancer Burden after neoadjuvant chemotherapy and long-term survival outcomes in breast cancer: a multi-center pooled analysis of 5161 patients

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ABSTRACT

Background: Recent studies have independently validated the prognostic relevance of residual cancer burden (RCB) after neoadjuvant chemotherapy (NAC). We utilized results from multiple independent cohorts in a pooled subject-level analysis to evaluate the relationship of RCB to long-term prognosis across phenotypic sub-types of breast cancer to assess its generalizability among a broader range of practice settings.

Method: Twelve institutes and trials, identified through personal communications, provided subject-level RCB results, clinical and pathologic stage, tumor subtype and grade, treatment and follow-up data in November 2019 from patients, aged 18 or older, with primary Stage I-III breast cancer . The association between the continuous RCB score and primary study outcome, event-free survival (EFS), were assessed using mixed-effects Cox models with the incorporation of random RCB effects and stratification to account for between-study heterogeneity and differences in baseline hazard across subtypes, respectively. The association was further evaluated within each subtype in multivariate analyses incorporating random RCB effects and adjustments for age, clinical T-category (cT), nodal status (cN), and grade. Kaplan-Meier estimates of EFS at 3, 5 and 10 years were computed for each RCB class within subtype.

Findings: We analyzed subject-level data from 5161 patients treated with NAC between 1994 to 2019 from 12 participating groups. There were 1164 EFS events during follow-up (median 56 months, IQR: 61 months). RCB score was prognostic within each subtype (Hazard Ratio per unit increase in RCB, 95% CI): HR-positive/HER2-negative (EFS: 1.55, 1.41-1.71), HR-positive/HER2-positive (EFS: 1.74, 1.51-2.00), HR-negative/HER2-positive (EFS: 2.13, 1.71-2.66), and HR-negative/HER2-negative (EFS:1.98, 1.82-2.15), and remained prognostic in multivariate models adjusting for age, grade, and cT, and cN category at diagnosis.

Interpretation: RCB score and class were independently and strongly prognostic in all subtypes, and generalizable to multiple practice settings.

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RESEARCH IN CONTEXT

Evidence before this study

The seminal CTNeoBC meta-analysis published in 2014 demonstrated that on an individual level, achieving a pathologic complete response (pCR) following neoadjuvant chemotherapy (NAC) is associated with better long-term survival outcomes. As of September 13th 2021, a search of the PubMed database using the term "pathological complete response and breast cancer prognosis" yielded 1531 published articles between 1st January 2014 and 31st December 2019. However, pCR does not provide distinction among patients with residual disease. The Residual Cancer Burden (RCB) method was proposed in 2007 as a standardized methodology to evaluate and quantitate the extent of residual disease in breast and axillary lymph nodes following NAC. Between 1st January 2007 and 31st December 2019, 166 published articles have been indexed on PubMed as related to "residual cancer burden and breast cancer prognosis". RCB has been validated as prognostic in single institution studies and multicenter trials.

Added value of this study

Individually, the cohorts in previous studies evaluating the prognostic value of RCB are too small to obtain accurate estimates within the various subtypes of breast cancer. By assembling a pooled cohort of >5000 patients across 12 participating groups from the United States and Europe representing a variety of clinical settings, our study was able to validate the prognostic value of RCB overall as well as within each hormone receptor/HER2 defined subtype. As well, by evaluating RCB as a continuous measure in a model that allows for non-linear effect within each subtype, we were able to better characterize how risk of recurrence or death changes with increasing RCB and contrast this relationship between subtypes.

Implications of all the available evidence

The prognostic importance of pCR (RCB=0) is well-established. RCB adds significantly to the binary assessment of pCR vs. residual disease in predicting long-term survival. The prognostic consistency of RCB collected across different countries and clinical settings highlights the generalizability of implementing the RCB methodology. There is a strong potential to use the RCB score in a subtype-specific context to predict a patient's residual risk after NAC in a prospective setting with standardized evaluation of post-treatment resection specimens, especially given the increasing options for adjuvant therapy in the setting of residual disease.

INTRODUCTION

Neoadjuvant chemotherapy (NAC) was introduced for patients with locally advanced inoperable breast cancer in the late 1970s.[1] NAC is at least as effective as adjuvant therapy and has several additional advantages.[2] It permits less extensive breast and axillary surgery by downstaging the tumor and allows monitoring of treatment response, which provides important prognostic information. Pathological complete response (pCR) to NAC, defined as the absence of residual invasive disease in breast and axilla, is strongly associated with improved long-term survival endpoints.[3-5] The influential CTNeoBC meta-analysis demonstrated that patients with pCR have improved survival, with the greatest prognostic value in patients with highly proliferative tumors.[4] Consequently, the Food and Drug Administration (FDA) and European Medicines Agency (EMA) issued guidance for the use of pCR as a regulatory endpoint for accelerated approval of new agents for NAC of breast cancer. Since then, contemporary trials have incorporated standardized pathologic assessments of surgical resection specimens and validated pCR as an excellent prognostic marker.[6] Increasingly, the presence or absence of residual disease is being used to guide adjuvant decisions following NAC.[7,8]

The binary outcome of pCR versus residual disease confers limited information, offering no distinction among patients with varied amounts of residual disease. Furthermore, methods to evaluate surgical specimens and report residual disease have not been adequately standardized within pathology practice. Residual Cancer Burden (RCB), first described in 2007, was designed to address these shortcomings by providing a standard methodology to evaluate and quantify the extent of residual disease in breast and axillary lymph nodes following NAC.[9] It yields a continuous score in which pCR is the equivalent of an RCB score of zero. Empirically derived cutpoints (0, 1.36, 3.28) are applied to the continuous score to define four RCB classes, RCB-0 through RCB-III that represent an increasing residual disease burden. RCB assessments are highly reproducible between pathologists;[10,11] and both RCB and its classes have been validated as prognostic in single-institution studies[12-15] and multicenter trials.[12,16-19] However, individually, these cohorts are too small to obtain accurate estimates of prognosis related to RCB within the various subtypes of breast cancer. Therefore, we performed a pooled subject-level analysis of multiple clinical trials and cohorts to evaluate the overall association between RCB and long-term outcomes, with emphasis on the breast cancer subtypes defined by hormone receptor (HR) and HER2 receptor status. Our goal was to understand the prognostic value of RCB relative to

pCR in the context of subtypes in order to optimize its interpretation and better inform patient management across a broad array of practice settings.

METHODS

Study Design and Patient Cohorts

For inclusion in the analysis, trials or cohorts were required to: (1) include patients with primary breast cancer (any phenotypic subtype) treated with NAC followed by surgery; and (2) have available data for RCB, and follow-up data to evaluate event-free survival (EFS) and distant relapse-free survival (DRFS). Investigators from institutions or trials that were known to have assessed and reported RCB in a pre-defined cohort were invited to participate (and all accepted). Participating investigators representing twelve groups (four trials and eight clinical cohorts) from the United States and Europe provided individual patient data.

The following trials were included: the I-SPY1 trial,[17] the I-SPY2 trial,[18,20] the ARTemis Trial,[16] and a trial led by the Instituto de Investigación Sanitaria Gregorio Marañón (IISGM; Madrid, Spain).[19] Two of the trials included investigational therapies: the ARTemis study, in which bevacizumab was the investigational agent; and I-SPY2, in which nine investigational drugs were adaptively randomized 4:1 against a concurrent control.[17,20] ISPY-1 and the IISGM trials were both observational, evaluating standard chemotherapies without any experimental arms.

The eight clinical cohorts were the MDACC cohort (MDACC-LAB98-240 and MDACC-LAB02-010 protocols) of the MD Anderson Cancer Center (Houston, TX, USA),[12] the NEOREP cohort (CNIL declaration number 157270) from Curie Institute (Paris, France),[10] the TNBC P.R.O.G.E.C.T registry of the University of Kansas Medical Center (KUMC; Kansas CIty, KS, USA),[13] the TransNEO cohort from University of Cambridge (Cambridge, UK, European Genome-Phenome number EGAS00001004582), and cohorts from the Edinburgh Breast Unit at the Western General Hospital (Edinburgh, UK; Edinburgh Cancer Information Programme Board reference number CIR21166)), the Mayo Clinic (Rochester, MN, USA), the Netherlands Cancer Institute (Amsterdam, the Netherlands)[14] and Yale University (New Haven, CT, USA).

After neoadjuvant treatment and surgery, patients received adjuvant endocrine, HER2 therapy and locoregional radiation per institution standard of care. For the remainder of the manuscript, we refer to these trials and clinical cohorts as 'cohorts'. Details on these cohorts, including eligibility criteria, type of consent, enrollment period and patient characteristics, are provided in appendix, pp 2-3. All patients identifiers were removed from data before the data were transferred and collated into a single dataset for the present analysis.

Procedures

RCB was assessed by breast cancer pathologists trained in using the standard methodology to evaluate and calculate RCB score and class.[9] RCB was evaluated prospectively for five of the twelve cohorts (KUMC, I-SPY2 trial, IISGM, Mayo Clinic, and Yale), while RCB was determined in a retrospective review in the other seven (appendix, pp 2-3). RCB values used in this analysis were based on reporting at the treating center and were not centrally reviewed.

RCB (or RCB score) is calculated as a continuous variable. To aid in interpretation, cutpoints are applied to define four RCB classes indicating progressively larger residual disease burden: RCB-0 (RCB score=0, equivalent to pCR), RCB-1 (RCB score: 0-1.36), RCB-II (RCB score: 1.37-3.28) and RCB-III (RCB score>3.28).

Evaluation of pre-treatment histological grade was performed according to the Elston-Ellis modification of the Scarff-Bloom-Richardson grading system.[21] ER (estrogen receptor, ESR1) and PR (progesterone receptor, PGR) status used in this analysis were as defined and provided by the institutions. Two cohorts (ARTemis and TransNeo) only recorded the ER but not PR status. Thus, for our analysis, HR status was determined based on ER and PR status if both were available; or ER status alone if PR status was not available. In the ARTemis trial, the TransNeo cohort and Edinburgh cohort, HR status was defined as positive using an Allred score ≥3. In other cohorts, HR status was defined by the percentage of cells stained positive on IHC at either 1% or 10% threshold, depending on the institution. HER2 (ERBB2) status was determined according to international guidelines.[22] HR and HER2 status were used to define four phenotypic subtypes (HR-negative/HER2-negative; HR-negative/HER2-positive HR-positive/HER2-positive and HR-positive/HER2-negative) for analysis.

Outcomes

The primary endpoint was event-free survival, adapted from the standardized definitions proposed in the CTNeoBC study, and measured as time from start of neoadjuvant treatment to the occurrence of an event.[4] Any loco-regional recurrence or distant recurrence or death from any cause was considered as an event, and patients without an event were censored at the date of last follow-up. The secondary endpoint was distant relapse-free survival, defined as time from start of neoadjuvant therapy to distant recurrence or death from any cause. Follow-up was calculated from the start date of neoadjuvant chemotherapy.

Statistical Analysis

The association between the RCB score and EFS/DRFS in the pooled population was assessed with mixed effects Cox models, which included random cohort and RCB effects to account for between-cohort heterogeneity and stratification to account for differences in baseline hazard across biological breast cancer subtypes. The significance of the association was determined by the significance of the mean hazard ratio associated with a 1 unit increase in RCB on a log-transformed scale, with $p<0.05$ as the significance threshold. Similar mixed effects models were used to assess RCB score-EFS associations within each subtype. In addition, multivariable mixed effects Cox analysis adjusting for age, pre-treatment T-category (T0/I, T3, T4 vs. T2), pre-treatment nodal status (positive vs. negative) and grade (III vs I/II) (as fixed effects) as covariates were performed (overall and within each subtype) to evaluate whether RCB remains significantly prognostic independent of these clinical covariates. We also evaluated associations within each participating cohort using fixed effects Cox models stratified by subtype. In addition, to evaluate the non-linear effect of RCB on survival, we used B-splines with 2 degrees of freedom in our mixed effects models and constructed relative event rate plots (with RCB score of 0 as reference) as a function of increasing RCB. Mixed-effects analysis was conducted with the coxme package in R (version 3.4.3). Kaplan-Meier plots of EFS and DRFS by RCB class, overall and within breast cancer subtypes, were constructed with survival times truncated at 12 years (a time at which around 10% of the smallest RCB group [RCB-I] remained at risk for an event); survival estimates at 3, 5 and 10 years were computed.

Legal Agreements

Contracts between the different institutes and groups were centralized and organized by the legal team at the University of California, San Francisco. Agreements between US and European institutions were based on the European Union General Data Protection Regulation (GDPR).[23] All data was stripped of patient identifiers prior to data transfer.

Role of the funding source

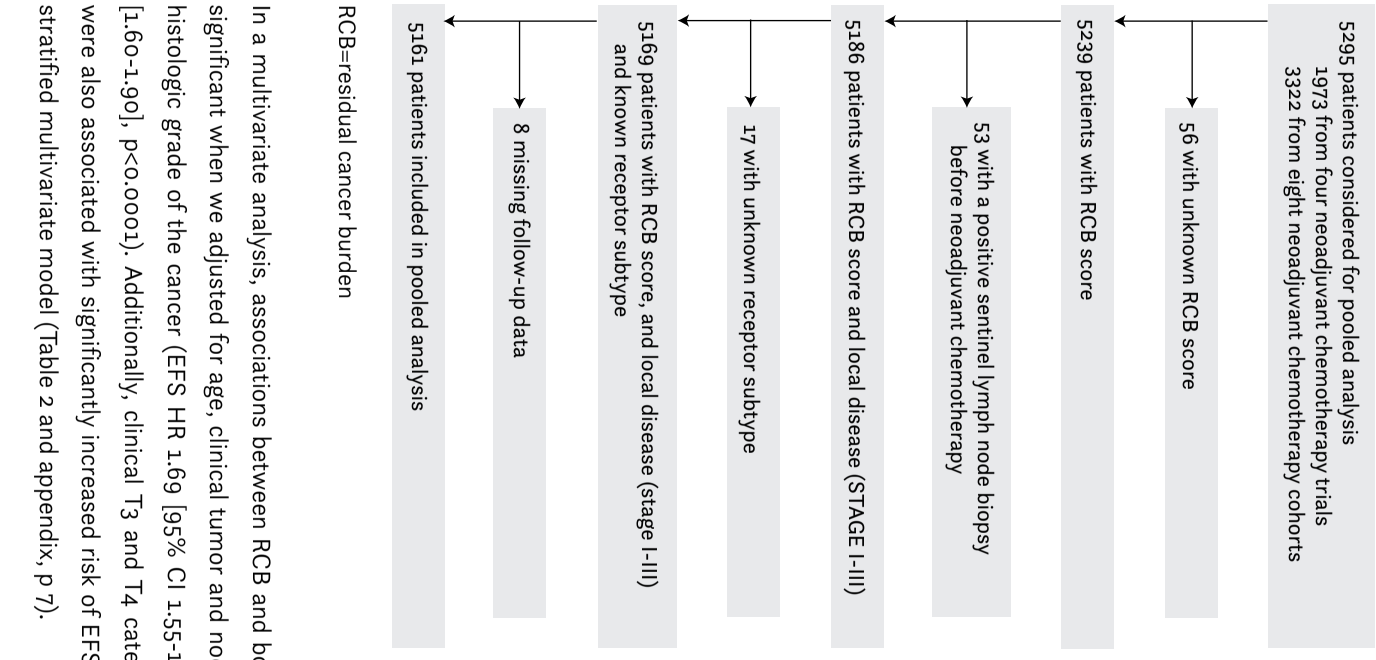
The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. CY, MOK. MO, MvdN, and SS had access to the raw data. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

RESULTS

5295 patients from 12 participating groups were identified for the pooled analysis. Patients with missing RCB score (n=56), a positive sentinel lymph node biopsy before NAC (n=53), unknown receptor subtype (n=17) or missing follow-up information (n=8) were excluded, yielding a total of 5161 patients for analysis (Figure 1).

Baseline patient and tumor characteristics, RCB class distribution and follow-up information are summarized in table 1 for the overall population and by breastt cancer subtype. In the overall population, median age was 49 years (IQR: 15). 466/5161 (9%) had a T1 tumor, 3139/5161 patients (61%) had a T2 tumor, 1026/5161 (20%) had a T3 tumor and 345/5161 (7%) had a T4 tumor. Lymph nodes were clinically involved in 2780/5161 patients (54%). There were 1774/5161 patients (34%) with HR-negative/HER2-negative disease, 1430/5161 patients (28%) had HER2-positive disease (of which 756/1439 (60%) were HR-positive and 488/1439 (40%) HR-negative) and 1957/5161 patients (38%) had HR-positive/HER2-negative tumors. 87% (1244/1430) of the HER2-positive patients received neoadjuvant HER2-targeted therapy. 93% (4790/5161) of tumors in our study were ductal or mixed ductal histology; only 4% (216/5161) were lobular. In the HR-positive/HER2-negative subset specifically, the fraction of lobular cancers in our study is only 8% (159/1957). Median follow-up time was 56 months (IQR: 61), with 1164 EFS events and 1072 DRFS events.

Figure 1. Study profile



RCB=residual cancer burden

In a multivariate analysis, associations between RCB and both EFS and DRFS remained highly significant when we adjusted for age, clinical tumor and nodal stage category at baseline, and histologic grade of the cancer (EFS HR 1.69 [95% CI 1.55-1.85], p<0.0001; and DRFS HR 1.75 [1.60-1.90], p<0.0001). Additionally, clinical T3 and T4 category, node positivity and grade III were also associated with significantly increased risk of EFS and DRFS events in this subtype-stratified multivariate model (Table 2 and appendix, p 7).

Table 1. Patient characteristics overall and by subtype

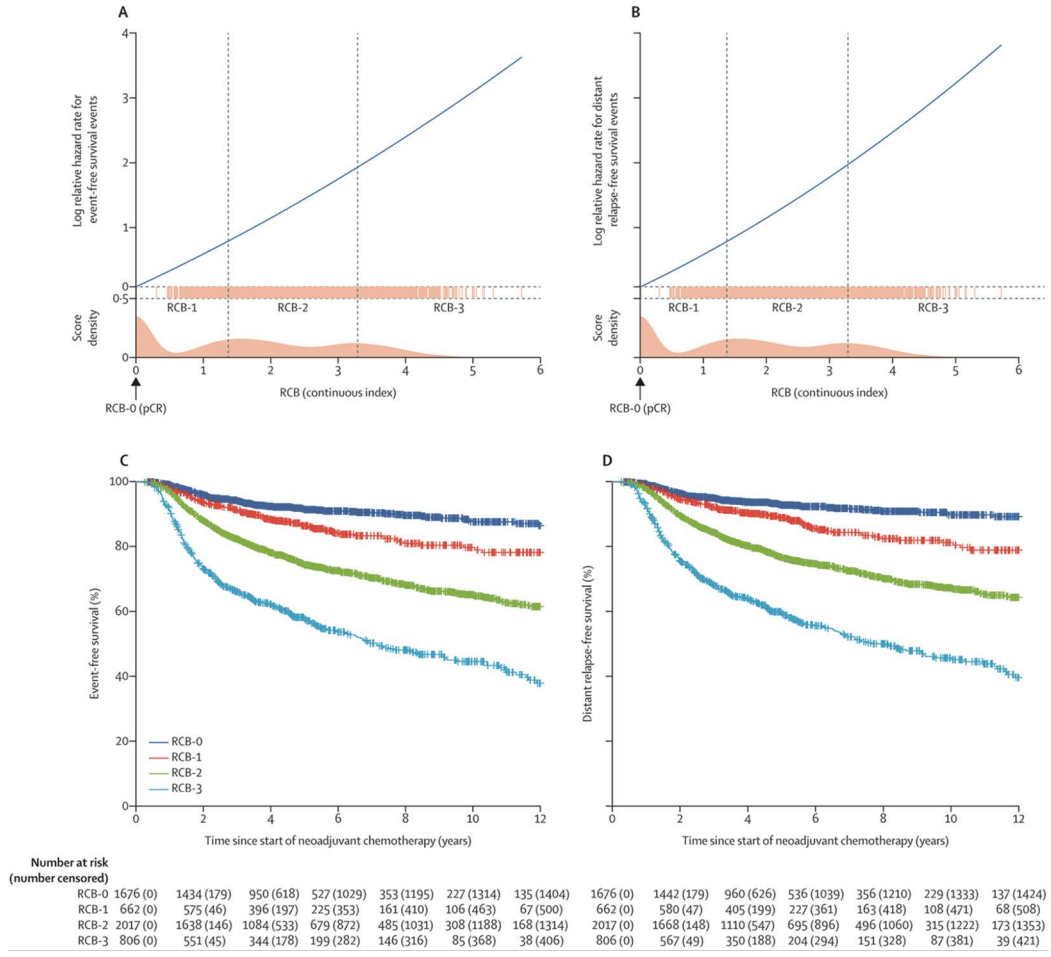
	Overall	HR-HER2-	HR-HER2+ (All)	HR-HER2+ (Neoadjuvant HER2-targeted)*	HR+HER2+ (All)	HR+HER2+ (Neoadjuvant HER2-targeted)*	HR+HER2-
N	5161	1774	572	488	858	756	1957
Baseline characteristics							
Age, median (IQR)	49 (15)	49 (16)	51 (14)	51 (14)	48 (16)	48 (16)	49 (15)
Baseline T category, N (%)							
0/1	466 (9.0%)	174 (9.8%)	56 (9.8%)	45 (9.2%)	84 (9.8%)	76 (10.1%)	152 (7.8%)
2	3139 (60.8%)	1132 (63.8%)	318 (55.6%)	277 (56.8%)	494 (57.6%)	444 (58.7%)	1195 (61.1%)
3	1026 (19.9%)	310 (17.5%)	138 (24.1%)	109 (22.3%)	172 (20.0%)	139 (18.4%)	406 (20.7%)
4	345 (6.7%)	106 (6.0%)	46 (8.0%)	43 (8.8%)	69 (8.0%)	59 (7.8%)	124 (6.3%)
Missing	185 (3.6%)	52 (2.9%)	14 (2.4%)	14 (2.9%)	39 (4.5%)	38 (5.0%)	80 (4.1%)
Baseline N positive, N (%)	2780 (53.9%)	806 (45.4%)	360 (62.9%)	298 (61.1%)	499 (58.2%)	429 (56.7%)	1115 (57%)
Histological Grade, N(%)							
I	130 (2.5%)	16 (0.9%)	3 (0.5%)	3 (0.6%)	8 (0.9%)	6 (0.8%)	103 (5.3%)
II	1668 (32.7%)	270 (15.2%)	151 (26.4%)	130 (26.6%)	356 (41.5%)	313 (41.4%)	911 (46.6%)
III	2945 (57.1%)	1348 (76%)	378 (66.1%)	317 (65%)	437 (50.9%)	381 (50.4%)	782 (40%)
Missing	398 (8.1%)	140 (7.9%)	40 (7%)	38 (7.8%)	57 (6.6%)	56 (7.4%)	161 (8.2%)
Receptor Status, N (%)							
HR-HER2-	1774 (34.4%)	1774 (100%)	0	0	0	0	0
HR-HER2+	572 (11.1%)	0	572 (100%)	488 (100%)	0	0	0
HR+HER2+	858 (16.6%)	0	0	0	858 (100%)	756 (100%)	0
HR+HER2-	1957 (37.9%)	0	0	0	0	0	1957 (100%)

Table 1. *Continued*

	Overall	HR-HER2-	HR+HER2+ (All)	HR+HER2+ (Neoadjuvant HER2-targeted)*	HR+HER2- (Neoadjuvant HER2-targeted)*
Histologic Type, N (%)					
Ductal or Mixed Ductal	4790 (92.8%)	1690 (95.3%)	542 (94.8%)	461 (94.5%)	814 (94.9%)	719 (95.1%)	1744 (89.1%)
Lobular	216 (4.2%)	19 (1.1%)	10 (1.7%)	9 (1.8%)	28 (3.3%)	24 (3.2%)	159 (8.1%)
Other	100 (1.9%)	42 (2.4%)	16 (2.8%)	14 (2.9%)	13 (1.5%)	10 (1.3%)	29 (1.5%)
Unknown/Missing	55 (1.1%)	23 (1.3%)	4 (0.7%)	4 (0.8%)	3 (0.3%)	3 (0.4%)	25 (1.3%)
Post Neoadjuvant Chemotherapy: RCB Classes, N (%)							
RCB-0	1676 (32.5%)	770 (43.4%)	376 (65.7%)	336 (68.9%)	313 (36.5%)	290 (38.4%)	217 (11.1%)
RCB-I	662 (12.8%)	212 (12%)	67 (11.7%)	55 (11.3%)	172 (20.1%)	153 (20.2%)	211 (10.8%)
RCB-II	2017 (39.1%)	590 (33.3%)	100 (17.5%)	76 (15.6%)	291 (33.9%)	250 (33.1%)	1036 (52.9%)
RCB-III	806 (15.6%)	202 (11.4%)	29 (5.1%)	21 (4.3%)	82 (9.6%)	63 (8.3%)	493 (25.2%)
Follow up Information							
Median follow-up (IQR) (months)	56 (61)	45 (45)	69 (71)	65 (61)	64 (63)	61 (56)	58 (64)
EFS Events	1164	450	95	62	154	118	465
DRFS Events	1072	417	79	53	135	100	441

Data are n, n (%), or median (IQR). Negative values for the lower IQR bound are truncated at 0. RCB=residual cancer burden.
* The subset who received neoadjuvant HER2-targeted therapy as neoadjuvant treatment in combination with chemotherapy.

Figure 2. Prognostic value of RCB score and RCB class in the overall pooled analysis cohort.



Plots of log relative hazard rate for event-free survival events (**A**) and distant relapse-free survival events (**B**) as a function of RCB score. Splines approximation of RCB with two degrees of freedom was used to allow for non-linear effect. A log linear increase in relative hazard rate implies that the hazard ratio associated with change in RCB remains constant over the range of RCB. Thresholds for corresponding RCB classes (RCB-0 to RCB-3) are shown for reference (vertical dashed lines). Vertical bars represent all RCB scores recorded on a continuous scale. Kaplan-Meier plots of event-free survival (**C**) and distant relapse-free survival (**D**) stratified by RCB class. Crosses denote patients censored. RCB=residual cancer burden. pCR=pathological complete response.

Table 2. Multivariate mixed effects Cox models of EFS as a function of RCB

Variable	All patients (n=4607)*	HR-HER2- (all patients, n=1585)*	HR-HER2+ (all patients; n=522)	HR-HER2+ (neoadjuvant HER2-targeted, n=440)*†	HR+HER2+ (all patients, n-773)*	HR+HER2+ (neoadjuvant HER2-targeted, n=674)*†	HR+HER2- (all patients, n=1727)*
RCB	1.69 (1.55-1.85)‡	1.93 (1.74-2.13)‡	2.09 (1.73-2.53)‡	2.1 (1.68-2.62)‡	1.66 (1.45-1.9)‡	1.69 (1.45-1.97)‡	1.52 (1.36-1.69)‡
Age	1 (0.99-1)	0.99 (0.98-1)	1 (0.98-1.02)	1 (0.97-1.03)	1 (0.99-1.02)	1 (0.98-1.02)	1 (0.99-1.01)
cT category (Reference: cT2)§							
T0/1	1.08 (0.85-1.37)	1.05 (0.69-1.6)	1.99 (1-3.96)‡	2.46 (1.03-5.87)‡	0.8 (0.4-1.61)	0.5 (0.2-1.26)	1.01 (0.69-1.46)
T3	1.28 (1.1-1.49)‡	1.73 (1.37-2.18)‡	1.6 (0.95-2.69)	1.64 (0.83-3.24)	1.02 (0.66-1.56)	0.88 (0.53-1.48)	1.08 (0.85-1.37)
T4	1.89 (1.55-2.31)‡	1.43 (1.02-2.01)	1.27 (0.6-2.68)	2.39 (1.02-5.58)‡	3.23 (2.07-5.03)‡	2.98 (1.81-4.9)‡	2.11 (1.53-2.91)‡
cN status (Reference: N-)							
N+	1.15 (1-1.32)‡	1.17 (0.94-1.44)	0.87 (0.52-1.45)	0.72 (0.38-1.35)	1.25 (0.84-1.86)	1.34 (0.85-2.11)	1.3 (1.04-1.62)‡
Grade (Reference: Grade I/II)							
Grade III	1.51 (1.33-1.72)‡	1.09 (0.85-1.4)	0.96 (0.58-1.59)	0.86 (0.46-1.63)	0.76 (0.55-1.06)	0.68 (0.46-0.99)‡	1.55 (1.27-1.89)‡

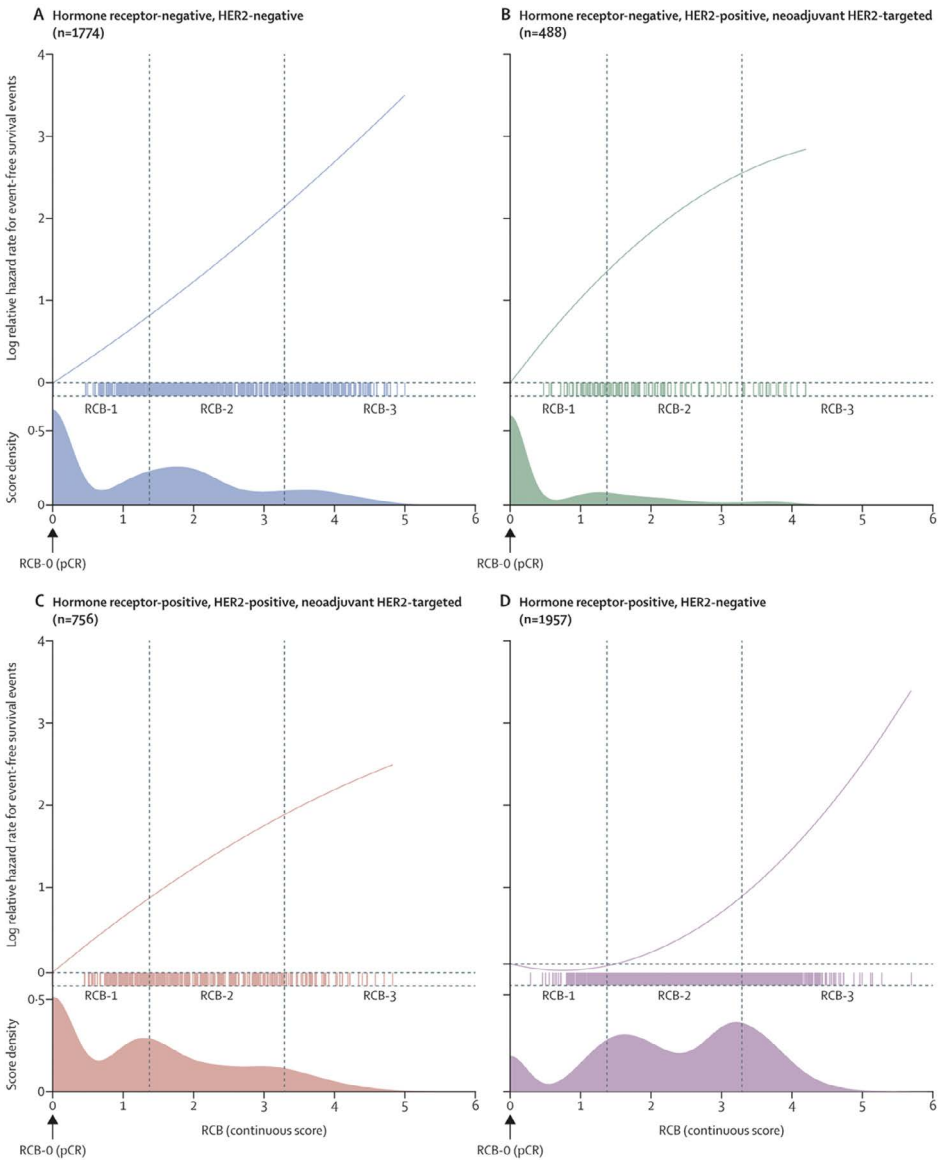
RCB was analysed as a continuous score, adjusting for age and pretreatment T category, nodal status, and grade (as fixed effects). Hazard ratios (95% CIs) are shown. All p values are shown in the appendix (p 7).
RCB=residual cancer burden.
* Patients with complete covariate data.
† The subset who received neoadjuvant HER2-targeted therapy as neoadjuvant treatment in combination with chemotherapy.
‡ Indicates significant p values less than 0·05.
§ T2 was used as the reference category due to the small sample size of the T0–1 group (particularly within the HER2-positive subtypes) in view of concern for the stability of the hazard ratio estimates.

Overall, the proportion of each RCB class was: 32% (1676/5161) RCB-0 (pCR), 13% (662/5161) RCB-I, 39% (2017/5161) RCB-II and 16% (806/5161) RCB-III (Table 1). RCB class was prognostic for both EFS (Figure 2C) and DRFS (Figure 2D), with clear prognostic separation between each class. EFS estimates for patients with RCB-0 were 94% (95% CI 93%-95%), 91% (90%-93%) and 88% (85%-90%) at 3, 5 and 10 years, respectively; compared with 91% (89%-93%), 86% (84%-89%), 80% (76%-84%) for RCB-I; 82% (81%-84%), 74% (72%-76%), 65% (62%-68%) for RCB-II; and 66% (63%-70%), 58% (54%-62%), 45% (40%-49%) for RCB-III (Figure 2C and appendix, pp 4-6). Similarly, DRFS estimates were 95% (95% CI 94%-96%), 93% (91%-94%) and 90% (88%-92%) for pCR at 3, 5 and at 10 years; compared with 92% (90%-94%), 89% (86%-91%) and 81% (77%-85%) for RCB-I; 84% (83%-86%), 77% (75%-79%) and 67% (65%-70%) for RCB-II; and 68% (65%-71%), 60% (56%-63%) and 46% (41%-51%) for RCB-III (Figure 2D and appendix pp 4-6).

Increased RCB score was significantly associated with worse EFS within all four sub-types, where the hazard ratio associated with one unit increase in RCB ranged from 1.55 (95% CI 1.41-1.71) in the HR-positive/HER2-negative subtype to 2.16 (95% CI 1.79-2.61) in the HR-negative/HER2-positive subtype; appendix pp 4-6). Similar findings were observed when considering only patients with HR-negative/HER2-positive (488/572) or HR-positive/HER2-positive (756/858) who also received neoadjuvant HER2-targeted therapies (appendix pp 5-6). Increasing RCB was associated with a near linear increase in log relative hazard rate among all subtypes, except for the HR-positive/HER2-negative subtype, where the log relative hazard remained near zero until RCB score ~1.5, close to the class threshold between RCB-I and RCB-II (Figure 3; appendix p 11). The results were similar for DRFS (and appendix, pp 4-6, 10-11).

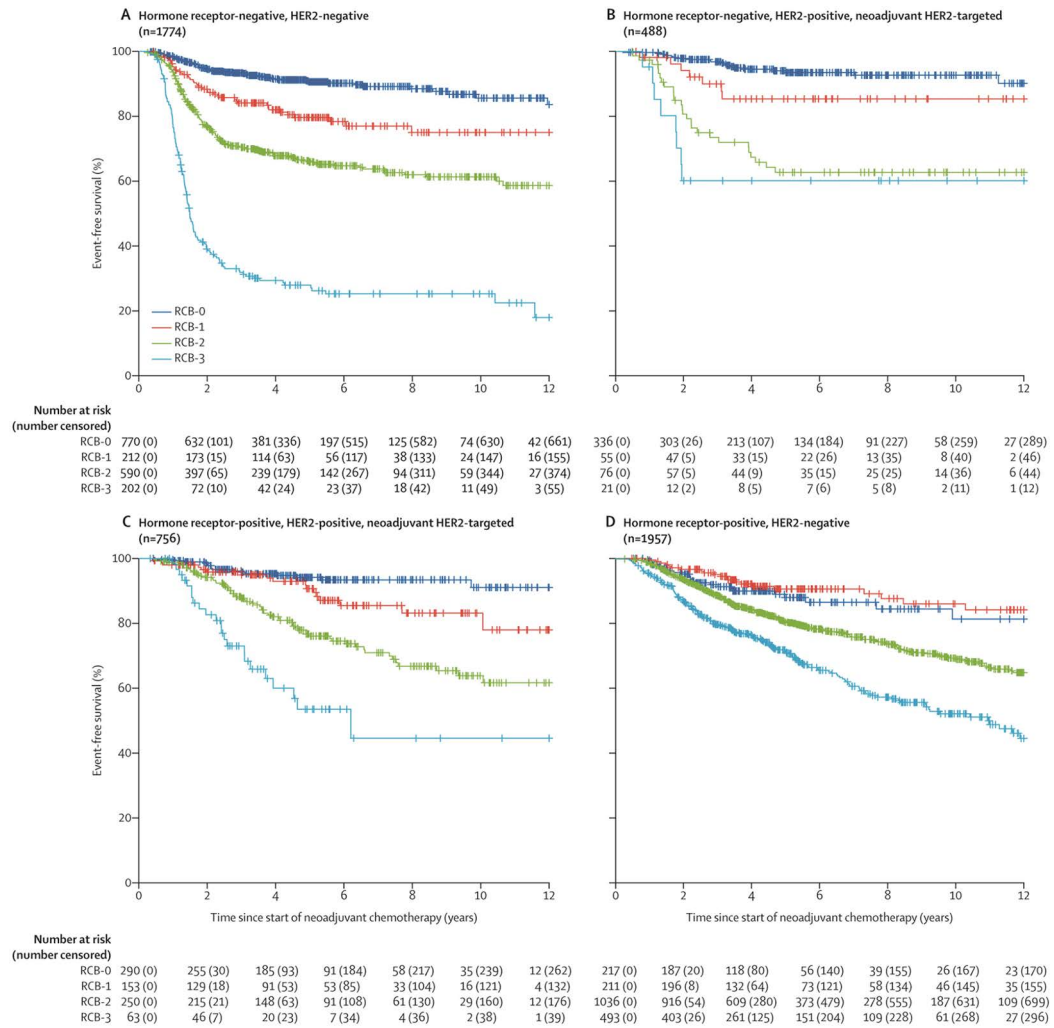
In the multivariate analysis, RCB score remained a significant independent predictor of EFS and DRFS in all subtypes when we adjusted for baseline characteristics (Table 2, appendix, pp 7-8). Clinical category T4 was significantly associated with increased risk of an event in all subtypes. In contrast, the presence of tumor-positive nodes at baseline and higher grade was associated with significantly worse outcomes only in the HR-positive/HER2-negative subtype (HR 1.3 [95% CI 1.04-1.62] and 1.55 [1.3-1.9] respectively; Table 2). Similar results were observed for the DRFS endpoint (appendix, p 8).

Figure 3. Prognostic value of RCB score within HR/HER2 subtypes.



Plots of log relative hazard rate for event-free survival events as a function of RCB score among breast cancer subtypes. For the two HER2-positive subtypes, plots of the subset of patients who received neoadjuvant HER2-targeted therapy are shown (plots for all HER2-positive patients, with or without HER2-targeted therapy, are presented in the appendix p 11). Splines approximation of RCB with two degrees of freedom was used to allow non-linear effect. A log linear increase in relative hazard rate implies that the hazard ratio associated with change in RCB remains constant over the range of RCB. Thresholds for corresponding RCB classes (RCB-0 to RCB-3) are shown for reference (vertical dashed lines). Vertical bars represent all RCB scores recorded on a continuous scale. RCB=residual cancer burden. pCR=pathological complete response.

Figure 4. Prognostic value of RCB class for hormone receptor and HER2 subtypes.



Kaplan-Meier plots of event-free survival by RCB classes among breast cancer subtypes. For the two HER2-positive subtypes, plots of the subset of patients who received neoadjuvant HER2-targeted therapy are shown (plots for all HER2-positive patients, with or without HER2-targeted therapy, are presented in the appendix p 13). Crosses denote patients censored. RCB=residual cancer burden; HR=hormone receptor.

Despite differences in the distribution of RCB class between subtypes, there was clear prognostic separation between patients with RCB-II and RCB-III disease from those who achieved a pCR (RCB-0) in all subtypes (Figure 4 and appendix, pp 4-6, 13). Significant prognostic differences were also observed between RCB-I patients within the HR-negative/HER2-negative and HR-positive/HER2-positive subtypes as compared to the RCB-0 group (appendix, pp 4-6). Notably, within the HR-positive/HER2-positive subtype, patients with RCB-0 and RCB-I showed similar EFS within the first five years (5-year EFS 94% [95% CI 91%-97%] and 91% (85%-96%) respectively) before their prognosis diverged; at 10 years, the EFS of RCB-0 patients was 91% (95% CI 86%-97%), compared with 83% (75%-92%) for RCB-I patients (post-hoc analysis; Figure 4C). Within the HR-positive/HER2-negative subtype, consistent with the non-linear relationship between EFS and continuous RCB, RCB-0 and RCB-I patients had similar EFS (HR 0.97 [0.57-1.65], p=0.90; Figure 4D, appendix pp 4-6). Results for the DRFS endpoint were similar to those for EFS (appendix, pp 4-6, 12).

DISCUSSION

In this pooled analysis, we showed that RCB is highly prognostic across twelve independently acquired cohorts, independent of pre-treatment clinical-pathological information and regardless of HR and HER2-defined subtype. Currently, there is no universally adopted standard methodology for the pathological evaluation of response to NAC in breast cancer.[24] In the past, the degree of residual invasive disease was not considered of critical importance for patient management, in part because mastectomy was the gold standard for patients with locally advanced breast cancer. Use of NAC increased as improved systemic therapies emerged and it became apparent that breast conservation following NAC led to similar outcomes as mastectomy.[25] Multiple studies have since demonstrated the strong prognostic relationship between the presence and/or extent of residual disease and the risk of loco-regional and distant recurrences.[9,10,17] In this analysis, the number of EFS and DRFS events was similar (1164 vs. 1072), demonstrating that distant recurrences are the predominant risk for patients selected for NAC. Our definitions of EFS and DRFS endpoints are consistent with the CTNeoBC meta-analysis[4] and the STEEP system, which recommends the date of first therapy as the starting point for time-to-event calculations.

Important aspects to the RCB method are that it provides both a standardized approach for pathological evaluation of post-treatment resection specimens and an algorithm that quantifies the extent of residual disease. Studies have reported highly reproducible measurements of RCB from different pathologists[10,11] and RCB's prognostic value has been validated in multiple single center studies and multicenter trials.[12-19] Indeed, in this pooled analysis, we observed significant association between RCB and EFS/DRFS in the population as a whole, within all subtypes and across all cohorts (except in the smallest cohort for EFS). Because our pooled cohorts represent a variety of clinical settings, this result implies a broad generalizability of the association between RCB and prognosis in the overall population and within each molecular subtype of breast cancer.

Importantly, the risk of a recurrence event increases with the extent of residual disease, regardless of subtype. Use of RCB, therefore, adds prognostic information when pCR is not achieved. As more post-neoadjuvant (adjuvant) therapy options become available for patients with residual disease, a more refined estimate of an individual's risk of recurrence, based on their subtype and RCB, may be useful for informing decisions on adjuvant treatment selection. Interestingly, unlike in the HR-negative and HR-positive/HER2-positive subtypes, the increase in risk with RCB appears non-linear in the HR-positive/HER2-negative subtype. One potential reason for this may be that patients with HR-positive/HER2-negative cancers usually go on to receive endocrine therapy for 5 years or more, the effects of which may not be dependent on response to NAC.[26] This highlights the importance of subtypes in prognostication and suggests that use of RCB for recurrence risk prediction after neoadjuvant therapy should be performed within a subtype-specific context.

The weakest association between RCB and survival was in patients with HR-positive/HER2-negative tumors, where the RCB-0 and RCB-I groups have similar EFS. This appeared to be driven by a handful of early recurrences in the RCB-0 group (16 within the first 3 years). Five of these early recurrences occurred in the bevacizumab arm of the ARTemis trial and may be attributable to a differential effect of bevacizumab, which increases pCR rates in the primary tumor but has less effect on micro-metastatic disease.[27] Variation in how hormone receptor positivity was defined across sites may also play an important role in the higher-than-expected early recurrence rates in the HR-positive/HER2-negative RCB-0 group. Three groups used Allred score, three groups defined positivity as more than 1% of cells with ER-positive staining, and others defined it as more than 10%, reflecting uncertainty on how to classify HR-low tumors.

Five of the early recurrences in the HR-positive/HER2-negative RCB-0 group were observed in ER negative (PR-low) or in ER low (PR negative) cases. Whether these HR-low cases were more similar to HR-negative tumors or their strongly HR-positive counterparts remains a question. Characterization using molecular subtypes, previously shown to associate with responsiveness to therapy and prognosis, may be informative.[28]

This study has several additional limitations. Patients received a range of neo-adjuvant therapies (chemotherapy per cohort's standard of care with/without additional targeted therapies) and we did not control for treatment type or duration in this analysis. However, an analysis of the I-SPY2 trial (cohort 2 in our analysis, appendix pp 2-3) suggests that the prognostic association of both pCR and RCB score is strong, regardless of type of chemotherapy-based treatment.[18,20] Additionally, not all participating groups performed extensive metastatic workup as part of standard clinical care prior to neoadjuvant therapy, and the length of follow-up differed among the included cohorts. In addition, the proportion of lobular cancers in our study is less than the proportion of lobular cases in the overall breast cancer population, likely reflecting the common belief among clinicians that lobular cancers do not respond well to neoadjuvant chemotherapy.

In this analysis, 7 of 12 groups calculated RCB retrospectively, some reviewing specimens only when RCB or its components were unavailable in the original pathology report or only when there was residual disease reported. It has been observed that pCR rate can decrease when the RCB method is incorporated into practice, possibly because a standardized and more focused pathologic evaluation of the original tumor bed can identify residual disease that might otherwise have been missed.[29] This is a shortcoming for retrospective pathology reviews because inaccurate sampling of the surgical specimen is the greatest potential source of residual disease misdiagnosed as pCR, and cannot be determined by reviewing the slides. That is particularly relevant in the HR-positive/HER2-negative subtype in which there is a higher preponderance of diffuse disease,[30] increasing the likeli-hood that sampling could affect the classification of RCB-0 and I. In addition, only the most recent series used clips as standard practice to mark the sites, assuring that the original tumor bed was sampled. Prospective assessment of RCB, along with careful identification of the initial site of disease, may improve the overall prognostic performance of RCB. This should particularly hold true in the setting of mastectomy, as it allows pathologists to identify the original site of disease using specimen radiographs and the clip placed during the biopsy at diagnosis for a more careful characterization of the tumor bed.

Despite these limitations, the consistency of the prognostic importance of RCB across participating groups in our study highlights the generalizability of implementing and standardizing the entire RCB methodology, from the stage of tissue acquisition to final pathology assessment, across different countries, treatments and clinical settings. Altogether, our findings suggest that there is a strong potential to calibrate the RCB score in a subtype-specific context to predict a patient's residual risk after neoadjuvant chemotherapy in a prospective setting with standardized evaluation of post-treatment resection specimens. Given the increasing options for escalation and de-escalation of adjuvant therapy in the setting of residual disease, prospective evaluation of RCB as part of standard pathology reporting following neoadjuvant therapy may be warranted.

Data Sharing

Data used in this study were made available under contract between the different institutes and groups and University of California, San Francisco. Agreements between US and European institutions were based on the European Union General Data Protection Regulation (GDPR). Requests for datasets should be made to the original investigators.

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Declaration of Interests

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Supplementary appendix

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9455620/

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Chapter 3

Pathologic response of ductal carcinoma in situ to neoadjuvant systemic treatment in HER2-positive breast cancer

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ABSTRACT

Purpose: The presence of extensive ductal carcinoma in situ (DCIS) adjacent to HER2-positive invasive breast cancer (IBC) is often a contra-indication for breast-conserving surgery, even in case of excellent treatment response of the invasive component. Data on the response of DCIS to neoadjuvant systemic treatment (NST) is limited. Therefore, we estimated the response of adjacent DCIS to NST containing HER2-blockade in HER2-positive breast cancer patients and assessed the association of clinicopathological and radiological factors with response.

Methods: Pre-NST biopsies were examined to determine presence of DCIS in all women with HER2-positive IBC treated with trastuzumab-containing NST ± pertuzumab between 2004-2017 in a comprehensive cancer center. When present, multiple DCIS factors, including grade, calcifications, necrosis, hormone receptor and Ki-67 expression were scored. Associations of clinicopathological and radiological factors with complete response were assessed using logistic regression models.

Results: Adjacent DCIS, observed in 138/316 patients with HER2-positive IBC, was eradicated after NST in 46% of patients. Absence of calcifications suspicious for malignancy on pre-NST mammography (Odds Ratio (OR) 3.75; 95% Confidence Interval (95% CI) 1.72-8.17), treatment with dual HER2-blockade (OR 2.36; 95% CI 1.17-4.75), a (near) complete response on MRI (OR 3.55; 95% CI 1.31-9.64), and absence of calcifications (OR 3.19; 95% CI 1.34-7.60) and Ki-67>20% in DCIS (OR 2.74; 95% CI 1.09-6.89) on pre-NST biopsy were significantly associated with DCIS response.

Conclusions: As DCIS can respond to NST containing HER2-blockade, the presence of extensive DCIS in HER2-positive breast cancer before NST should not always indicate a mastectomy. The predictive factors we found could be helpful when considering breast-conserving surgery in these patients.

BACKGROUND

Neoadjuvant systemic therapy (NST) that contains trastuzumab in addition to neoadjuvant chemotherapy leads to high pathologic complete response (pCR) rates in patients with human epidermal growth factor receptor 2 (HER2)-positive invasive breast cancer (IBC).[1-3] Even higher pCR rates are seen when a trastuzumab-containing regimen is combined with the HER2-targeted antibody pertuzumab (i.e. dual HER2-blockade), with pCR rates of up to 80% reported in the HER2-positive/hormone receptor (HR)-negative subtype.[4-9] These excellent response rates allow for frequent conversion from mastectomy to breast-conserving surgery (BCS).

The presence of ductal carcinoma in situ (DCIS) adjacent to IBC, observed in 57-72% of HER2-positive breast cancer patients, may however impede this de-escalation of surgery, as DCIS is considered insensitive to systemic treatment.[10-17] A lower proliferative state, more intact physiological resistance mechanisms compared to IBC and a less receptive microenvironment to chemotherapeutic agents due to a protective basal membrane and less dense microvasculature have been put forward as potential causes for this therapy resistance.[18-20] Therefore the presence of a large area of calcifications on mammography or non-mass enhancement on MRI, both of which may be associated with DCIS, and/or extensive DCIS adjacent to IBC in pre-NST biopsies are often considered contra-indications for BCS, even in patients with radiological complete response of the tumor on breast MRI.[21,22]

However, data on the response of DCIS to NST is limited. A few studies have shown that DCIS may sometimes respond to NST.[14,23-25] Two retrospective studies evaluating response of DCIS adjacent to HER2-positive breast cancer found that 36-51% of these DCIS lesions were eradicated after trastuzumab-containing NST combined with pertuzumab in a small subgroup.[24,25]

It is however not possible to predict which DCIS lesions adjacent to HER2-positive IBC will respond to NST. Imaging studies have difficulties to identify residual DCIS after NST, as the extent of calcifications on mammography after NST is very poorly associated with the pathologic response or residual size of invasive or in situ components.[14,16,26,27] Therefore, performing BCS in patients with extensive DCIS is challenging, even when an excellent treatment response of their IBC has been achieved. To facilitate potential de-escalation of surgery in the future in this patient group, we aim to estimate the response of adjacent DCIS to NST containing HER2-blockade in a large series of HER2-positive breast cancer patients and to identify clinicopathological and radiological factors that predict response.

METHODS

Patient and data collection

All women ≥18 years diagnosed with HER2-positive IBC who received NST containing HER2-blockade at the Netherlands Cancer Institute (NKI) between January 2004 and November 2017 were selected from the prospectively maintained NKI's tumor registry.

Detailed patient, imaging, tumor and treatment characteristics were extracted from medical records. HER2 and HR status of IBC were assessed in all patients according to the Dutch guidelines. HR status was considered positive when ≥10% of luminal epithelial cells showed nuclear estrogen receptor (ER) expression, irrespective of progesterone receptor (PR) expression.[22,28] Ki-67 in IBC was categorized into low (≤20% of expression) and high (>20% expression) proliferation. Neoadjuvant chemotherapy regimens were categorized into taxane-based, anthracycline plus taxane-based or other. Type of HER2-blockade was registered (i.e., trastuzumab alone or dual HER2-blockade with trastuzumab and pertuzumab). Patients underwent both mammography and MRI pre-NST. All lesions were assessed by radiologists according to the BI-RADS lexicon.[29] For each tumor the size of the largest mass lesion, i.e., the index lesion, was reported as the largest diameter in the axial plane. In addition, the extent of the tumor was reported, being the size of the tumor area including surrounding satellites and non-mass enhancement. The presence and extent of calcifications suspicious for malignancy on pre-NST mammography was noted. A dedicated breast radiologist (RMM) reassessed mammographic images when relevant information regarding the presence or level of suspicion of calcifications (i.e., whether the calcifications were considered benign or suspicious for malignancy) was missing in the original report.

Tumor response was assessed on MRI after completion of NST, since MRI is superior to mammography in determining the presence and size of residual disease, and was categorized into (near) complete versus partial or no radiological response.[30] Radiological complete response was defined as no residual enhancement within the original tumor bed after NST. Near complete

response was reported when only minimal residual enhancement (either some foci, or a diffuse glow) was visualized within the original tumor bed, without any components that were clearly identifiable as part of the original tumor. Post-NST mammography was not performed.

For women treated with breast-conserving surgery, the tumor was marked with a clip marker and localized with use of radio-guided occult lesion localization in the earlier years of our study cohort. In some patients, localization of the tumor was done with use of a wire. From 2007 the tumor was typically marked with an iodine seed prior to NST.[31] Breast-conserving surgery was planned using post-NST MRI findings. Specimen radiography was performed for all lumpectomies and for mastectomy specimens if a substantial pre-NST DCIS component was present to guide tissue sampling.

This study was approved by the institutional review board of the NKI.

Pathology review

A dedicated breast pathologist (EJG) re-examined all pre-NST biopsies, blinded for response, to determine whether DCIS was present adjacent to IBC. These pre-NST biopsies mostly targeted the invasive component and were preferentially obtained under ultrasound guidance using a 14G core biopsy needle. In lesions that were ultrasound occult or presented as mammographic calcifications only, stereotactic biopsy was performed using a 9G vacuum needle. The number of available tissue cores was documented. If adjacent DCIS was present, the following histopathological DCIS features were scored: number of DCIS ducts, grade (1,2 or 3) according to Holland criteria, dominant growth pattern (clinging, (micro-)papillary, cribriform, or solid), presence of calcifications, necrosis, periductal lymphocytic infiltrate, (type of) periductal fibrosis and mitotic activity (see scoring form in Supplementary methods).[32] When slides originally stained with ER, PR, HER2 and Ki-67 contained DCIS, their expression was scored in the DCIS component (see details on antibodies in Supplementary methods). HER2 and HR status of DCIS were determined similarly as for IBC. As little is known about the distribution of Ki-67 in DCIS, Ki-67 in DCIS was categorized into two categories with the median used as cut-off value: low proliferation when ≤20% of cells showed expression and high proliferation when >20% of cells showed expression.

Response of DCIS was defined as complete eradication of DCIS after NST. Data on the presence of residual DCIS in post-NST surgical specimens was retrieved from pathology reports. The number of slides that were originally examined was also noted. When no residual DCIS was described in the reports from women in whom adjacent DCIS was found in pre-NST biopsies, pathology slides were re-examined to affirm the eradication of DCIS.

Statistical analysis

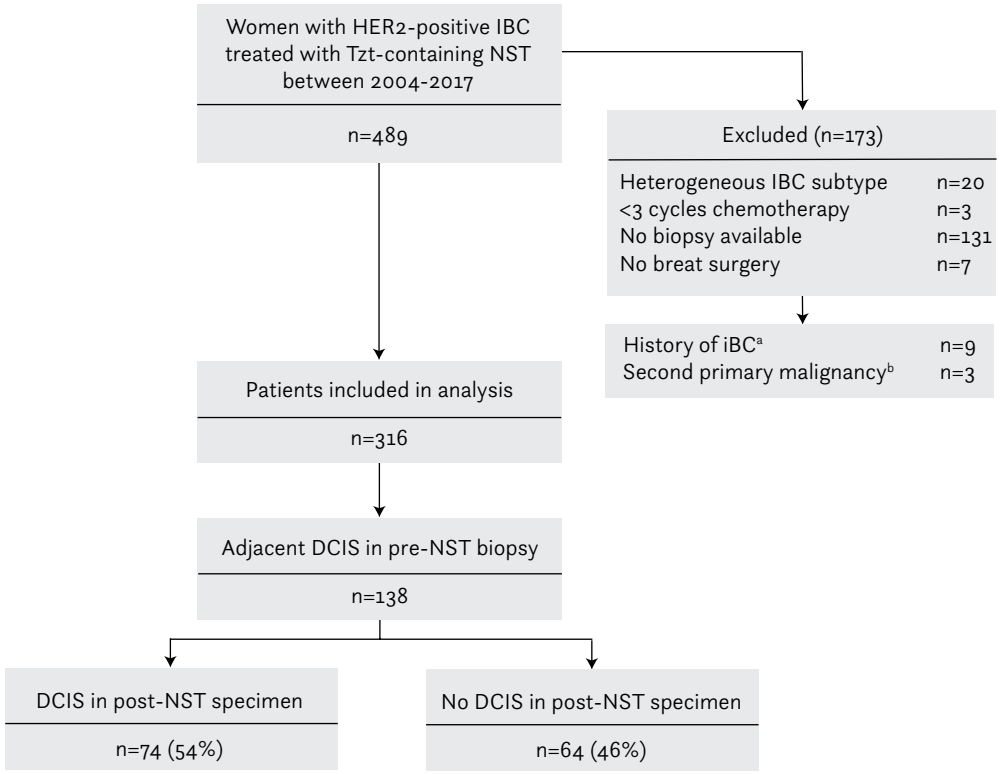
Descriptive statistics were used for patient, imaging, tumor and treatment characteristics. Included and excluded patients were compared, as were included patients with and without adjacent DCIS on pre-NST biopsy, using Pearson's chi-squared test for categorical values and Wilcoxon rank-sum test or t-test for continuous variables. Adjacent DCIS was defined as any presence of DCIS on pre-NST biopsy.

Associations of clinicopathological and radiological factors with the response of DCIS to NST were assessed using logistic regression models. A stepwise regression was undertaken using forward selection. Variables were entered in multivariable models, based on a P value ≤0.05 in univariable analyses with elimination of variables at a threshold P value of >0.05 in the multivariable analysis. Missing data on these eligible variables were imputed using chained equations (MICE) creating 50 datasets. Frequency of missingness was 1% for suspicious calcifications on mammography, 5% for tumor response on MRI, 5% for calcifications in DCIS in the biopsy, and 44% for Ki-67 expression in DCIS. Estimates from the imputed data sets were pooled using Rubin's rule.[33] All tests were two-sided and P values ≤0.05 were considered statistically significant. All statistical analyses were performed using Stata/SE (version 13.1, Statacorp).

RESULTS

During the inclusion period, 489 patients with HER2-positive IBC received NST containing HER2-blockade at the Netherlands Cancer Institute. After exclusion of 173 patients, mainly because their pre-NST biopsies were not available for review (76%), 316 patients were available for further analyses (see flow diagram for patient selection and exclusions in Figure 1). Included patients more often had lower-stage disease and were more frequently treated by a taxane-only regimen than excluded patients (see Supplementary Table 1, demonstrating clinicopathological characteristics of included and excluded patients).

Figure 1. Flow diagram for patient selection and exclusions.



IBC=invasive breast cancer; Tzt=trastuzumab; NST=neoadjuvant systemic therapy; n=number; iBC=ipsilateral breast cancer; [a]in situ and invasive breast cancer; [b]Second primary malignancies, for which treatment may interfere with response evaluation of DCIS to NST

Adjacent DCIS was observed in pre-NST biopsies from 138 out of 316 patients (44%). In 63 patients (20%) multiple biopsies were taken; in ten of these patients these biopsies targeted an area of calcifications or non-mass enhancement suspicious for an adjacent DCIS component. The remainder was targeted at the IBC only. Presence of adjacent DCIS increased with the number of examined tissue cores (P=0.001), decreased with age (P=0.047), was more frequent when suspicious calcifications were present on mammography (P=0.005) and, in those with suspicious calcifications, increased when the extent of calcifications on the mammography was larger (P=0.022; Table 1). Although patients with adjacent DCIS more often had a lower grade (grade 1+2 versus grade 3) of IBC, this association did not reach statistical significance (P=0.054). At histopathological re-examination of pre-NST biopsies, DCIS was assigned grade 1 in 2% of patients, grade 2 in 45% and grade 3 in 53%. The HER2 status of DCIS could be assessed in 86/138 patients and was positive in 92%, equivocal in 7% (in these patients no SISH was available) and negative in 1% of patients. HR status of DCIS was positive in 63.5% and negative in 36.5% out of the 85 patients for whom HR stains were available. In 82% of these 85 patients, HR status of DCIS and IBC was concordant. In case of discordancy, a combination of HR-positive DCIS adjacent to HR-negative IBC was most frequently observed. In 9 out of 34 patients with HR-negative IBC the DCIS component was HR positive (26%), of these patients 67% showed a complete response (6/9 patients). Conversely, in the 6 (12%) out of 51 patients with HR-positive IBC with adjacent HR-negative DCIS, the response rate was 50%.

Table 1. Clinico-radiological and IBC factors in patients with and without adjacent DCIS.

Factors	**DCIS n (%)[a] n=138 (43.7)**	**No DCIS n (%) n=178 (56.3)**	**P**
Age at diagnosis, years, median (IQR)	45.9 (39.5-53.7)	48.6 (40.9-56.7)	**0.047**
Age at diagnosis			**0.040**
≤50 years	91 (65.9)	97 (54.5)	
>50 years	47 (34.1)	81 (45.5)	
cT			0.54
T1	23 (16.7)	28 (15.8)	
T2	74 (53.6)	101 (57.1)	
T3	38 (27.5)	40 (22.6)	
T4	3 (2.2)	8 (4.5)	
cN			0.19
Node negative	50 (36.2)	52 (29.2)	
Node positive	88 (63.8)	126 (70.8)	
cM			0.19
Mo	129 (93.5)	172 (96.6)	
M1	9 (6.5)	6 (3.4)	
Tumor size MRI before NST[b]			0.45
0-35 mm	73 (54.1)	88 (49.2)	
36-120 mm	62 (45.9)	89 (50.3)	
MRI size, mm, median (IQR)	34 (24-60)	36 (24-52)	0.66
Suspicious calcifications Mx			**0.005**
Absent	41 (29.9)	79 (45.4)	
Present	96 (70.1)	95 (54.6)	

Table 1. *Continued*

Factors	DCIS n (%)[a] n=138 (43.7)	No DCIS n (%) n=178 (56.3)	P
Extent of suspicious calcifications[b]			**0.031**
5-55 mm	23 (41.8)	32 (62.8)	
56-140 mm	32 (58.2)	19 (37.3)	
Area suspicious calcifications, mm, median (IQR)	60 (35-88)	50 (20-70)	**0.022**
IBC subtype			0.003
No special type[c]	133 (96.4)	150 (84.8)	
Lobular	2 (1.5)	15 (8.5)	
Other	3 (2.2)	12 (6.8)	
Grade IBC[d]			0.054
Grade 1+2	71 (52.2)	70 (41.2)	
Grade 3	65 (47.8)	100 (58.8)	
HR status IBC			0.58
HR negative	60 (43.5)	83 (46.6)	
HR positive	78 (56.5)	95 (53.4)	
Ki-67 IBC, %			0.45
Low, ≤20	40 (39.2)	44 (34.4)	
High, >20	62 (60.8)	84 (65.6)	
Chemotherapy			0.79
Taxanes	111 (80.4)	147 (82.6)	
Anthracyclines+Taxanes	26 (18.8)	29 (16.3)	
Other	1 (0.7)	2 (1.1)	
HER2 blockade			0.37
Tzt	84 (60.9)	117 (65.7)	
Tzt+Ptz	54 (39.1)	61 (34.3)	
Type of surgery			0.11
Breast conserving surgery	73 (52.9)	110 (61.8)	
Mastectomy	65 (47.1)	68 (38.2)	
Response on MRI			0.096
No/partial response	24 (18.3)	43 (26.5)	
(Near)complete response	107 (81.7)	119 (73.5)	

IBC=invasive breast cancer; n=number; [a]one woman had bilateral breast cancer; P=P value; IQR=interquartile range; NST=neoadjuvant systemic therapy; [b]Tumor size on MRI before NST and extent of suspicious calcifications on mammography were categorized into two groups with the median in this group of 316 patients used as cut-off value; Mx=mammography; [c]formerly known as invasive ductal carcinoma; [d]Grade IBC: only 1 patient had IBC grade 1 and did not have adjacent DCIS; HR=hormone receptor; Tzt=trastuzumab; Ptz=pertuzumab

Of the 138 patients with adjacent DCIS on pre-NST biopsy, 80% were treated with a taxane-based regime, 19% with an anthracycline plus taxane-based regime and in 1% with another regime. Sixty-one percent of patients received trastuzumab and 39% received dual HER2-blockade with trastuzumab and pertuzumab. A (near) complete radiological response on MRI was observed in 82% of patients. Seventy-seven patients were initially treated by lumpectomy and 61 by mastectomy. Resection margins were free in 87% of the women treated by breast-conserving surgery (67/77). Margins were involved in 10 patients due to irradically removed DCIS (n=6), IBC (n=1) or both (n=3). Re-surgery was performed in 6 patients (re-lumpectomy in 2 and mastectomy in 4 patients) leading to a final free margin status. In the remaining 4 patients, who all showed only focally involved margins, no re-surgery was performed.

The median number of slides examined from post-NST surgical specimens for women with adjacent DCIS was 10 (interquartile range 8-14). After NST, DCIS was eradicated in 64 out of 138 patients (46%). The number of examined slides did not differ between patients with or without residual DCIS (P=0.20). In 59% of patients who showed DCIS response, breast-conserving surgery was performed (without considering other pre-NST factors), while in the non-responder group this was 47% (P=0.16). In women with residual DCIS after NST, DCIS was found without IBC in 39/74 women (53%; Table 2). In contrast, in women with residual IBC, IBC without DCIS was found only in 9 out of 44 patients (20%). Among the 178 patients in whom adjacent DCIS was not found on pre-NST biopsy, 61 patients (34%) had DCIS after NST based on pathology reports, which was associated with residual IBC in 38 patients (62%).

Table 2. Pathologic findings after NST in patients with and without DCIS in pre-NST biopsy

	DCIS in pre-NST biopsy n (%) n=138 (43.7)		No DCIS in pre-NST biopsy n (%) n=178 (56.3)	
	DCIS post-NST	No DCIS post-NST	DCIS post-NST	No DCIS post-NST
IBC post-NST	35 (47.3)	9 (14.1)	38 (62.3)	38 (32.5)
No IBC post-NST	39 (52.7)	55 (85.9)	23 (37.7)	79 (67.5)
Total n	74	64	61	117

NST=neoadjuvant systemic therapy; n=number; IBC=invasive breast cancer

Association between clinicopathological and radiological factors and response of DCIS to NST

The clinico-radiological factors, absence of suspicious calcifications on mammography (Odds Ratio (OR) 3.75; 95% Confidence Interval (95% CI) 1.72-8.17), treatment with dual HER2-blockade (OR 2.36; 95% CI 1.17-4.75) and a (near) complete response on MRI (OR 3.55; 95% CI 1.31-9.64) were associated with DCIS response in univariable analysis (Table 3a-b), as were the histopathological factors absence of calcifications in DCIS on pre-NST biopsy (OR 3.19; 95% CI 1.34-7.60) and Ki-67 expression >20% in DCIS (OR 2.74; 95% CI 1.09-6.89). Grade and HR status of IBC or DCIS was not associated with DCIS response. The number of patients with HER2-negative DCIS was too small to allow an informative analysis on the association of HER2 status in DCIS with treatment response.

Table 3a. Associations of clinico-radiological and IBC factors with response[a] of DCIS to NST in univariable analysis

Clinico-radiological factors	Total n(%)	Response n(%) n=64 (46.4)	No response n(%) n=74 (53.6)	OR[b] (95% CI)[c]	P[d]
Age at diagnosis					
≤50 years	91 (65.9)	37 (57.8)	54 (73.0)	**REF**	
>50 years	47 (34.1)	27 (42.2)	20 (27.0)	1.97 (0.97-4.02)	0.061
Chemotherapy					
Taxanes	111 (80.4)	50 (78.1)	61 (82.4)	**REF**	
Anthracyclines+Taxanes	26 (18.8)	13 (20.3)	13 (17.6)	1.22 (0.52-2.87)	
Other	1 (0.7)	1 (1.6)		NA	0.65
HER2 blockade					
Tzt	84 (60.9)	32 (50.0)	52 (70.3)	**REF**	
Tzt + Ptz	54 (39.1)	32 (50.0)	22 (29.7)	2.36 (1.17-4.75)	**0.015**
Tumor size MRI before NST[e]					
7-34 mm	69 (50.0)	34 (53.1)	35 (47.3)	1.24 (0.63-2.44)	0.53
35-110 mm	66 (47.8)	29 (45.3)	37 (50.0)	**REF**	
Unknown	3 (2.2)	1 (1.6)	2 (2.7)		
Suspicious calcifications Mx					
Absent	41 (29.7)	28 (43.8)	13 (17.6)	3.75 (1.72-8.17)	
Present	96 (69.6)	35 (54.7)	61 (82.4)	**REF**	**0.001**
Unknown	1 (0.7)	1 (1.6)			

Table 3a. *Continued*

Clinico-radiological factors	Total n(%)	Response n(%) n=64 (46.4)	No response n(%) n=74 (53.6)	OR[b] (95% CI)[c]	P[d]
Extent of suspicious calcifications[e]					
13-60 mm	28 (29.2)	10 (28.6)	18 (29.5)	**REF**	
61-140 mm	27 (28.1)	11 (31.4)	16 (26.2)	1.24 (0.42-3.68)	0.70
Unknown	41 (42.7)	14 (40.0)	27 (44.3)		
Response on MRI					
No/partial response	24 (17.4)	6 (9.4)	18 (24.3)	**REF**	
(Near)complete response	107 (77.5)	58 (90.6)	49 (66.2)	3.55 (1.31-9.64)	**0.008**
Unknown	7 (5.1)		7 (9.5)		
IBC factors					
Grade					
Grade 1+2	71 (51.5)	37 (57.8)	34 (46.0)	1.63 (0.83-3.22)	
Grade 3	65 (47.1)	26 (40.6)	39 (52.7)	**REF**	0.16
Unknown	2 (1.5)	1 (1.6)	1 (1.4)		
HR status					
HR negative	60 (43.5)	32 (50.0)	28 (37.8)	1.64 (0.83-3.24)	0.15
HR positive	78 (56.5)	32 (50.0)	46 (62.2)	**REF**	
Ki-67, %					
Low, ≤20	40 (29.0)	18 (28.1)	22 (29.7)	**REF**	
High, >20	62 (44.9)	30 (46.9)	32 (43.2)	1.15 (0.52-2.54)	0.74
Unknown	36 (26.1)	16 (25.0)	20 (27.0)		

IBC=invasive breast cancer; [a]response is defined as complete eradication of DCIS after neoadjuvant systemic therapy; NST=neoadjuvant systemic therapy; n=number; OR=Odds Ratio; [b]Missings were not taken into account as a separate category; CI=Confidence Interval; [c]Confidence Interval is Wald-based; P=P value; [d]P value is based on the LR-based test statistic; REF=reference; NA=not applicable; Tzt=trastuzumab; Ptz=pertuzumab; [e]Tumor size on MRI before NST and extent of suspicious calcifications on mammography were categorized into two groups with the median used as cut-off value; Mx=mammography; HR=hormone receptor

Table 3b. Associations of DCIS factors with response[a] of DCIS to NST in univariable analysis

DCIS factors	Total n(%) n=138	Response n(%) n=64 (46.4)	No response n(%) n=74 (53.6)	OR[b] (95% CI)[c]	P[d]
Grade[e]					
Grade 1+2	63 (45.7)	27 (42.2)	36 (48.7)	**REF**	
Grade 3	72 (52.2)	37 (57.8)	35 (47.3)	1.41 (0.71-2.78)	0.32
Unknown	3 (2.2)		3 (4.1)		
Growth pattern[f]					
(Non)solid	22 (15.9)	8 (12.5)	14 (18.9)	**REF**	
Solid	110 (79.7)	54 (84.4)	56 (75.7)	1.69 (0.66-4.34)	0.27
Unknown	6 (4.4)	2 (3.1)	4 (5.4)		
Calcifications					
Absent	99 (71.7)	55 (85.9)	44 (59.5)	3.19 (1.34-7.60)	**0.006**
Present	32 (23.2)	9 (14.1)	23 (31.1)	**REF**	
Unknown	7 (5.1)		7 (9.5)		
Necrosis					
Absent	69 (50.0)	39 (60.9)	30 (40.5)	1.98 (0.99-3.95)	0.053
Present	63 (45.7)	25 (39.1)	38 (51.4)	**REF**	
Unknown	6 (4.4)		6 (8.1)		
Mitoses					
Sparse	82 (59.4)	38 (59.4)	44 (59.5)	**REF**	
Many	48 (34.8)	23 (35.9)	25 (33.8)	1.07 (0.52-2.17)	0.86
Unknown	8 (5.8)	3 (4.7)	5 (6.8)		
Periductal fibrosis					
Absent + subtle	71 (51.5)	32 (50.0)	39 (52.7)	**REF**	
Prominent	53 (38.4)	27 (42.2)	26 (35.1)	1.27 (0.62-2.58)	0.52
Unknown	14 (10.1)	5 (7.8)	9 (12.2)		
Type fibrosis[g]					
Sclerotic	41 (46.1)	17 (42.5)	24 (49.0)	**REF**	
Myxoid	47 (52.8)	23 (57.5)	24 (49.0)	1.35 (0.58-3.15)	0.48
Unknown	1 (1.1)		1 (2.0)		
Lymphocytic infiltrate					
Absent + subtle	99 (71.7)	45 (70.3)	54 (73.0)	**REF**	
Prominent	27 (19.6)	14 (21.9)	13 (17.6)	1.29 (0.55-3.03)	0.56
Unknown	12 (8.7)	5 (7.8)	7 (9.5)		

Table 3b. Associations of DCIS factors with response[a] of DCIS to NST in univariable analysis

DCIS factors	Total n(%) n=138	Response n(%) n=64 (46.4)	No response n(%) n=74 (53.6)	OR[b] (95% CI)[c]	P[d]
HR status					
HR negative	31 (22.5)	15 (23.4)	16 (21.6)	1.17 (0.48-2.84)	0.73
HR positive	54 (39.1)	24 (37.5)	30 (40.5)	**REF**	
Unknown	53 (38.4)	25 (39.1)	28 (37.8)		
Ki-67, %					
Low, ≤20	39 (28.3)	14 (21.9)	25 (33.8)	**REF**	
High, >20	38 (27.5)	23 (35.9)	15 (20.3)	2.74 (1.09-6.89)	**0.030**
Unknown	61 (44.2)	27 (42.2)	34 (46.0)		

[a]response is defined as complete eradication of DCIS after neoadjuvant systemic therapy; NST=neoadjuvant systemic therapy; n=number; OR=Odds Ratio; [b] Missings were not taken into account as a separate category; CI=Confidence Interval; [c] Confidence Interval is Wald-based; P=P value; [d] P value is based on the LR-based test statistic; REF=reference; [e] Grade DCIS: only 2 patients had grade 1; [f] (Non)solid=clinging, (micro)papillary, cribriform; [g] Type of fibrosis was only scored when periductal fibrosis was present; HR=hormone receptor

All above-mentioned, eligible factors except Ki-67 expression >20% in DCIS, were also independently associated with DCIS response in multivariable analysis (see Supplementary Table 2). After multiple imputation, Ki-67 expression >20% in DCIS no longer reached the significance level set for entry into multivariable analysis.

DISCUSSION

We have demonstrated that a part of the DCIS lesions adjacent to HER2-positive breast cancer can be eradicated after NST. To the best of our knowledge, this is the largest study that examined the response of DCIS, found adjacent to HER2-positive IBC, to NST containing HER2-blockade and the first study that assessed the association of clinicopathological and radiological factors with response. The response evaluation of adjacent DCIS is highly relevant, as NST containing HER2-blockade frequently results in pCR of HER2-positive IBC, but the presence of extensive, clinically detectable DCIS pre-NST often precludes performing BCS. Therefore, it would be most relevant to know in which patients adjacent DCIS will respond to NST to eventually increase the conversion rate of mastectomy to breast-conserving surgery. We have identified several factors associated with the response of DCIS to NST that can aid towards selection of a subgroup

among HER2-positive breast cancer patients with extensive DCIS that could be treated by breast-conserving surgery.

In this study, we analyzed 316 women with HER2-positive IBC of whom 138 (44%) had adjacent DCIS in their pre-NST biopsies. Our incidence rate of DCIS was in the same range as reported by others who also evaluated the presence of adjacent DCIS in pre-NST biopsies, i.e. 37-46% in HER2-positive IBC.[14,24,25] However, a higher incidence rate of adjacent DCIS is seen in studies assessing its presence in surgical specimens of patients undergoing upfront surgery, i.e. 57-72% in HER2-positive IBC.[13,15,17] Our finding of residual DCIS after NST in 61 out of 178 patients (34%) without adjacent DCIS in their pre-NST biopsies underlines that identifying patients with adjacent DCIS in biopsies, targeting the invasive component, is less accurate.

Studies have suggested that IBC with adjacent DCIS is associated with less aggressive behavior compared to IBC without DCIS with significantly better overall survival (5-year overall survival, 89% versus 86%, p<0.001).[13,15] Compared to IBC without DCIS, IBC with adjacent DCIS was associated with a lower Ki-67 expression and grade, ER/PR/HER2-positivity, lower tumor and nodal stage, and was more frequently found in pre-menopausal women.[13,15] In our study, IBC with adjacent DCIS was associated with a younger age and the presence of suspicious calcifications on pre-NST mammography. In addition, DCIS was more often found adjacent to IBC grade 1+2, but this association did not reach statistical significance. Two other studies that evaluated the sensitivity of DCIS to NST did not find a correlation between the presence of adjacent DCIS and age, nodal status, IBC grade, HR status or Ki-67.[14,24] As these studies, like ours, were performed in women treated by NST partly focusing on HER2-positive IBC alone, and likely suboptimally identifying IBC with adjacent DCIS in pre-NST biopsies, associations may be different.

We found that DCIS was eradicated after NST in 64 out of 138 women with adjacent DCIS in their pre-NST biopsies (46%). Our results are in line with those of a smaller study by von Minckwitz et al, in which DCIS was eradicated in 30/59 patients (51%) with HER2-positive IBC who were treated with a neoadjuvant regimen including anthracyclines, taxanes and trastuzumab with or without capecitabine.[24] A slightly lower, but still comparable response rate of 36% was found in a study, which also focused on adjacent DCIS in HER2-positive IBC, in which patients were treated with taxane-based chemotherapy plus trastuzumab and also pertuzumab in a small subgroup.[25] Another study showed a pCR of DCIS, found adjacent to IBC of all subtypes, in 10 out of 30 patients (33%) treated with anthracycline–taxane-containing NST (plus trastuzumab when the HER2-receptor was overexpressed).[14]

Absence of suspicious calcifications on pre-NST mammography, dual HER2-blockade, a (near) complete response on MRI, the absence of calcifications in DCIS on pre-NST biopsy and a Ki-67 expression in DCIS of >20% were associated with response of DCIS to NST in univariable analysis. The results for Ki-67 expression in DCIS should be interpreted with some caution due to the large proportion of missings. Reports on response of invasive HER2-positive breast cancer have identified similar factors, as complete response is more frequently observed in patients treated with dual HER2-blockade compared to trastuzumab alone, in patients with a (near) complete response on MRI or in IBC with a high Ki-67 expression.[4-6,8,34,35] A recent review concerning HER2-positive IBC showed that three factors are associated with an increased pCR rate: (1) high HER2 combined with low estrogen receptor 1 gene expression levels, (2) a 'HER2-enriched' PAM50 intrinsic subtype, and (3) higher levels of tumor infiltrating lymphocytes.[36] Although we did not perform gene expression analysis, we evaluated HR status of IBC and DCIS, but did not find a higher response rate for HR-negative IBC or DCIS. It could be that response rates of HR-negative versus HR-positive DCIS does not parallel the situation for IBC in HER2-positive breast cancer patients. In our study cohort, women with HR-negative IBC did not differ from women with HR-positive IBC in terms of age, treatment, and grade or proliferation of IBC. There was a trend towards smaller tumor size in HR-positive IBC compared to HR-negative IBC based on T-stage and MRI size at baseline (p=0.065 and p=0.074 respectively), but this does not imply an association with a smaller size of the DCIS component per se. Perhaps a discordancy in HR-status between DCIS and IBC may play a role here, but this seems unlikely when considering the small subset of such patients found in our cohort, of whom HR-negative IBC patients with adjacent HR-positive DCIS showed a higher response rate than HR-positive IBC patients with adjacent HR-negative DCIS (response rate 67% versus 50%). Lastly, HR-status of DCIS was missing for 38% of all cases included that could mask an underlying difference in response rates between HR-positive versus HR-negative DCIS.

Our study has several limitations. One limitation is a lack of thorough radiological correlation with DCIS prior to NST, which would enable more accurate identification of patients with (extensive) DCIS, allowing for more accurate assessment of true response. A second limitation is intrinsic to the way in which IBC is diagnosed and classified prior to NST, i.e., by taking a biopsy

targeted on the IBC and pathologic evaluation thereof. This implies that the aim of most biopsies is not to assess the presence of adjacent DCIS. This may compromise adequate evaluation of the response of DCIS to NST, as there is a risk of missing adjacent DCIS in pre-NST biopsies. Expanding our analysis by including patients who only showed (residual) DCIS after NST would enable rightful recognition of these 'non-complete responders'. However, this would also lead to an underestimation of DCIS response because patients without DCIS in pre-NST biopsies who had a complete response would not be considered. In this context it is also important to note that in clinical practice DCIS can be occult on imaging, representing a subset of patients in whom adjacent DCIS was only identified after NST in our study. For these patients, prediction of DCIS response will not change surgical treatment decisions. A third potential minor limitation might be that the diagnostic biopsy procedure results in complete removal of a small component of adjacent DCIS, compromising response evaluation. Yet, as feasibility issues for breast-conserving surgery particularly arise in patients with extensive adjacent DCIS, it is unlikely that this will impact clinical practice.

In conclusion, we demonstrated in this exploratory study that complete response of DCIS to NST can be achieved in almost half of the patients with confirmed DCIS adjacent to HER2-positive IBC in pre-NST biopsies. Further research is needed to validate our findings within HER2-positive breast cancer patients with clinically detectable, extensive DCIS, while carefully correlating radiology and pathology of the DCIS component pre- and post-NST. Within such a context, the conversion rate of mastectomy to breast-conserving surgery, and recurrence and survival rates related to DCIS response could be evaluated. For now, our study indicates that the presence of extensive DCIS in HER2-positive breast cancer before NST should not always indicate a mastectomy, and the predictive factors we found could be helpful when considering BCS in these patients.

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Conflicts of interest

Dr Sonke reports receiving institutional research support from AstraZeneca, Merck, Novartis, and Roche and Dr Mann from Siemens Healthineers, Medtronic, Bayer Healthcare, BD, Screenpoint Medical, Seno Medical and Transonic Imaging. For the remaining authors none were declared.

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Section II

Reducing local treatment of the breast after neoadjuvant systemic therapy

Chapter 4

Breast conserving therapy in patients with cT3 breast cancer with good response to neoadjuvant systemic therapy results in excellent local control: a comprehensive cancer center experience

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SYNOPSIS

Breast-conserving therapy (BCT) could be performed in 82% of cT3 breast cancer patients in whom BCT appeared feasible on MRI after neoadjuvant systemic therapy. Seven-year local recurrence free interval in this group was 96%. In patients with hormone-receptor positive tumors, non-mass enhancement on baseline MRI or lobular carcinoma, the risk of positive margins should be considered pre-operatively.

ABSTRACT

Background: Many cT3 breast cancer patients are treated with mastectomy, regardless of response to neoadjuvant systemic therapy (NST). We evaluated local control of cT3 patients undergoing breast-conserving therapy (BCT) based on MRI evaluation post-NST. In addition, we analyzed predictive characteristics for positive margins after breast-conserving surgery (BCS).

Methods: All cT3 breast cancer patients who underwent BCS after NST between 2002-2015 at the Netherlands Cancer Institute were included. Local recurrence free interval (LRFI) was estimated with the Kaplan-Meier method. Predictors for positive margins were analyzed with univariable analysis and multivariable logistic regression.

Results: Of 114 patients undergoing BCS post-NST, 75 had negative margins, 16 focally positive and 23 positive margins. Of those with (focally) positive margins, 12 underwent radiotherapy, 6 re-excision and 21 mastectomy. Finally, 93/114 patients were treated with BCT (82%) with a LRFI of 95.9% (95% CI 91.5-100%) after a median follow-up of 7 years. Predictors for positive margins in univariable analysis were HR+/HER2- subtype, lobular carcinoma and non-mass enhancement (NME) on pre-NST MRI. MRI response was not correlated to positive margins. In multivariable regression, odds of positive margins were decreased in patients with HER2-positive (OR 0.27, 95% CI 0.10-0.73, p=0.01) and TN tumors (OR 0.17, 95% CI 0.03-0.82, p=0.028). A trend toward positive margins was observed in patients with NME (OR 2.38, 95% CI 0.98-5.77, p=0.055).

Conclusion: BCT could be performed in 82% of cT3 patients in whom BCT appeared feasible on post-NST MRI. Local control in these patients was excellent. In those with HR+/HER2- tumors, NME on MRI, or ILC the risk of positive margins should be considered pre-operatively.

INTRODUCTION

Systemic therapy in breast cancer patients is increasingly administered prior to surgery. Although no survival advantage of neoadjuvant systemic therapy (NST) over adjuvant systemic therapy has been demonstrated,[1-4] the neoadjuvant treatment approach has several advantages. Most importantly, NST enables down-staging of the primary tumor and metastatic lymph nodes.[5-7] In addition, it allows response monitoring, facilitates research by identifying predictors for response, and enables evaluation of new systemic treatment strategies by using pathologic complete response (pCR) as an early surrogate endpoint that correlates with survival.[8-12] Systemic regimens are adapted to patient and tumor characteristics, resulting in pCR rates as high as 65% in patients with triple negative (TN) breast cancer and 75% in patients with human epidermal growth receptor 2 (Her2)-positive tumors.[13-16]

The increased use and efficacy of NST appears not to lead to higher rates of breast conserving therapy (BCT; breast conserving surgery [BCS] and radiation treatment).[7,17] Especially in patients with large breast tumors, BCT remains controversial. A recent large study showed that administering NST increased the likelihood of BCT for patients with clinical T3 (cT3) breast cancer.[17] However, 80% of cT3 patients still underwent mastectomy after NST. Additionally, there was a trend toward mastectomy in younger women over time.

An explanation for the reserved attitude towards BCT after NST could be a concern about the safety of not removing the entire original tumor area after NST. Since tumor-positive margins after breast-conserving surgery (BCS) are associated with a higher risk of local recurrence,[18-20] the selection of patients for BCS should be based on whether tumor-free margins can be achieved. However, adequate evaluation of the extent of residual disease after NST by clinical examination and imaging is challenging. To this end, magnetic resonance imaging (MRI) has been demonstrated to be superior to physical examination, ultrasound and mammography.[21-23]

In this study, we analyzed local recurrence free interval (LRFI) in cT3 breast cancer patients who were selected for BCT (BCS + radiation treatment) based on MRI evaluation before and after NST. In addition, we analyzed predictive characteristics for positive margins after BCS.

METHODS

Patient selection

We included all primary breast cancer patients with a cT3 tumor (cN1-3) who underwent initial BCS after NST between 2002 and 2015 at the Netherlands Cancer Institute (NKI). Clinical T3 was defined as a breast tumor >50 mm in greatest dimension at the initial enhancement series on pre-NST contrast enhanced MRI (CE-MRI). Patients who did not undergo CE-MRI before and after NST, and patients with distant metastatic disease at diagnosis or a BRCA mutation were excluded.

Patients were identified from the NKI's tumor registry. Patient, imaging, tumor and treatment characteristics were extracted from the medical records. This study was approved by the institutional review board of the NKI.

MRI examination and evaluation

At the NKI, patients undergo MRI before NST and during or after NST for response evaluation. Until April 2007 a 1.5-T Magnetom Vision scanner with dedicated bilateral phased array breast coil was used (Siemens, Erlangen, Germany). Thereafter, a 3.0-T Achieva scanner with a 7-elements sense breast coil was used (Philips Medical Systems, Best, the Netherlands). Images were acquired with the patient in prone position with both breasts imaged at the same time. First, an unenhanced coronal 3D fast field echo (thrive) sense T1-weighted sequence was performed. A bolus (14 mL) of gadolinium containing contrast (0.1 mmol/kg) was injected intravenously followed by a bolus of 30 mL saline salt. Hereafter, dynamic imaging was performed in five consecutive series at 90-s intervals with voxel size 1.21 x 1.21 x 1.69 mm^3 (1.5-T) or 1.1 x 1.1 x 1.2 mm^2 (3.0 T).

For this study, a dedicated breast radiologist (I.I.) reviewed all MR images before, during and after NST, without knowledge of tumor subtype and final pathology outcome. An independent dedicated breast radiologist (J.v.U.) performed secondary review in case of doubt. The largest diameter (LD) of the tumor was assessed at the initial and late enhancement series in three planes (i.e, sagittal, axial and coronal). According to BI-RADS criteria lesions were categorized as mass, non-mass enhancement (NME) or a combination of these.[24] Distribution of mass enhancement was categorized in three groups: unifocal, multifocal or multicentric mass.

Response assessment was performed according to the Response Evaluation Criteria In Solid Tumors (RECIST):[25] radiologic complete response (rCR) was defined as a complete absence of pathologic contrast enhancement in the original tumor bed at MRI, radiologic partial response (rPR) as ≥30% decrease in tumor diameter and stable disease as neither sufficient shrinkage to qualify for rPR or progressive disease (at least 20% increase in diameter). In addition, the shrinkage pattern of the tumor was denoted in five categories: 'shrinking mass', 'diffuse reduction', 'small foci in original tumor bed', 'no enhancement' (=rCR) and 'no reduction/progression'.

Additional diagnostics at baseline

Core needle biopsies from the primary tumor were obtained prior to NST to determine histological subtype and receptor status. Scoring for estrogen receptor (ER), progesterone receptor (PR) and HER2 was done according to the Dutch guidelines.[26] All patients underwent axillary ultrasound and fine needle aspiration (FNA) was performed in case of a suspect node. Performance of ^{18}F-FDG PET/CT prior to NST for detection of regional and distant metastases was optional, but performed in the majority of patients.

NST and surgical treatment

NST was administered according to institutional guidelines. From 2003, patients with HER2-positive tumors were offered trastuzumab. Eligibility for BCS was assessed at multidisciplinary meetings with dedicated breast cancer specialists and discussed with the patient. In general, BCS was considered for patients with rCR or rPR on MRI, taking into account the volume ratio of the breast and tumor after NST. Contra-indications for BCS were the presence of suspect calcifications in more than two quadrants of the breast, patients desire for a mastectomy or insufficient shrinkage of the breast tumor.

In the earlier years of our study cohort, the tumor was marked with a clip marker and localized with use of radioguided occult lesion localization (ROLL). In some patients localization of the breast tumor was done by palpation or with use of a wire. From 2007 the tumor was marked with an iodine seed prior to NST (radioactive seed localization; RSL).[27-29] Multiple seeds were used in patients with multifocal or multicentric tumors. Adequate position of the clip marker, wire or iodine seed(s) was confirmed with mammography and/or ultrasound.

Axillary lymph node dissection was performed until 2004 in the majority of patients. Since 2004, sentinel lymph node biopsy was performed before or after NST in cN0 patients. From 2014, cN+ patients underwent the MARI-procedure (Marking Axillary lymph nodes with Radioactive Iodine seeds), in which a tumor-positive axillary lymph node is marked before NST and selectively removed after NST.[30] ALND was only performed in patients with extensive axillary disease prior to NST and a tumor-positive MARI-node after NST.[31,32]

Pathology review

Specialized breast pathologists assessed all surgical specimens. Volume and weight of the specimen was collected from the pathology report. A positive margin was defined as ink on invasive or in situ carcinoma, either focally positive (up to 4 mm) or more than focally positive (>4 mm or multiple small areas). Pathologic response was evaluated according to the Dutch guidelines, based on Pinder et al.[33] Pathologic complete response was defined as the absence of any residual invasive carcinoma, regardless of the presence of ductal carcinoma in situ. Conform Dutch guidelines re-excision is indicated only in patients with more than focally positive margins.[26] In patients with focally positive margins, whole breast irradiation (WBI) with boost is indicated.

Adjuvant radiation treatment

Until 2010 whole breast irradiation (WBI) comprised 25 fractions of 2 Gray (Gy) per fraction with a boost of 16 Gy in 8 fractions, or with an integrated boost to 64.4 Gy in 28 fractions. In those with positive margins at BCS requiring secondary mastectomy, post-mastectomy irradiation consisted of 25 x 2Gy with in select cases a boost dose of 10-16 Gy in 5-18 fractions. From 2010 onwards, Dutch guidelines were conformed to the fractionation scheme used in the Canadian trial,[34] with an adjustment for a simultaneously integrated boost (SIB): 16 x 2.66Gy or 21 x 2.20Gy whole breast irradiation and 21 x 2.66Gy SIB to the tumor bed. Indications for a boost included age ≤50 years, grade 3, lymphovascular invasion, tumor size ≥3 cm after NST and focally positive margins.

Statistics

LRFI and distant recurrence free interval (DRFI) were calculated from the date of surgery. A local recurrence was defined as any recurrence in the ipsilateral breast, including second primary breast cancer. Probabilities were estimated with the Kaplan-Meier method where patients without local recurrence or distant recurrence were censored at last follow-up or time of death. The median of specimen weight and volume in different time periods were compared with the Mann-Whitney U Test. In univariable analysis, predictive characteristics for tumor-positive margins were analyzed using Fishers exact test. Multivariable logistic regression was performed to identify independent

characteristics correlated with tumor-positive margins. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. P-values ≤0.05 were considered statistically significant. For this study, a tumor-positive margin was defined as a focally or more than focally positive margin, as the Society of Surgical Oncology-American Society for Radiation Oncology advises re-excision in patients with any ink tumor.[35]

RESULTS

Patient, tumor and MRI characteristics

After all exclusions, 114 patients fulfilled criteria for analysis (Supplementary figure 1). Table 1 summarizes patient, tumor and MRI characteristics. The majority of patients had a ductal carcinoma (IDC; 81%) and an HR+/HER2- tumor (53%). Median tumor size on MRI prior to NST was 60 mm (range 51-120 mm). Baseline MRI showed mass enhancement in 57% of patients, NME in 29% and a combination of mass enhancement and NME in 14%. Of patients with mass enhancement, 35% had a unifocal mass, 49% a multifocal mass and 16% had multicentric masses. IDC presented as mass enhancement on baseline MRI more frequently (64%), whereas lobular carcinoma (ILC) more often presented as NME or a combination of mass enhancement and NME (73%; p=0.003).

Table 1. Patient, tumor and MRI characteristics.

Characteristic	Total N (%)	
All patients	114	
Median age (range)	48	(29-74)
Histology		
Ductal	92	(80.7)
Lobular	22	(19.3)
Subtype		
HR+/HER2-	60	(52.6)
HER2+ (HR+/-)	34	(29.8)
TN	20	(17.5)
Clinical nodal stage at diagnosis		
cNo	39	(34.2)
cN1	50	(43.9)
cN2	9	(7.9)
cN3	16	(14.0)
Neoadjuvant therapy Chemotherapy		
ddAC	58	(50.9)
ddAC + taxanes	6	(5.3)
ddAC + taxanes + carboplatin	4	(3.5)
ddAC + CD	13	(11.4)
CD	2	(1.8)
ddAC + mini-CTC	2	(1.8)
Chemotherapy + HER2 targeted therapy		
PTC	24	(21.1)
PTC + Ptz	2	(1.8)
FECT-Ptz + PTC-Ptz	1	(0.9)
ddAC + taxanes + trastuzumab	2	(1.8)
Adjuvant systemic therapy		
None	19	(16.7)
Hormonal therapy	47	(41.2)
HER2-blockage	16	(14.0)
Chemotherapy	4	(3.5)
Chemotherapy + hormonal therapy and/or HER2-blockage	15	(13.2)
Hormonal therapy + HER2-blockage	13	(11.4)
Median size of tumor on MRI in mm pre-NST (range)	60.0	(51-120)
Type of enhancement on MRI		
Mass enhancement	65	(57.0)
Non-mass enhancement	33	(28.9)
Mass + non-mass enhancement	16	(14.0)
Distribution of mass enhancement on MRI		
Unifocal	28	(34.6)
Multifocal	40	(49.4)
Multicentric	13	(16.0)
Only non-mass enhancement	33	

Table 1. *Continued*.

Characteristic	Total N (%)	
Clinical tumor stage post-NST (ycT)		
ycT0	52	(45.6)
ycT1	35	(30.7)
ycT2	25	(21.9)
ycT3	2	(1.8)
Median size of tumor on MRI in mm post-NST (range)	4.0	(0-58)
Radiologic response breast tumor on MRI (RECIST)		
Complete response	52	(45.6)
Partial response (>30%)	56	(49.1)
Stable disease (<30%)	6	(5.3)
Pattern of tumor reduction on MRI		
No reduction	1	(0.9)
Shrinking mass	19	(16.7)
Diffuse reduction	18	(15.8)
Small foci in original tumor area	24	(21.1)
No enhancement (complete response)	52	(45.66)

HR=hormone receptor; HER2=human epidermal growth factor receptor 2; TN=triple negative; ddAC=dose-dense doxorubicine, cyclophosphamide; CD=Capecitabine, Docetaxel; PTC=Paclitaxel, Trastuzumab, Carboplatin; Ptz=pertuzumab; FEC-T=Fluorouacil, Epirubicine, Cyclofosfamide, Trastuzumab, NST=neoadjuvant chemotherapy; RECIST=Response Evaluation Criteria In Solid Tumors

After NST, median tumor size on MRI was 4 mm (range 0-58 mm). MRI showed rCR in 45%, rPR in 50% and stable disease in 5% of patients according to RECIST. In 17% of patients the tumor presented as a shrinking mass on post-NST MRI, in 16% a diffuse reduction was observed, in 21% small foci in the original tumor area were observed and in 1% no reduction was observed.

Pathology results

In 61% of patients, the tumor was localized with RSL, in 24% ROLL was used, in 14% the tumor was removed by palpation and in 2% a wire was placed (Table 3). Overall median weight of the specimen was 50 grams, with a lower median weight after the introduction of RSL in 2008 compared to older localization techniques (<2008: 92 grams, ≥2008: 40 grams; p<0.001). The median volume of the specimen was 108 cm^2, with 220 cm^2 <2008 and 84 cm^2 ≥2008 and (p<0.001). Overall, pCR in the breast was achieved in 33% of patients, with 8% in HR+/HER2- tumors, 40% in TN tumors and 59% in HER2+ (HR+/-) tumors (p<0.001). Median size of the invasive tumor at pathology was 15 mm (range 0-70 mm) and DCIS was present in 39% of patients.

Margins after breast conserving surgery

Of 114 patients undergoing BCS, 75 had negative margins (65%), 16 (14%) had focally positive margins for either the invasive tumor (n=15) or DCIS (n=1), and 23 (21%) had more than focally positive margins for either the invasive tumor (n=18) or the in situ component (n=5). All 75 patients with negative margins were treated with WBI ± boost. Of 16 patients with focally positive margins, 11 received WBI with boost and in five patients a mastectomy was performed. Three out of these five patients had focally positive margins for ILC. At mastectomy, pathology showed microscopic (1 mm, 2 mm and 4 mm) residual ILC. In two patients mastectomy was performed because of the patient's request: one patient had focally positive margins for IDC and one patient had focally positive margins for DCIS. Pathology results at mastectomy showed no residual disease in both patients.

Of 23 patients with more than focally positive margins, re-excision with negative margins was performed in 6 patients and mastectomy in 16 patients. One patient underwent mastectomy because of unexpected inflammatory breast cancer. One patient with positive margins for DCIS received WBI with boost and no additional surgery, because extensive nodal involvement in this patient was considered to be more prognostic for overall survival.

Overall, of 39 patients with positive margins (16 focally positive and 23 more than focally positive) after initial BCS, 12 underwent WBI with boost, 6 underwent re-excision and in 21 patients mastectomy was performed. Consequently, the final surgical treatment was BCT in 82% (93 out of 114) patients.

Local recurrence and distant recurrence free survival

The median follow-up was 7.2 years (range 0.7-17.0 years). Of 93 patients who were finally treated with BCT, two had a local recurrence and two had ipsilateral second primary breast cancer (7-year LRFI 95.9% [95% CI 91.5-100%]) (Figure 1). Sixteen out of 93 patients developed distant metastasis (7-year DRFI 85.2% [95% CI 78.1-93.0%).

Table 2. Surgery and pathology after NST.

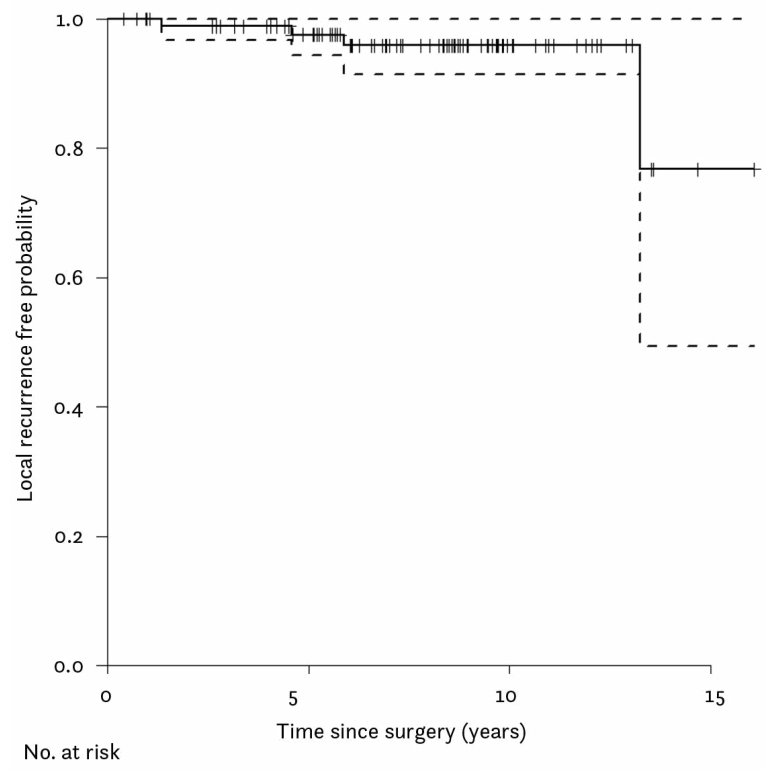
	Total	(%)	P value
Localization of tumor			n.a.
I-125 seed	69	(60.5)	
ROLL	27	(23.7)	
Palpation	16	(14.0)	
Wire	2	(1.8)	
Pathologic complete response breast			<0.001
Overall	33	(28.9)	
HR+/HER2-	5	(8.3)	
HER2+ (HR+/-)	20	(58.8)	
TN	8	(40.0)	
Pathologic tumor stage post-NST			n.a.
ypT0	33	(28.9)	
ypT1	39	(34.2)	
ypT2	34	(29.8)	
ypT3	7	(6.1)	
ypT4	1	(0.9)	
Median size of invasive tumor in mm (range)	15.0	(0-70)	n.a.
DCIS			n.a.
Not present	70	(61.4)	
Present	44	(38.6)	
Margins			n.a.
Negative	75	(65.8)	
Focally positive	16	(14.0)	
More than focally positive	23	(20.2)	
Type of axillary surgery			n.a.
SLNB	34	(29.8)	
MARI procedure	14	(12.3)	
ALND	66	(57.9)	

Table 2. *Continued*.

	Total	(%)	P value
Nodal stage after NST			n.a.
ypN0	60	(52.6)	
ypN1	38	(33.3)	
ypN2	12	(10.5)	
ypN3	4	(3.5)	

*If a re-lumpectomy was performed, the weight of the re-lumpectomy was added to the weight of the first lumpectomy. Patients who underwent secondary mastectomy because of positive margins were excluded for this analysis.
I-125=radioactive iodine; ROLL=radioactive occult lesion localization; HR=hormone receptor; HER2=human epidermal growth factor receptor 2; TN=triple negative; DCIS=ductal carcinoma in situ; SLNB=sentinel lymph node biopsy; MARI=Marking Axillary lymph nodes with Radioactive Iodine seeds; ALND=axillary lymph node dissection; NST=neoadjuvant chemotherapy

Figure 1. Local recurrence free interval.



Local recurrence free interval probability with 95% confidence interval of cT3 breast cancer patients treated with breast-conserving therapy after neoadjuvant systemic therapy.

Predictors for tumor-positive margins

In univariable analysis (Table 3), patients with HR+/HER2- tumors were more likely to have positive margins than patients with HER2+ or TN tumors (respectively 50%, 21% and 10%; p=0.001). In addition, patients with lobular carcinoma (ILC) had a higher positive margin rate (55%) than patients with IDC (29%; p=0.043). Higher rates of positive margins were also observed in patients with non-mass enhancement on baseline MRI (49% versus 23% in patients without non-mass enhancement; p=0.003). Tumor distribution on baseline MRI, response and pattern of tumor reduction on MRI post-NST, and tumor localization method at surgery were not associated with tumor-positive margins.

Table 3. Univariable analysis of predictors for positive margins after initial breast conserving surgery

Characteristic	Total	Positive margins		P value
Total	114	39	(34.2)	n.a
Histology				0.043
Ductal	92	27	(29.3)	
Lobular	22	12	(54.5)	
Subtype				0.001
HR+/HER2-	60	30	(50.0)	
HER2+ (HR+/-)	34	7	(20.6)	
TN	20	2	(10.0)	
Distribution of mass on MRI pre-NST				0.802
Unifocal	28	7	(25.0)	
Multifocal	40	13	(32.5)	
Multicentric	13	4	(30.8)	
Only non-mass enhancement	33			
NME on MRI pre-NST				0.005
Present	49	24	(49.0)	
Absent	65	15	(23.1)	
Clinical tumor stage post-NST (ycT)				0.941
ycT0	52	35	(67.3)	
ycT1	35	22	(62.9)	
ycT2	25	17	(68.0)	
ycT3	2	1	(50.0)	
Response on MRI post-NST				0.763
Complete response	51	17	(33.3)	
Partial response	57	21	(36.8)	
Stable disease	6	1	(16.7)	
Pattern of tumor reduction				0.989
No reduction	1	0	(0)	
Shrinking mass	19	6	(31.6)	
Diffuse reduction	18	6	(33.3)	
Small foci in original tumor area	24	9	(37.5)	
No enhancement	51	18	(34.6)	
DCIS in surgical specimen				0.426
Not present	70	26	(37.1)	
Present	44	13	(29.5)	
Localization of tumor				0.826
I-125 seed	69	25	(36.2)	
ROLL	27	8	(29.6)	
Palpation	16	5	(31.3)	
Wire	2	1	(50.0)	

HR=hormone receptor; HER2=human epidermal growth factor receptor 2; TN=triple negative; MRI=magnetic resonance imaging; NST=neoadjuvant chemotherapy; NME=non-mass enhancement; DCIS=ductal carcinoma in situ; I-125=radioactive iodine; ROLL=radioactive occult lesion localization

In multivariable logistic regression after correction for confounders (Table 4), the odds of tumor-positive margins were significantly lower in patients with HER2-positive (OR 0.27, 95% CI 0.10-0.73, p=0.01) and TN breast cancer (OR 0.17, 95% CI 0.03-0.82, p=0.028). A borderline significant trend toward higher tumor-positive margins was observed in patients with NME on baseline MRI (OR 2.38, 95% CI 0.98-5.77, p=0.055).

Table 4. Multivariable logistic regression

Characteristic	OR (95% confidence interval)		P value
Histology			
Ductal	**Ref**		
Lobular	1.647	(0.58-4.68)	0.349
Subtype			
HR+/HER2-	**Ref**		
HER2+ (HR+/-)	0.27	(0.10-0.73)	0.01
TN	0.17	(0.03-0.82)	0.028
Non-mass enhancement on MRI pre-NST			
Not present	**Ref**		
Present	2.38	(0.98-5.77)	0.055

HR=hormone receptor; HER2=human epidermal growth factor receptor 2; TN=triple negative; MRI=magnetic resonance imaging; NST=neoadjuvant chemotherapy

DISCUSSION

Although NST can achieve downsizing or even pCR of the tumor in breast cancer patients, BCT after NST in patients with large tumors remains controversial. This study showed that BCT could be performed in 82% of cT3 patients in whom BCT appeared feasible on post-NST MRI. Local control in this group was excellent with a 7-year local recurrence free interval of 95.9% (95% CI 91.5-100%), which is similar to previous reported local control rates of cT3 breast cancer patients treated with upfront BCT.[36,37] The majority of patients with focally positive margins did not undergo re-excision but received WBI with boost, which did not compromise local control. In addition, we found that HR+ subtype and the presence of NME on baseline MRI were correlated with tumor-positive margins.

Our findings mirror results from a meta-analysis that compared BCS with mastectomy after NST in locally advanced breast cancer.[38] In this meta-analysis, there was no difference in LR between BCT and mastectomy after good response to NST (OR 0.83; 95% CI 0.60-1.15; p=0.26). Despite the excellent local control that is associated with BCT after NST, many patients who are eligible for BCS are still treated with mastectomy. In the United States, there has even been an increase in mastectomy rates over time.[17,39] Although the selection of patients with cT3 breast cancer for BCS after NST is challenging, it should be considered in patients with good response, taking into account the patient, tumor and MRI characteristics, and marking of the tumor before NST.

Recently, an EBCTCG meta-analysis with individual patient data of 4765 women randomized to NST or adjuvant chemotherapy showed that NST was associated with a 15-year absolute LR increase of 5.5% (95% CI 2.4-8.6).[2] However, this increase is most likely not a result of NST itself, but a result of less extensive surgery or omission of surgery in patients in whom this should not have been attempted. In two trials included in this meta-analysis in which the 10-year increase in local recurrence was largest (13.3% [95% CI 5.5-21.1), the majority of patients did not undergo surgery post-NST. In the remaining trials, surgery was scheduled irrespective of response (10-year LR increase 3.2% [0.6-5.8%]. In our opinion, BCS should only be attempted when adequate imaging such as MRI shows sufficient reduction of the tumor volume.

MRI has been demonstrated to be superior to other imaging methods in determining the shape and extent of residual disease.[21-23] MRI however has a lower accuracy in predicting response to NST in HR+ tumors, which could explain the higher rate of positive margins in HR+ tumors in our study. Loo et al. demonstrated that changes on MRI correlated well with pathology results for TN and HER2+, but not for HR+/HER2- tumors.[8] In addition, studies have demonstrated that HR+ tumors often show a non-concentric shrinkage on MRI,[40,41] which could impede determining surgical margins. Last, higher positive margin rates in patients with HR+ tumors could also be a result of lower response rates to NST in this subtype, as compared to the TN or HER2+ subtypes.[42]

A borderline significant trend toward higher tumor-positive margin rates was also observed in patients with NME on baseline MRI in multivariable analysis. This finding is in accordance with the study by Bahl et al,[43] in which positive margins were found in 34% in patients with NME as compared to 17% in patients with mass enhancement (p<0.01). Also in the study by Kim et al, higher positive margin rates were observed in patients with NME.[44] In our study, ILC presented as NME in 73% of patients, which could be an explanation for the higher probability of surgery with positive margins in patients with NME. ILC is associated with a higher risk of positive margins in the upfront surgery setting, as well as in the neoadjuvant setting.[45-49] A few studies showed that use of MRI has the potential to lower positive margin rate in the upfront surgery setting.[50,51] However, in our study use of MRI still resulted in a higher rate of surgery with positive margins

in ILC (55%) as compared to IDC (29%; p=0.043). Similarly, Vriens et al[52] demonstrated a more than fourfold higher positive margin rate after NST in ILC as compared to IDC (OR 4.53, 95% CI 2.67-7.67; p<0.001), despite the fact that breast MRI was more frequently used in patients with ILC. Consequently, use of MRI did not result in a reduced mastectomy rate in patients with ILC. It could be hypothesized that ILC shows a more scattered pathologic response that is harder to visualize with use of MRI, in contrast to a more pathologic concentric response in IDC.[45,47] In patients with extensive ILC, BCS after NST is often challenging and should be advised with caution.

The SSO-ASTRO guideline on margins for BCS advises re-excision in patients with ink on invasive tumor or DCIS.[35] In our study, a positive margin was therefore defined as any ink on tumor (either focally positive or more than focally positive). The SSO-ASTRO guideline was developed based on a meta-analysis that found that positive margins were associated with a two-fold increase in the risk of ipsilateral breast tumor recurrence compared with negative margins.[35] However, the median year of patient recruitment of studies included in the meta-analysis was 1990, and only 26% and 38% of patients received chemotherapy and endocrine therapy, respectively. Over the past 20 years, the use and efficacy of systemic therapy in the treatment of breast cancer has increased dramatically. Many large randomized trials showed that systemic therapy reduces the risk of local recurrence by half and suggest that its use might have a greater impact on local control than margin width.[53-57] Moreover, it has been demonstrated that WBI with boost for patients with focally positive margins results in adequate local control and overall survival.[19,58,59] Therefore, in Dutch guidelines re-excision is only indicated for those with more than focally positive margins.[26] In our study, 5 patients with focally positive margins underwent mastectomy. Retrospectively, we believe these patients could have been safely treated with WBI with boost, foregoing re-excision according to Dutch guidelines.

Several comments on this study need to be noted. First, it is essential to know that the breast team at the NKI considers the possibility of BCT for all cT3 breast cancer patients with partial or complete response on MRI after NST, even though we know that rCR on MRI is not equal to pCR. This explains why the overall positive margin rate was relatively high. Therefore, patients with rCR are unambiguously informed about the possibility of incomplete resection and, as a consequence, have a risk of a second, more extensive surgical procedure. Second, this is a single-centre study with a relatively small sample size, urging for validation of our findings in a larger multi-center cohort. In addition, selection bias may have occurred at the time of surgery. In this retrospective study substantiation for the choice of BCS or mastectomy could not always be retrieved. Last, a control group with cT3 patients that underwent mastectomy after NST is lacking.

In conclusion, this study demonstrates that BCT based on MRI evaluation before and after NST in cT3 breast cancer patients is feasible, with a local recurrence free survival of 95.9%. BCT should therefore be considered in cT3 breast cancer patients with adequate response on MRI after NST. In patients with HR+/HER2- tumors, non-mass enhancement on baseline MRI, or ILC the risk of positive margins should be considered pre-operatively.

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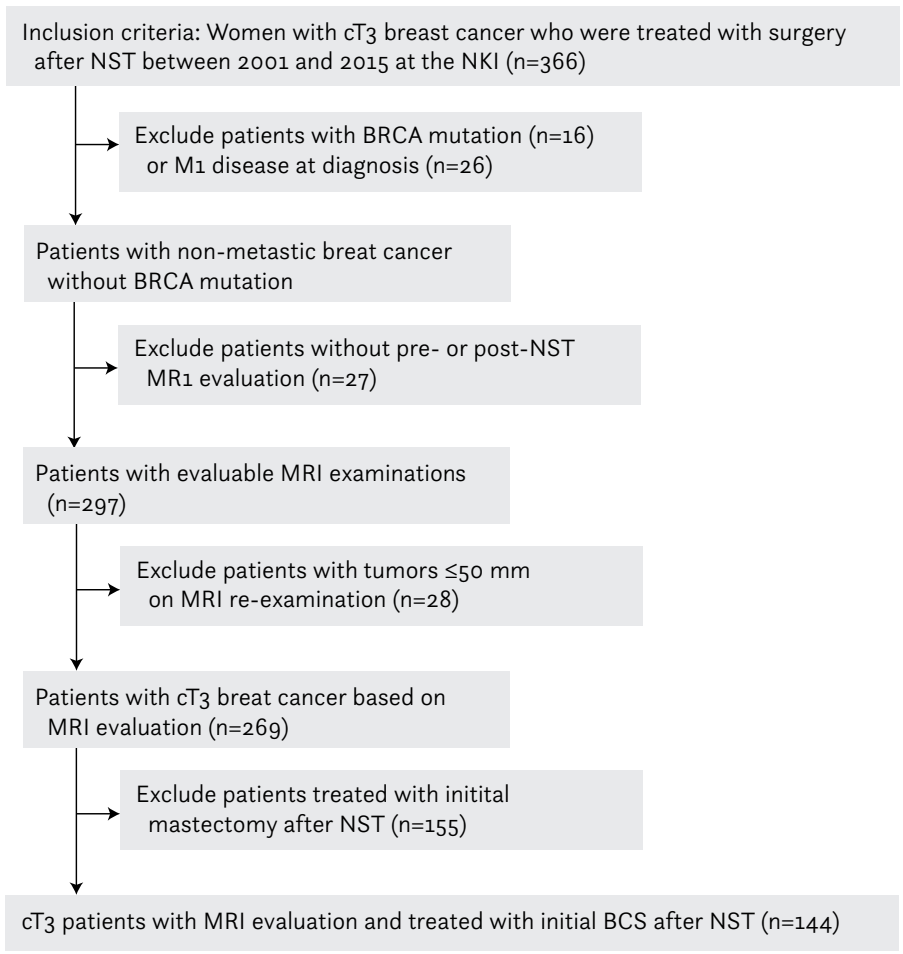
SUPPLEMENTARY FIGURE



cT3=clinical T3; NST=neoadjuvant systemic therapy; NKI=Netherlands Cancer Institute; MRI=magnetic resonance imaging; BCS=breast conserving surgery

Chapter 5

Towards omitting breast surgery in patients with a pathologic complete response after neoadjuvant systemic therapy: study design and feasibility of the MICRA trial (**M**inimally **I**nvasive **C**omplete **R**esponse **A**ssessment)

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ABSTRACT

Purpose: Improvements in neoadjuvant systemic therapy (NST) for breast cancer patients have led to increasing rates of pathologic complete response (pCR). The MICRA trial (NTR6120) aims at identifying pCR with post-NST biopsies. Here, we report the study design and feasibility of the study.

Methods: The MICRA-trial is a multi-center prospective cohort study. Patients with a pre-NST placed marker and radiologic complete (rCR) or partial response response on MRI after NST are eligible for inclusion. Ultrasound guided biopsy of the original tumor area is performed. Pathology results of the biopsies and surgery specimens are compared. The primary endpoint is false-negative rate of biopsies in identifying pCR.

Results: During the first year of the trial 58 patients with rCR were included. One patient was a screening failure and excluded for analysis. Twenty-one percent had hormone receptor(HR)+/HER2- tumors, 21% HR+/HER2+ tumors, 18% HR-/HER2+ tumors and 40% TN tumors. Overall pCR was 68%. In seven patients biopsies could not be obtained: in 6 patients, the marker could not be identified on ultrasound in the OR and in 1 patient there were technical difficulties. A median of eight biopsies was obtained (range 4-9). The median of histopathological representative biopsies was 4 (range 1-8).

Conclusion: Ultrasound guided biopsy of the breast in patients with excellent response on MRI after NST is feasible. Accuracy results of the MICRA trial will be presented after inclusion of 525 patients to determine if ultrasound guided biopsy is an accurate alternative to surgical resection for assessment of pCR after NST.

INTRODUCTION

Breast cancer management is increasingly focused on patient-tailored and de-escalating therapy, to prevent overtreatment. Systemic treatment is adapted to patient and tumor characteristics and is often administered before surgery with increasing rates of pathologic complete response (pCR). To what extent downsizing of the tumor occurs is largely dependent on breast cancer subtypes: for triple negative (TN) and human epidermal growth factor receptor 2 (HER2)-positive subtypes, treatment with new regimens results in pCR rates as high as 40-80% are achieved.[1-5]

Studies have demonstrated that local-regional recurrence (LRR) rates after breast-conserving surgery (BCS) following NST are comparable to LRR rates in patients treated with primary surgical treatment (when margins are clear and BCS is followed by radiotherapy).[6-9] BCS after NST is therefore considered safe, despite the fact that the original tumor bed is not entirely excised. It can therefore be hypothesized that breast surgery could be omitted in patients achieving pCR.

Breast surgery is associated with limited short-term morbidity (wound infection, hematoma) in approximately 5% of patients. However, moderate to severe long-term morbidity such as pain, fibrosis, loss of flexibility, asymmetry and decreased psychosocial function is present in up to 45% of patients.[10-15]

In patients with pCR, resection of (part of) the original tumor area is performed as a diagnostic procedure, since imaging methods are not sufficiently accurate to identify pCR.[16-18] Magnetic imaging resonance (MRI) is accurate in determining tumor size after NST, but unable to identify pCR with sufficient reliability to replace surgical excision.[17,19,20] When NST was emerging, some studies already investigated the possibility of local-regional therapy without surgery.[21-23] In these studies, patients with a clinical complete response (cCR) (no palpable disease and/or absence of residual tumor on mammography and/or ultrasound) were treated with radiotherapy only. Results showed unacceptable high rates of local recurrence (LR) (21-47%). As later studies showed that presence of pCR cannot reliably be assessed by clinical examination or conventional imaging, it can be assumed that in a significant number of patients with a cCR there was no concurrent pCR.[24,25] In another study, biopsies were obtained without image guidance in patients with cCR after NST. Tumors were not marked prior to NST and 6-10 biopsies per quadrant were obtained.

Patients with pCR in the biopsies were treated with radiotherapy only.[26] After a follow-up of 34 months LR in these patients was 13%.

Since current practice consists of marking the breast lesion prior to NST and pCR rates are increasing, a renewed and justified interest has emerged in the possibility to omit surgery after NST. The MICRA trial (Minimally Invasive Complete Response Assessment) is designed to determine the value of ultrasound guided biopsy of the breast in identifying pCR after NST. The ultimate aim of our study is to eliminate surgery of the breast in patients who achieve pCR, consequently improving quality of life of these patients. Here, we present the study protocol and the feasibility.

METHODS AND MATERIALS

Study protocol

Patient selection and recruitment

This multi-center prospective observational cohort study was approved by the medical ethical committee of the Netherlands Cancer Institute (NKI). Eligible patients are ≥18 years old, diagnosed with invasive primary breast cancer, treated with NST and have a radiologic partial (rPR) or complete response (rCR) on MRI after NST (Table 1). Patients with all tumor subtypes (HR+/HER2-, HR+/HER2+, HR-/HER2+, TN) and all histological subtypes are eligible for participation.

Initially, inclusion was limited to patients with rCR. With this strict inclusion criterion, a considerable number of patients with pCR will not be identified since not all patients with pCR have a concordant rCR.[19] Therefore, an amendment was adopted to include a separate group of patients with radiologic partial response (rPR) on MRI. In the preliminary results in this manuscript, only patients with rCR were included. Written informed consent is obtained from all patients. Data collection was planned prospectively. Study specifications are published on trialregister.nl (NTR6120).[27]

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Women with invasive breast cancer >18 years (all histological subtypes and all tumor subtypes)	DCIS as shown by core biopsy prior to NST
Tumor histology and receptor status established by pre-NST core biopsy	Women with distant metastatic disease
Suitable for response evaluation with MRI	History of ipsilateral breast cancer
Complete or partial response on post-NST MRI	
Marker placed in tumor prior to NST	
Correct position of marker confirmed by mammography or ultrasound	

NST=neoadjuvant systemic therapy; MRI=magnetic resonance imaging; DCIS=ductal carcinoma in situ

Diagnostic work-up and tumor localization prior to NST

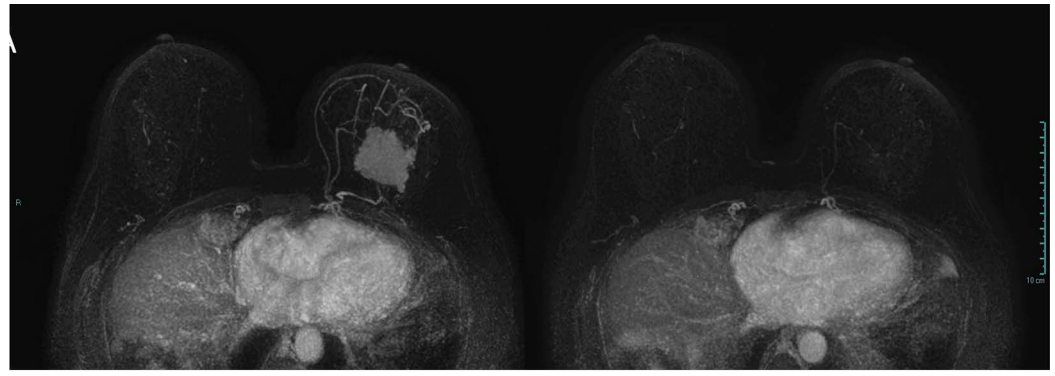
Mammography, ultrasound and MRI are used for assessment of the primary tumor. All patients undergo axillary ultrasound and fine needle aspiration (FNA) is performed in case of a suspect lymph node. An ^{18}F-FDG PET/CT prior to NST for detection of regional and distant metastases is optional. Core needle biopsies from the primary tumor are obtained to determine histological subtype and receptor status. Scoring for ER, PR and HER2 is done according to the Dutch guidelines.[28] Before the start of NST, the center of the breast lesion is localized with a marker (iodine seed, clip marker, hydro marker) under ultrasound guidance. Adequate position of the marker is confirmed by mammography and/or ultrasound.

NST is administered according to national or institutional guidelines. At NKI, patients with HR-positive/HER2-negative tumors are treated with four cycles of two-weekly cyclophosphamide and doxorubicin, followed by 12 weekly administrations of paclitaxel. Patients with TN tumors in addition receive carboplatin concurrent with paclitaxel. Patients with HER2-positive tumors either receive nine cycles of paclitaxel, carboplatin, trastuzumab and pertuzumab (PTC-Ptz) or three cycles FEC-trastuzumab-pertuzumab followed by 6 cycles of PTC-Ptz.[29]

Response evaluation

All patients undergo MRI of the breasts before NST and just before or after the last course of NST. MRI examination during NST is optional. Breast MRI is performed with a 1.5 or 3-T system by using a dedicated phased array bilateral breast coil. Images are acquired in the axial plane with the patient in prone position. The MRI protocol consists of a dynamic contrast-enhanced T1-weighted sequence, a diffusion-weighted sequence and optionally a fast dynamic sequence. The diffusion-weighted sequence is acquired with a minimum of three b-values 0, 150, 800 (1.5T) and optionally 1200 (3.0T) sec/mm^2. The protocol starts with an unenhanced T1-weighted sequence. The dynamic contrast-enhanced protocol consists of five consecutive series at 80-120 second intervals. A bolus of gadolinium-containing contrast (0.1 ml/kg) is injected intravenously at a rate of 2 mL/sec and followed by a bolus of 30 mL saline salt. Subsequently, five consecutive series are acquired with a maximum voxel size of 1 mm^3. All MRI examinations are assessed by breast radiologists. Radiologic complete response is defined as complete absence of pathologic (i.e. non-physiological) contrast enhancement in the original tumor area at MRI after NST (Figure 1). Radiologic partial response is defined as 0.1-2.0 cm contrast enhancement and ≥30% decrease in tumor size, according to RECIST criteria.[30]

Figure 1. Radiologic complete response on MRI after NST. MRI before (left) and after (right) neoadjuvant systemic treatment.



Maximum Intensity Projection (MIP) enhancement at initial enhancement (90 s); MIP images after NST show no pathologic enhancement in the left breast. Radiologically assessed as complete response.

Biopsies after NST and surgery

Ultrasound guided biopsy and surgery are performed <6 weeks after the last NST course. In all patients eight 14G biopsies of the original tumor area are obtained by a breast radiologist. Biopsies are obtained concentrically around the marker: four central biopsies (<0.5 cm) and four

peripheral biopsies (0.5-1.5cm), to determine whether both the central and peripheral biopsies are required for pathologic response assessment (Figure 2).

Preferably, since the procedure does not confer benefit for the patient, biopsies are obtained under general anesthesia in the operating room (OR) to minimize patient discomfort. Immediately hereafter, conventional surgery is performed, which may consist of BCS or mastectomy. In hospitals where ultrasound guided biopsy in the OR is not feasible because of logistic difficulties, the procedure may be performed in the outpatient clinic. In this scenario, biopsies are obtained under ultrasound or stereotactic guidance after injection of a local anesthetic. To minimize the number of biopsy scars, a pre-existing biopsy scar is used and/or the biopsy route is discussed with the surgeon. Axillary surgery, if indicated, is left to the discretion of the surgeon. At NKI, axillary staging after NST is performed with sentinel lymph node biopsy in cN0 patients and the MARI-procedure[31,32] in cN+ patients.

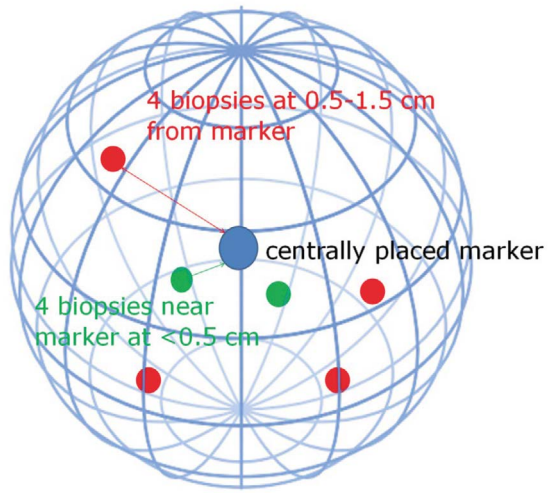
Pathology review

Pathology findings are scored using Miller-Payne criteria.[33] To evaluate the quality and representativeness of the biopsies, the biopsies are categorized according to length and pathology results. The length of biopsies are categorized as <4 mm, 4-7 mm and ≥8 mm. Pathology results are categorized as follows: residual tumor cells or (parts) of the former tumor bed present (I=histopathological representative), only breast tissue present (II) and only fatty tissue present (III). Tissue is classified as former tumor bed when it contains fibrosis, fibroelastosis, (edematous) stroma cell reactions, residues of atypical ductal epithelial proliferation, chemotherapy-associated reactive changes or inflammatory cell infiltrates that include macrophages, hemosiderphages, lymphocytes or plasma cells.

Statistical analysis and power calculation

The primary endpoint of the MICRA trial is the false-negative rate (FNR) of the biopsy procedure, i.e. the proportion of patients with non-pCR in the surgical specimen but with pCR in the biopsy specimens. Secondary endpoints are specificity, sensitivity, positive predictive value and negative predictive value. It was calculated that if the true FNR is 3%, 130 patients without pCR in specimen are sufficient to show that the FNR does not exceed 8% using a one-sided binomial test with a significance α-level of 0.05. With an expected average pCR rate of 65%, 375 patients with rCR will be included. In the rPR-group the expected pCR rate is 12% and therefore 150 patients will be included. Patients who do not meet the inclusion criteria or whose biopsy procedure fails are excluded from analysis.

Figure 2. Schematic representation of marker and biopsy locations within surgical specimen.



Green dots represent 4 central biopsies; red dots represent 4 peripheral biopsies.

Preliminary results

Patient and tumor characteristics

During the first year of the MICRA trial, fifty-eight patients with rCR on MRI were included at NKI. One patient did not meet the inclusion criteria, because there was DCIS in the pre-NST biopsy (screening failure). Table 2 lists patients and tumor characteristics of the remaining 57 patients. The median age of the patients was 48 years (range 24-68). The median size of the tumor on MRI before NST was 25 mm (range 12-95 mm). Eighty-one percent of patients had a unifocal tumor and 19% had a multifocal or multicentric tumor. Twenty-one percent had HR-positive/HER2-negative tumors, 21% HR+/HER2+ tumors, 18% HR-/HER2+ tumors and 40% TN tumors. The majority of patients had a ductal carcinoma (97%). Forty percent of patients were clinical node-positive prior to NST.

Table 2. Patient and tumor characteristics (n=57).

Characteristic	Number (%)	
Median age, years (range)	47.8	range 24.3-68.4
Initial largest median tumor size on MRI, mm (range)	25.0	range 12.0-95.0
Focality of tumor on MRI		
Unifocal	46	(80.7)
Multifocal	6	(10.5)
Multicentric	5	(8.8)
Clinical T stage		
T1	14	(24.6)
T2	34	(59.6)
T3	9	(15.8)
Clinical N stage		
No	35	(61.4)
N1-3	22	(38.6)
Histology		
Ductal carcinoma	55	(96.5)
Lobular carcinoma	2	(3.5)
Tumor receptor subtype		
HR+/HER2-	12	(21.1)
HR+/HER2+	12	(21.1)
HR-/HER2+	10	(17.5)
TN	23	(40.4)
Nuclear grade		
2	15	(26.3)
3	38	(66.7)
Unknown	4	(7.0)
Type of marker		
Iodine seed	55	(96.5)
Clip	2	(3.5)
Radiologic complete response on MRI	57	(100)
Type of breast surgery		
Lumpectomy	47	(82.5)
Mastectomy	10	(17.5)

Table 2. *Continued*.

Characteristic	Number (%)	
ypT stage		
ypTo	39	(68.4)
ypTis	3	(5.3)
ypT1	15	(26.3)
Pathologic complete response per subtype (ypTo)		
HR+/HER2-	3	(25.0)
HR+/HER2+	9	(75.0)
HR-/HER2+	8	(80.0)
TN	19	(82.6)

MRI=magnetic resonance imaging; HR=hormone receptor; HER2=human epidermal growth factor receptor 2; TN=triple negative

Response after NST

All patients had rCR on CE-MRI after NST (Table 3). In the majority of patients, BCS was performed (83%). Overall pCR of the breast (ypTo) was 68% (39/57 patients). Of 18 patients with residual disease (32%), 3 had DCIS (that was not present in pre-NST biopsies) and 15 had invasive disease. PCR rates were 25% in HR+/HER2- tumors (3/12 patients), 75% in HR+/HER2+ tumors (9/12 patients), 80% in HR-/HER2+ tumors (8/10 patients) and 83% in TN tumors (19/23 patients).

Of 39 patients with breast pCR, 26 were initially node-negative. Of these patients, 5 underwent SLNB prior to NST which were all tumor-negative. Twenty-one cNo patients underwent SLNB after NST and in 20/21 patients the SLN could be identified. These 20 patients all had tumor-negative SLNs. Thirteen out of 39 patients with breast pCR were initially node-positive and underwent the MARI-procedure. In 1 patient, the MARI-node could not be identified. Of the remaining 12 patients, 11 had a pCR of the MARI node (91%). In one patient, isolated tumor cells were found in the MARI-node.

Table 3. Outcome after NST (n=57).

Characteristic	Number (%)	
Radiologic complete response on MRI	57	(100)
Type of breast surgery		
Lumpectomy	47	(82.5)
Mastectomy	10	(17.5)
ypT stage		
ypTo	39	(68.4)
ypTis	3	(5.3)
ypT1	15	(26.3)
Pathologic complete response per subtype (ypTo)		
HR+/HER2-	3	(25.0)
HR+/HER2+	9	(75.0)
HR-/HER2+	8	(80.0)
TN	19	(82.6)

Of 18 patients with residual disease (ypTis/1) in the breast, 9 patients were initially node-negative. Eight of these patients had a tumor-negative sentinel node (89%) after NST and in 1 patient microscopic disease was found in the sentinel node. Nine patients with residual disease in the breast were initially node-positive. Of these, 7 had residual disease in the MARI-node.

Feasibility of the biopsy procedure

Of the total group of 57 patients, seven patients were not evaluable: in 6 patients, the marker that was placed prior to NST could not be identified on ultrasound in the OR and in 1 patient there were technical problems with the ultrasound equipment. In total, ultrasound guided biopsy was successful in 50 patients (88%). All procedures were performed while the patient was under general anesthesia. The median number of total post-NST biopsies was 8 (range 4-9), of central biopsies 4 (range 0-8) and of peripheral biopsies 4 (range 0-8). In one patient, only four biopsies were obtained because the marker was extracted from the breast with the biopsy needle. No hemorrhages occurred that affected surgery.

The median number of histopathological representative biopsies was 4 (range 1-8), with a median of 3 central (range 0-4) and 2 peripheral (range 0-4) histopathological representative biopsies. The length of biopsies was ≥8 mm in a median of 6 biopsies (range 0-8) per patient.

DISCUSSION

Due to improvement in chemotherapy regimens and targeted therapies, pCR is achieved in an increasing number of breast cancer patients when administered in a neoadjuvant setting. In patients with TN and Her2-positive tumors, pCR rates as high as 80% can be reached.[1,3,5,34] Achievement of pCR is associated with improved long-term disease free and overall survival in patients with TN and Her2-positive tumors.[4,35] As survival after breast cancer is increasing, it is imperative that the necessity, benefits and adverse consequences of proposed treatments are continuously assessed. In patients who achieve pCR after NST it is unlikely that breast surgery is of added therapeutic value. However, since imaging modalities are insufficiently accurate to predict the absence or presence of pCR after NST, the need for surgery is unchanged.

In the MICRA trial, we evaluate the accuracy of ultrasound guided biopsy after NST in identifying pCR. Tumors are marked prior to the start of NST and response evaluation is performed with CE-MRI. The ultimate aim of the MICRA trial is to select patients with a pCR of the breast after NST in whom surgery may be omitted. In this manuscript, we present the study protocol and the feasibility of the first 57 patients. In 19/57 patients (32%) with rCR on MRI residual disease was present in the surgical specimen (16 patients with invasive carcinoma and 3 patients with DCIS), confirming the need for post-NST tissue analysis. The preliminary results of the MICRA trial indicate that post-NST tissue analysis is feasible with biopsies: in 50/57 patients, the biopsy procedure was successful. In total, 375 patients with rCR and 150 patients with rPR on MRI will be included. Results of the MICRA trial will be presented after inclusion of 525 patients to determine if ultrasound guided biopsy is an accurate alternative to surgical resection for assessment of pCR after NST. If the primary endpoint if the MICRA trial is answered, we will start a prospective registration trial in which surgery of the breast is omitted in patients with pCR in biopsies. In this registration study, patient will be followed for 10 years and a questionnaire including patient-reported outcomes will be used to evaluate quality of life of these patients.

Since the start of our trial, two amendments have been made. Initially, inclusion was limited to patients with rCR after NST. With this strict inclusion criterion, a considerable number of patients with pCR will not be identified since not all patients with pCR have a concordant rCR.[19] Therefore, an amendment was adopted to include a separate group of patients with rPR on MRI after NST. In addition, we will document ultrasound findings of the tumor area at the post-

NST biopsy procedure as this may contribute to future patient selection. We will evaluate the accuracy of both MRI and ultrasound in assessing (non-)pCR.

A limitation of our study is the fact that biopsies are performed in the OR. Our preliminary results indicate that in 6/57 patients the marker was not visible on ultrasound. Conditions for ultrasound guided biopsy in the OR are suboptimal, since patients are under general anesthesia and therefore uncooperative. However, if our trial has a positive outcome and results can be implemented in daily practice, biopsies will be obtained in the outpatient clinic. In this setting, stereotactic guidance can be used when the marker is not visible on ultrasound.

Recently, a meta-analysis from the EBCTCG including individual patient data for 4765 women randomly allocated to NST or adjuvant systemic therapy showed that neoadjuvant chemotherapy (NAC) resulted in a 15-year absolute LR increase of 5.5% (95% CI 2.4-8.6) (REF). However, this increase is most likely not a result of NAC itself, but a result of inadequate selection of patients in whom BCS can be performed or surgery can be omitted at all. In two trials, in which the 10-year increase in local recurrence was largest (13.3% [95% CI 5.5-21.1), the majority of patients did not undergo surgery after NAC. In the other eight trials, surgery was scheduled irrespective of response to NAC (10-year LR increase 3.2% [95% CI 0.6-5.8). BCS in patients with larger tumors should only be considered after NST when adequate imaging shows tumor response, and surgery can only be omitted in patients achieving pCR. In addition, the trials included in this meta-analysis were performed from 1983 until 2002. Pathologic complete response rates were considerably lower in these years, and patients with HER2+ tumors did not receive trastuzumab. We hypothesize that omitting surgery in patients in whom pCR is accurately identified, local control will not be impacted.

Several other groups started similar trials to investigate the possibility of omitting surgery after NST. MDACC recently finished a single-center pilot study with 40 patients with TN or Her2-positive tumors who did not require a radiologic partial or complete response.[36] A median of twelve 9G vacuum-assisted core biopsies (VACB) was obtained. Overall pCR (ypT0) was 47.5%. FNR of VACB was 10% and 5% when combined with FNA. Based on these results, this group started a phase-2 study in which breast surgery is omitted in patients with T1-2 Her2-positive and TN tumors, ≤4 lymph nodes and pCR in a minimum of 12 VACBs (NCT02945579). Patients are treated with radiotherapy only. The primary endpoint for this trial is 5-year LRR. If the ipsilateral local recurrence rate exceeds 7%, the study will be ended. A major difference with our study is the amount of tissue examined with biopsies. In the MICRA trial, eight 14G core-biopsies are obtained whereas in patients in the MDACC study a minimum of twelve 9G VACBs are obtained. With twelve 9G biopsies, a large volume of tissue is removed which is associated with more patient discomfort, and higher chance of post-biopsy pain and hematoma. We aim to predict pCR by removing as little tissue as possible, while maintaining safety in terms of local-regional control.

Another feasibility study was performed at the University of Heidelberg.[37] In 50 patients with clinical or radiologic partial or complete response, VACB was performed after NST. A median of 8 VACBs was obtained with an overall FNR of 26%. When only histopathological representative biopsies were considered, the FNR was 4.8% (n=38). The high overall FNR could be due to suboptimal marking of the tumor prior to NST: a marker was placed in 80% of patients and markers were not always visible on ultrasound. In the MICRA trial, biopsies are only obtained when the breast radiologist is certain of the visibility of the marker. The Heidelberg group started a prospective multi-center trial (RESPONDER trial: NCT02948764) including 600 patients with partial or complete response (on mammography, ultrasound or MRI) to NST. Another trial (NOSTRA trial) will include 150 patients with TN or Her2-positive tumors receiving NST (regardless of clinical or radiologic response).[38] A minimum of 6 ultrasound-guided biopsies is obtained. Similarly, the NRG-BR005 multicenter trial will evaluate the accuracy of 6-8 11-gauge VACBs in 175 patients with ductal carcinoma and a clinical (near-)complete response (on mammogram, ultrasound or MRI). The primary endpoint of the RESPONDER, NOSTRA and NRG-BR005 is a FNR of <10%.[38]

CONCLUSION

Our preliminary results show that ultrasound-guided biopsy of the breast after NST is feasible. In the future, a biopsy procedure might be able to replace surgical resection for assessment of pCR after NST. In this scenario, local therapy in patients with pCR in biopsies would be restricted to radiotherapy. Overtreatment in these patients will be prevented and quality of life will be improved. Results of the MICRA trial are expected in 2021.

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Conflicts of interest

None

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Chapter 6

Minimally Invasive Complete Response Assessment of the breast after neoadjuvant systemic therapy for early breast cancer (MICRA trial): interim analysis of a multicenter observational cohort study

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SYNOPSIS

The therapeutic effect of surgery in breast cancer patients with pathological complete response (pCR) after neoadjuvant therapy is questionable. We evaluated the FNR of core-biopsies assessing pCR in patients with good response on MRI, aiming to identify pCR without surgery.

ABSTRACT

Background: The added value of surgery in breast cancer patients with pathological complete response (pCR) after neoadjuvant systemic therapy (NST) is uncertain. The accuracy of imaging identifying pCR for omission of surgery, however, is insufficient. We investigated the accuracy of ultrasound-guided biopsies identifying breast pCR (ypT0) after NST in patients with radiological partial (rPR) or complete response (rCR) on MRI.

Methods: We performed a multicenter, prospective single-arm study in three Dutch hospitals. Patients with T1-4(N0 or N+) breast cancer with MRI rPR and enhancement ≤2.0 cm or MRI rCR after NST were enrolled. Eight ultrasound-guided 14G core biopsies were obtained in the operating room before surgery close to the marker placed centrally in the tumor area at diagnosis (no attempt was made to remove the marker), and compared with the surgical specimen of the breast. Primary outcome was the false-negative rate (FNR).

Results: Between April 2016 and June 2019, 202 patients fulfilled eligibility criteria. Pre-surgical biopsies were obtained in 167 patients, of whom 136 had rCR and 31 had rPR on MRI. Forty-three (26%) tumors were hormone receptor (HR)-positive/HER2-negative, 64 (38%) were HER2-positive, and 60 (36%) were triple-negative. Eighty-nine patients had pCR (53%; 95% CI 45-61) and 78 had residual disease. Biopsies were false-negative in 29 (37%; 95% CI 27-49) of 78 patients. Multivariable associated with false-negative biopsies was rCR (FNR 47%; OR 9.81, 95% CI 1.72-55.89; p=0.01); a trend was observed for HR-negative tumors (FNR 71% in HER2-positive and 55% in triple-negative tumors; OR 4.55, 95% CI 0.95-21.73; p=0.058) and smaller pathological lesions (6mm vs. 15mm; OR 0.93, 95% CI 0.87-1.00; p=0.051).

Conclusion: The MICRA trial showed that ultrasound-guided core biopsies are not accurate enough to identify breast pCR in patients with good response on MRI after NST. Therefore, breast surgery cannot safely be omitted relying on the results of core biopsies in these patients.

INTRODUCTION

With systemic treatments becoming increasingly effective, the number of breast cancer patients undergoing breast conserving surgery after neoadjuvant systemic therapy (NST) has increased, and pathological complete response (pCR) occurs more frequent.[1-3] Previous studies have demonstrated that excision of the residual disease, rather than the entire initial tumor bed, does not compromise the recurrence rate in patients undergoing breast conserving treatment after NST.[4,5] It can thus be questioned whether any surgical resection was needed in patients with pCR in the surgical specimen.

A major challenge in pursuing a surgery-free treatment strategy for patients with pCR, is the identification of pCR without surgery. Current imaging modalities such as ultrasound, MRI and ^{18}F-FDG PET/CT-scan are not sufficiently accurate to identify pCR.[6,7] Minimally invasive biopsies to detect the presence of residual tumor in the breast after NST have been explored in several pilot studies.[8-14] The primary outcome of these studies was the false-negative rate (FNR), defined as the proportion of patients with residual disease in the surgical specimen of the breast that had tumor-negative biopsies after NST. Promising FNRs were achieved in some of these studies, leading to the initiation of new trials with a 10% cut-off for the FNR of biopsies assessing pCR [Suppl. table].[8,9,13,14]

We designed the MICRA trial (**M**inimally **I**nvasive **C**omplete **R**esponse **A**ssessment of the breast after NST) to determine whether ultrasound-guided core biopsies of the breast are sufficiently accurate to differentiate between breast pCR and residual disease (irrespective of nodal status) in patients with a radiological complete or partial response on MRI.[15] Here, we present the results of the interim analysis.

METHODS

Study design and participants

This multicenter, prospective, single-arm study included women aged 18 years or older with stage I–III invasive breast cancer of any subtype receiving NST. Key eligibility criteria were placement of a marker centrally in the tumor before start of NST and a radiological complete (rCR) or partial response (rPR, residual size ≤2.0 cm and ≥30% decrease in tumor size) on dynamic contrast-enhanced (DCE)-MRI after NST according to RECIST criteria.[16] Exclusion criteria were histopathological confirmed DCIS before start of NST and a history of ipsilateral breast surgery and/or radiotherapy. Patients were enrolled in three Dutch hospitals (the Netherlands Cancer Institute, Deventer Hospital, and Rijnstate Hospital). The medical ethical committee of the Netherlands Cancer Institute approved the conduct of the study.

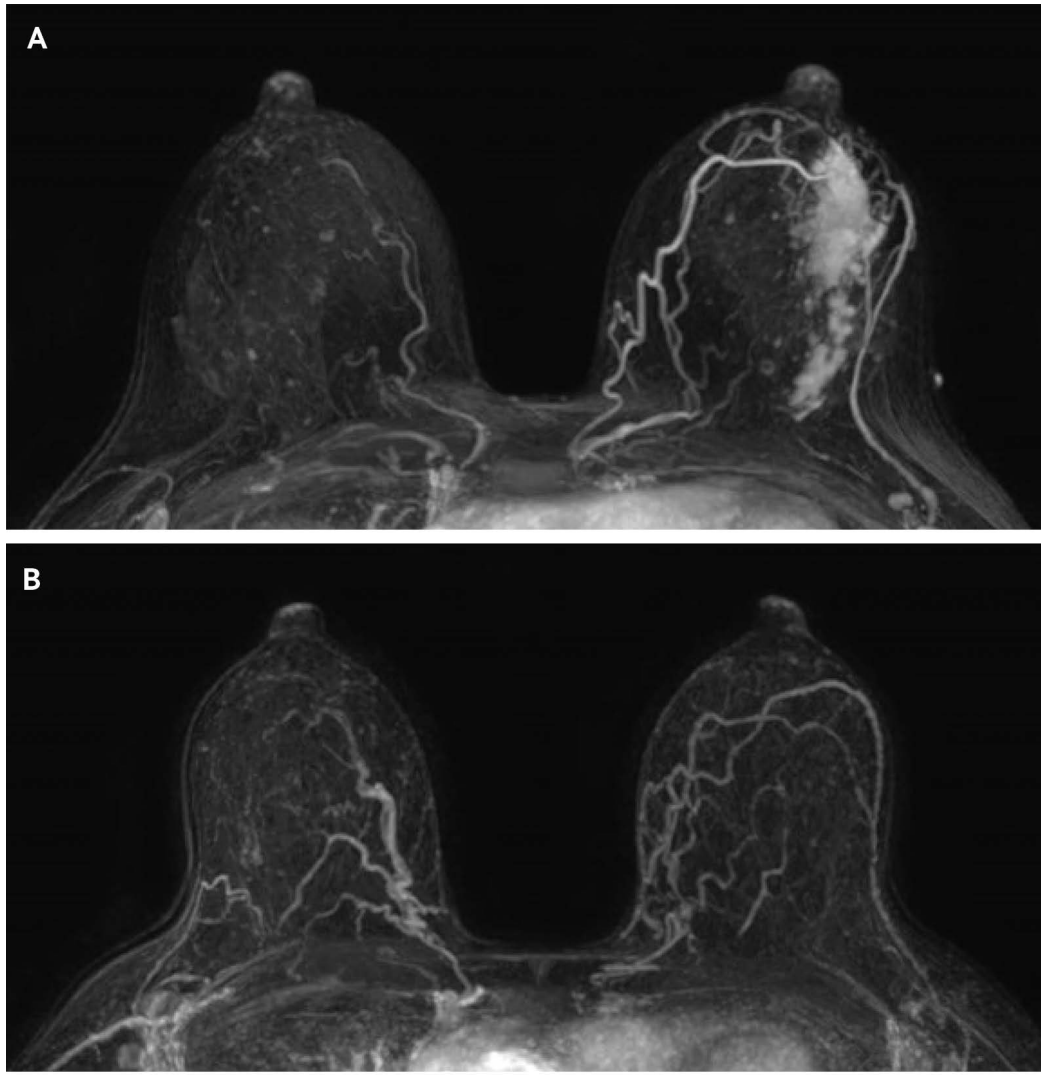
Procedures

Mammography, ultrasound and DCE-MRI were used for assessment of the primary tumor and axillary nodes prior to NST. Core needle biopsies (14G) from the primary tumor were obtained to determine breast cancer subtype and grade (according to the modified Bloom-Richardson system) and fine needle aspiration (FNA) was performed of suspect lymph nodes. Estrogen receptor and progesterone receptor were defined as positive if expression was ≥10% and immunohistochemistry assessment of HER2 overexpression was regarded positive if 3+ or 2+ with positive in-situ hybridization, according to ASCO-CAP guidelines. Before the start of NST, the breast lesion was localized with a marker (e.g., iodine seed, clip, hydromarker, twist marker) followed by mammography and/or ultrasound to confirm adequate position of the marker.

Patients with hormone receptor (HR)-positive/HER2-negative tumors were treated with four cycles of two-weekly cyclophosphamide and doxorubicin, followed by 12 weekly administrations of paclitaxel. Patients with triple-negative tumors in addition received carboplatin concurrent with paclitaxel. Patients with HER2-positive tumors received nine cycles of paclitaxel, carboplatin, trastuzumab and pertuzumab (PTC-Ptz), or three cycles of 5-fluorouracil, epirubicin, cyclo-phosphamide, trastuzumab, pertuzumab (FEC-T-Ptz), followed by six cycles PTC-Ptz.[2] Patients with cT1N0 HER2-positive disease received twelve weekly cycles of paclitaxel and trastuzumab. All patients underwent DCE-MRI before the start and at the end of NST with a 1.5-T system (in 18 patients, GE healthcare, Eindhoven, The Netherlands) and a 3.0-T system (in 201 patients, Philips Medical Systems, Best, The Netherlands) using dedicated phased array bilateral breast coils. Images were acquired in the axial plane with the patient in prone position. The MRI protocol consists of a DCE T1-weighted sequence, a diffusion-weighted sequence and optionally a fast dynamic sequence as previously described.[15] MRI examinations were assessed by breast radiologists. Radiological complete response (rCR) was defined as complete absence of pathological (i.e. non-physiological) contrast enhancement in the original tumor

area. Radiological partial response was defined as 0.1-2.0 cm contrast enhancement and ≥30% decrease in tumor size, according to RECIST 1.1 criteria[16] (Figure 1).

Figure 1. Radiological complete response on dynamic contrast-enhanced MRI after neoadjuvant systemic therapy. Breast MRI in a patient with left-sided breast cancer before the start of neoadjuvant systemic therapy (A) and after neoadjuvant systemic therapy (B). Maximum Intensity Projection (MIP) images after treatment show no pathologic enhancement in the left breast, radiologically assessed as a complete response.



Other radiologic features analyzed were presence of non-mass enhancement and multifocality on MRI, and presence of calcifications on mammography.

Biopsies and the surgical procedure were performed within six weeks after NST. Specialized breast radiologists obtained a maximum of eight ultrasound-guided biopsies of the initial tumor area with a 14-gauge (14G) automated needle device and a 22-mm-throw biopsy gun (Bard Magnum biopsy Instrument, Covington, GA, USA), concentrically around a pre-NST placed marker: four central biopsies close to the marker, and four more peripheral biopsies. In patients with multifocal or multicentric tumors, more than one marker may be used to facilitate breast conserving surgery in patients with good NST response. In these patients, biopsies were obtained from the index lesion or from the largest marked residual lesion, and compared with pathology analysis of this lesion only. To minimize patient discomfort, all biopsies were performed in the operating room under general anesthesia. The surgical procedure was performed immediately hereafter. Breast and axillary surgery were left to the discretion of the institute.

Outcomes

The primary outcome of the MICRA trial was the FNR of the biopsy procedure, i.e. the proportion of patients with residual disease in the surgical specimen of the breast in whom the biopsies were tumor-negative. Histopathological analyses of the biopsies were categorized as (1) histopathological representative, containing residual tumor cells or signs of the former tumor bed, (2) unknown, containing normal breast-, fatty- or connective tissue and (3) non-representative, containing small non-assessable tissue.[15] A pathological complete response (pCR) was defined as absence of invasive and in-situ carcinoma in the breast, irrespective of nodal status (ypT0). Response of the breast was assessed according to the Pinder classification system.[17,18]

Secondary outcome measures were specificity, sensitivity, positive predictive value and negative predictive value of the biopsy procedure. In addition, patient-, tumor- and imaging characteristics were collected to evaluate correlations with a false-negative outcome.

Statistical analysis

We hypothesized that the true FNR was 3%. The null hypothesis was a FNR of 8%. It was calculated that 130 patients with residual disease in the surgical specimen were sufficient to show, with 80% power, that the FNR would not exceed 8% using a one-sided binomial test with

a significance α-level of 0.05. Based on published data, a pCR rate of 65% is expected among patients with a rCR and a pCR rate of 12% among patients with a rPR.[7,19] Therefore, 375 patients with rCR and 150 patients with rPR would be required. Taking into account an approximate 10% biopsy failure rate due to technical difficulties, we required inclusion of 575 patients at final analysis.[15] An interim analysis for futility was planned after inclusion of 150 patients with rCR on MRI.

The two-sided 95% confidence intervals for the FNR and for proportions of patients with pCR were calculated using the Clopper-Pearson exact method. Patients in whom biopsies could not be obtained were excluded from analysis.

Differences between patients with false-negative and true-positive biopsies were tested using Kruskal-Wallis rank sum test, Fisher's Exact Test and Pearson's Chi-squared test. Subgroup analyses were prespecified for histopathological classification, Bloom-Richardson grade, hormone receptor status, tumor size on MRI, presence of non-mass enhancement or multifocality on MRI, presence of microcalcifications on mammography, and clinical tumor and nodal stage. Post-hoc analyses, including size of the residual lesions at pathology analysis, were also performed. Logistic regression was used to identify factors associated with a false negative result. Statistical significance for comparisons between groups was defined as $p<0.05$. The conditional power calculations were performed with PASS software version 15.0.4. All other statistical analyses were done using R (version 3.5.0). This study is registered with the Netherlands Trial Register, number NTR6120.

RESULTS

Study participants

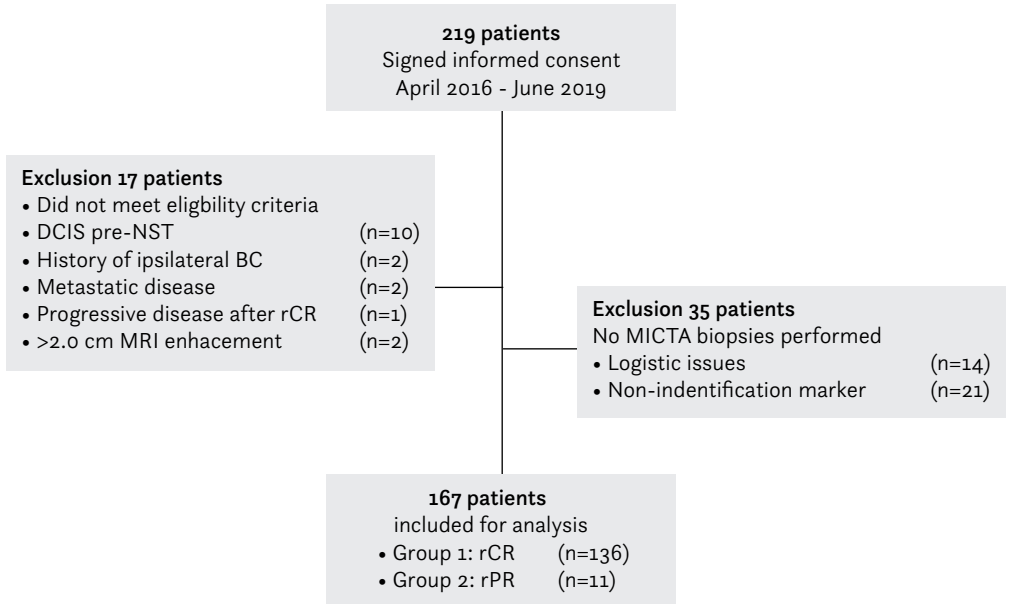
Between April, 2016, and June, 2019, we enrolled 219 patients of which 202 patients fulfilled eligibility criteria. Protocol violations were identified in 17 patients, mainly due to missed DCIS in pre-NST obtained diagnostic biopsies. In 35 patients, post-NST biopsies were not performed. This was in 21 patients due to non-identification of the marker and in 14 patients due to logistic reasons. Thus, a total of 167 (76%) patients were included for interim analysis (Figure 2).

Table 1. Baseline characteristics by radiological response group.

	Complete response MRI (n=136)		Partial response MRI (n=31)		Total (n=167)	
Age	48	(42-56)	50	(43-56)	49	(42-56)
Clinical tumor stage						
T1	32	(24%)	4	(13%)	36	(21%)
T2	87	(64%)	20	(65%)	107	(64%)
T3	17	(12%)	6	(19%)	23	(14%)
T4	0		1	(3%)	1	(1%)
Clinical nodal stage						
N+	68	(50%)	16	(52%)	84	(50%)
Imaging features						
Multifocal	31	(23%)	9	(29%)	40	(24%)
Non-mass	27	(20%)	6	(19%)	33	(20%)
Calcifications	36	(27%)	9	(29%)	45	(27%)
Tumor size (mm)	27	(20–40)	27	(22–40)	27	(21–40)
Histology						
Ductal	121	(89%)	25	(81%)	146	(88%)
Lobular	10	(7%)	4	(13%)	14	(8%)
Other	5	(4%)	2	(6%)	7	(4%)
Tumor subtype						
HR+ / HER2-	32	(24%)	11	(35%)	43	(26%)
HR+ / HER2+	36	(26%)	5	(16%)	41	(24%)
HR- / HER2+	21	(15%)	2	(7%)	23	(14%)
Triple-negative	47	(35%)	13	(42%)	60	(36%)
Tumor grade						
Grade 1	7	(5%)	0		7	(4%)
Grade 2	41	(30%)	15	(48%)	56	(34%)
Grade 3	80	(59%)	15	(48%)	95	(57%)
Unknown	8	(6%)	1	(3%)	9	(5%)

Data are median (IQR) or n (%). All baseline characteristics were assessed before administration of neoadjuvant systemic therapy. Calcifications were assessed on mammography, other imaging features were assessed on MRI.

Figure 2. Flowchart. Patient inclusion at interim analysis.



rCR=radiological complete response; rPR=radiological response; NST=neoadjuvant systemic therapy; BC=breast cancer

Median age was 49 years (IQR 42-56). Tumor histology was invasive ductal carcinoma (IDC) in 146 patients, invasive lobular carcinoma in 14 patients and other special type carcinomas in 7 patients. Distribution of tumor subtype by hormone receptor and HER2-expression was HR-positive/HER2-negative in 43 (26%) patients, HR-positive/HER2-positive in 41 (24%) patients, HR-negative/HER2-positive in 23 (14%) patients and triple-negative in 60 (36%) patients. Mean tumor size on DCE-MRI prior to NST was 27mm (IQR 21-40). Fifty percent (84 of 167; 95% CI 42-58) of patients were clinically node-positive prior to NST. Post-NST MRI showed rCR in 136 of 167 (81%, 95% CI 75-87) patients and rPR in 31 of 167 (19%; 95% CI 13–25) patients. Baseline patient characteristics are listed in Table 1.

Pathology analysis

Post-NST, a median of eight (IQR 8-8) 14G ultrasound guided biopsies per patient were obtained, followed by breast conserving surgery in 140 (84%) patients and mastectomy in 27 (16%) patients. Biopsies were representative in 151 (90%) patients, not representative in eight (5%) patients and representativeness was unknown in eight (5%) patients.

Table 2. Pathological response assessment by radiological response group.

	Complete response MRI (n = 136)		Partial response MRI (n = 31)		Total (n = 167)	
Pathological response surgical specimen						
no residual carcinoma (1i)	81	(60%)	8	(26%)	89	(53%)
no residual invasive but DCIS (1ii)	8	(6%)	0		8	(5%)
minimal residual disease, <10% (2i)	31	(23%)	8	(25%)	39	(23%)
10-50% of tumor remaining (2ii)	11	(8%)	12	(39%)	23	(14%)
>50% of tumor remaining (2iii)	3	(2%)	3	(10%)	6	(4%)
no-evidence of response (3)	1	(1%)	0		1	(1%)
Only LVSI present	1	(1%)	0		1	(1%)
Pathological response biopsies						
Tumor-negative	107	(79%)	11	(35%)	118	(71%)
Tumor-positive	29	(21%)	20	(65%)	49	(29%)

Data are n (%). LVSI=Lymphovascular invasion.

In total, 89 (53%, 95% CI 45-61) of 167 patients had pCR in the surgical specimen, while 78 had residual disease. Eighty-one (91%) of the 89 patients with breast pCR had no axillary metastases (ypT0N0). The pCR rate was 60% (81 of 136) in patients with rCR on MRI and 26% (8 of 31) in patients with rPR on MRI (Table 2).

The false-negative rate of the biopsy procedure

In 29 of the 78 patients without pCR in the surgical specimen, the residual disease was not present in the biopsies. Thus, the FNR of the biopsies assessing pCR was 37% (29 of 78; 95% CI 27-49). Sensitivity of the biopsies was 63% (49 of 78, 95% CI 51-74), specificity was 100% (89 of 89, 95% CI 0.96-1), positive predictive value was 100% (49 of 49, 95% CI 0.93-1) and negative predictive value was 75% (89 of 118, 95% CI 67-83) (Table 3). Biopsies had been scored as non-representative in two of 29 patients with false-negative biopsies and representativeness was unknown in four patients.

The FNR differed per response group and tumor subtype. In the rCR group, the FNR was 47% (26 of 55; 95% CI 34-61) and in the rPR group, the FNR was 13% (3 of 23; 95% CI 3-34) (p=0.005). The FNR was 24% (8 of 34; 95% CI 11-41) in HR-positive/HER2-negative tumors, 29% (5 of 17; 95% CI 10-56) in HR-positive/HER2-positive tumors, 71% (5 of 7; 95% CI 29-96) in HR-negative/HER2-positive tumors and 55% (11 of 20; 95% CI 32-77) in triple-negative tumors (p=0.025).

Table 3. False-negative rate of biopsies identifying pathological complete response of the breast.

	Residual Disease in Surgical Specimen											
	No (n=89)						Yes (n=78)					
Biopsies	**rPR**		**rCR**		**Total**		**rPR**		**rCR**		**Total**	
Tumor-neg	8	(9%)	81	(91%)	89	(100%)	3	(4%)	26	(33%)	29	(37%)
Tumor-pos	0		0		0		20	(26%)	29	(37%)	49	(63%)
Total	8	(9%)	81	(91%)	89	(100%)	23	(29%)	55	(71%)	78	(100%)

Data are n (%). rCR=radiologic complete response on MRI; rPR=radiologic partial response on MRI.

All characteristics of patients with false-negative biopsies and patients with true-positive biopsies are listed in Table 4. Baseline radiological features (calcifications, multifocality and non-mass) did not differ between the groups. Compared with patients that had true-positive biopsies, patients with false-negative biopsies more often had HR-negative tumors (55% vs. 22%, p=0.0006), a higher Bloom-Richardson grade (66% vs. 33% grade 3, p=0.006), rCR (90% vs. 59 %, p=0.005) and less residual invasive disease and/or DCIS in the specimens (6 mm [IQR 3-9] vs. 15 mm [IQR 9-29], p<0.001). The residual disease in patients with false-negative biopsies was more frequent DCIS only (ypTis, 21% vs. 4%) than residual invasive disease and DCIS (14% vs. 41%) or invasive disease only (65% vs. 55%) (p=0.009). In multivariable analysis, only rCR was significantly associated with false-negative biopsies (OR 9.81, 95% CI 1.72-55.89; p=0.01). A trend was seen for HR-negative tumors and smaller size of the residual disease (size in mm) (OR 4.55, 95% CI 0.95-21.73; p=0.058 and OR 0.93, 95% CI 0.87-1.00; p=0.051) (Table 5).

Table 4. Characteristics and MICRA assessment in patients with residual disease.

	False-negative biopsies (n=29)		True-positive biopsies (n=49)		P value*
Imaging features pre-NST					
Tumor size (mm)	25	(20-31)	32	(23-58)	0.028
Multifocal	5	(17%)	18	(37%)	0.078
Non-mass	7	(24%)	14	(29%)	0.794
Calcifications	12	(41%)	20	(41%)	1.000
Histology pre-NST					
Ductal	26	(90%)	39	(80%)	0.423
Lobular	3	(10%)	7	(14%)	
Other	0		3	(6%)	
Tumor subtype pre-NST					
HR+ / HER2-	8	(28%)	26	(53%)	0.025
HR+ / HER2+	5	(17%)	12	(25%)	
HR- / HER2+	5	(17%)	2	(4%)	
Triple-negative	11	(38%)	9	(18%)	
Tumor grade pre-NST					
Grade 1	1	(3%)	3	(6%)	0.006
Grade 2	7	(24%)	29	(59%)	
Grade 3	19	(66%)	16	(33%)	
Unknown	2	(7%)	1	(2%)	
Radiological response					0.005
Complete	26	(90%)	29	(59%)	
Partial	3	(10%)	20	(41%)	
Pathology post-NST					
Tumor size (mm)	6	(3-9)	15	(9–29)	<0.001
DCIS or invasive carcinoma					
No DCIS	19	(65%)	27	(55%)	0.009
DCIS and invasive	4	(14%)	20	(41%)	
DCIS only	6	(21%)	2	(4%)	

*kruskal-walis rank sum test, Fisher's exact test. Data are median (IQR) or n (%). NST=neoadjuvant systemic therapy. All baseline characteristics were assessed before administration of neoadjuvant systemic therapy. Calcifications were assessed on mammography, other imaging features were assessed on MRI.

Table 5. Predictive factors for false negative MICRA biopsies (n=78)

	Univariable			Multivariable		
	OR	95% CI	P value	OR	95% CI	P value
Imaging features pre-NST						
Tumor size (mm)	0.98	0.95-1.00	0.066	0.98	0.94-1.01	0.23
Multifocal	0.36	0.12-1.11	0.074			
Non-mass	0.80	0.28-2.28	0.67			
Calcifications	1.02	0.40-2.60	0.96			
Histology pre-NST						
Ductal	1					
Lobular	0.64	0.15-2.72	0.55			
Other	0.00	0.00-Inf.	0.99			
HR ≥10% pre-NST						
Positive	4.25	1.58-11.48	0.0043	4.55	0.95-21.73	0.058
Subtype pre-NST						
HR+ / HER2-	1					
HR+ / HER2+	1.35	0.37-5.02	0.65			
HR- / HER2+	8.12	1.31-50.21	0.024			
triple-negative	3.97	1.21-12.99	0.023			
Radiological response						
Partial	1					
Complete	5.98	1.59-22.46	0.008	9.81	1.72-55.89	0.01
Pathology post-NST						
Tumor size (mm)	0.88	0.81-0.95	0.0006	0.93	0.87-1.00	0.051
DCIS or invasive carcinoma						
No DCIS	1					
DCIS and invasive	0.28	0.08-0.97	0.044	0.51	0.12-2.11	0.35
DCIS only	4.26	0.78-23.44	0.095	2.39	0.23-24.37	0.46

Univariable and multivariable logistic regression. HR=hormone receptor expression; NST=neoadjuvant systemic therapy. All baseline characteristics were assessed before administration of neoadjuvant systemic therapy. Calcifications were assessed on mammography, other imaging features were assessed on MRI.

Adverse events

Adverse events related to the biopsy procedure were observed in 11 of 167 (7%; 95% CI 3-11) patients. In these patients, the radioactive iodine seed (I-125) used for localization of the tumor area was accidently removed during the biopsy procedure. Removal of the iodine seed led to minor adjustments of the surgical procedure in five patients with planned lumpectomy: in one patient the iodine seed was directly replaced by a new iodine seed, three patients had guided wire localization and in two patients the local excision was widened.

DISCUSSION

The MICRA trial showed that ultrasound-guided 14G core biopsies of the breast failed to detect residual disease in approximately one third of patients with a radiological complete or partial response to NST on DCE-MRI. The MICRA trial was the first trial that studied the accuracy of MRI and ultrasound-guided biopsies of the breast after NST to identify pCR of the breast.

Minimally invasive methods aiming to identify patients with pCR of the breast are currently investigated by several groups.[9,14,20] The published literature before this study showed promising results.[20] In three smaller pilot studies with 20 to 50 patients, FNRs of 5% to 26% were achieved.[9,13,14] A larger multicenter exploratory analysis of 164 patients performed by the German Breast Group demonstrated an overall FNR of 49%. In this study, not all patients had a pre-NST placed marker (63%) and biopsy methods were not standardized.[8] A post-hoc analysis in 16 patients with mammographic-guided vacuum-assisted biopsies (VAB) found a FNR of 0%. In the pilot study performed by the University of Heidelberg, the FNR was lowered from 26% to 5% when patients in whom biopsies showed neither tumor cells nor (signs of) the initial tumor bed at histopathological analysis were excluded.[14] None of these studies used DCE-MRI to selected patients with response, as we did in the MICRA trial.

Updated results including a multi-institutional pooled analysis (MDACC, Seoul National University Hospital[21] and The Royal Marsden[22]), results of the RESPONDER trial[23] (NCT02948764, University of Heidelberg) and results of the NRG-BR005 trial[10] (NRG Oncology) were recently presented.[24,25,26] The multi-institutional pooled analysis included patients with a partial or complete radiological response on ultrasound, mammography or MRI, of which 51% had pCR

in the surgical specimen.[24] Vacuum-assisted biopsies (86%) or core-cut biopsies (14%) were performed under ultrasound (78%) or stereotactic (22%) guidance at which a median of six (2-18) 10G (7-14) biopsies were obtained. The overall FNR was 19% in 159 patients. Post-hoc analysis of patients with a residual imaging abnormality of ≤2 cm who had at least six image-guided representative VABs showed a FNR of 3% (n=76).[24]

In the RESPONDER trial,[23] 398 patients were evaluated at interim analysis in which a median of seven 7-8G VABs per patient had been obtained. The FNR was 18%: residual disease was missed in 37 of 208 patients without pCR in the surgical specimen.[25]

The NRG-BR005 trial assessed the accuracy of six to eight 11G biopsies in patients with ductal carcinoma and a clinical (near) complete response with tri-modality imaging after NST: <1 cm residual mass on mammography (no calcifications), <2 cm residual mass on ultrasound, no rapid rise or washout kinetics on a 1.5-T post-NST MRI.[10] At planned interim analysis, 36 out of 98 evaluable patients had residual disease at surgery, of which 18 patients were not correctly identified by post-NST biopsies (FNR of 50%).[26]

Compared to the RESPONDER trial and the multi-institutional pooled analysis, we found a relatively high FNR for biopsies detecting residual disease. Key differences in the study designs were patient selection criteria and biopsy technique. The MICRA trial and the NRG-BR005 trial are the only trials that used DCE-MRI to select patients with therapy response. The NRG-BR005 trial, however, only assessed therapy response on post-NST MRI, whereas both pre- and post-NST MR-images were used in the MICRA trial for adequate response evaluation. As DCE-MRI is more accurate in selecting patients with a (near) pCR compared to conventional imaging, the proportion of patients with substantial residual disease in the studies that used conventional imaging for response monitoring might be higher, which will lower the reported FNR.

We found a significantly higher FNR in patients with no rCR on MRI than in patients with residual enhancement (47% vs. 13%). Patients with false-negative biopsies had less residual disease in the surgical specimens than patients with true-positive biopsies and tumors were more often triple-negative and HR-negative/HER2-positive, which are the subtypes that respond well to NST. Hence, these factors that are predictive for a false-negative outcome represent the same causal mechanism: sampling errors occur more frequently in patients with minimal residual disease after NST.

The results of the MICRA trial and those of the previous studies underline that current imaging modalities, including MRI, are not accurate enough to identify patients with pCR for omission of surgery.[6,7] We found residual disease in the surgical specimens of 40% patients with rCR. In the patients with rPR, 26% did achieve pCR at time of surgery.

One major difference between the previous mentioned studies and the MICRA study is the quantity of tissue obtained and examined with biopsies. In the MICRA trial core biopsies were performed, whereas vacuum-assisted biopsies were used in most other trials. With 9G to 10G vacuum-assisted biopsies, approximately seven times as much tissue per biopsy is obtained compared with 14G core needle biopsies, making assessment more reliable.[27,28] However, VAB procedures are also associated with more patient discomfort and may be associated with more severe bleeding events.[29]

Another limitation of the MICRA trial was that all biopsies were obtained immediately before breast surgery in the operating room, with the patient under general anesthesia. This procedure minimized patient discomfort, but most likely affected the accuracy of the biopsies. The ultrasound equipment used for the biopsy procedure in the operating room was sometimes inferior to that of the radiology department. Optimal positioning of the patient under general anesthesia in an operating room was more difficult compared to the normal setting at the radiology department, resulting in more difficult biopsy angles. However, biopsies were not performed if the marker could not be visualized during the procedure (21 patients) and parts of (former) tumor area were seen in at least one of the biopsies obtained in almost all patients.

In 89% of all patients, at least eight biopsies could be obtained. Only six (4%) patients underwent less than six biopsies. Representativeness of the biopsies was marked as “unknown” (i.e., sufficient material for analysis, but no signs of therapy response or tumor) in eight (5%) patients. In four of these patients, residual disease was found in the surgical specimen. Another eight patients were found to have insufficient biopsy specimens for a pathological diagnosis, of which two patients had residual disease. Excluding these patients from the analysis, however, would not have resulted in a significantly improved FNR (32% vs. 37%).

The ultimate aim of the MICRA trial was to develop an accurate minimal invasive method that would identify pCR in patients with a radiological response and thereby potentially allow omission of surgery of the breast in these patients. At the same time, it is important to accurately identify

patient who do not achieve pCR, as patients with residual disease after NST have a significant worse prognosis and may benefit from additional systemic treatment.[30-32] In addition, altough the correlation is strong, pCR of the breast (ypT0) does not entirely exclude the presence of lymph node metastases (ypN+).[33] Several studies are currently investiagting the de-escalation of axillary surgery after NST.[34,35] If breast surgery after NST in patients with pCR could be omitted in the near future, simulteneous de-escalation of axillary surgery will be essential.

The optimal cut-off value for the FNR of biopsies (and type and extent of the errors) identifying pCR for a clinically acceptable recurrence rate, is yet unknown. Investigators from the MDACC have already started a trial (NCT02945579) in which breast surgery is omitted in early-stage triple-negative or HER2-positive breast cancer patients who have at least 12 tumor-negative VABs. The primary outcome is 5-year locoregional recurrence-free survival.[20]

Although the minimal invasive method developed in the MICRA trial may not be used for omission of surgery, the interim results contribute to the development of more accurate methods for detection of pCR in patients with an excellent response on MRI after NST. The risk of sampling errors in patients who are most likely to have limited residual disease after NST may be reduced by obtaining larger, vacuum-assisted biopsies under optimal conditions at the radiology department. The development of non-invasive response prediction models incorporating biomarkers and MRI radiomics using machine-learning, on the other hand, may eventually outperform minimally invasive pCR detection methods. Regardless of the methods used to identify pCR, it will be essential to decide to what extent a possibly increased risk of local recurrence outweighs the benefits of elimination of breast surgery. We will continue to investigate minimally invasive techniques predicting pCR to ultimately achieve an operation-free treatment strategy for patients with pCR after NST.

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Section III

Reducing axillary treatment after neoadjuvant systemic therapy

Chapter 7

Toward omitting sentinel lymph node biopsy after neoadjuvant chemotherapy in patients with clinically node-negative breast cancer

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ABSTRACT

Background: The nodal positivity rate after neoadjuvant chemotherapy (ypN+) in patients with clinically node-negative (cN0) breast cancer is low, especially in those with a pathological complete response of the breast. The aim of this study was to identify characteristics known before surgery that are associated with achieving ypN0 in patients with cN0 disease. These characteristics could be used to select patients in whom sentinel lymph node biopsy may be omitted after neoadjuvant chemotherapy.

Methods: This cohort study included patients with cT1-3 cN0 breast cancer treated with neoadjuvant chemotherapy followed by breast surgery and sentinel node biopsy between 2013 and 2018. cN0 was defined by the absence of suspicious nodes on ultrasound imaging and PET/CT, or absence of tumour cells at fine-needle aspiration. Univariable and multivariable logistic regression analyses were performed to determine predictors of ypN0.

Results: Overall, 259 of 303 patients (85.5%) achieved ypN0, with high rates among those with a radiological complete response (rCR) on breast MRI (95.5%). Some 82% of patients with hormone receptor-positive disease, 98% of those with triple-negative breast cancer (TNBC) and all patients with human epidermal growth factor receptor 2 (HER2)-positive disease who had a rCR achieved ypN0. Multivariable regression analysis showed that HER2-positive (odds ratio (OR) 5.8, 95% CI 1.9 to 23.1) and TNBC subtype (OR 11.7, 2.9 to 106.9) were associated with ypN0 status. In addition, there was a trend toward ypN0 in patients with a breast rCR (OR 2.4, 0.95 to 6.77).

Conclusion: The probability of nodal positivity after neoadjuvant chemotherapy was less than 3% in patients with TNBC or HER2-positive disease who achieved a breast rCR on MRI. These patients could be included in trials investigating the omission of sentinel node biopsy after neoadjuvant chemotherapy.

INTRODUCTION

Sentinel lymph node biopsy (SLNB) has replaced axillary lymph node dissection (ALND) in patients with clinically node-negative (cN0) disease. Several trials[1-4] have demonstrated the accuracy and safety of SLNB alone when the sentinel lymph node (SLN) is tumour-free. Multiple trials[5-8] have verified that the risk of axillary recurrence is not increased when ALND is omitted in patients with low-volume metastasis in the sentinel node who are treated with breast-conserving therapy (BCS) followed by whole-breast radiotherapy. According to the American Society of Clinical Oncology,[9] ALND should not be offered to patients with early-stage breast cancer and one or two positive sentinel nodes who undergo BCS and whole-breast radiotherapy. There is more controversy regarding patients undergoing mastectomy because radiotherapy is not routinely administered in this setting.

The appropriate management of the axilla in the context of neoadjuvant chemotherapy (NACT) remains a topic of debate. Axillary lymph node status is one of the most important prognostic factors for breast cancer survival, with the best survival in patients with cN0 disease and those who achieve a pathological complete response (pCR) of the axillary lymph nodes.[10,11] NACT is effective, with nodal pCR rates of 65-74% in human epidermal growth factor receptor 2 (HER2)-positive breast cancer and 50-67% in triple-negative breast cancer (TNBC).[12–15]

Adequate staging before NACT is required to select candidates for less extensive axillary surgery afterwards. Axillary ultrasound imaging and PET/CT have better sensitivity than physical examination in determining axillary lymph node status.[16-20] PET/CT has a positive predictive value of 77-98% in detecting axillary metastases and may also detect occult regional node involvement.[19-21] Koolen and colleagues[21] showed that PET/CT detected occult N3 disease in 11% of patients with normal findings on physical examination or ultrasonography. Patients with node-positive disease initially are at higher risk of having tumour-positive axillary nodes after NACT.[15,22] In these patients, axillary staging methods, such as the marking axillary lymph nodes with radioactive iodine seeds (MARI) procedure[23–25] or targeted axillary dissection,[26,27] are increasingly being used.

In patients with cN0 tumours, SLNB can be performed accurately after NACT. Although the risk of co-morbidity associated with SLNB is lower than that of ALND, co-morbidities such as paraesthesia, numbness and pain are reported in 5-34% of patients after SLNB. Lymphoedema occurs significantly less frequently after SLNB compared with ALND, but is still noted in up to 5% of patients.[28]

After NACT, the rate of nodal positivity (ypN+) is low in patients with cN0 disease.[22,29-31] In those with TNBC or HER2+ disease and a pCR in the breast, ypN+ rates lower than 2% have been demonstrated.[15,22] In these patients, the value of surgical axillary staging after NACT may be limited. Whether a breast pCR has been achieved is not known before surgery. In the present study, the association between breast pCR and ypN0 status was validated. In addition, predictive characteristics of ypN0 after NACT that are known before surgery were investigated in patients with cN0 disease.

METHODS

Data used in the study were derived from the tumour registry of the Netherlands Cancer Institute (NKI). All patients with cT1–3 cN0 breast cancer who received NACT ± anti-HER2 treatment followed by breast and nodal surgery between January 2013 and June 2018 were identified. At NKI, patients with breast cancer receiving NACT routinely undergo both axillary ultrasound imaging and PET/CT, with fine-needle aspiration (FNA) performed in patients with suspicious axillary lymph nodes. cN0 status was defined as the absence of suspicious nodes on ultrasonography and PET/CT, or the absence of tumour cells at FNA in patients with suspicious nodes. Patients who underwent SLNB after NACT were included. Patients who did not have both axillary ultrasound examination and PET/CT were excluded, as were those with distant metastases, synchronous contralateral breast cancer or with a history of ipsilateral breast cancer. This study was approved by the institutional review board of NKI.

Diagnostic procedures before and after neoadjuvant chemotherapy

Core needle biopsies were obtained from the tumour before NACT to determine the histological subtype, and HER2 and hormone receptor (HR) status. Scoring for oestrogen receptor (ER), progesterone receptor (PR) and HER2 was done according to Dutch guidelines.[32] Staining of at least 10% of tumour cells on immunohistochemistry was considered positive for ER and PR. MRI was performed to determine the size and extent of the breast tumour, and all tumours were

marked with an iodine seed.[33] Axillary staging before NACT involved both ultrasound imaging and PET/CT (Philips Gemini TF; Philips, Cleveland, Ohio, USA), in accordance with institutional guidelines. A lymph node was defined as normal on ultrasonography if oval in shape with a plump echogenic hilum and a cortex of less than 2 mm that was thickened uniformly. For regional staging and the detection of distant metastases, total-body PET (3 min per bed position) was performed with the patient in the supine position. PET acquisition was preceded by low-dose CT (40 mA, 2-mm slices). A lymph node was regarded as normal when nodal uptake did not exceed the uptake in the blood pool activity. PET/CT images in which nodal uptake exceeded that of the blood pool activity were reviewed by a nuclear physician, and the axillary lymph nodes categorized as normal, reactive (marginal uptake, standardized uptake value (SUV) 2.6 or less), malignant (SUV over 2.6), or not evaluable (breast tumour showing no fluorodeoxyglucose (FDG) uptake). FNA was performed in patients with abnormal nodes on ultrasound imaging and/or PET/CT. If FNA was unrepresentative, it was repeated.

The radiological response of the tumour was evaluated with MRI during and/or after NACT. A radiological complete response (rCR) was defined by the absence of contrast enhancement in the original tumour bed (during or after NACT). For patients in whom MRI during NACT showed residual disease, and in whom MRI not undertaken after NACT, the presence of rCR was categorized as unknown.

Neoadjuvant chemotherapy

NACT was administered according to institutional guidelines. In short, patients with HR+/HER2– tumours were either treated with six cycles of biweekly cyclophosphamide and doxorubicin (ddAC), or with four cycles of biweekly ddAC followed by weekly administration of paclitaxel for 12 weeks. Patients with TNBC received four cycles of biweekly ddAC, followed by weekly administration of carboplatin and paclitaxel for 12 weeks, regardless of BRCA status. Before 2014, the majority of patients with HER2-positive tumours received paclitaxel, trastuzumab and carboplatin weekly for 24 weeks.[34] From 2014, patients with HER2-positive tumours received either nine cycles of paclitaxel, carboplatin, trastuzumab and pertuzumab (PTC-Ptz), or three cycles of FEC (fluorouracil, epirubicin and cyclophosphamide) with trastuzumab and pertuzumab, followed by six cycles of PCT-Ptz.[35] From 2016, patients with stage I HER2-positive breast cancer received weekly paclitaxel and trastuzumab for 12 weeks.[36]

Sentinel lymph node biopsy and pathological evaluation

On the day before surgery, ^{99m}Tc-labelled nanocolloid was injected into the tumour on palpation, or near the iodine seed under ultrasound guidance in patients without palpable disease. SLNs detected on lymphoscintigraphy were marked on the skin. Under general anaesthesia, blue dye was injected if no SLNs were detected on scintigraphy. SLNs were then identified using a γ probe or visualization of blue-coloured lymph drainage pathways. Before breast surgery, all SLNs as well as nodes considered suspicious on palpation during surgery were removed based on the judgement of the surgeon.

All SLNs were fixed in formalin overnight and parallel sections 2 mm thick were cut starting with a section through the hilum. Haematoxylin and eosin and cytokeratin staining was then undertaken at a single level. For this study, ypN0 was defined by the absence of viable tumour cells. Isolated tumour cells (ITCs), micrometastases and/macrometastases were considered as residual tumour. The pathological response of the breast was assessed according to European Society of Breast Cancer Specialists (EUSOMA) guidelines.[37,38]

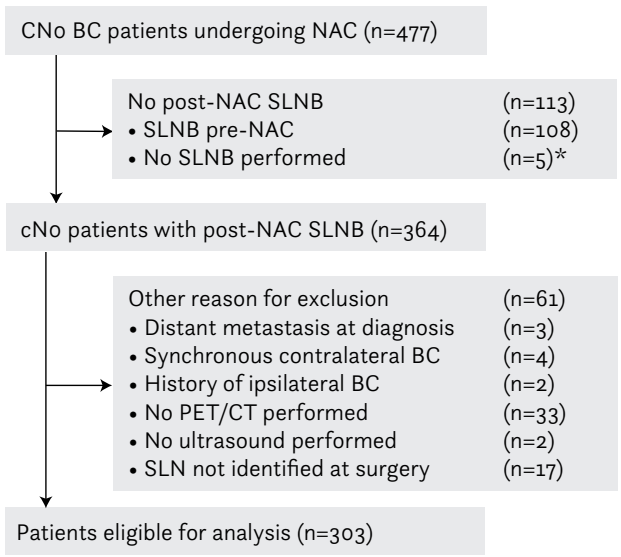
Statistical analysis

Univariable analysis was carried out by calculating the percentage of patients with ypN0 status overall and within each tumour subgroup. The 95% confidence interval of the percentage was calculated using the Clopper–Pearson method, and percentages in the subgroups were compared by means of Fisher's exact test.

To identify patients in whom SLNB potentially can be omitted after NACT, only characteristics known before surgery were used to create a multivariable logistic regression model. Firth's penalization method of logistic regression was used to address the quasi-complete separation of the SLN response (tumour-negative versus -positive).[39] A stepwise backward selection procedure was adopted as follows: variables with $p<0.100$ in the univariable analyses were entered into a multivariable logistic regression model using Firth's penalized maximum likelihood method. Variables were then removed one by one, and the resulting hierarchically nested models were compared on the basis of their penalized likelihood ratio statistics. The variable with the lowest contribution to the likelihood was removed and this process was repeated until all variables left in the model reached significance at the level of 0.100 (on multiple degrees of freedom, if applicable). To retain patients with missing data in the model, missing values were considered

as a separate category. Confidence intervals and p values were calculated using the profile likelihood. P<0.050 was considered statistically significant.

Figure 1. Study flow chart.



Four of five patients had cN+ disease on secondary review. Axillary staging was therefore performed by marking axillary lymph nodes with radioactive iodine seeds.[23] Sentinel lymph node biopsy (SLNB) was not done in the other patient for technical reasons. NACT, neoadjuvant chemotherapy; SLN, sentinel lymph node.

RESULTS

A total of 303 patients with cT1–3 cNo breast cancer treated with NACT followed by breast and nodal surgery were identified (Figure 1). Patient and tumour characteristics of the study cohort are shown in Table 1. The majority of patients had an invasive carcinoma of no special type (85.8%) and a grade II or III tumour (44.6 and 42.9% respectively). Some 18.2% had cT1, 59.4% cT2 and 22.4% cT3 disease. Tumours were HR-positive/HER2-negative in 44.9% and HER2-positive (HR+/–) in 31.0%, and 24.1% of patients had TNBC.

Table 1. Patient and tumour characteristics.

	No. of patients* (n=303)
Age (years)†	48.4 (18.0–78.0)
Histology	
Invasive cancer NST	260 (85.8)
Invasive lobular cancer	43 (14.2)
Subtype	
HR+/HER2–	136 (44.9)
(HR+/–)/HER2+	94 (31.0)
TNBC	73 (24.1)
Tumour grade	
I	14 (4.6)
II	135 (44.6)
III	130 (42.9)
Unknown	24 (7.9)
cT category	
T1	55 (18.2)
T2	180 (59.4)
T3	68 (22.4)
Tumour focality	
Unifocal	194 (64.0)
Multifocal/multicentric	109 (36.0)
Axillary nodes on ultrasonography	
Normal	200 (66.0)
Abnormal	103 (34.0)
Axillary nodes on PET/CT	
Normal	194 (64.0)
Suspect for reactive node	43 (14.2)
Suspect for malignant node	18 (5.9)
Not evaluable (breast tumour not FDG-avid)	48 (15.8)
MRI of breast tumour after NACT	
rCR	134 (44.2)
Non-rCR	149 (49.2)
Unknown	20 (6.6)

Table 1. *Continued*.

	No. of patients* (n=303)
Breast surgery	
Breast-conserving surgery	174 (57.4)
Mastectomy	129 (42.6)
No. of SLNs removed‡	1.6(0.9)
1	180 (59.4)
2	78 (25.7)
3	30 (9.9)
>3	15 (5.0)
ypT category after NACT	
ypTo	89 (29.4)
ypTis	31 (10.2)
ypT+	183 (60.4))
Pathology of SLNs	
Tumour-negative	259 (85.5)
Tumour-positive	44 (14.5)
Macrometastasis	20 (6.6)
Micrometastasis	13 (4.3)
ITCs	11 (3.6)

*With percentages in parentheses unless indicated otherwise; values are †median (range) and ‡mean(s.d.). NST=no special type; HR=hormone receptor; HER2=human epidermal growth factor receptor 2; TNBC=triple-negative breast cancer; FDG=fluorodeoxyglucose; NACT=neoadjuvant chemotherapy; rCR=radiological complete response; SLN=sentinel lymph node; ITC=isolated tumour cell.

Ultrasound imaging before NACT showed normal axillary lymph nodes in 200 patients (66.0%). Ten of these underwent secondary targeted ultrasonography and FNA because of abnormal axillary nodes on PET/CT. FNA showed non-malignant lymphoid cells in all ten patients. Ultrasound examination in 103 patients (34.0$) showed abnormal axillary nodes, but all were tumour-negative on FNA.

Some 57.4% of patients underwent lumpectomy followed by breast irradiation and 42.6% had a mastectomy. After mastectomy, patients with positive resection margins or cT3 and/or ypT3 lobular carcinoma received local radiation to the thoracic wall. A median of 1.6 (SD 1-5) sentinel nodes were removed. Patients with micrometastases or macrometastases in the sentinel nodes received locoregional radiation, whereas those with ITCs did not. One patient with two tumour-positive sentinel nodes underwent ALND and locoregional radiation.

Radiological and pathological response after neoadjuvant chemotherapy

MRI showed a breast rCR during or after NACT in 149 patients (49.2%), whereas this was not achieved in 134 patients (44.2%) (Table 1). The radiological response could not be evaluated in 20 patients (6.6%) as there was no MRI after the last course of NACT.

A pCR in the breast (ypTo/is) was observed in 130 patients overall (40%); the pCR rate was only in 6 among patients with HR-positive/HER2-negative disease but 76% in those with HER2-positive tumours and 55% in patients with TNBC patients (p<0.001). Overall, 259 patients (85.5%) had tumour-negative SLNs, 37 (12.2%) had one tumour-positive SLN and seven (2.3%) had two tumour-positive SLNs. Of 44 patients with ypN+ status, 20 had residual macrometastases, 13 had micrometastases and 11 had ITCs. Thirty-nine of the patients had HR-positive/HER2-negative disease, three had HER2-positive tumours, and two had TNBC.

Predictors of ypNo

In univariable analysis, breast pCR was a strong significant predictor of negative axillary nodes after NACT (ypNo) (Table 2). ypNo was achieved in all patients with a breast pCR compared with 79.4% of patients with residual breast disease (p<0.001).

The strongest predictors of ypNo known before surgery were tumour subtype, tumour grade and breast rCR on MRI. Higher ypNo rates were observed in TNBC and HER2-positive breast cancer than HR-positive tumours (97, 97 and 71.3% respectively) (p<0.001). In addition, the ypNo rate was higher in patients with grade III than those with grade I or II tumours (96.9, 71 and 77.8% respectively; p<0.001). Patients with a breast rCR were more likely to achieve ypNo than those with residual disease on MRI (95.5 versus 77.9% respectively; p<0.001). In an analysis of patients with a breast rCR stratified by tumour subtype, ypNo was achieved in 82% of patients with HR-positive disease (versus 71% with HR-positive disease without rCR; p=0.34), all patients with HER2-positive tumours (versus 86% with HER2-positive tumours without rCR; p=0.015) and 98% of patients with TNBC (versus all patients with TNBC without rCR; p=1.000) (Table 3).

Table 2. Univariable analysis of predictors for negative sentinel lymph nodes after neoadjuvant chemotherapy.

	No. of patients	Negative SLN	Negative SLN rate (%)	P*
All patients	303	259	85.5 (81.0, 89.2)	
Histology				0.035
Invasive cancer, NST	260	227	87.3 (82.6, 91.1)	
Invasive lobular cancer	43	32	74 (59, 87)	
Tumour subtype				<0.001
HR+/HER2-	136	97	71.3 (62.9, 78.7)	
(HR+/-)/HER2+	94	91	97 (91, 99)	
TNBC	73	71	97 (91, 98)	
Tumour grade				<0.001
I	14	10	71 (42, 92)	
II	135	105	77.8 (69.8, 84.5)	
III	130	126	96.9 (92.3, 99.2)	
Unknown	24			
T category				0.017
T1	55	52	95 (85, 99)	
T2	180	155	86.1 (80.2, 90.8)	
T3	68	52	77 (65, 86)	
Tumour focality				0.310
Unifocal	194	169	87.1 (81.6, 91.5)	
Multifocal/multicentric	109	90	82.6 (74.1, 89.2)	
Axillary nodes on ultrasonography				0.864
Normal	200	170	85.0 (79.3, 89.6)	
Abnormal	103	89	86.4 (78.2, 92.4)	
Axillary nodes on PET/CT				0.102
Normal	194	172	88.7 (83.3, 92.8)	
Suspicious for reactive node	43	36	84 (69, 93)	
Suspicious for malignant node	18	15	83 (59, 96)	
Not evaluable (breast tumour not FDG-avid)*	48	36	75 (60, 86)	0.041†
FNA of axillary nodes				0.501
Not performed	190	160	84.2 (78.2, 89.1)	
No tumour cells	113	99	88 (80, 93)	
MRI of breast tumour after NACT				<0.001
rCR	134	128	95.5 (90.5, 98.3)	
Non-rCR	149	116	77.9 (70.3, 84.2)	
Unknown	20			
ypT category after NACT				<0.001
pCR (ypT0)	89	89	100 (96, 100)	
ypTis	31	29	94 (79, 99)	
ypT+	183	141	77.0 (70.3, 82.9)	

Values in parentheses are 95% confidence intervals. SLN=sentinel lymph node; NST=no special type; HR=hormone receptor; HER2=human epidermal growth factor receptor 2; TNBC=triple-negative breast cancer; FDG=fluorodeoxyglucose; FNA=fine-needle aspiration; NACT=neoadjuvant chemotherapy; rCR=radiological complete response; pCR=pathological complete response. *Fisher's exact test; †not evaluable versus all evaluable.

Table 3. ypN0 status by tumour subtype in patients with a complete or incomplete radiological response on breast MRI

	rCR			Non-rCR			Total	P*
	n	ypN0	ypN0 rate (%)	n	ypN0	ypN0 rate (%)		
HR+/HER2–	27	22	82 (62, 94)	103	73	71 (61, 79)	130	0.34
(HR+/–)/HER2+	65	65	100 (93, 100)	22	19	86 (64, 97)	87	0.015
TNBC	42	41	98 (87, 100)	24	24	100 (86, 100)	66	1.000
Total	134	128	95.5 (90.5, 98.3)	149	116	77.9 (70.3, 84.2)	283	

Values in parentheses are 95% confidence intervals. rCR=radiological complete response; HR=hormone receptor; HER2=human epidermal growth factor receptor 2; TNBC=triple-negative breast cancer. *Fisher's exact test.

Overall, the PET/CT findings before NACT were not significantly associated with ypN0 (P=0.102). Patients for whom axillary lymph node status could not be evaluated by PET/CT were, however, less likely to achieve ypN0 than those in whom the axillary nodes were evaluable (75 versus 87.5%; P=0.044). Other significant characteristics associated with ypN0 were tumour histology (ductal carcinoma 87.3%, lobular carcinoma 74%; P=0.035) and T category (95, 86.1 and 77% For T1, T2 and T3 respectively; P=0.017). In multivariable analysis, the odds ratio (OR) for ypN0 was 5.8 (95% CI 1.9 to 23.1; P=0.001) for HER2-positive tumours, 11.7 (2.9 to 106.9; P<0.001)

for TNBC and 2.4 (95 0.95 to 6.8; P=0.06) for patients with a breast rCR on MRI (Table 4). After a median follow-up of 24 (range 1-64) months, there were no isolated regional recurrences. One patient with cT2 N0, ypT1 N0 TNBC and a TP53 mutation had a synchronous local (T4) and regional (N2) recurrence.

Table 4. Multivariable logistic regression model including characteristics for predicting tumour-negative sentinel lymph nodes known before surgery

	Odds ratio	P
Tumour subtype		
HR+/HER2–	1.00 (reference)	
HER2+	5.8 (1.9, 23.1)	0.001
TNBC	11.7 (2.9, 106.9	<0.001
MRI of breast tumour after NACT		
Non-rCR	1.00 (reference)	
rCR	2.4 (0.95, 6.8)	0.06

Values in parentheses are 95% confidence intervals. HR=hormone receptor; HER2=human epidermal growth factor receptor 2; TNBC=triple-negative breast cancer; NACT=neoadjuvant chemotherapy; rCR=radiological complete response. Histology, grade and clinical tumour category were not independently associated with tumour-negative sentinel lymph nodes in multivariable regression.

DISCUSSION

This study identified factors known before operation that predict tumour-negative sentinel nodes after NACT in patients with cN0 breast cancer. By identifying such characteristics, it would be possible to select patients in whom axillary staging by SLNB could safely be omitted after NACT. At the authors' institute, patients with breast cancer receiving NACT routinely undergo both axillary ultrasound imaging and PET/CT, and FNA is performed on suspicious nodes.

Both tumour subtype and rCR on breast MRI were found to be strong predictors of tumour-negative SLNs after NACT. Tumour-negative SLNs were found in 97% of patients with HER2-positive tumours and 97% of those with TNBC. Overall, 95.5% of patients with a breast rCR had tumour-negative sentinel nodes (ypN0) after NACT. When stratified by subtype, a breast rCR on MRI was significantly associated with ypN0 in patients with HER2-positive tumours. In patients with HR-positive/HER2-negative disease or TNBC, breast rCR was not significantly associated with ypN0. In patients with TNBC, subtype was such a strong predictor of ypN0 that breast rCR on MRI did not further contribute to prediction of nodal disease.

Tadros and colleagues[22] similarly showed that 131 of 132 patients (99.2%) with cT1-2 cN0 HER2-positive tumours and 149 of 158 patients (94.3%) with cT1-2 cN0 TNBC achieved ypN0 after NACT. All patients with cN0 disease and a pCR of the breast tumour had tumour-negative axillary lymph nodes. These results were recently validated by a large study[15] using data from the National Cancer Database (30 821 patients), which reported nodal positivity rates of less than 2% in patients with cN0 HER2-positive tumours or TNBC with a breast pCR. Murphy and co-workers[40] identified tumour subtype as the strongest predictor of ypN0 in patients with cN0 disease, with an OR of 5.2 for ER-negative/HER2-positive, 3.9 for ER-negative/HER2-negative and 2.4 for ER-positive/HER2-positive tumours, each versus ER-positive/HER2-negative tumours (p<0.001). Overall, the ypN0 rate was 78% in that study. The performance of routine axillary ultrasound imaging was not documented, which could explain the lower ypN0 rate than the 85.5% observed in the present study. The addition of axillary ultrasonography (+/– FNA) to physical examination has been shown to be more reliable and sensitive in determining axillary lymph node status.[16-18] Moreover, PET/CT was performed in all patients in the present study, which has also been demonstrated to be an accurate and sensitive regional staging method.[19-21] The ability of PET/CT to identify nodal metastases is dependent on adequate FDG uptake by the breast tumour. Correspondingly, patients in whom the breast tumour was not FDG-avid on PET/CT had a lower ypN0 rate than those with FGD-avid tumours in the present study (75 versus 87.5%; p=0.044). Other imaging methods, such as ultrasonography, should be considered in patients without an FDG-avid tumour on PET/CT.

Only patients with cT1-2 tumours were included in the studies of Tadros et al.[22] and Barron and co-workers.[15] The present study also included 68 patients with cT3 tumours. These patients had lower ypN0 rates than those with cT1 or cT2 tumours (77, 95 and 86.1% respectively; p=0.017). The ypN0 rates were, however, very high in all patients with an HER2-positive or TNBC, and in all patients achieving a breast rCR or pCR, regardless of T category. This indicates that omitting axillary staging could also be considered in selected patients with cT3 tumours.

Several trials are currently investigating the need for SLNB in patients with cN0 breast cancer. The SOUND (Sentinel node versus Observation after axillary UltraSouND trial)[41] is randomizing patients with cN0 disease (negative axillary ultrasonography or after cytology of a single suspicious node on ultrasound imaging) who are treated with upfront BCS and radiotherapy to SLNB ± ALND or no axillary surgical staging. In the BOOG 2013-08 trial,[42] patients with cT1-2 N0 tumours (negative axillary ultrasound imaging or negative cytology/histology) who undergo lumpectomy and whole-breast irradiation are randomized to SLNB or no SLNB. Patients treated with NACT are also eligible for inclusion in BOOG 2013-08, regardless of the timing of SLNB.

In the present study, only one of 44 patients with tumour-positive SLNs underwent ALND and the remaining patients received axillary radiotherapy. According to Dutch National Guidelines,[43] a tumour-positive SLN after NACT can be treated with either radiotherapy or ALND. At the authors' institute, radiotherapy is the standard of care in patients with limited axillary disease after NACT.[44]

A few comments on the present study are warranted. In this study, ITCs were considered tumour-positive, in contrast to the SENTINA[45] and American College of Surgeons Oncology Group (ACOSOG) Z071[46] trials in which they were considered tumour-negative. Results regarding the association between ITCs and locoregional control and survival are conflicting.[47-49] As the aim is to omit axillary staging after NACT, the strictest definition of ypN0 was used here, in which ITCs are considered tumour-positive. In addition, in the present study, the mean number of SLNs removed was low, which could have had a negative impact on the false-negative rate. Moreover, because of the very low rate of nodal positivity in some subgroups, the confidence intervals of the percentages of patients with tumour-negative SLNs were relatively large. Finally, the study cohort comprised a selected group, as all patients underwent both axillary ultrasound imaging and PET/CT. Although the diagnostic effectiveness of PET/CT has been demonstrated, applying these results could be challenging in a setting where PET/CT is not routinely used for axillary staging before NACT. Validation of the present results in a cohort in which ultrasonography is used for axillary staging before NACT is therefore warranted.

The need for surgery is being investigated in patients with a pCR of the breast. The MICRA (Minimally Invasive Complete Response Assessment) trial (NTR6120), RESPONDER (NCT02948764), NRG BR005 (NCT03188393) and several other trials are currently evaluating the accuracy of NACT biopsies after NACT in identifying breast pCR.[50-52] When these trials reach their primary endpoint, axillary staging by SLNB may also be omitted in patients in whom a pCR of the breast tumour is shown on biopsy after NACT.

Tumour subtype, rCR of the breast on MRI and pCR of the breast were strong predictive characteristics for the presence of tumour-negative sentinel nodes after NACT in patients with clinically node-negative breast cancer. Omitting SLNB may be considered in patients with TNBC or HER2-positive tumours, or who achieve a breast rCR on MRI. Based on the results of the present study, the prospective non-inferiority single-arm ASICS trial (Avoiding Sentinel lymph node biopsy In select Clinical node negative breast cancer patients after neoadjuvant Systemic therapy; NCT04225858) was initiated at NKI. In this study, SLNB is being omitted in selected patients with cN0 disease (cT1-3 HER2-positive tumours or TNBC) who achieve a rCR on MRI after NACT. The primary endpoint is the incidence of axillary recurrence. Secondary endpoints are breast cancer-specific quality of life, level of cancer worry, and recurrence-free, overall and disease-specific survival.

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Chapter 8

Combined PET-CT and MARI procedure for tailored axillary treatment in node-positive breast cancer after neoadjuvant therapy

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ABSTRACT

Background: The treatment of axillary lymph node metastases after neoadjuvant systemic therapy (NST) remains debatable and axillary lymph node dissection (ALND) is still the standard of care. The MARI procedure (Marking Axillary lymph nodes with Radioactive Iodine seeds) is accurate in restaging the axilla after NST (false-negative rate 7 per cent). Here, the potential of tailored axillary treatment, determined by combining the results of PET/CT before NST with those of the MARI procedure after NST, was analysed.

Methods: A cohort of axillary node-positive patients was used to construct a hypothetical treatment algorithm based on a combination of PET/CT and the MARI procedure. In the algorithm, the number of fluorodeoxyglucose (FDG)-avid axillary lymph nodes (1-3 versus ≥4) before NST and the tumour status of the MARI node (positive versus negative) after NST were used to tailor axillary treatment. All patients in the cohort underwent ALND, allowing estimation of potential overtreatment and undertreatment.

Results: A total of 93 patients were included in the study. Between one and three FDG-avid axillary lymph nodes were observed in 59 patients, and four or more in 34 patients. The MARI node was tumour-negative in 32 patients and showed residual disease in 61. Treatment according to the constructed algorithm would have resulted in 74% of patients avoiding an ALND, with potential undertreatment in three patients (3%) and overtreatment in 16 (17%).

Conclusion: Tailored axillary treatment after NST in node-positive patients, by combining PET/CT before NST and the MARI procedure after NST, has the potential for ALND to be avoided in 74% of patients.

INTRODUCTION

Neoadjuvant systemic therapy (NST) has traditionally been reserved for patients with locally advanced breast cancer, but is increasingly being used in the setting of early breast cancer. One of the important aims of NST is tumour size reduction, allowing a higher rate of breast-conserving therapy.[1] Additionally, achieving a pathological complete response (pCR) is associated with improved long-term disease-free and overall survival in patients with human epidermal growth factor receptor 2 (Her2)-positive and triple-negative disease.[2,3] Furthermore, initially tumour-positive (axillary) lymph nodes can be converted to ypN0, thus possibly obviating the need for axillary lymph node dissection (ALND) or axillary radiotherapy in this subgroup of patients.

Identification of reduction of tumour load in the breast after NST by contrast-enhanced MRI is well established.[4-6] However, there is no consensus on the optimal method and timing of nodal staging and axillary treatment in the NST setting. Traditionally, sentinel lymph node biopsy (SLNB) has been performed in patients with clinically node-negative status, and ALND recommended after NST in case of a positive sentinel node or in patients with tumour-positive nodes before NST.[7-9]

The pCR rates have increased over recent decades with improvements in chemotherapy regimens and targeted therapies according to tumour subtype, especially in patients with triple-negative and Her2-positive disease. Recent studies[10-12] have reported a pCR in the axilla in 40-75% of patients with tumour-positive axillary lymph nodes at diagnosis. It is not known how the axilla should be treated when an axillary pCR is achieved. For patients who are treated primarily with surgery, the European Organisation for Research and Treatment of Cancer AMAROS (After Mapping Of The Axilla: Radiotherapy Or Surgery) trial has shown that axillary radiotherapy gives equal locoregional control and less morbidity compared to ALND in patients with clinically node-negative disease and a positive sentinel node.[13] Therefore, it might be safe to treat patients with limited axillary disease before NST with axillary radiotherapy, and the same might be the case for patients with more extensive axillary disease who achieve an axillary pCR after NST. To select patients for more conservative treatment of the axilla, thus sparing them the substantial short- and long-term morbidity of ALND, axillary staging needs to be adequate both before and after completion of NST.[14,15]

Physical examination and imaging modalities such as ultrasonography, PET combined with CT using [^{18}F]fluorodeoxyglucose (FDG) or MRI have insufficient sensitivity and specificity to discriminate between residual disease and a pCR in the axilla after NST.[16-18] SLNB after chemotherapy in patients with proven metastatic lymph nodes before NST is under debate, because a wide variation in identification rate (68-100%) and false-negative rate (FNR) (5-30%) has been reported.[19-22] The FNR can be lowered with extra attention to patient selection and technical details. In patients with cN1 disease, the FNR can be reduced to 8-10% by using both radioactive tracer and patent blue for sentinel node mapping, by harvesting more than two sentinel nodes and by performing ultrasound imaging of the axilla.[20,21,23]

The MARI procedure (Marking Axillary nodes with Radioactive Iodine seeds) was developed at the Netherlands Cancer Institute, to stage the axilla after NST in patients presenting with clinically tumour-positive nodes before NST (cN1-3).[24] In this technique, before NST, the largest of the cytology-proven positive axillary lymph nodes is marked with a radioactive iodine seed (MARI node). After completion of NST and during surgery, the MARI node is removed selectively with radiographic guidance using a γ-probe. This technique resulted in a 97% identification rate and a 7% FNR in predicting the response in the additional axillary lymph nodes[25]. Marking one of the positive nodes with a conventional marker before NST followed by placement of an iodine seed after NST, just before surgery, has also been described.[26,27] The combination of SLNB and the MARI procedure is currently being explored as well.[12] Thus, at present it is possible to stage the axilla adequately after NST with a FNR of below 10%, a criterion for acceptable change of practice.

Recent studies[28-30] have shown the value of [^{18}F]FDG PET–CT in patients with breast cancer, particularly in staging regional and distant metastases. The positive predictive value for detecting axillary lymph node metastases with PET/CT before NST is 98%.[31] Furthermore, it enables an accurate determination of the amount of FDG-avid axillary nodes.[32] As the number of tumour-positive nodes before treatment is a significant risk factor for locoregional recurrence and an important indicator of the need for regional radiotherapy,[33-36] results of PET/CT before NST can be useful as a surrogate marker for the clinical N status and as a discriminator regarding the risk of locoregional recurrence.[32]

The hypothesis of the present study was that overtreatment of the axilla could be reduced by combining the results of [^{18}F]FDG PET/CT and the MARI procedure. A treatment algorithm for tailored axillary treatment after NST was developed and tested in a cohort of axillary node-positive patients.

METHODS

Between October 2008 and November 2012, patients were asked to participate in a prospective study in which the value of marking cytology-proven tumour-positive axillary nodes with an ^{125}I-labelled seed (I-125 seed) for axillary response monitoring was investigated[25]. The study was approved by the local ethics committee and written informed consent was obtained from all patients.

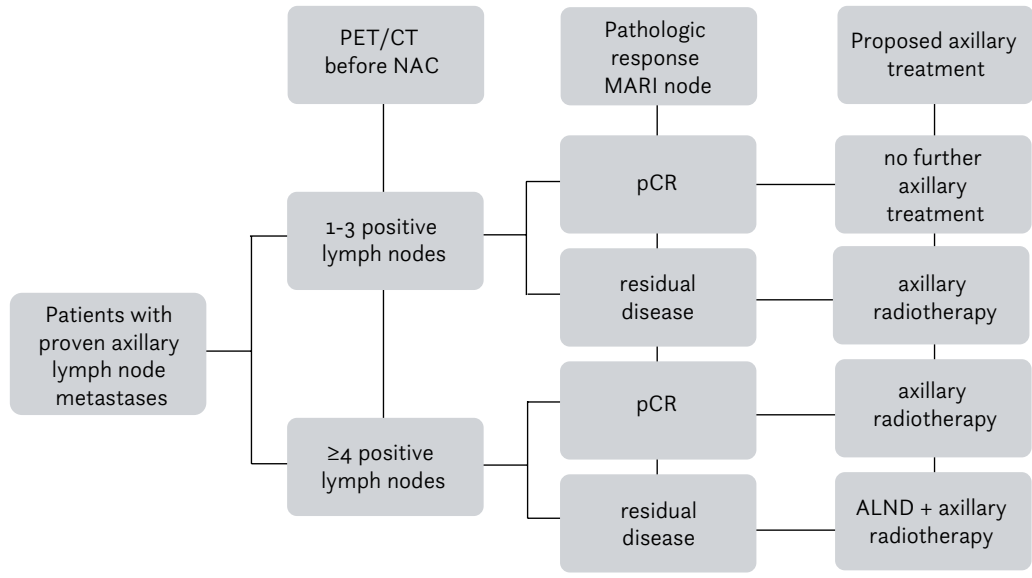
Treatment algorithm

Based on the number of FDG-avid nodes on PET–CT before chemotherapy, disease was classified as cN1 (1-3 positive axillary lymph nodes) or cN2 (4 or more positive axillary lymph nodes). In addition, according to the pathological response to NST in the MARI node (pCR or residual disease), four groups were generated and an algorithm for tailored treatment of the axilla after NST proposed (Figure 1). Axillary treatment would be omitted in patients with between one and three FDG-avid axillary lymph nodes on PET/CT and a tumour-negative MARI node. Those with one to three FDG-avid axillary lymph nodes and a tumour-positive MARI-node would receive axillary radiotherapy, as would patients with four or more FDG-avid axillary lymph nodes and a tumour-negative MARI node. An ALND would be performed only in patients with four or more FDG-avid axillary lymph nodes and a tumour-positive MARI node after NST. The finding of 95% of patients being treated correctly with this strategy would be acceptable for change of practice.

Patients and diagnostic methods before systemic therapy

Patients with invasive breast cancer larger than 3 cm in diameter and/or at least one tumour-positive axillary lymph node (stage II-III breast cancer) were offered NST. Mammography, ultrasonography and MRI were used for assessment of the primary tumour in all patients. Axillary ultrasonography was undertaken in all patients, and fine-needle aspiration cytology (FNAC) if there was cortical thickening (at least 2.3 mm) or other features of suspected lymph nodes. Whole-body [^{18}F]FDG PET/CT was carried out before chemotherapy for detection of regional and distant metastases. As described previously for locoregional staging[37], PET/CT of the thorax (3.00 min per bed position) was performed with the patient in the prone position and with hanging breasts. Low-dose CT (40 mAs, 2-mm slices) preceded PET acquisition. The number of FDG-avid lymph nodes was assessed visually (Figure 2).

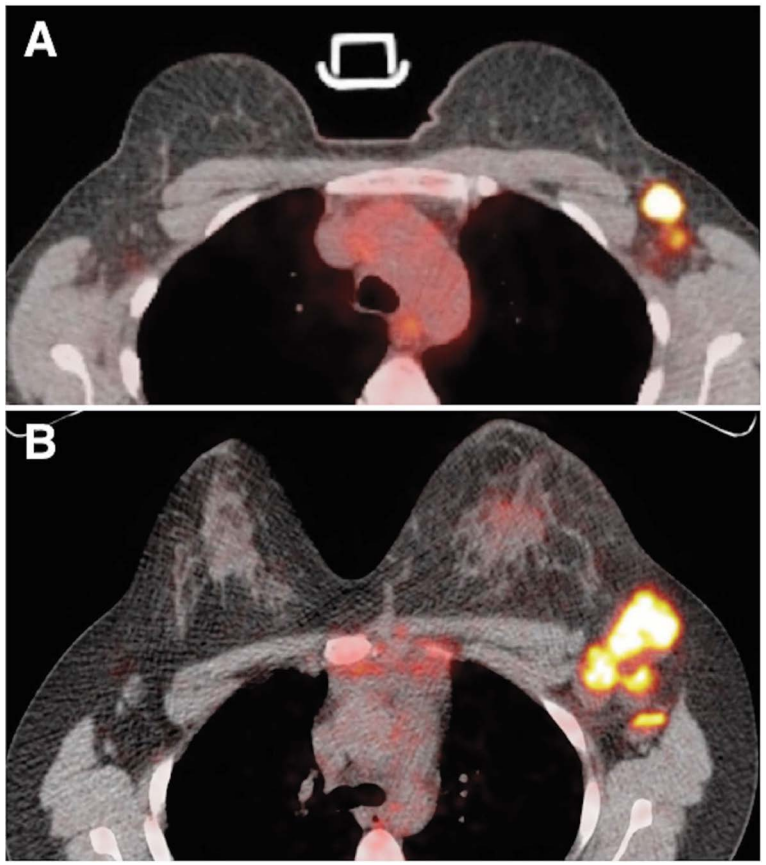
Figure 1. Proposed axillary treatment after combining [^{18}F]fluorodeoxyglucose PET/CT before neoadjuvant systemic therapy (NST) and the MARI (Marking the Axilla with Radioactive Iodine seeds) procedure after NST ALND, axillary lymph node dissection. .



PET/CT=positron emission tomography combined with computed tomography; PST=primary systemic treatment; MARI=Marking the Axilla with Radioactive Iodine Seeds; pCR=pathologic complete response; ALND=axillary lymph node dissection

For this study, when FNAC showed tumour-positive axillary nodes, an I-125 seed was placed in the largest proven tumour-positive lymph node under ultrasound guidance. This node is further referred to as the MARI node.

Figure 2. Transverse PET–CT of two different patients with (A) two and (B) ≥four [^{18}F]fluorodeoxyglucose (FDG)-avid pathological lymph nodes in the left axilla



Neoadjuvant systemic therapy regimens

Core biopsies from the primary tumour were used to determine the histology and for immunohistochemical staining. Tumours were considered oestrogen receptor (ER)- and progesterone receptor-positive when at least 10% of tumour cells stained positive for these receptors. Samples were scored as Her2-positive when either strong membrane staining (3+) was observed, or chromogenic in situ hybridization revealed amplification in samples with moderate (2+) membrane staining. NST was administered according to institutional guidelines. Briefly, Her2-positive tumours were treated with paclitaxel (70 mg/m^2), trastuzumab (70 mg/m^2) and carboplatin (3 AUC mg per ml per min) administered weekly in three cycles of eight administrations. In weeks 7 and 8 of each cycle, only trastuzumab was given. Her2-negative tumours were treated with three cycles of AC (cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2) in a dose-dense schedule (every 2 weeks), after which patients were switched to capecitabine (1000 mg/m^2) and docetaxel (75 mg/m^2) every 3 weeks, or continued with dose-dense AC if the response was good.

Axillary treatment and histopathological evaluation

After completion of NST, during surgery the MARI node was removed selectively guided by a gamma probe on the ^{125}I setting, followed by an ALND in all patients. Breast surgery was performed in the same session.

In the pathology department, the I-125 was extracted from the MARI node, after which the node was bisected and embedded completely. Paraffin blocks were cut at three levels with at least 150-µm intervals. The MARI node was assessed according to routine pathological assessment for SLNB procedures (haematoxylin and eosin routinely; immunohistochemical keratin staining if tumour-negative on haematoxylin and eosin staining). Lymph nodes in the ALND specimen were evaluated at one level and stained with haematoxylin and eosin. A specialized breast pathologist reviewed all MARI nodes and classified the response to systemic treatment. For this study, pCR was defined by an absence of vital tumour cells in the MARI node and additional nodes in the ALND, irrespective of the response in the breast.

RESULTS

A total of 125 patients with clinically node-positive disease were included in the study. One of the histologically proven metastatic axillary lymph nodes was marked with an I-125 seed in each patient. [^{18}F]FDG PET–CT was carried out before the start of NST in 110 of the 125 patients. Seventeen patients were excluded because the MARI node was not identified (3), ALND was not performed at the request of the patient (13) or axillary lymph nodes on PET/CT were not FDG-avid (1). This resulted in 93 patients eligible for analysis.

Table 1 summarizes patient and tumour characteristics of the 93 patients before NST. Median age at the time of enrolment was 49 (range 24-67) years and most patients had a T1 or T2 tumour. The majority of the patients (87, 94%) had an invasive ductal carcinoma. Twenty-eight patients had a Her2-positive tumour, 45 tumours were ER-positive and 20 tumours triple-negative. Between one and three FDG-avid axillary lymph nodes were observed in 59 of the 93 patients (63%), and four or more in the remaining 34 patients (37%).

Table 1. Patient- and tumour-related characteristics.

	No. of patients* (n=93)
Age (years)†	49 (24–67)
Radiological tumour category before systemic therapy	
To	1 (1)
T1	21 (23)
T2	46 (49)
T3	17 (18)
T4	8 (9)
Axillary lymph node stage before systemic therapy‡	
cN1 (1–3 positive nodes)	59 (63)
cN2-3 (≥ 4 positive nodes)	34 (37)
Tumour histopathology	
Ductal carcinoma	87 (94)
Lobular carcinoma	6 (6)
Receptor-based subtype§	
ER–/PgR–/Her2–	20 (22)
ER+/Her2–	45 (48)
Her2+	28 (30)
Neoadjuvant systemic therapy regimen	
Doxorubicin–cyclophosphamide	63 (68)
Capecitabine–docetaxel	4 (4)
Paclitaxel–trastuzumab–carboplatin	25 (27)
Other	1 (1)

*With percentages in parentheses are unless indicated otherwise; †values are median (range). ‡Determined by PET–CT; §Established on histological biopsy before neoadjuvant systemic therapy. ER=oestrogen receptor, PgR=progesterone receptor; Her2=human epidermal growth factor receptor 2.

Surgical and pathological outcome of the MARI node and axillary lymph node dissection

At the time of removal of the MARI node, the I-125 seed had been in situ for a median of 18 (range 9-31) weeks and showed an apparent radioactivity varying from 0.006 to 0.06 mCi (0.2-2.1 MBq). In 32 of the 93 patients (34%) a pCR was observed in the MARI node. After removal of the MARI node, ALND was performed in all patients.

Nodal outcome in patients with between one and three positive axillary lymph nodes on PET/CT

Of 59 patients with one to three positive lymph nodes seen on PET/CT before the start of NST, 22 (37%) had a pCR in the MARI node (Table 2). In three of these, the ALND specimen revealed additional metastasis (false-negative MARI node). Two had a solitary macrometastasis in one of the additional axillary nodes, and in one patient isolated tumour cells (ITC) were found in one additional node.

In the remaining 37 patients (63%), the MARI node contained residual tumour. No additional metastasis was found in the ALND specimen in 13 patients. In 24 patients, a median of 2 (range 1-12) additional metastatic lymph nodes was seen. Three of these patients had four or more tumour-positive lymph nodes in the ALND specimen (4, 11 and 12 additional positive nodes).

Nodal outcome in patients with four or more positive axillary lymph nodes on PET/CT

Of 34 patients with four or more positive axillary lymph nodes on PET/CT before the start of NST, ten (29%) showed a pCR in the MARI node. In two of these ten patients, additional nodal metastases were found in the ALND specimen (false-negative MARI node). There were two additional macrometastatic nodes in one patient, and five nodes with ITC in the other.

Among twenty-four patients with a positive MARI node, three had no additional metastases in the ALND specimen. In the remaining 21 patients, a median of 6 (range 1-23) additional metastatic nodes was found.

Consequences of tailoring axillary treatment based on the proposed algorithm

Tailoring the axillary treatment based on the proposed algorithm would lead to omission of ALND in 74% of the patients. Consequences of adjusting the protocol to the proposed algorithm are shown in Table 2. In the present cohort, 74 patients (80%) would have received the correct treatment. Thirteen patients (with 1-3 involved nodes before NST) would have received axillary radiation treatment, although the only remaining positive node was the removed MARI node. Three patients with more than three FDG-avid nodes on PET/CT before NST would have undergone an ALND without additional positive nodes being found after ALND; thus, in total 16 patients would potentially have been overtreated. Three patients (3%) with three or fewer suspected axillary lymph nodes on PET/CT and a false-negative MARI node would have been undertreated.

Table 2. Pathological status of the MARI node and additional axillary lymph node dissection, and consequences of tailoring axillary treatment according to proposed algorithm.

FDG-avid axillary lymph nodes before NST	MARI node after NST		Proposed axillary treatment according to algorithm	Additional ALND after NST	
1-3 (n=59)	Negative	22	No further axillary treatment	Negative	19
				Positive	3*§
	Positive	37	Axillary radiotherapy	Negative	13¶
				Positive	24
≥4 (n=34)	Negative	10	Axillary radiotherapy	Negative	8
				Positive	2†
	Positive	24	Axillary lymph node dissection + axillary radiotherapy‡	Negative	3¶
				Positive	21

*Solitary macrometastasis in one of the additional axillary nodes (2 patients); isolated tumour cells (ITC) in one additional node (1). †Two additional macrometastatic nodes (1 patient); five nodes with ITC (1). ‡Axillary radiotherapy given when the axillary lymph node dissection (ALND) still shows residual disease. §Undertreatment; ¶Potential overtreatment. FDG=fluorodeoxyglucose; NST=neoaduvant systemic therapy; MARI=Marking the Axilla with Radioactive Iodine seeds.

DISCUSSION

This study has shown that combining [^{18}F]FDG PET–CT before NST with the MARI procedure after NST could lead to 74% of patients avoiding ALND, with limited risk of undertreatment.

As NST is increasingly being used in patients with breast cancer, and chemotherapy regimens and targeted therapies have improved, rates of pCR of the primary breast cancer and metastatic axillary lymph nodes are rising. In the ongoing development of patient-tailored treatment, critical appraisal of the current standard practice of completion ALND in these patients is warranted.

In patients with a radiologically complete response, surgical resection of either the original primary tumour area or pretreatment tumour-positive lymph nodes is performed to confirm the absence or presence of residual tumour. In absence of residual cancer (pCR), this surgical procedure would most likely not have contributed to locoregional control. Surgical resection in these patients could be considered overtreatment. It is therefore imperative that patients with a pCR of the primary tumour and/or axilla are reliably identified, using adequate staging methods before and after NST.

PET/CT using [^{18}F]FDG provides optimal nodal staging before the start of NST. Nodal staging is considered important because more extensive nodal involvement is associated with poorer prognosis and indicates a need for more extensive regional nodal irradiation.[38,39] In general, worldwide, patients with more than three positive nodes (pN2 and pN3) are considered candidates for postoperative locoregional radiotherapy, with the aim of increasing locoregional control and survival.[40]

The MARI procedure provides a minimally invasive measurement of the pathological nodal response to NST, with a low FNR of 7%.[25] In countries with regulatory issues regarding radiation safety, a clip marker can be placed in an axillary lymph node before NST. After completion of NST and just before surgery, the clipped node is localized with an I-125 seed.[27] SLNB in combination with removal of an I-125 seed-marked tumour-positive lymph node has also been explored, demonstrating FNRs lower than 5%.[41,42] With the MARI procedure, only one lymph node is removed, whereas several lymph nodes are excised when SLNB and the MARI procedure are combined.

Controversy still exists regarded how to incorporate the ypTNM stage in daily practice. It is agreed that patients with a pCR in the breast need less extensive surgery, and converting from ablative to breast-conserving surgery has not led to an increased locoregional recurrence rate[1]. Both the Netherlands Cancer Institute and MD Anderson Cancer Center are now even exploring whether and how breast surgery may be omitted in patients with a pCR[18] (trialregister.nl; NTR6120), because surgical excision of the original tumour bed in patients with a pCR is not likely to contribute to locoregional control. Consequently, it is imperative to investigate whether axillary treatment in patients with proven lymph node metastasis and a pCR after NST can be reduced. During the St Gallen conference in 2015, 90% of the attendees voted that axillary clearance can be avoided in a patient who is clinically node-positive at presentation but with disease downstaged to ypN0 after NST.[43] However, 90% of the attendees also voted that axillary clearance could not be avoided if there is still residual tumour in one or more lymph nodes. Schwartz and colleagues[40] showed no axillary recurrence after a median follow-up of 62 months in patients with cN1-2 disease who had an axillary pCR and no further axillary treatment. However, Kim and co-workers reported a significant difference in the disease-free survival rate between patients with an axillary pCR treated with SLNB alone or ALND.[44] However, they used SLNB only to select patients with a pCR, and the reliability of this strategy is being debated.[20,21]

It remains questionable whether the axillary nodes can be left untreated in patients with an axillary pCR who had a more extensive tumour load in the axilla before the start of NST (4 or more tumour-positive lymph nodes on PET/CT). In the present algorithm, these patients are proposed to be treated with adjuvant axillary radiation therapy if the MARI node is tumour-negative. Future research should determine whether further axillary treatment could be omitted in this group. Similarly, a point of debate is how to treat ITCs in axillary nodes after NST. In the adjuvant setting, ITCs are considered node-negative and need no further treatment. This is daily practice, irrespective of whether patients are receiving adjuvant chemotherapy and/or endocrine therapy. It is currently unknown whether ITCs would cause locoregional recurrence in the neoadjuvant setting and further research is needed.

According to the present algorithm, patients with one to three positive lymph nodes on PET/CT before NST and a tumour-positive MARI node after NST would be treated with axillary radiotherapy. In the AMAROS study, 25% of patients in the axillary clearance group had between one and three positive lymph nodes, and this is most probably equal to the percentage of patients with one to three positive lymph nodes in the axillary radiotherapy group.[13] There were no significant differences in locoregional recurrence or survival between the treatment arms, leading to the conclusion that this patient group may safely be treated with axillary radiotherapy.[13] A difference between the present study population (patients with 1-3 suspected axillary lymph nodes on PET–CT and a tumour-positive MARI node) and those in the AMAROS trial is that the former patients had already received chemotherapy.

There are currently two trials investigating axillary treatment after NST in patients with clinically node-positive disease. In the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-51/ RTOG Radiation Therapy Oncology Group (RTOG) 1304 trial, patients with clinically node-positive disease who achieve an axillary pCR (assessed by ALNB, or SLNB with or without ALND) are randomized between nodal radiotherapy or no nodal radiotherapy (clinicaltrials.gov; NCT01872975). In the Alliance A11202 study, patients with clinical N1 disease and a tumour-positive SLNB after NST are randomized to ALND plus breast/chest wall and nodal radiotherapy (without radiotherapy to the dissected axilla) or solely breast/chest wall and nodal radiotherapy (clinicaltrials.gov; NCT01901094). These trials will also provide more information on the long-term follow-up of patients with clinically node-positive disease in whom radiotherapy and/or ALND is omitted.

A few comments on the present study are warranted. The cost of [^{18}F]FDG PET/CT is much lower in the Netherlands than in other countries, such as the USA. Therefore, implementation of the algorithm proposed here would be more challenging in countries where costs of PET/CT are high or not reimbursed for this indication. Additionally, in the present study, patients with one to three suspected axillary lymph nodes on PET/CT and a false-negative MARI node would be undertreated (2 patients with a solitary macrometastasis and 1 with ITC), although it is debatable whether untreated ITC would increase the risk of locoregional recurrence. In addition, there were two patients with at least four FDG-avid lymph nodes on PET/CT and a tumour-negative MARI node, but with residual disease in the ALND specimen. Most likely these patients could be treated safely with radiotherapy, but if the MARI node is false-negative and there is more residual disease in the additional lymph nodes, these patients might also be undertreated. After implementation of the proposed strategy, these patients need to be followed prospectively to demonstrate safety in terms of locoregional control. Furthermore, in the group of patients with three or fewer positive lymph nodes on PET/CT before NST and a positive MARI node, there

was a median of 2 (range 1-12) additional positive lymph nodes found on ALND. In three patients, PET–CT apparently underestimated the axillary staging before NST. These patients might be undertreated by omitting ALND.

This study has shown that combining [^{18}F]FDG PET/CT before NST with the MARI procedure after NST can lead to omission of ALND in 74% of patients with minimal undertreatment. This strategy is now being implemented at the authors' institute for patients with clinically node-positive breast cancer undergoing neoadjuvant systemic therapy and included in a prospective study.

Disclosure

The authors declare no conflict of interest.

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Chapter 9

Major reduction in axillary lymph node dissections after neoadjuvant systemic therapy in node-positive breast cancer by combining PET/CT and the MARI procedure (Marking Axillary lymph nodes with Radioactive Iodine Seeds)

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SYNOPSIS

Combining axillary staging pre-NST with PET/CT and staging post-NST with use of the MARI-procedure (Marking Axillary lymph nodes with Radioactive Iodine seeds) resulted in a reduction of 82% ALNDs in cN+ breast cancer patients at the Netherlands Cancer Institute.

ABSTRACT

Purpose: Axillary lymph node dissection (ALND) is frequently performed in node-positive (cN+) breast cancer patients. Combining PET/CT pre-NST and the MARI-procedure (Marking Axillary lymph nodes with Radioactive Iodine seeds) after neoadjuvant systemic therapy (NST) has the potential to avoid unnecessary ALNDs. In the present study, we present the results of the implementation of this strategy.

Patients and methods: All breast cancer patients treated with NST at the Netherlands Cancer Institute who underwent a PET/CT and MARI-procedure from July 2014 until July 2017 were included. All patients underwent tailored axillary treatment according to a protocol based on the combined results of the PET/CT pre-NST and the MARI-procedure post-NST. In this protocol, patients with 1-3 FDG-avid axillary lymph nodes (ALNs) on PET/CT (cN<4) and a tumor-negative MARI-node receive no further axillary treatment. cN(<4) patients with a tumor-positive MARI-node receive local-regional radiotherapy, as well as patients with ≥4 FDG-avid ALNs (cN(4+) and a tumor-negative MARI-node after NST. An ALND is only performed in cN(4+) patients with a tumor-positive MARI-node.

Results: Data of 159 patients who received a PET/CT pre-NST and a MARI-procedure post-NST was analyzed, of whom 110 patients had 1-3 and 49 patients had ≥4 FDG-avid ALNs on PET/CT prior to NST. ALND was omitted in 130 patients (82%). Local-regional radiotherapy was administered in 91 patients (57%) and 39 patients (25%) received no further axillary treatment.

Conclusion: Combining pre-NST axillary staging with PET/CT and post-NST staging with the MARI-procedure resulted in a reduction of 82% of ALNDs in cN+ breast cancer patients.

INTRODUCTION

Neoadjuvant systemic therapy (NST) is increasingly administered in breast cancer patients and is often used in patients with clinical node-positive (cN+) disease. After NST, conversion of cN+ disease into pathological node-negative disease occurs in 5-75% of patients, depending on tumor subtype.[1-3] The majority of patients who achieve axillary pathologic complete response (pCR) have improved local-regional and survival outcomes.[4-7] Axillary lymph node dissection (ALND) is still frequently performed in cN+ patients, regardless of response to NST. ALND is associated with significant morbidity[8,9] and in patients with an axillary pCR the therapeutic effect of ALND should be questioned.

To select patients in whom less extensive axillary treatment is safe, adequate staging of the axilla before and after NST is required. [^{18}F]FDG positron emission tomography computed tomography (PET/CT) is an optimal method for nodal staging prior to NST with a positive predictive value (PPV) of 77-98% for detecting axillary lymph node (ALN) metastases.[10,11] In addition, the number of FDG-avid ALNs can reliably be determined.[12,13]

For axillary restaging after NST, non-invasive methods (physical examination, ultrasound, MRI, PET/CT) cannot discriminate accurately enough between residual disease and axillary pCR.[14-16] False-negative rates (FNR) of sentinel lymph node biopsy (SLNB) after NST range from 5-30% and therefore SLNB is only useful in select patients: the FNR can be reduced to <10% in cN1-2 patients, when ultrasound after NST shows no suspect ALNs, when both technetium-99m-nanocolloid and blue dye are used, and when ≥3 SLNs can be retrieved and examined.[17-20]

At the Netherlands Cancer Institute (NKI), an alternative technique was introduced for axillary staging after NST: the MARI-procedure (Marking Axillary lymph nodes with Radioactive Iodine seeds).[21] In this technique, a tumor-positive ALN is marked with an iodine seed before NST and selectively removed after NST with a FNR of 7% in predicting pCR in the additional ALNs.[22]

Recently, we published a feasibility study to demonstrate that combining PET/CT before NST with the MARI-procedure can reliably select patients in whom an ALND can be replaced by axillary radiotherapy (ART) or even by omitting all axillary treatment.[23] In that study, we revised PET/CT and MARI-procedure data of patients who underwent ALND, to recalculate the proportion of ALNDs considered necessary when information of pre-NST PET/CT and the MARI-procedure is combined. We showed that this tailored axillary treatment could potentially prevent ALND in 74% of cN+ patients, with minimal risk of undertreatment (3%).

In the present study, we present the results of the implementation of this axillary treatment protocol in cN+ patients, in which treatment is based on results of PET/CT pre-NST in combination with results of the MARI-procedure post-NST (Figure 1).

METHODS

Patient selection

At NKI, treatment of all breast cancer patients is discussed in multidisciplinary meetings with dedicated breast cancer specialists. Since July 2014, in cN+ patients who undergo NST, a PET/CT (in supine and prone position) is performed prior to NST. Patients with evaluable FDG-avid ALNs are treated according to the protocol presented in Figure 1. NST is administered according to institutional guidelines. All data of patients treated with NST at NKI is entered in a database maintained by the Department of Biometrics. This database was queried to select patients who were treated according to the protocol from July 2014 until July 2017.

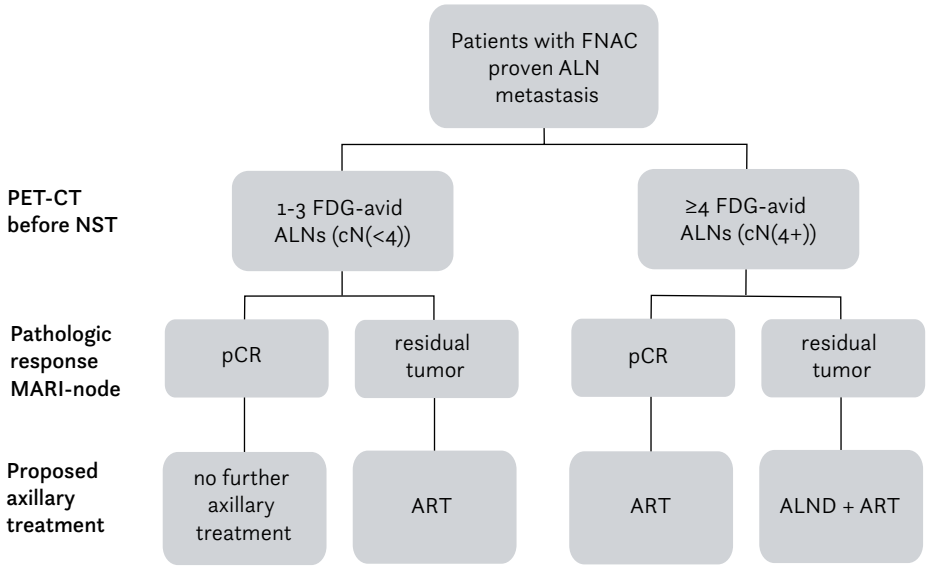
Diagnostics prior to NST

Core needle biopsies are taken from the breast lesion to determine histological subtype and HER2 and hormone receptor status. Scoring for estrogen receptor, progesterone receptor and HER2 is performed according to the Dutch guidelines.[24] The size and extent of the primary tumor were routinely assessed by mammography, ultrasound and MRI. All patients undergo axillary and peri-clavicular ultrasound and fine needle aspiration (FNA) is performed in case of a suspect lymph node.

In all patients undergoing NST at NKI, a whole body [^{18}F]FDG PET/CT (Philips Gemini, Cleveland, USA) is routinely performed for the detection of regional lymph node metastases and distant metastases. A total body PET scan (3.00 min per bed position) is performed with the patient in supine position; in the same procedure a PET scan of the breast is performed in prone position with hanging breast for local-regional staging. PET acquisition is preceded by a low-dose CT

scan (40 mAs, 2-mm slices). The uptake of FDG-avid ALNs is visually evaluated by experienced nuclear medicine physicians. A lymph node is regarded as highly suspicious for metastasis when the uptake is higher than the blood pool activity. To stage the axilla, we use the quantity of FDG-avid ALNs, as an alternative to the clinical TNM classification in which the N-classification also refers to internal mammary and peri-clavicular nodes.

Figure 1. Axillary treatment protocol at the Netherlands Cancer Institute for patients presenting with axillary disease prior to NST.



NST=neoadjuvant systemic therapy; FNAC= fine-needle aspiration cytology; ALN=axillary lymph node; PET/CT=positron emission tomography combined with computed tomography; MARI=Marking Axillary lymph nodes with Radioactive Iodine Seeds; pCR=pathologic complete response; ALND=axillary lymph node dissection; ART=axillary radiotherapy

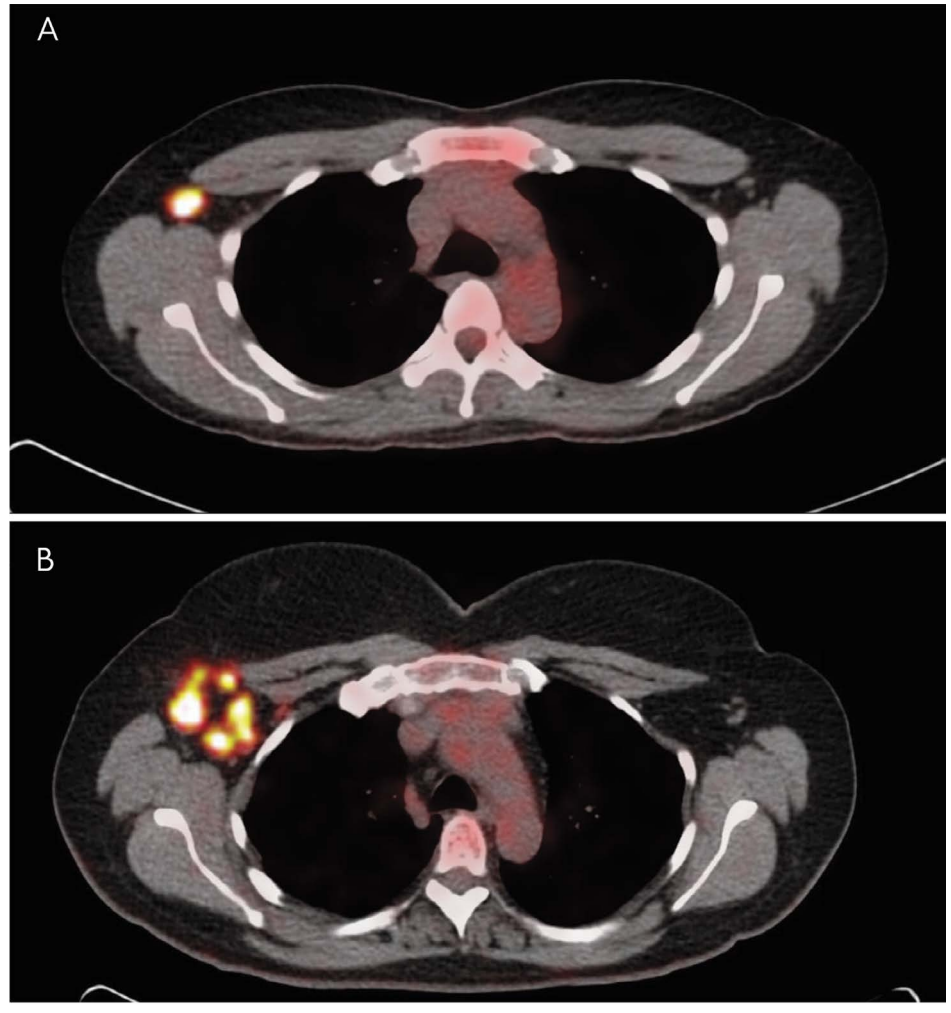
Iodine seed localization

The MARI-procedure and the relevant radiation safety protocols have been described in detail previously.[21,25] In short, in all cN+ patients an iodine seed is placed in the largest tumor-positive ALN under ultrasound guidance (MARI-node). In pregnant women or in women with children <1 year, a clip is placed in a positive ALN before NST. Just before surgery, the clipped node is localized with an iodine seed.

Surgical axillary management

At NKI, we have implemented an axillary treatment protocol that is based on two factors: first, the number of FDG-avid ALNs on PET/CT prior to NST (Figure 2) and second, the presence or absence of tumor cells in the MARI-node after NST. Based on these factors, patients are categorized into four groups (Figure 1).

Figure 2. [^{18}F]FDG PET/CT (prone position with hanging breast) of a patient with 1 FDG-avid ALN cN(<4) (A) and of a patient with ≥4 FDG-avid ALNs cN(4+) (B).



PET/CT=positron emission tomography combined with computed tomography; ALN=axillary lymph node

After NST, selective removal of the MARI-node, breast surgery and, if required, ALND is performed. A gamma-probe is used to guide the excision of the MARI-node. In case of ≥4 suspect ALNs on PET/CT pre-NST (cN(4+)), an intra-operative frozen section of the MARI-node is performed. When the frozen section is tumor-positive, ALND is performed.

Histopathological evaluation of the MARI-node

First, the iodine seed is removed from the MARI-node at gross examination of the specimen at the pathology department. If during surgery pathological evaluation of the MARI-node is indicated, 2 mm tissue slices were made from which 5 µm H&E frozen sections were prepared. After microscopic examination of these frozen sections, the tissue is fixed in formalin overnight after which a new H&E and a cytokeratin stain at single level were performed. If no perioperative MARI-node evaluation is indicated, the tissue was formalin-fixed overnight and processed according to routine diagnostic histology procedures, i.e. H&E and cytokeratin staining. For this study, pCR is defined as the absence of vital tumor cells in the ALNs, irrespective of the response in the breast. Isolated tumor cells and micro/macro-metastases are considered residual tumor.

Radiation therapy

Axillary levels I-IV are irradiated in patients with 1-3 FDG-avid ALNs (cN(<4)) and a tumor-positive MARI-node (ypN+(MARI)), as well as in cN(4+) patients and a tumor-negative MARI-node (ypN0(MARI)). After ALND (in cN(4+);ypN+(MARI) patients), partial or complete irradiation to axillary levels is administered. Delineation of level I-IV was performed according to the Danish national delineation guidelines and from January 2015 according to the ESTRO consensus guidelines.[26,27] A dose of 42.56 Gy in 16 fractions of 2.2 Gy was prescribed, or 46.2 Gy in 21 fractions of 2.2 Gy if a simultaneous boost dose was given to the tumor bed in the breast.

Statistical analysis

Outcome and tumor characteristics were analyzed using the Chi-square test for categorical variables. Two-sided p-values were reported with values <0.05 considered as statistically significant.

RESULTS

Clinical-pathological features and systemic therapy

Between July 2014 and July 2017, we treated 159 patients according to the protocol in which results of pre-NST PET/CT are combined with results of the MARI-node. Table 1 lists the clinical-pathological features. Forty-six percent of patients had HR-positive tumors, 29% HER2+ and 25% triple negative (TN) tumors.

In 110 patients (69%), 1-3 FDG-avid ALNs were detected on PET/CT and in 49 patients ≥4 ALNs (31%). All patients received NST followed by local-regional treatment (surgery and radiotherapy). Adjuvant systemic therapy (hormonal therapy and/or trastuzumab and/or chemotherapy) was administered in 80% of patients.

MARI-procedure, ALND and breast surgery

At the time of surgery, the axillary iodine seed was in situ for a median of 151 days (range 0-258 days). Frozen section of the MARI-node was performed in 49 patients (all cN(4+) patients). Additional ALND was performed in 28/49 patients because of a positive frozen section. In 1 patient, ALND was performed in a separate procedure because results of frozen section of the MARI-node and final pathology were discordant.

Table 1. Clinical-pathological features and systemic therapy.

Variable	No. (%)
No. of patients	159
Median age, years	49.3, range 23-80
Number of FDG-avid axillary lymph nodes on PET/CT	
1-3 (cN<4)	110 (69.2)
1	62
2	30
3	18
≥4 (cN4+)	49 (30.8)

Table 1. *Continued.*

Variable	No. (%)
Tumor histology	
Ductal	143 (89.9)
Lobular	16 (10.1)
Tumor nuclear grade	
1	3 (1.9)
2	78 (49.1)
3	69 (43.4)
Unknown	9 (5.7)
Tumor receptor subtype	
HR+ (ER and/or PR+)/HER2-	73 (45.9)
HER2+	46 (28.9)
TN	40 (25.2)
Neoadjuvant systemic treatment	
ddAC	29 (18.2)
ddAC + paclitaxel	50 (31.4)
ddAC + CP	28 (17.6)
ddAC + miniCTC	7 (4.4)
PTC-P	32 (20.1)
FEC-T + PTC-P	13 (8.2)
Breast surgery	
Breast conserving surgery	99 (62.3)
Mastectomy	59 (37.1)
Only axillary surgery	1 (0.6)
Adjuvant systemic therapy	
hormonal therapy	60 (37.7)
trastuzumab	19 (11.9)
chemotherapy	8 (5.0)
hormonal therapy + trastuzumab	20 (12.6)
hormonal therapy + chemotherapy	19 (11.9)
trastuzumab + chemotherapy	1 (0.6)
no adjuvant systemic therapy	32 (20.1)

PET/CT=positron emission tomography combined with computed tomography; HR=hormone receptor; ER=estrogen receptor; PR=progesterone receptor; HER2=human epidermal growth factor receptor 2; TN=triple negative; ddAC=doxorubicine and cyclophosphamide (dose dense); CP=carboplatin and paclitaxel; miniCTC=carboplatin; thiotepa and cyclophosphamide with Peripheral Blood Progenitor Cell harvest and reinfusion, PTC-P=paclitaxel; trastuzumab, carboplatin and pertuzumab; FEC-T=5-fluoruoracil, epirubicin, cyclophosphamide and trastuzumab

Sixty-two percent of patients underwent BCS and 37% underwent mastectomy. In one patient only a MARI-procedure was performed because of an occult breast tumor.

pCR of the MARI-node

Overall, 37% of patients achieved axillary pCR (n=59) (Table 2). In patients with HR-positive tumors an axillary pCR of 6% was achieved, in patients with HER2-positive tumors 67% and in patients with TN tumors 60% (p=<0.001). Of patients with 1-3 FDG-avid nodes on PET/CT, 35% had axillary pCR and of patients with ≥4 nodes, 41% had axillary pCR (p=0.518).

Table 2. Pathological outcome MARI-node.

Variable	No. (%)	P value
Time of axillary I-125 seed in situ (median)	151 days, range 0-258	n.a.
Overall outcome MARI-node		
pCR	59 (37.1)	n.a.
residual disease	100 (62.9)	
Macro-metastasis	83	
Micro-metastasis	10	
Isolated tumor cells	7	
pCR MARI-node per subtype		
HR+ (ER and/or PR+)/HER2-	4 (5.5)	<0.001
HER2+	31 (67.4)	
TN	24 (60.0)	
pCR MARI-node per subgroup		
cN(<4)	39 (35.1)	0.518
cN(4+)	20 (40.8)	

MARI=Marking Axillary lymph nodes with Radioactive Iodine seeds; I-125=Iodine-125; pCR=pathologic complete response; ALND=axillary lymph node dissection; ART =axillary radiotherapy

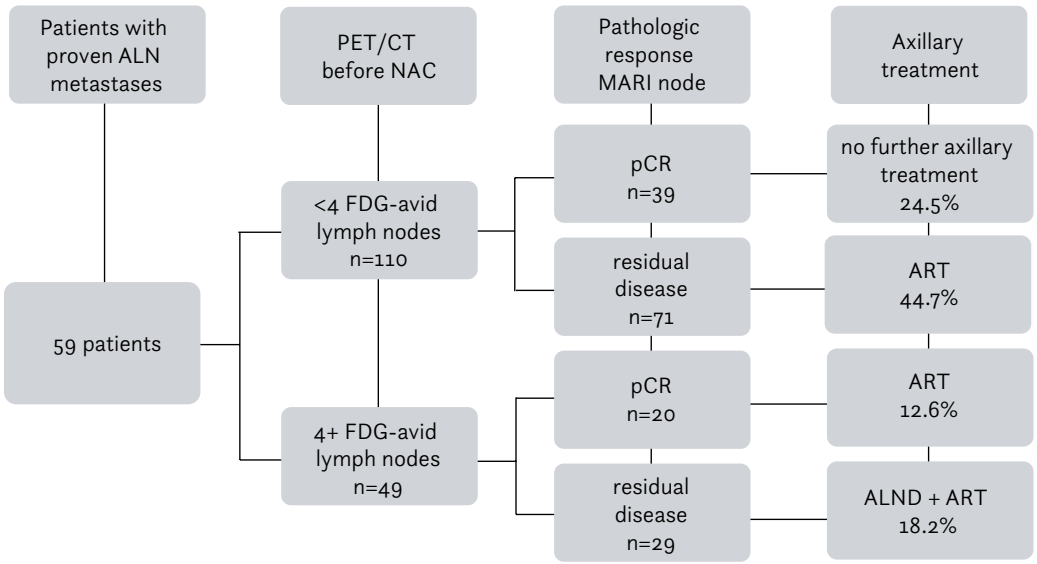
Tailored axillary treatment

In Figure 3, axillary treatment is presented. Of 110 cN(<4) patients, 39 were ypN0(MARI) and therefore received no further axillary treatment (25% of entire cohort). The remaining 71 patients in this group were ypN+(MARI) and were treated with ART. Forty-three patients were cN(4+)

before NST, of whom 20 achieved pCR of the MARI-node and were treated with ART. In total, ART was administered in 91 patients (57%). Twenty-nine cN(4+) patients were ypN+(MARI) and underwent ALND (18%). A median of 5 tumor-positive ALNs was found in the ALND specimens (range 0-14). ALND was followed by ART in all patients. In 17 patients, all axillary levels including the lateral axilla (level I-II) and peri-clavicular nodes were irradiated (level III-IV), and in 12 patients only the peri-clavicular nodes were irradiated whereby the lateral extent of the target volume bordered on the surgical resection volume.

During a median follow-up of 16 months (range 1-36), 1 cN(<4);ypN+(MARI) patient who underwent local-regional radiotherapy developed an axillary, parasternal and mediastinal recurrence. Two cN(4+);ypN+(MARI) patients who underwent ALND and ART had a supraclavicular recurrence.

Figure 3. Tailored axillary treatment by combining pre-NST PET/CT and the MARI-procedure post-NST.



NST=neoadjuvant systemic therapy; PET/CT=positron emission tomography combined with computed tomography; MARI=Marking the Axilla with Radioactive Iodine Seeds; ALN=axillary lymph node; pCR=pathologic complete response; ALND=axillary lymph node dissection

DISCUSSION

Clinical nodal status prior to NST and pathologic response to NST are both important prognostic predictors for LRR and survival in breast cancer patients.[4-7] In patients who achieve axillary pCR, the additional therapeutic effect of local axillary treatment should be questioned.

To select patients in whom axillary treatment after NST can be omitted, adequate staging of the axilla is required before as well as after NST. In previous studies, PET/CT was shown to be an optimal local-regional staging method before NST with a high PPV for detecting ALN metastases and assessment of the number of FDG-avid ALNs.[10,12,28-30] In addition, PET/CT has superior accuracy in the detection of distant metastasis when compared to (the combination of) conventional methods.[11,13,31]

Performing ALND after NST has been the standard of care for cN+ patients, since imaging methods and SLNB after NST have a wide variety in FNRs.[14,17-19] Alternative techniques to restage the axilla have been introduced. At NKI, the MARI-procedure was developed in which a tumor-positive ALN is marked with an iodine seed before NST and selectively removed after NST. The identification rate of the MARI-node is 97% and the FNR is 7% when ITC are considered tumor-positive.[21,22] The FNR is 4% when ITC are considered tumor-negative, which is standard in the SENTINA and ACOSOG Z071 trials.[17,18] Alternatively, in countries with regulatory issues regarding radiation safety, a clip is placed in a tumor-positive ALN before NST. After NST, the clipped node is localized with an iodine seed.[32] This technique can also be combined with SLNB, demonstrating similar low FNRs.[3,33]

Because adequate axillary restaging after NST is currently feasible, axillary treatment should be adapted accordingly. In the present study, we demonstrate that a new protocol based on results of PET/CT before NST and the MARI-procedure[23] resulted in a substantial decrease (82%) in the performance of ALNDs at our institute.

With our protocol, cN(<4);ypN0(MARI) patients receive no further axillary treatment. In the upfront surgery setting, the ACOSOG Z0011 trial demonstrated excellent regional control in patients with positive SLNs who did not undergo further axillary treatment.[34,35] A difference between our cN(<4);ypN0(MARI) patients and the Z0011 patients is that our patients initially

had limited axillary disease detected by PET/CT, whereas the Z0011 patients had limited axillary disease detected by SLNB. However, our cN(<4);ypN0(MARI) patients achieved pCR and the best relative disease free survival is seen in patients who pCR.[36] In the Z0011 patients, effect of adjuvant systemic therapy is unknown. Follow-up of our cN(<4);ypN0(MARI) patients who receive no further axillary treatment will have to demonstrate safety of this strategy in terms of local-regional control. In addition, the ongoing NSABP B-51/RTOG 1304 trial evaluates LRR and survival of cN1 patients who become ypN0 after NST and are treated with regional nodal radiotherapy or no radiotherapy.[37]

In our treatment protocol, cN(<4);ypN+(MARI) patients are treated with ART. Twenty-five percent of patients in the AMAROS trial who received ALND had 1-3 tumor-positive ALNs, and it can be expected that patients who were randomized to ART had a comparable number of tumor-positive ALNs.[38] Both treatment arms had excellent and comparable local-regional control and survival rates. A difference between our patients and the AMAROS trial is that our patients already received chemotherapy. However, most of our patients are treated with adjuvant endocrine treatment or HER2-blockage. The Alliance A11202 will provide us with more information on the long-term follow-up of cN1 patients with a tumor-positive SLNB after NST. These patients are randomized to either ALND plus breast/chest wall and nodal radiotherapy or radiotherapy only.[39]

Higher rates of LRR have been described in patients with advanced nodal disease.[40,41] Therefore in our protocol cN(4+);ypN0(MARI) patients receive ART. This strategy prevents the significant morbidity of combined ALND and radiotherapy that is still routinely administered in these patients.

In addition to the crucial issues that have been addressed, a few comments on the current study are warranted. The aim of this study was to present the reduction in ALNDs in cN+ breast cancer patients at our institute. We acknowledge that the safety of our axillary treatment protocol in terms of local-regional control has to be confirmed by longer follow-up of our patients and by results of other trials. Patients will be followed for 10 years. In addition, Koolen et al. demonstrated that PET/CT underestimated the number of tumor-positive ALNs prior to NST in 3/93 patients.[42] These patients might be at risk of undertreatment when treated according to our protocol. Furthermore, costs of performing PET/CT in the Netherlands are much lower than in other countries, such as the United States. In the Netherlands, costs of performing whole body PET/CT are estimated at €1100 by the Dutch Healthcare Authority.[24] PET/CT has superior accuracy in the detecting (the number of) ALNs and distant metastasis.[43] Thus, diagnostic effectiveness has been demonstrated in breast cancer care. However, the high costs of PET/CT and the lack of randomized trials into cost-effectiveness could be a challenging issue when implementing our treatment protocol elsewhere.

In conclusion, combining axillary staging prior to NST with PET/CT and staging after NST with use of the MARI-procedure has resulted in a major reduction of 82% ALNDs in cN+ breast cancer patients at our institute. Furthermore, 25% of patients received no further axillary treatment.

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Chapter 10

Three-year follow-up of de-escalated axillary treatment after neoadjuvant systemic therapy in clinically node-positive breast cancer: the MARI-protocol

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ABSTRACT

Purpose: In clinically node-positive (cN+) breast cancer patients, evidence supporting response-guided treatment after neoadjuvant systemic therapy (NST) instead of axillary lymph node dissection (ALND) is increasing, but follow-up results are lacking. We assessed three-year axillary recurrence-free interval (aRFI) in cN+ patients with response-adjusted axillary treatment according to the 'Marking Axillary lymph nodes with Radioactive Iodine seeds' (MARI)-protocol.

Methods: We retrospectively assessed all stage II-III cytologically proven cN+ breast cancer patients who underwent the MARI-protocol between July 2014 and November 2018. Pre-NST axillary staging with FDG-PET/CT (less- or more than four suspicious axillary nodes; cALN<4 or cALN≥4) and post-NST pathological axillary response measured in the pre-NST largest tumor-positive axillary lymph node marked with an iodine seed (MARI-node; ypMARI-neg or ypMARI-pos) determined axillary treatment: no further treatment (cALN<4, ypMARI-neg), axillary radiotherapy (ART) (cALN<4, ypMARI-pos and cALN≥4, ypMARI-neg) or ALND plus ART (cALN≥4, ypMARI-pos).

Results: Of 272 women included, the MARI-node was tumor-negative in 56 of 174 (32%) cALN<4 patients and 43 of 98 (44%) cALN≥4 patients. According to protocol, 56 (21%) patients received no further axillary treatment, 161 (59%) received ART and 55 (20%) received ALND plus ART. Median follow-up was 3.0 years (IQR 1.9-4.1). Five patients (one no further treatment, four ART) had axillary metastases. Three-year aRFI was 98% (95% CI 96-100). The overall recurrence risk remained highest for patients with ALND (HR 4.36; 95% CI 0.95-20.04, p=0.059).

Conclusions: De-escalation of axillary treatment according to the MARI-protocol prevented ALND in 80% of cN+ patients with an excellent three-year aRFI of 98%.

INTRODUCTION

In clinically node-positive (cN+) breast cancer patients, axillary lymph node dissection (ALND) is still widely considered the standard of care.[24,35,36] The ongoing shift from adjuvant to neoadjuvant systemic therapy (NST) however, allows consideration of less extensive axillary surgery for cN+ patients.[42,46] Currently, a pathologic complete response (pCR) of the axilla (ypN0) is seen in one-third of cN+ patients with NST, with pCR rates of more than 50% in triple-negative and HER2-positive patients.[43] Patients with axillary pCR are unlikely to benefit from ALND, while facing surgical complications and long-term morbidity such as lymphedema and limitation of shoulder motion. Therefore, strategies to de-escalate axillary treatment in cN+ patients are currently investigated.[7,9,53]

At the Netherlands Cancer Institute, the Marking Axillary Lymph Nodes with Radioactive Iodine seeds (MARI)-procedure[16] was developed to re-stage the axilla after NST. The largest -positive axillary lymph node (ALN) was marked with an iodine seed pre-NST (MARI-node) and selectively removed and assessed post-NST.[30] This procedure was found to be a reliable measurement of axillary response with a false-negative rate of only 7%.[16,30,51] Hereafter, an axillary treatment algorithm was developed (i.e., MARI-protocol) which combined the outcome of the MARI-procedure (ypMARI-neg or ypMARI-pos) with a pre-NST acquired fluorodeoxyglucose (FDG)-positron emission tomography/computed tomography (PET/CT)[30,51] scan to determine the presence of less or more than four (cALN<4 or cALN≥4) tumor-positive ALNs prior to NST. Patients staged cALN<4, ypMARI-neg received no further axillary treatment, patients staged cALN<4, ypMARI-pos and cALN≥4, ypMARI-neg received axillary radiotherapy (ART) and patients staged cALN≥4, ypMARI-pos received ALND plus ART.[51]

Long-term outcomes of patients treated according to the MARI-protocol have not yet been reported. In this study we assessed three-year follow-up results and in particular axillary recurrence-free interval (aRFI) of clinically node-positive breast cancer patients who underwent tailored and de-escalated axillary treatment after NST according to the MARI-protocol.

METHODS

Patient selection

This is a single-center cohort study including prospectively registered patients. We included all women, 18 years or older, with stage II–III pathologically proven axillary cN+ breast cancer of any subtype, who underwent the MARI-protocol between July 2014 and November 2018 at the Netherlands Cancer Institute. Exclusion criteria were history of breast cancer and non-FDG-avid breast cancer. This study was approved by the institutional review board of the Netherlands Cancer Institute.

Diagnostic procedures

Core needle biopsies of the breast tumor were obtained to determine histological subtype, hormone receptor and HER2- status. Hormone receptor status was defined as positive if estrogen expression was ≥10%, and HER2-status was regarded positive if 3+ or 2+ with positive in-situ hybridization, according to ASCO-CAP guidelines.[54] Tumor grade was determined according to the modified Bloom-Richardson method.[18] The size and extent of the primary tumor were assessed by mammography, ultrasound and dynamic contrast-enhanced (DCE) MRI. All patients underwent axillary and peri-clavicular ultrasound. Ultrasound-guided fine needle aspiration (FNA) was performed in case of suspect lymph nodes.

A whole body FDG-PET/CT (Philips Gemini, Cleveland, OH, USA) was performed for regional staging and detection of distant metastasis. PET acquisition was followed by a low-dose CT scan (40 mAs, 2 mm slices). Additional PET/CT images in prone position were acquired if patients were scanned at the Netherlands Cancer Institute. The uptake of FDG-positive ALNs was assessed by experienced nuclear medicine physicians and was discussed during multidisciplinary consultations. A lymph node was regarded as highly suspicious for metastasis when the uptake was higher than the blood pool activity. For axillary staging according to the MARI-protocol, the number of FDG-positive ALNs was used rather than the clinical TNM classification. Patients with less than four FDG-positive axillary nodes on PET/CT were defined as cALN<4 and patients with more than three FDG-positive axillary nodes were defined as cALN≥4, regardless of presence of peri-clavicular or internal mammary chain nodes.

Radioactive seed localization

In all patients, an Iodine seed (STM1251, Bard Brachytherapy Inc., Carol Stream, IL) with an apparent activity varying from 0.2 to 1.0 MBq at time of implementation was placed under ultrasound guidance in the largest pathology proven tumor-positive axillary lymph node (i.e., MARI-node) prior to the start of the first NST cycle. The activity of Iodine seeds used for MARI-node localization is lower than for breast tumor localization (apparent activity 1.0-7.6 Mbq)[4,15] to minimize irradiation of the node. Marking of the breast tumor was performed during the same procedure. Adequate position of the markers in the breast and axilla was confirmed by ultrasound and/or mammography. A comprehensive description of the MARI-procedure and radiation safety protocols has been described previously.[49]

Treatment and response evaluation

Neoadjuvant systemic therapy was administered according to institutional guidelines as previously described.[30] After completion of NST, surgery of the breast and selective removal of the MARI-node was performed. A gamma probe was used to guide the localization of the Iodine seeds and surgical resection. Additional axillary nodes were removed when a lymph node was located directly adjacent to the MARI-node.

In cALN<4 patients, the MARI-node was formalin-fixed overnight followed by hematoxylin and eosin (H&E) and cytokeratin staining at a single level. An intraoperative frozen section of the MARI-node was obtained in all cALN≥4 patients. For intraoperative frozen sections, 2 mm tissue slices ware made from which 5 µm H&E sections were prepared and assessed. Hereafter, the tissue was also fixed in formalin overnight followed by a new H&E and a cytokeratin stain at a single level.

Pathological complete response of the axilla was defined as the absence of vital tumor cells in the removed axillary lymph node(s) (ypN0). A pCR of the breast was defined as absence of invasive and in-situ carcinoma in the breast (ypT0).

Tailored and de-escalated axillary treatment

All cALN<4 patients with pCR of the MARI-node (ypMARI-neg) received no further axillary treatment. Axillary levels I to IV were irradiated in patients staged cALN<4, ypMARI-pos and cALN≥4, ypMARI-neg. ALND and ART was performed in all patients staged cALN≥4, ypMARI-pos. The ALND was performed in a second operation in patients with a false-negative intraoperative frozen section of the MARI-node.

Patients with ART underwent irradiation to the axillary and infra/supraclavicular nodes, and in case of FDG-positive nodes in the internal mammary chain (IMC), the IMC was included. Delineation of lymph node levels was performed according to the Danish national delineation guidelines, and from January 2015, according to the European Society for Radiotherapy and Oncology consensus guidelines. A dose of 42.56 Gy in 16 fractions of 2.66 Gy was prescribed, or 46.2 Gy in 21 fractions of 2.2 Gy if a simultaneous boost dose was given to the tumor bed in the breast. The radiotherapy technique used was either static field Intensity Modulated RadioTherapy (IMRT) or Volumetic Modulated Arc Therapy (VMAT) planning. Deep Inspiration Breath Hold Technique was applied for all left sided breast tumors.

Patients received adjuvant systemic treatment according to institutional guide-lines. Patients with hormone-receptor positive tumors received adjuvant hormonal therapy and all patients with HER2-positive tumors received adjuvant HER2-directed therapy. Following the publication of the CREATE-X trial in 2017,[38] adjuvant Capecitabine was administered in all patients with triple-negative breast cancer with residual disease and a selection of estrogen receptor-positive tumors with residual disease.

Outcomes

The primary endpoint was three-year axillary recurrence-free interval (aRFI), defined as tumor recurrence in lymph nodes in the ipsilateral axilla. Secondary outcomes were local-, regional-, distant and overall- RFI rates and overall survival. Axillary recurrence-free interval was defined as time from the MARI-procedure to axillary recurrence or death from any cause. Patients who died without axillary recurrence or were lost to follow-were censored in the analysis. Patients who developed (and received treatment) for another event (e.g. local recurrence, distant metastases, or new primary) before axillary recurrence were censored in the analysis, except if it was a synchronous event (i.e., diagnosed at subsequent disease staging). In addition,

three-year RFI was assessed in the pre-specified treatment groups (i.e., no further treatment [cALN<4, ypMARI-neg] ART [cALN<4, ypMARI-pos and cALN≥4, ypMARI-neg] and ALND plus ART [cALN≥4, ypMARI-pos], as well as factors influencing disease recurrence (i.e., age, clinical stage, subtype and pathological response) were evaluated.

Statistical Analysis

Recurrence-free interval and overall survival of the four treatment groups were estimated by the Kaplan-Meier method and compared with log-rank tests. All survival estimates were reported with their 95% confidence intervals. To evaluate associations between patient characteristics, axillary treatment and recurrence-free interval, Cox proportional-hazards models were used. The two-sided 95% confidence intervals for proportions were calculated using the exact Clopper-Pearson method. Baseline characteristics were compared between patients staged cALN<4 and cALN≥4 with an independent sample t-test for sample means and with Pearson Chi-square or Fisher's exact test for categorical variables. Statistical significance for comparisons between groups was defined as $p<0.05$. All statistical analyses were performed in IBM SPSS Statistics, version 25.0.

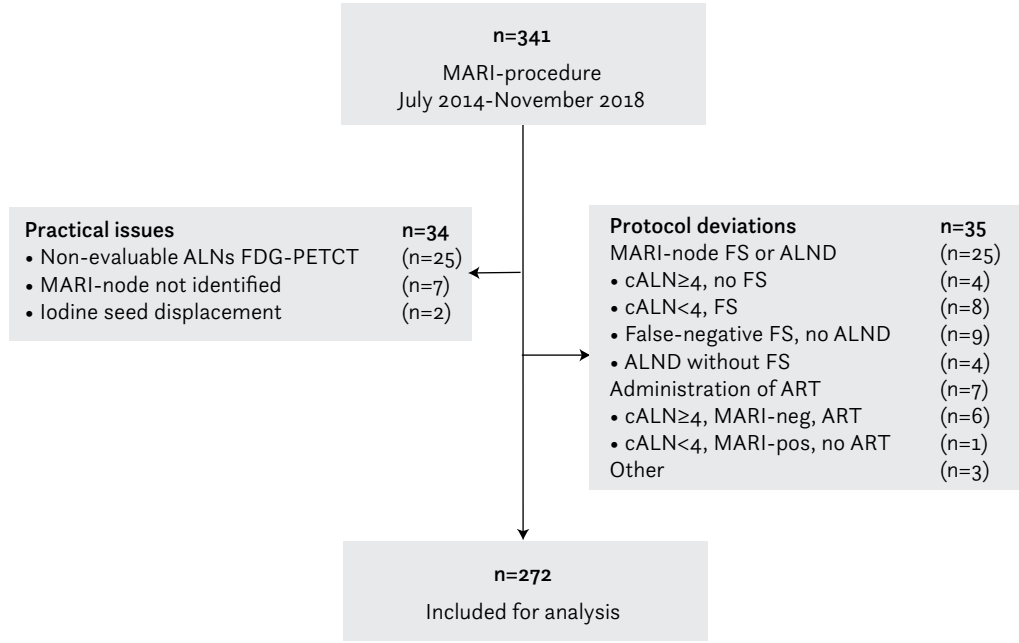
RESULTS

Patient characteristics

Between July 2014 and November 2018, 272 (80%) of 341 prospectively registered patients who underwent the MARI-procedure fulfilled eligibility criteria (Figure 1). Reasons for exclusion were practical issues (N=34) (e.g. non-FDG avid or clustered, indistinguishable ALNs) or protocol deviations (N=35) (e.g. false-negative intraoperative frozen section not followed by ALND).

Baseline characteristics are shown in Table 1. Median age was 48 years (range 22-79) and the majority of patients had invasive carcinoma of no special type (89%). Staging with FDG-PET/CT prior to NST categorized 174 (64%) patients as cALN<4 and 98 (36%) patients as cALN≥4. Baseline characteristics differed between the groups: more HER2-positive tumors (38% vs. 23%) and less HR-positive/HER2-negative tumors (43% vs. 57%) were found in cALN≥4 patients compared to cALN<4 patients (p=0.012, Table 1).

Figure 1. Patient inclusion.



MARI=Marked axillary lymph node with radioactive iodine seed; FDG-avid=fluorodeoxyglucose (FDG)- positron emission tomography/computed tomography (PET/CT)-positive; ALNs=Axillary lymph nodes; FS=frozen section; ALND=axillary lymph node dissection; cALN<4=less than four FDG-PET/CT-positive axillary lymph nodes; cALN≥4=more than four FDG-PET/CT positive axillary lymph nodes; ART=axillary radiotherapy

The MARI-procedure

The total number of ALNs removed during the MARI-procedure ranged from one to six, with a median of one (IQR 1-2). A pCR of the MARI-node (ypMARI-neg) was found in 56 (32%) of 174 cALN<4 patients and in 43 (44%) of 98 cALN≥4 patients (p=0.054) and varied per subtype, with rates of 9% (13 of 140) in HR-positive/HER2-negative tumors, 59% (27 of 46) in HR-positive/HER2-positive tumors, 94% (30 of 32) in HR-negative/HER2-positive tumors and 54% (29 of 54) in triple-negative tumors (p<0.001). In all patients with a tumor-negative MARI-node, the additionally removed ALNs were negative as well.

Breast pCR occurred in 78 (29%; 95% CI 23-34) patients and 64 (24%; 95% CI 19-29) patients had both pCR of the breast and the MARI-node (ypT0N0).

Table 1. Baseline patient and tumor characteristics

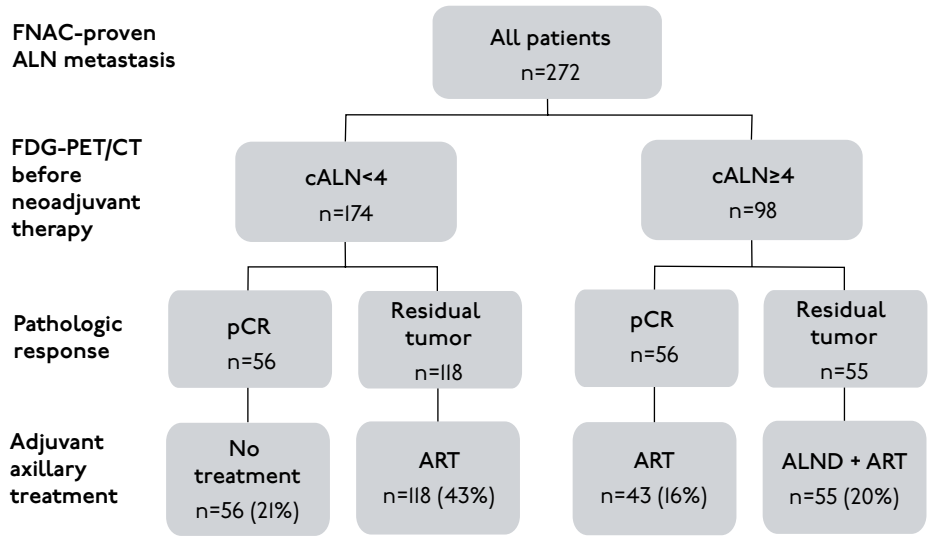
	Total n=272		cALN<4 n=174		cALN≥4 n=98		P value
Age (y)	48	(41-56)	48	(40-55)	49	(42-56)	0.981
Diagnostic imaging							
Tumor size MRI (mm)	32	(22-50)	31	(22-46)	36	(24-55)	0.109
PET/CT-positive ALNs	2	(1-4)	1	(1–2)	5	(4-7*)	<0.001
Histology							**0.797**
No special type*	242	(89%)	153	(88%)	89	(91%)	
Lobular	29	(11%)	20	(11%)	9	(9%)	
Other	1	(1%)	1	(1%)	0	–	
Tumor subtype							**0.012**
HR+/HER2-	140	(51%)	99	(57%)	41	(43%)	
HR+/HER2+	46	(17%)	27	(15%)	19	(19%)	
HR-/HER2+	32	(12%)	13	(8%)	19	(19%)	
Triple-negative	54	(20%)	35	(20%)	19	(19%)	
Bloom-Richardson Grade							**0.565**
Grade 1	9	(4%)	7	(4%)	2	(2%)	
Grade 2	135	(53%)	90	(55%)	45	(51%)	
Grade 3	110	(43%)	68	(41%)	42	(47%)	
Unknown	18	–	9	–	9	–	

Data are median (IQR) or N (%). *The number of ALNs was reported as 'multiple' in 26 patients. *formerly known as invasive ductal carcinoma. All characteristics were assessed before administration of neoadjuvant systemic therapy. Abbreviations: cALN<4=less than four FDG-PET/CT-positive axillary lymph nodes; cALN≥4=more than four FDG-PET/CT positive axillary lymph nodes; MARI=marked axillary lymph node with radioactive iodine seed; ALNs=axillary lymph nodes;, ALND=axillary lymph node dissection.

Tailored axillary treatment

Axillary treatment according to the MARI-protocol is presented in Figure 2 and resulted in omission of ALND in a total of 217 (80%) patients: no further axillary treatment was administered in 56 (21%) patients (cALN<4, ypMARI-neg) and 161 (59%) patients (118 cALN<4, ypMARI-pos and 43 cALN≥4, ypMARI-neg) received ART. Fifty-five (20%) cALN≥4 patients had residual tumor in the MARI-node underwent ALND plus ART. Adjuvant systemic therapy was administered in 228 (84%) patients and included chemotherapy in 44 (16%) patients, HER2-directed therapy in 80 (29%) patients and hormonal therapy in 183 (67%) patients.

Figure 2. Tailored adjuvant axillary treatment strategy according to the MARI protocol.



Abbreviations: FNAC=fine needle aspiration cytology; cALN<4=less than four FDG-PET/CT-positive axillary lymph nodes; cALN≥4=more than four FDG-PET/CT positive axillary lymph nodes; MARI=marked axillary lymph node with radioactive iodine seed; pCR=pathological complete response; ALN=Axillary lymph node; ALND=axillary lymph node dissection; ART=axillary radiotherapy.

Axillary recurrence

Median follow-up was 3.0 years (IQR 1.9-4.1, range 0.3-5.4). Axillary recurrences occurred in a total of five (1.8%) patients, and three-year aRFI was 98% (95% CI 96-100). All five were cALN<4 patients with synchronous other metastases. Subtype was triple-negative in four patients and HR-positive/HER2negative in one. One of the five patients had pCR of the MARI-node and therefore received no further axillary treatment. In this patient, extensive metastases were found in the axilla, lower neck and cervical region. The remaining four patients had residual disease in the MARI-node and underwent radiation treatment. Of these, one patient had axillary and IMC metastases, one patient had axillary metastases with concurrent metastases in the breast/thoracic wall, supraclavicular nodes and in the IMC, and two patients had axillary metastases with synchronous distant metastases.

Secondary outcomes

In total, 27 (9.9%) patients developed one or more recurrences (distant, regional or local). Distant metastases were found in 19 (7.0%) patients, regional nodal recurrences (including the five patients with axillary metastases) occurred in 10 (3.7%) patients and a local recurrence was detected in 6 (2.2%) patients. The corresponding overall three-year RFI and distant, regional, and local RFI rates were 90% (95% CI 86-94), 93% (95% CI 90-96), 96% (95% CI 94-99) and 98% (95% CI 95-100), respectively. Sixteen (5.9%) patients died, al due to breast cancer recurrence, resulting in a three-year overall and breast cancer survival of 95% (95% CI 91-98).

The first documented site(s) of recurrence by axillary treatment group are shown in Table 2. In total, fewest recurrences (5%) occurred in cALN<4, ypMARI-neg patients with no further axillary treatment. Nine percent recurrences were found in both ART groups (cALN<4 and cALN≥4) and 18% in the ALND group (Table 2). The corresponding three-year RFI rates were 100% (95% CI: n.a.), 91% (95% CI: 85-97), 88% (95% CI: 76-100) and 79% (95% CI: 66-92) (Figure 3). In an exploratory analysis, the trend in increased risk of disease recurrence for cALN≥4, ypMARI-pos patients remained after adjusting for age, subtype and pathological response of the breast (HR 4.36, 95% CI 0.95-20.04, p=0.059).

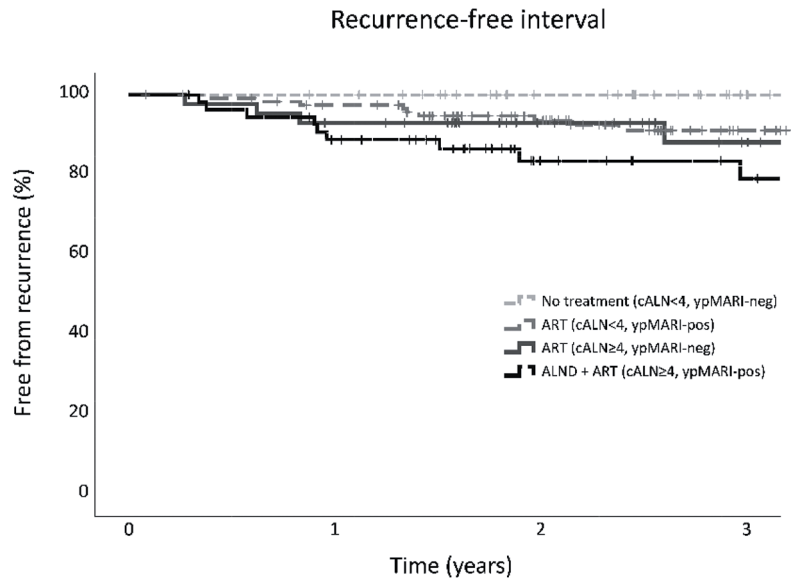
Baseline characteristics associated with increased risk of disease recurrence in univariate analysis were clinical stage cALN≥4 (HR 2.25, 95% CI 1.05-4.79, p=0.036) and triple-negative breast cancer (HR 2.89, 95% CI 1.23-6.81, p=0.015) (Table 3). In multivariate analysis, triple-negative breast cancer (HR 4.32, 95% CI 1.74-10.53, p=0.002) and residual tumor in the MARI-node (HR 3.13, 95% CI 1.02–9.68, p=0.047) were significantly associated with disease recurrence.

Table 2. Locations of breast cancer recurrence by response adjusted axillary treatment group.

	cALN<4		cALN≥4		Total
	MARI pCR No treatment	MARI tumor+ ART	MARI pCR ART	MARI tumor+ ALND + ART	n=272
	n=56	n=118	n=43	n=55	
Total patients with event per treatment group*					
Axillary + Local	0	1	0	0	1
Axillary + Regional	1	1	0	0	2
Axillary + Distant	0	2	0	0	2
Local	1	0	0	2	3
Local + Regional	0	0	0	1	1
Local + Distant	0	0	1	0	1
Regional	0	0	0	1	1
Regional + Distant	0	0	1	2	3
Distant	1	6	2	4	13
Total	3 (5.4%)	10 (8.5%)	4 (9.3%)	10 (18.2%)	27 (9.9%)
Total patients with event by location					
Axillary	1	4	0	0	5 (1.8%)
Local	1	1	1	3	6 (2.2%)
Regional (incl. axilla)	1	4	1	4	10 (3.7%)
Distant	1	8	4	6	19 (7.0%)

*Axillary recurrences were reported separately from non-axillary regional nodal metastases; Lower neck/cervical metastases were considered regional metastases. Abbreviations: cALN<4=less than four FDG-PET/CT-positive axillary lymph nodes; cALN≥4=more than four FDG-PET/CT positive axillary lymph nodes; MARI=marked axillary lymph node with radioactive iodine seed; pCR=pathological complete response; tumor+=tumor-positive; ART= axillary radiotherapy; ALND=axillary lymph node dissection

Figure 3. Overall recurrence-free interval by axillary staging and treatment.



No. at risk				
No treatment (cALN<4, ypMARI-neg)	55	46	24	18
ART (cALN<4, ypMARI-pos)	118	110	85	56
ART (cALN≥4, ypMARI-neg)	43	37	25	16
ALND + ART (cALN≥4, ypMARI-pos)	56	54	43	29

Abbreviations: cALN<4=less than four FDG-PET/CT-positive axillary lymph nodes; cALN≥4=more than four FDG-PET/CT positive axillary lymph nodes; MARI=marked axillary lymph node with radioactive iodine seed; ypMARI-neg/ypMARI-pos=pathology analysis of MARI-node after neoadjuvant systemic therapy tumor-negative/tumor-positive; ART=axillary radiotherapy; ALND=axillary lymph node dissection.

Table 3. Cox regression analysis for overall recurrence-free interval.

	Events		Univariate			Multivariate		
	n	(%)	HR	95% CI	P value	HR	95% CI	P value
Age, years	27	(10%)	1.01	0.98-1.05	0.517	1.01	0.97-1.05	0.582
Subtype								
HR+/HER2-	10	(7%)	REF.					
HR+/HER2+	3	(7%)	0.99	0.27-3.58	0.981	1.57	0.40-6.10	0.519
HR-/HER2+	3	(9%)	1.33	0.37-4.84	0.666	3.39	0.63-18.12	0.154
Triple-negative	11	(20%)	2.89	1.23-6.81	0.015	4.28	1.74-10.53	0.002
Clinical tumor stage								
≤cT1	2	(4%)	REF.					
cT2	16	(10%)	2.72	0.63-11.85	0.182	2.91	0.66-12.81	0.157
≥cT3	9	(14%)	4.06	0.88-18.82	0.073	3.68	0.78-17.49	0.101
Clinical ALN group								
cALN<4	13	(8%)	REF.					
cALN≥4	14	(14%)	2.25	1.05-4.79	0.036	1.96	0.88-4.35	0.100
Pathology MARI node(s)								
Tumor-negative	7	(7%)	REF.					
Tumor-positive	20	(12%)	1.67	0.71-3.95	0.244	3.13	1.02-9.68	0.047
Pathology breast								
Residual disease	23	(12%)	REF.					
Complete response	4	(5%)	0.45	0.15-1.29	0.137			
Adjuvant axillary treatment*								
No further treatment	3	(5%)	REF.					
ART (cALN<4)	10	(9%)	1.64	0.45-5.97	0.451			
ART (cALN≥4)	4	(9%)	2.04	0.46-9.13	0.351			
ALND plus ART	10	(18%)	4.18	1.15-15.22	0.030			

*Adjuvant axillary treatment was not included in multivariate analysis due to collinearity with clinical axillary lymph node group and pathology MARI node(s) (R^2≥0.6). Abbreviations: HR=hazard ratio; cALN<4=less than four FDG-PET/CT-positive axillary lymph nodes; cALN≥4=more than four FDG-PET/CT positive axillary lymph nodes; MARI=marked axillary lymph node with radioactive iodine seed; ART=axillary radiotherapy; ALND=axillary lymph node dissection.

DISCUSSION

This study demonstrates that tailored de-escalated axillary treatment after NST according to the MARI-protocol in cN+ breast cancer patients is safe with an 80% reduction in ALNDs and excellent three-year aRFI and regional RFI of 98% and 96%, respectively. As axillary recurrences occur at a median of two years following treatment,[20,26,41] the high aRFI of 98% we found at a median follow-up of three years can be considered a significant result.

Previously reported regional RFS rates in cN+ patients who underwent complete ALND after NST included rates of 96% at three years follow-up,[34] 94%-96% at five years follow-up10,[25,28,29,50] and 91%-95% at ten years follow-up.[37] Notably, the number of cN2-3 patients we included was generally higher (36% cALN≥4 patients), and the high RFS we found is therefore less likely to result from a more favorable patient selection. Several studies have established the significance of clinical stage and especially pathological axillary response as prognostic factors.[13,19,25,37,52] Accordingly, we found fewest recurrences in cALN<4 patients with MARI-node pCR and most recurrences in patients staged cALN≥4, ypMARI-pos who underwent ALND plus ART. Baseline factors associated with disease recurrence in multivariable analysis were residual tumor in the MARI-node (HR 3.1) and triple-negative subtype (HR 4.3).

Post-NST axillary staging strategies for cN+ patients other than the MARI-procedure include the post-NST sentinel lymph node biopsy (SLNB) and targeted axillary dissection (TAD),[9] which combines removal of a pre-NST clipped node with SLNB.[42,46] The accuracy of the post-NST SLNB is a much-debated topic. While the MARI-procedure has a false-negative rate (FNR) of 7% with a risk of undertreatment in only 3% of patients,[16,30] FNRs of 8% to 40% have been reported for the post-NST SLNB.[6,7,33,46] A clinically considered acceptable FNR of ≤10% was only achieved when three or more sentinel nodes (SNs) were removed and dual-tracer mapping was used.[7,33] In the ACOSOG Z1071 and SENTINA trial, retrieval of three or more SNs occurred only in 56% and 34% of patients, respectively.[7,33]

The FNR of TAD was reported to be as low as 2-4%,[9,16,47,53] and could be lower than the FNR of the MARI-procedure due to assessment of more ALNs. In the study by Caudle et al,[9] three or more ALNs were removed in 47% (63 of 134) of patients, while a median of only one (IQR 1-2) ALN is removed with the MARI-procedure. Compared to the MARI-procedure, TAD also requires an additional visit to the outpatient clinic for both the localization of the clipped node and the sentinel-node procedure.

Although the removal of more ALNs may decrease the FNR, it also increases the risk of lymphedema.[14] Moreover, it is important to note that lowering the FNR of post-NST axillary staging methods further below 10% may not significantly lower the axillary recurrence rate. With the MARI-procedure, we found an excellent three-year aRFI of 98%.

Several other studies indicate that limited axillary residual disease may safely be left in situ without compromising aRFI. In patients treated with SLNB in the primary surgery setting, five- to ten year axillary recurrence rates of 0% to 2% were found, which is lower than expected based on the reported FNRs of 5% to 10%,[21-23,26,27,42,48] and the ACOSOG Z0011 and IBCSG 23-01 trials reported excellent locoregional control in patients with limited disease at SLNB without further axillary treatment.[21,26] In addition, the AMAROS trial found that ART was as effective as ALND for the treatment patient with tumor-positive SLN's (5-year axillary recurrence of 1.2% vs. 0.4%).[17] Of note, four or more tumor-positive ALNs (pN2) were found in 8% of the patients in the ALND-arm, which supports the efficacy of ART even in patients with higher axillary tumor load.

Reports on axillary recurrence after de-escalated locoregional axillary treatment in cN+ patients with NST are limited. Four- and five year recurrence rates of 2% and 0% were described in cN1 patients with a tumor-negative post-NST SLNB in whom ALND was omitted.[11,23,45] Results of comprehensive trials investigating the impact of de-escalated axillary treatment after NST such as the ongoing NSABP B-51/RTOG 1304 (NCT01872975)[2] and the Alliance A011202 trial (NCT01901094),[1] are currently unknown. In addition, whether ALND can be avoided after NST in patients with cN2-3 disease is not investigated in these trials.[40] Notably, in the present study we showed that the MARI-protocol is not only an effective method for de-escalation of axillary treatment in cN1 patients, but also for patients with more extensive axillary disease prior to NST.

Limitations to implementation of the MARI-protocol could be the use of radioactive iodine seeds. Although iodine seeds are increasingly being used for tumor localization due to improved surgical planning and diminished patient discomfort,[15] extensive regulations often apply for handling and disposal of the seeds. According to our protocol, iodine seeds should be allowed to remain in situ for the duration of NST.

Furthermore, FDG-PET/CT it is not yet part of the diagnostic work-up for cN+ breast cancer patients in several countries. The costs (+/- €1,260[8] [$1,545[12]]) may therefore not always be fully covered by health insurance.[5,8,12] Staging breast cancer patients with FDG-PET/CT however can replace diagnostic imaging with CT, chest X-ray and ultrasound with higher diagnostic accuracy and cost-effectiveness.[3,39] In addition, the diagnostic accuracy of FDG-PET/CT for axillary staging is higher compared to other modalities and therefore essential when tailoring axillary treatment.[31,32,44]

Limitations of this study are its single-center character and prospective registration design. Ten percent of the patients undergoing tailored axillary treatment after NST according to the MARI-protocol were excluded from analysis due to deviations from the protocol. The type of protocol violations varied, and included both patients with overtreatment (e.g. cALN≤4 patients with intraoperatively assessed extensive residual axillary disease treated with ALND) as well as patient who were undertreated (no ALND or ART in case of a tumor-positive MARI-node) according to protocol.

In conclusion, in this study we demonstrated that the MARI-protocol is an effective axillary staging and treatment algorithm which resulted in omission of ALND in 80% of cN+ patients undergoing NST while maintaining excellent three-year axillary- and regional RFI rates of 98% and 96%. Therefore, the MARI-protocol may be considered a suitable method to de-escalate axillary treatment in selected patients. Longer follow-up is needed to evaluate these results at five- and ten years follow-up.

DECLARATIONS

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Conflicts of interest

GS received institutional research support from AstraZeneca, Merck, Novartis, and Roche outside the scope of this manuscript. All other authors declare no competing interests

Ethics approval

This retrospective chart review study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The institutional review board of the Netherlands Cancer Institute approved this study.

Informed consent

This study was conducted retrospectively from data obtained for clinical purposes. An official waiver of ethical approval was granted from the institutional review board.

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Section IV

Concluding remarks

Chapter 11

General discussion and future perspectives

Over the last century, the treatment of breast cancer patients has evolved dramatically, from a strictly surgical approach to a multidisciplinary one including radiotherapy and systemic therapy (chemotherapy, immunotherapy, endocrine therapy and targeted therapy). As a result, breast cancer survival has greatly improved and interest in de-escalating treatments to decrease morbidity and preserve quality of life has grown.[1]

To be able to select patients for de-escalation of locoregional treatments, adequate assessment of response is mandatory. In this thesis, multiple minimally and non-invasive strategies to de-escalate locoregional treatments in patients with exceptional response to neoadjuvant systemic therapy (NST) are investigated.

In this thesis, the following questions relevant in the context of de-escalation of locoregional treatments are discussed:

1. What is the prognostic value of residual cancer burden (RCB), a standardized pathological methodology that evaluates and quantitates the extent of residual disease following NST?
2. Can predictors of response of ductal carcinoma in situ (DCIS) after NST in patients with HER2-positive breast cancer be defined?
3. Is breast conserving therapy feasible in patients with large breast tumors who show good response to NST?
4. Can patients with a pathologic complete response (pCR) of the breast after NST be identified without surgery?
5. For which patients is de-escalated locoregional treatment of the axillary lymph nodes a safe option?

The prognostic value of residual cancer burden

Pathologic complete response to NST is strongly associated with improved long-term survival.[2,3] However, with the binary outcome of pCR, valuable incremental response information is lost. Therefore, the RCB method, that quantifies the amount of residual disease, was developed.[4] RCB has been validated as prognostic in several single-institution studies and multicenter trials.[5-12] However, individually, these cohorts were too small to evaluate accurate estimates of prognosis within the various subtypes of breast cancer. Therefore, in **chapter 2,** the results of a pooled subject-level analysis of multiple clinical cohorts and trials are presented, to evaluate the association between RCB and long-term outcomes with emphasis on breast cancer subtypes.

Indeed, in this pooled analysis, we observed significant association between RCB and EFS/DRFS in the population as a whole, within all subtypes and across all cohorts (except in the smallest cohort for EFS). Additionally, RCB remained prognostic in multivariate models adjusting for age, grade, and cT, and cN status at diagnosis. Importantly, the risk of a recurrence event increases with the extent of residual disease, regardless of subtype. Therefore, use of RCB adds prognostic information when pCR is not achieved. As more adjuvant therapy options become available for patients with residual disease, a more refined estimate of an individual's risk of recurrence, based on their subtype and RCB, can be useful for decisions on adjuvant treatment selection.

Response of ductal carcinoma in situ following neoadjuvant systemic therapy

When selecting patients for de-escalating local treatment after NST, patients with DCIS are often not considered, as it is believed that the DCIS component is insensitive to NST.[13,14] Therefore, performing breast-conserving therapy (BCS) in patients with extensive DCIS is challenging, even when an excellent treatment response of the invasive component has been achieved. It would be most relevant to know in which patients adjacent DCIS will respond to NST to eventually increase the conversion rate of mastectomy to BCS. As HER2-positive breast cancer responds well to NST and adjacent DCIS is frequently found (57-72%), we estimated the response of DCIS following NST containing HER2-blockade in this breast cancer subtype and evaluated clinicopathological and radiological factors that are associated with response in **chapter 3**. To the best of our knowledge, this is the largest study that examined the response of DCIS to NST in HER2-positive breast cancer patients and the first study that evaluated the association of clinicopathological and radiological factors with response.

Overall, DCIS was eradicated after NST in 46% of patients. Absence of calcifications on pre-NST mammography, treatment with dual HER2-blockade, a (near) complete response on MRI and absence of calcifications and Ki-67>20% in DCIS on pre-NST biopsy were significantly associated with DCIS response. As concerns on the safety of BCS especially arise in patients with a high likelihood of extensive DCIS, a subgroup analysis was also performed in patients with suspicious calcifications on mammography, as well as adjacent DCIS in pre-NST biopsy. In this subgroup, the same factors were associated with DCIS response, while additionally the absence of necrosis in DCIS in the pre-NST biopsy was associated with DCIS response. Higher response rates were also observed in those with HR-negative tumors and grade I and II breast cancer in this subgroup, but these associations were not statistically significant.

In conclusion, chapter 3 indicates that the presence of extensive DCIS in HER2-positive breast cancer before NST should not always indicate a mastectomy, and the predictive factors described in this thesis could be helpful when considering BCS in these patients.

Section II: Reducing local treatment of the breast after neoadjuvant systemic therapy

At this point, the increased use and efficacy of NST does not always result in de-escalation of local treatment of the breast. Although breast conserving therapy (BCT; BCS + radiation therapy) showed similar survival to mastectomy in the neoadjuvant and adjuvant setting, a meta-analysis suggested that less extensive surgery after NST may be associated with higher local recurrence rates.[15] This effect could be attributed to two trials from 1983 to 1985 that did not perform any surgery after NST. In addition, missing data on margin and axillary status may have contributed to higher local recurrence rates as well. Additionally, more recent evidence suggests that the risk of local recurrence is driven by tumor biology rather than neoadjuvant versus adjuvant therapy.[16]

Nonetheless, BCS after NST remains controversial, especially in patients with large breast tumors. In **Chapter 4**, it is shown that BCT following MRI evaluation before and after NST in cT3 breast cancer patients is feasible with a success rate of 82%. Local control in these patients is excellent with a 7-year local-recurrence free survival of 96%. Patients with HR-positive/HER2-negative or lobular tumors, or tumors that initially presented as non-mass enhancement on MRI are more likely to have positive margins at BCS. In this study, we confirm the safety of BCS after NST in cT3 breast cancer patients with a good response to NST, despite the fact that the original tumor bed is not entirely excised in these patients. It can be hypothesized that it is safe to refrain from removing tissue that originally contained tumor but is free of tumor after NST. Extrapolating this concept, it may be possible to omit breast surgery at all in patients with pCR of the breast tumor following NST. However, until now, no method other than complete pathological assessment of the surgical breast specimen after NST has been demonstrated to be accurate at determining the presence of pCR. Magnetic imaging resonance (MRI) is accurate in determining tumor size after NST, but unable to identify pCR with sufficient reliability to replace surgical excision.[17-19] When NST was emerging, some studies already investigated the possibility of local-regional therapy without surgery.[20-22] In these studies, patients with a clinical complete response (cCR) (no palpable disease and/or absence of residual tumor on mammography and/or ultrasound) were treated with radiotherapy only. Results showed unacceptable high rates of

local recurrence (21-47%). In another study, biopsies were obtained without image guidance in patients with cCR after NST.[23] Tumors were not marked prior to NST and 6-10 biopsies per quadrant were obtained. Patients with pCR in the biopsies were treated with radiotherapy only. After a follow-up of 34 months local recurrence in these patients was 13%. Since current practice consists of marking the breast lesion prior to NST and pCR rates are increasing, a renewed and justified interest has emerged in the possibility to omit surgery after NST. At the Netherlands Cancer Institute (NKI), we initiated the MICRA trial (Minimally Invasive Complete Response Assessment) to determine whether core biopsies of the breast are sufficiently accurate at differentiating between pCR and residual disease in patients with an excellent response on MRI after NST. In **Chapter 5** presents the study design and feasibility of this study. The MICRA trial is a multi-center prospective cohort study including patients with pre-NST placed marker and a partial or complete response on MRI after NST. In all patients, ultrasound-guided 14-gauge core biopsies of the original tumor bed are obtained prior to breast surgery. Pathology results of the biopsies and surgical specimens are compared. The primary endpoint of the MICRA trial is the false-negative rate of the biopsies in identifying pCR. During the first year of the trial, performing ultra-sound guided biopsy of the breast appeared feasible. In the majority of patients, the marker could be identified. A median of 8 biopsies could be obtained and the median of histopathological representative biopsies was 4. Although several other pilot studies showed promising results[24], the interim analysis of the MICRA trial could not confirm these results (**chapter 6**). It was found that 14-gauge biopsies were not accurate enough at differentiating patients with pCR from those with residual disease (FNR 37%). Other larger prospective trials also failed to confirm a sufficiently high diagnostic accuracy of biopsies to replace surgery, with FNRs ranging from 18-50%.[25-28] Two aspects of the design of the MICRA trial could explain the high FNR. First, by using MRI for response monitoring, patients with the highest odds of achieving pCR were selected. It can be expected that sampling errors are more likely to occur in patients with minimal residual disease. In the MICRA trial, a significantly higher FNR was found in patients with complete response on MRI compared to patients with residual enhancement (47% vs. 13%). Additionally, patients with false-negative biopsies had less residual disease in the surgical specimens than those with true-positive biopsies. Another limitation of the MICRA trial is the quantity of the tissue obtained and examined with biopsies, as 14-gauge core biopsies were used. Several other studies used 9 to 10-gauge vacuum-assisted biopsies that obtain approximately seven times as much tissue per biopsy.[25,29] However, at this point the conclusion is that the FNR of both core and vacuum assisted biopsies far exceed the clinically estimated acceptable threshold of 5-10%.

Reducing regional treatment after neoadjuvant systemic therapy

Axillary staging and treatment after completion of NST remains an area of controversy. In patients with clinically node-negative disease (cN0), the nodal positivity rate (ypN+) after NST is low, especially in those with pCR of the breast. In these patients, the value of performing surgical axillary staging after NST could be limited. However, presence of breast pCR is not routinely known prior to surgery. In **chapter 7,** we validate the correlation of breast pCR and the absence of tumor-positive lymph nodes (ypN0) after NST in cN0 patients. In addition, we investigate preoperatively known predictive characteristics for ypN0, to be able to select patients for omission of sentinel lymph node biopsy (SLNB) after NST. It was found that the probability of nodal positivity after NST was less than 3% in patients with triple negative or HER2-positive disease who achieved a radiological complete response of the breast on MRI. These patients could be included in trials investigating the omission of SLNB after NST. In all patients that are described in chapter 9, axillary ultrasound and PET/CT was performed prior to the start of NST, and FNA was performed on suspicious nodes. Applying the results of this study could be challenging in a setting where PET/CT is not routinely used for axillary staging before NST. Validation of the present results in a cohort in which ultrasound is used for axillary staging before NACT is therefore warranted.

For patients with proven metastatic lymph nodes before NST (cN+), SLNB after NST remains a topic of discussions because a wide variation in identification rate (68-100%) and false-negative rate (5-30%) has been reported. The MARI procedure (Marking Axillary lymph nodes with Radioactive Iodine seeds) was developed at the Netherlands Cancer Institute (NKI) to stage the axilla after NST in cN+ breast cancer patients. In this technique, a tumor-positive axillary lymph node is marked with an iodine seed before NST and selectively removed after NST with a false-negative rate of 7% in predicting pCR in the additional lymph nodes. In **chapter 8,** we evaluated the potential of tailored axillary treatment, determined by combining the results of PET/CT before NST with those of the MARI procedure after NST. A cohort of cN+ patients was used to construct a hypothetical treatment algorithm based on a combination of results of the PET/CT and MARI procedure. In the algorithm, the number of FDG-avid axillary lymph nodes (1-3 versus ≥4) before NST and the tumor status of the MARI-node (positive versus negative) after NST are used to tailor axillary treatment. Patients with 1-3 FDG-avid axillary lymph nodes on PET/CT (cALN<4) and a tumor-negative MARI-node receive no further axillary treatment. Patients with 1-3 FDG-avid lymph nodes and a tumor-positive MARI-node receive local-regional

radiation treatment, as well as patients with ≥4 FDG-avid lymph nodes (cALN≥4) and a tumor-negative MARI node after NST. ALND is only performed in patients with ≥4 FDG-avid lymph nodes and a tumor-positive MARI-node. All patients in the cohort underwent ALND, allowing estimation of potential overtreatment and undertreatment. Results show that the algorithm would have resulted in 74% of patients foregoing ALND, with potential undertreatment in 3% of patients. This tailored axillary treatment protocol for cN+ patients was implemented at the NKI in 2014. In **chapter 9,** the results of the implementation of the protocol are described. From July 2014 until July 2017, 159 patients were treated according to the protocol. ALND was omitted in 130/159 patients (82%), local-regional radiotherapy was administered in 91 patients (57%) and 39 patients (25%) received no further axillary treatment. In **chapter 10**, the three-year axillary recurrence free interval (aRFI) was assessed of cN+ patients that underwent tailored and de-escalated treatment according to the MARI-protocol. After a median follow-up of three years, axillary recurrences occurred in a total of 5/272 patients (1.8%). As axillary recurrences especially occur at two years following treatment,[30-32] the high aRFI of 98% we found at a median follow-up of three years can be considered a significant result. Notably, pCR of the MARI-node occurred as least as frequently in cN2 patients as in patients with earlier stage disease (32% of cALN<4 vs. 44% cALN≥4; p=0.054). For cN2 patients, a combination of ALND and regional radiotherapy is still often recommended. When treating patients according to the MARI protocol, many patients can be spared the significant morbidity of ALND. Importantly, this thesis demonstrated that patients with the least favourable prognostic characteristis (cALN≥4 and residual disease post-NST) had the poorest survival compared to those with more favourable prognostic characteristics, despite extensive locoregional treatment consisting of ALND plus radiation treatment. While escalated surgery may contribute to improved locoregional disease control, most of the survival gain is to be expected of targeted systemic therapies.

FUTURE PERSPECTIVES

Response evaluation after neoadjuvant systemic therapy

Given the increasing options for de-escalation of locoregional therapy and escalation and de-escalation of adjuvant therapy, it is imperative to continue evaluating the optimal methods for response prediction and pathological assessment post-NST. RCB provides additional information compared to the binary outcome of pCR versus residual disease by quantifying the amount of residual disease. To optimize individualization of adjuvant therapy, prospective evaluation of RCB as part of standard pathology reporting after NST may be warranted. In poor or non-responders, treatment could be escalated by administering additional adjuvant systemic therapy (capecitabine and T-MD1). Contrary, it can be hypothesized that patients with an early complete response on imaging may not benefit from additional chemotherapy. In the TRAIN-3 trial, patients with stage II-III HER2-positive breast and with radiologic complete response on MRI after 3 or 6 cycles of NST proceed to surgery early.[33] This concept is also being studied in the current protocol of the adaptive I-SPY2 trial.[34]

Omission of surgery of the breast

Reliable assessment of residual disease is essential when considering omission of surgery, as breast cancer patients with residual disease post-NS could benefit from adjuvant systemic therapy. To this point, studies have not been able to demonstrate an acceptable low FNR of core and vacuum assisted biopsies in diagnosing pCR of the breast after NST. The FNR can be reduced by obtaining at least six large, representative vacuum-assisted biopsies under optimal imaging conditions. Although there are concerns about the diagnostic accuracy of breast biopsies, one trial has already started omitting surgery in excellent responders.[35] In this phase-2 study, breast surgery is omitted in patients with T1-2 Her2-positive and TN tumors, ≤4 lymph nodes and pCR in a minimum of 12 vacuum-assisted biopsies. Primary endpoints are 5-year ipsilateral breast tumor recurrence-free survival and overall survival. Preliminary results, presented at the American Society of Breast Surgeons 23rd Annual Meeting (2022), showed an early ipsilateral breast recurrence-free survival of 100%.

We will continue to investigate minimally invasive techniques predicting pCR. However, non-invasive response prediction models incorporating biomarkers and advanced MRI analysis may eventually outperform minimally invasive pCR detection methods. Studies have shown that

increased levels of tumor-infiltrating lymphocytes (TILs) are associated with higher response rates to NST and improved prognosis in patients with HER2-positive and triple negative breast cancer.[36] Also circulating tumor DNA (ctDNA) is increasingly being used a biomarker to predict response and prognosis. In the I-SPY2 trial, lack of ctDNA clearance was a significant predictor of poor response and metastastic recurrence, while clearance was associated with improved survival even in patients who did not achieve pCR.[37] Importantly, besides contributing to assessment of treatment response, ctDNA may help to fine-tune pCR as a surrogate endpoint of survival. In addition, genetic tumour profiling (e.g., 70-gene signature test [MammaPrint] and OncotypeDX) may be used as a prognostic biomarker. These signature tests have shown to accurately identify patients with a clinically high-risk but low genomic risk and can guide systemic treatment decisions in early breast cancer.[38-40] Potentially genetic tumour profiling could also be used to improve the selection of patients for omission of surgery by determining which patients have a high likelihood of achieving pCR and/or a low risk of disease recurrence.

The search into the optimal strategy for pCR prediction for emission of surgery of the breast is ongoing. One could argue that the lowest FNR possible should be pursued. However, it could be possible that missed minimal residual disease can be sufficiently controlled by either radiation treatment and/or adjuvant treatment, which is the current standard approach, even in patients with pCR. Moreover, when selecting patients for omission of surgery, the role of other factors that contribute to disease recurrence such as clinical tumor stage and involvement of lymph nodes should be taken into account.

Omission of axillary surgery

Axillary nodal stage (before and after NST) is an important prognostic predictor for recurrence and survival in breast cancer patients. Although breast pCR and axillary pCR are highly correlated, pCR of the breast does not guarantee pCR of the axillary nodes. In cN0 patients, SLNB can be performed accurately after NST. However, as demonstrated in this thesis, the probability is nodal positivity in cN0 patients after NST is low, especially in those with triple-negative of HER2-positive disease who achieve radiologic complete response (rCR) of the breast on MRI. To confirm the safety of omitting SLNB in these patients, we initiated the ASICS trial: Avoiding Sentinel lymph node biopsy In select Clinical node negative breast cancer patients after neoadjuvant Systemic therapy.[41] The primary endpoint is to evaluate whether SLNB can be safely omitted in cT1-3N0 breast cancer patients with HER2+ or triple-negative tumors who achieve rCR of the breast on MRI after NST, without compromising the 5-year axillary recurrence rate.

For cN+ patients, the optimal staging method after NST remains unclear. It is of great importance that residual axillary disease is detected, as omitting standard ALND is accompanied by the risk of leaving chemotherapy-resistant disease in situ. In addition, administering adjuvant systemic treatment in those with residual disease improves prognosis.[42,43] Conversely, patients with a pCR of the axillary lymph nodes are not expected to benefit from ALND. Even though the staging and treatment of the axilla in cN+ patients after NST remains an area of controversy, less invasive axillary strategies are the preferred policy in many institutions in the Netherlands. Randomized controlled trials that compare ALND to less invasive methods are therefore no longer feasible. However, exact strategies still vary widely among hospitals, indicating the need for evidence on the appropriate strategy for patients with axillary pCR or residual disease after NST.[44] The MINIMAX study is a Dutch registration study that will evaluate the oncologic safety at 5 and 10 years of different minimal and more invasive axillary staging and treatment protocols in cN1-3M0 patients undergoing NST. Patients who are treated according to MARI procedure are also included.

Omission of radiotherapy

Whole breast irradiation after breast-conserving therapy has long been known to contribute a substantial and significant reduction in locoregional recurrence.[45] The addition of a boost dose to the tumor bed provides a further benefit on the risk of recurrence, with an absolute reduction particularly evident in patients with unfavorable risk factors such as young age, high-grade tumors and involved surgical margins.[46,47] However, not all patients have a locoregional recurrence after BCS alone. In fact, it has been suggested that in specific subgroups, the 15-year risk of death due to breast cancer after omitting radiotherapy is extremely low.[47] Therefore, as with the omission of breast and axillary surgery, an interest has emerged in the omission or radiotherapy. Several studies are investigating the possibility of de-escalation of radiotherapy in selected low-risk breast cancer patients, primarily in elderly patients and those with node-negative and HR+ disease. These trials are of great importance, as radiotherapy of the breast and regional nodes can cause significant morbidity such as skin toxicity, lymphedema, pneumonitis and ischemic heart disease.[48,49] Current research focusing on de-escalation of radiotherapy includes partial breast irradiation, in which the target volume is reduced compared to whole-breast irradiation.[50]

Partial breast irradiation lowers the complication rate and improves cosmetic outcomes. In the TOP-1 study, radiotherapy after BCS is omitted in very-low risk cN0 patients of 70-years and older with small, low grade, HR-positive/HER2-negative tumors. The primary endpoint is locoregional recurrence at 5 years.

Other low-risk patients in whom radiotherapy may be de-escalated are patients who achieve pCR after NST. Although data is still limited, a large retrospective study demonstrated excellent 5-year local control after omission of radiotherapy in early-stage breast cancer patients with pCR after NST.[51] The DESCARTES study (De-ESCAlating RadioTherapy in breast cancer patients with pathologic complete response to neoadjuvant systemic therapy) was developed to investigate 5-year local recurrence in cT1-2N0 patients (all tumor subtypes) in whom radiotherapy is omitted when BCS and SLNB show pCR of the breast and lymph nodes after NST.[52]

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Chapter 12

Summary

Samenvatting

Summary

Breast cancer is the most frequently occurring cancer among women worldwide. Over the past three decades, the incidence rates of invasive breast cancer in the Netherlands have doubled. However, breast cancer mortality rates continue to decrease due to early detection and improvements in tailored systemic treatments. Systemic treatments in breast cancer patients are increasingly administered before surgery (i.e. neoadjuvant systemic therapy [NST]). The most important advantage of NST is the potential down-staging of the primary tumour and metastatic lymph nodes, permitting less extensive surgery in selected patients with good response to NST. Systemic treatments are adapted to patient and tumour characteristics, resulting in pathologic complete response (pCR) rates as high as 60% for triple-negative tumours and up to 90% for hormone-receptor (HR) negative, HER2-positive tumours.

Because of the improved breast cancer survival, it is imperative that the necessity and benefits of proposed treatments are continuously weighted up against the adverse consequences of these treatments. In patients with excellent response after NST, surgical de-escalation should be considered. To be able to select patients for de-escalation of locoregional treatments, adequate assessment of response is mandatory. In this thesis, multiple minimally and non-invasive strategies to de-escalate locoregional treatments in patients with exceptional response to NST are investigated. The ultimate aim of de-escalation of locoregional treatment is to improve quality of life of breast cancer patients without compromising locoregional disease control and survival.

Pathologic complete response after NST is strongly associated with improved long-term survival outcomes. However, with the binary outcome of pCR, value response information is lost. Therefore, the Residual Cancer Burden (RCB) method, that quantifies the amount of residual disease, was developed. RCB has been validated as prognostic in several single-institution studies and multicenter trials. In **chapter 2,** the results of a pooled subject-level analysis of multiple clinical cohorts and trials are presented. The aim was to evaluate the association between RCB and long-term outcomes with emphasis on breast cancer subtypes. Indeed, in this pooled analysis, we observed significant association between RCB and event free survival (EFS)/ disease recurrence free survival (DRFS) in the population as a whole, within all subtypes and

across all cohorts (except in the smallest cohort for EFS). Additionally, RCB remained prognostic in multivariate models adjusting for age, grade, and cT, and cN status at diagnosis. Importantly, the risk of a recurrence event increases with the extent of residual disease, regardless of subtype. These results emphasize the importance of measuring the extent of residual disease after NST, rather than only determining the binary outcome of pCR. As more adjuvant therapy options become available for patients with residual disease, a more refined estimate of an individual's risk of recurrence, based on their subtype and RCB, can be useful for decisions on adjuvant treatment selection.

Whereas increasing rates of pCR of invasive breast cancer (IBC) are being observed, ductal carcinoma in situ (DCIS) is considered insensitive to systemic treatment. Therefore, presence of DCIS adjacent to IBC may impede de-escalation of surgery. To facilitate potential de-escalation of surgery in the future in patients with adjacent DCIS, in **chapter 3**, we aim to estimate the response of adjacent DCIS to NST containing HER2-blockade in a large series of HER2-positive breast cancer patients. Furthermore we aim to identify clinicopathological and radiological factors that predict response of DCIS. Overall, DCIS was eradicated after NST in 46% of patients. Absence of calcifications on pre-NST mammography, treatment with dual HER2-blockade, a (near) complete response on MRI and absence of calcifications and Ki-67>20% in DCIS on pre-NST biopsy were significantly associated with DCIS response. The presence of extensive DCIS in HER2-positive breast cancer before NST should not always indicate a mastectomy, and the predictive factors that were found in this thesis could be useful when considering breast-conserving surgery (BCS) in these patients.

While mastectomy used to be standard of care in patients with breast cancer, BCS is nowadays recommended for most patients with early-stage breast cancer. However, in patients with large breast tumours, BCS after NST remains controversial. The selection of patients for BCS should be based on whether tumour-free margins can be achieved. Therefore, reliable assessment of residual disease is essential. Magnetic resonance imaging (MRI) has been demonstrated to be the most adequate imaging modality to evaluate the presence or extent of residual disease after NST. In **chapter 4**, the safety of breast conserving therapy (BCS + radiation treatment) after NST in cT3 breast cancer patients was investigated. In all patients, MRI was used to assess the presence of residual tumour during and after NST. It is demonstrated that breast-conserving therapy (consisting of BCS and radiation treatment) following MRI evaluation before and after NST in cT3 breast cancer patients is feasible with a success rate of 82%. Local control in these patients is excellent with a 7-year local-recurrence free survival of 96%. Patients with HR-positive/HER2-negative or lobular tumors, or tumors that initially presented as non-mass enhancement on MRI are more likely to have positive margins at BCS. With this study, we confirm the safety of BCS after NST in cT3 breast cancer patients with a good response to NST, despite the fact that the original tumor bed is not entirely excised in these patients. It can be hypothesized that it is safe to refrain from removing tissue that originally contained tumor but is free of tumor after NST. Extrapolating this concept, it may be possible to omit breast surgery at all in patients with pCR of the breast. However, until now, no method other than complete pathological assessment of the surgical breast specimen after NST has been demonstrated to be accurate at determining the presence of pCR. The MICRA trial (Minimally Invasive Complete Response Assessment) was designed to determine whether core biopsies of the breast are sufficiently accurate at differentiating between pCR and residual disease in patients with an excellent response on MRI after NST. In **chapter 5** of this thesis the study design and feasibility is presented. In the MICRA trial, patients with a pre-NST placed marker and a partial or complete response on MRI after NST are included. In all patients, ultrasound-guided 14G core biopsies of the original tumor bed are obtained prior to breast surgery. Pathology results of the biopsies and surgical specimens are compared. The primary endpoint of the MICRA trial is the false-negative rate of the biopsies in identifying pCR. During the first year of the trial, performing ultra-sound guided biopsy of the breast appeared feasible. In the majority of patients, the marker could be identified. A median of 8 biopsies could be obtained and the median of histopathological representative biopsies was 4. Preliminary results of the MICRA trial are demonstrated in **chapter 6**. Unfortunately, core biopsies are not accurate enough to allow safe omission of surgery. Residual disease was missed in 37% of patients without breast pCR.

As with breast surgery, axillary surgery has undergone multiple changes in the last few decades. In patients with clinically node-negative (cN0) breast cancer, sentinel lymph node biopsy (SLNB) has replaced axillary lymph node dissection (ALND). However, the nodal positivity rate (ypN+) after NST is low, especially in those with pCR of the breast. In these patients, the value of performing surgical axillary staging after NST could be limited. However, presence of breast pCR is not routinely known prior to surgery. In **chapter 7**, the correlation of breast pCR and the absence of tumor-positive lymph nodes (ypN0) after NST in cN0 patients is validated. In addition, we investigate preoperatively known predictive characteristics for ypN0. It was found

that the probability of nodal positivity after NST was less than 3% in patients with triple negative or HER2-positive disease who achieved a radiological complete response of the breast on MRI.

In patients with positive lymph nodes before NST (cN+), a wide variation in identification rate (68-100%) and false-negative rate (5-30%) of SLNB has been reported. The MARI procedure (Marking Axillary lymph nodes with Radioactive Iodine seeds) was developed to stage the axilla after NST in cN+ breast cancer patients. In this technique, a tumor-positive axillary lymph node (ALN) is marked with an iodine seed before NST and selectively removed after NST with a false-negative rate of 7% in predicting pCR in the additional lymph nodes. In **chapter 8**, we evaluated the potential of tailored axillary treatment, determined by combining the results of PET/CT before NST with those of the MARI procedure after NST. A cohort of cN+ patients was used to construct a hypothetical treatment algorithm based on a combination of results of the PET/CT and MARI procedure. In the algorithm, the number of FDG-avid ALNs (1-3 versus ≥4) before NST and the tumor status of the MARI-node after NST are used to tailor axillary treatment. Patients with 1-3 FDG-avid ALNs on PET/CT and a tumor-negative MARI-node receive no further axillary treatment. Patients with 1-3 FDG-avid ALNs and a tumor-positive MARI-node receive local-regional radiation treatment, as well as patients with ≥4 FDG-avid ALNs and a tumor-negative MARI node after NST. ALND is only performed in patients with ≥4 FDG-avid ALNs and a tumor-positive MARI-node. All patients in the cohort underwent ALND, allowing estimation of potential overtreatment and undertreatment. Results show that the algorithm would have resulted in 74% of patients foregoing ALND, with potential undertreatment in 3% of patients. In **chapter 9**, the results of the implementation of the protocol are described. From July 2014 until July 2017, 159 patients were treated according to the protocol. ALND was omitted in 130 patients (82%). Local-regional radiotherapy was administered in 91 patients (57%) and 39 patients (25%) received no further axillary treatment. In **chapter 10**, we assess the 3-year axillary recurrence free interval (ARFi) of cN+ patients that were treated according to the MARI-protocol. After a median follow-up of three years, axillary recurrences occurred in 5/272 patients (1.8%), while the overall recurrence risk remained highest for patients with ALND (hazard ratio 4.36). These results show that de-escalation of axillary treatment is possible in node-positive patients with good response to NST.

Samenvatting

Borstkanker is wereldwijd de meest voorkomende vorm van kanker onder vrouwen. Gedurende de laatste drie decennia is de incidentie van borstkanker in Nederland verdubbeld. Door vroegtijdige detectie met behulp van screening en verbeteringen in systemische therapie (hormonale, chemo- en immunotherapie) is de borstkanker mortaliteit echter gedaald. Indien er bij patiënten met borstkanker een indicatie bestaat voor systemische therapie, wordt deze steeds vaker voorafgaand aan de operatie toegediend (neoadjuvante systemische therapie; NST). Het belangrijkste voordeel van NST is het verkleinen of zelfs een pathologisch complete respons (pCR) van de tumor in de borst en axillaire lymfekliermetastasen. Systemische behandelingen worden toegespitst op patiënt- en tumorkarakteristieken, waarmee stijgende percentages pathologisch complete respons (pCR) worden bereikt. Bij patiënten met triple-negatieve tumoren wordt in 60% een pCR gevonden en bij patiënten met hormoon-receptor (HR)-negatieve, HER2-positieve tumoren wordt tot 90% pCR gezien.

Aangezien de overleving van patiënten met borstkanker verbetert, is het tegenwoordig steeds belangrijker om de kwaliteit van leven van patiënten voorop te stellen. Daartoe moeten de noodzakelijkheid en voordelen van behandelingen af worden gewogen tegen de nadelige gevolgen. Bij patiënten met goede respons op NST moet de-escalatie van de locoregionale behandeling overwogen worden. Om te kunnen beoordelen bij welke patiënten de-escalatie van locoregionale therapie mogelijk is, is nauwkeurige evaluatie van respons noodzakelijk. In dit proefschrift worden verschillende minimaal en non-invasieve methodes voor responsbeoordeling onderzocht, om de-escalatie van locoregionale therapie na NST mogelijk te maken. Het ultieme doel is om de kwaliteit van leven van patiënten met borstkanker te verbeteren met behoud van locoregionale ziektecontrole, en zonder de overleving in gevaar te brengen.

Een pCR is geassocieerd met een betere overleving. De uitkomst van pCR is echter binair (residuale ziekte versus geen residuale ziekte) en hiermee gaat veel waardevolle informatie verloren. In tegenstelling tot pCR, kwantificeert de “Residual Cancer Burden” (RCB) de hoeveelheid residuale ziekte. RCB is gevalideerd als een voorspeller voor prognose in een aantal uni- en multicenter studies. In **hoofdstuk 2** van dit proefschrift worden de resultaten van een gepoolde analyse naar RCB gepresenteerd. Het doel van deze studie was om de associatie tussen RCB en prognose

bij de verschillende subtypes van borstkanker te valideren. Er werd inderdaad een significante associatie tussen RCB en overleving waargenomen in het gehele cohort en bij alle subtypes van borstkanker. Het risico op een recidief nam toe naarmate de hoeveelheid residuale ziekte toenam. Ook bleef RCB een prognostische voorspeller in een multivariate analyse waarbij werd gecorrigeerd voor leeftijd, tumorgradering en cT en cN status voorafgaand aan NST. Deze studie benadrukt het belang van het kwantificeren van residuale ziekte na NST.

Waar er in toenemende mate een pCR wordt gezien, wordt er aangenomen dat ductaal carcinoma in situ (DCIS) niet reageert op NST. De aanwezigheid van DCIS wordt dan ook vaak gezien als een contra-indicatie voor borstsparende chirurgie, ook al is de invasieve component geheel verdwenen na NST. Om te evalueren of de-escalatie van lokale chirurgie in de toekomst mogelijk is bij vrouwen met DCIS, wordt in **hoofdstuk 3** onderzocht of DCIS reageert op trastuzumab-bevattende NST bij patiënten met HER2-positieve tumoren. Daarnaast is er onderzocht of bepaalde klinische en weefsel-factoren geassocieerd zijn met respons na NST. Bij 46% van de patiënten werd een complete pathologische respons van de DCIS component gezien. Afwezigheid van verdachte verkalkingen op mammografie, behandeling met zowel trastuzumab als pertuzumab, een (bijna) complete respons op MRI, en afwezigheid van calcificaties en Ki-67>20% in het biopt voorafgaand aan NST waren significant geassocieerd met DCIS respons. De resultaten van deze studie geven aan dat DCIS wel degelijk respondeert bij patiënten met HER2-positieve tumoren, en dat uitgebreide DCIS voorafgaand aan NST niet altijd een mastectomie impliceert.

Bij patiënten met een vroeg-stadium borstkanker is regelmatig een borstsparende operatie mogelijk. Bij patiënten met grotere tumoren blijft borstsparende therapie (BST) na NST echter controversieel. Om patiënten te kunnen selecteren bij wie BST mogelijk is, is adequate evaluatie van de aanwezigheid en hoeveelheid residuale ziekte noodzakelijk. Het is aangetoond dat MRI de meest betrouwbare beeldvorming is om de uitgebreidheid van residuale ziekte aan te tonen. In **hoofdstuk 4** wordt de veiligheid van BCT na NST bij cT3 borstkankerpatiënten onderzocht. Bij alle patiënten werd gebruik gemaakt van MRI om de respons van de tumor te beoordelen. Er kon worden geconcludeerd dat BCT na NST bij 82% van de cT3 patiënten mogelijk was, met een 7-jaars lokaal recidief-vrije overleving van 96%. Patiënten met hormoon-receptor positieve/HER2-negatieve tumoren of lobulaire tumoren hadden een hoger risico op positieve snijvlakken, evenals tumoren met “non-mass enhancement” op MRI voorafgaand aan NST. Deze studie bevestigt dat het veilig is om BCT uit te voeren bij patiënten met cT3 tumoren met goede respons, ondanks dat het complete originele tumorbed niet wordt verwijderd. Op basis van deze studie zou kunnen worden aangenomen dat het veilig is om weefsel waar zich oorspronkelijk tumor bevond, maar na NST vrij is van tumor, in situ te laten. In dat geval zou het ook mogelijk moeten zijn om mammachirurgie compleet achterwege te laten in geval van een pCR van de borst. Tot nu toe is een operatie van de borst echter nog de enige manier om betrouwbaar de mate van respons vast te stellen. Daarom is er behoefte aan een nieuwe techniek die minder invasief of niet-invasief patiënten met een pCR kan identificeren. In de MICRA studie (“Minimally Invasive Complete Response Assessment”) wordt onderzocht of biopten betrouwbaar de aanwezigheid van een pCR kunnen vaststellen bij patiënten met een zeer goede respons van de borsttumor op MRI na NST. Bij alle patiënten wordt voorafgaand aan NST een jodiummarker centraal in het tumorbed geplaatst. Na NST werden meerdere 14-gauge echogeleide biopten verkregen dichtbij de marker, gevolgd door lokale excisie. De belangrijkste uitkomstmaat was het fout-negatief percentage (FNR) van de biopten in het beoordelen van een pCR. In **hoofdstuk 5** wordt het studieprotocol en de uitvoerbaarheid van de studie gepresenteerd. Tijdens het eerste jaar van de studie bleek dat de studie goed uitvoerbaar was. Bij de meerderheid van de patiënten kon de marker worden gedetecteerd. Er werden gemiddeld 8 biopten afgenomen, waarvan 4 biopten histopathologisch representatief waren. In de interim-analyse van de studie (**hoofdstuk 6**) bleek helaas dat biopten niet nauwkeurig genoeg pCR voorspellen om een operatie veilig achterwege te kunnen laten. Residuale tumor werd gemist bij 37% van de patiënten zonder pCR.

Er is niet alleen sprake van toename van borstsparende chirurgie bij borstkankerpatiënten; ook de behandeling van de axilla is de afgelopen decennia veranderd. Waar aanvankelijk bij iedere patiënt een okselklierdissectie (OKD) werd uitgevoerd, heeft de schildwachtklier (SWK)-procedure de OKD vervangen bij patiënten zonder verdachte axillaire lymfeklieren (cN0). Bij cN0 patiënten die NST ondergaan is het percentage patiënten met positieve klieren na NST echter laag, met name bij patiënten met een pCR van de borsttumor. Bij deze patiënten heeft het uitvoeren van chirurgische axillaire stadiering na NST weinig toegevoegde waarde. We weten echter niet exact bij welke patiënten een pCR na NST optreedt. In **hoofdstuk 7** hebben we de associatie tussen pCR en de afwezigheid van tumor-positieve klieren na NST gevalideerd. Daarnaast wordt er aangetoond dat <3% van de patiënten met triple-negatieve of HER2-positieve tumoren bij wie tevens sprake was van een radiologisch complete respons van de borsttumor op MRI, tumor-positieve axillaire klieren hadden.

Over de behandeling van de axilla na NST bij patiënten met axillaire lymfeklier-metastasen (cN+) voorafgaand aan NST bestaat nog veel discussie, aangezien de SLNB bij cN+ patiënten minder betrouwbaar is. Om deze reden wordt een OKD mogelijk vaker uitgevoerd dan nodig. In het Antoni van Leeuwenhoek Ziekenhuis (AvL) is een nieuwe techniek geïntroduceerd om de axilla te restadiëren na NST bij cN+ patienten: “de MARI-procedure” (Marking Axillary lymph nodes with Radioactive Iodine seeds). Voor aanvang van NST wordt de grootste van de aangedane axillaire lymfeklieren echogeleid gemarkeerd met een radioactieve jodiumbron (MARI-klier). Na voltooien van NST wordt de MARI-klier selectief verwijderd met behulp van een gamma-probe. In een eerdere studie is aangetoond dat de MARI-klier een zeer betrouwbare voorspeller van respons in de additionele axillaire lymfeklieren is, met een fout-negatief percentage van 7%. In het AvL is een nieuw behandelprotocol geïmplementeerd voor cN+ patiënten, waarbij de behandeling is gebaseerd op resultaten van de PET/CT voorafgaand aan NST, in combinatie met resultaten van de MARI-procedure na NST. Patiënten met 1-3 FDG-avide axillaire lymfeklieren voorafgaand aan NST en een tumornegatieve MARI-klier ondergaan geen aanvullende axillaire behandeling (radiotherapie en/of OKD). Patiënten met 1-3 FDG-avide lymfeklieren voorafgaand aan NST en een tumorpositieve MARI-klier na NST worden behandeld met axillaire radiotherapie (ART), evenals patiënten met >3 FDG-avide lymfeklieren en een tumor-negatieve MARI-klier. Patiënten met >3 FDG-avide lymfeklieren voorafgaand aan NST en een tumorpositieve MARI-klier na NST ondergaan een OKD. In een cohort van 93 patiënten behandeld met NST, hebben wij bovenstaand protocol geanalyseerd (**hoofdstuk 8**). In totaal zouden 74 patiënten correct zijn behandeld (80%), 3 patiënten onderbehandeld (3%) en 16 patiënten mogelijk overbehandeld (17%). Naar aanleiding van deze analyse is bovenstaand protocol in 2014 geïmplementeerd in het AvL. Waar aanvankelijk alle cN+ patiënten na NST een OKD ondergingen, laat een nieuwe analyse zien dat het aantal OKD’s bij deze patiënten met 82% is gereduceerd (**hoofdstuk 9**). Bovendien werd bij ongeveer een kwart van de patiënten geen aanvullende okselbehandeling meer gegeven. Het drie-jaars recidiefvrije interval van de axilla (aRFI) wordt gepresenteerd in **hoofdstuk 10.** Na een mediane follow-up van 3 jaar trad een axillair recidief op bij slechts 2% van de patiënten (n=5). Het totale recidief percentage was het hoogst voor patiënten met een ALND. Hoewel de meeste locoregionale recidieven optreden binnen drie jaar na behandeling, zal een langere follow-up nodig zijn om deze resultaten te bevestigen.

PhD portfolio

List of publications

Appendices

Dankwoord

Curriculum vitae

PHD PORTFOLIO

PhD Student	M.E.M. van der Noordaa
PhD Period	November 2015 – January 2020
PhD Supervisors	Prof. E.J.T. Rutgers
	Dr. M.T.F.D. Vrancken Peeters
	Dr. F.H. van Duijnhoven
	Prof. L.J. van 't Veer

PHD TRAINING

General courses	**Year**	**ECTS**
OOA Retreat	2016	1
Basic Medical Statistics	2016	1,5
Good Clinical Practice	2016	0,5
Pre-IMPAKT Training Course: Translational Research	2017	1,5
Seminars, workshops and master classes		
Medical Business Masterclass	2016	0,9
Value Based Healthcare Masterclass	2017	0,9
Workshop ^{125}I-guided Breast Surgery	2017	0,3
Athena Breast Health Network Retreat	2018	0,6
I-SPY 2.2 Retreat	2018	0,6
I-SPY 2 Retreat	2018	0,9
Food and Drug Administration (FDA) Workshop	2018	0,9
Breast Oncology Program Retreat	2020	0,9
Quantitative Biology of the Cancer Cell Symposium	2020	0,3

Presentations (oral)		
"Selective elimination of axillary surgery after NAC in cN+ breast cancer by combining PET/CT and the MARI procedure" *Bossche Mamma Congres, Sint-Michielsgestel, the Netherlands*	2016	0,5
"Chirurgie op maat na neoadjuvant chemotherapie bij het mammacarcinoom" *Refereeravond chirurgie NKI-AVL, Amsterdam*	2016	0,5
"Selective elimination of axillary surgery after neoadjuvant systemic therapy in cN+ breast cancer by combining PET/CT and the MARI procedure" *European Society of Surgical Oncology (ESSO) Congress, Krakow, Poland*	2016	0,5
"MICRA trial: Minimally Invasive Complete Response Assessment of the breast after neoadjuvant systemic therapy" *Dutch Breast Surgeons Course, Amsterdam, the Netherlands*	2017	0,5
"MICRA trial: Minimally Invasive Complete Response Assessment of the breast after neoadjuvant systemic therapy" *European Congress of Radiology (ECR), Vienna, Austria*	2017	0,5
"MICRA trial: Minimally Invasive Complete Response Assessment of the breast after neoadjuvant systemic therapy" *Nederlandse Vereniging voor Heelkunde (NVvH) Chirurgendagen, Veldhoven*	2017	0,5
"Sentinel node procedure bij cNo patienten na neoadjuvante systeem-therapie; werkt het?" *Mammacongres Harderwijk, Harderwijk*	2018	0,5
"Towards Omitting sentinel lymph node biopsy after neoadjuvant systemic therapy in selected cNo breast cancer patients" *European Breast Cancer Conference (EBCC), Barcelona, Spain*	2018	0,5
"Breast conserving therapy after neoadjuvant systemic treatment in patients with cT3 breast cancer is feasible" *Bossche Mamma Congres, Sint-Michielsgestel*	2018	0,5
"Can we identify ypNo breast cancer patients prior to surgery?" *I-SPY Retreat, Washington, United States*	2018	0,5
"Residual cancer burden after neoadjuvant therapy and long-term survival outcomes in breast cancer: a multi-center pooled analysis" *San Antonio Breast Cancer Conference (SABCS), San Antonio, USA*	2019	0,5
"Assessing prognosis after neoadjuvant therapy: A comparison between anatomic ypAJCC staging, Residual Cancer Burden Class and Neo-bioscore" *SABCS, San Antonio, USA*	2020	0,5

Presentations (poster)

"Towards omitting sentinel lymph node biopsy after neoadjuvant systemic therapy in selected cN0 breast cancer patients" *European ESSO Congress, Krakow, Poland*	2016	0,5
"Selective elimination of axillary surgery after neoadjuvant systemic therapy in cN+ breast cancer by combining PET/CT and the MARI procedure" *Oncology Graduate School Amsterdam (OOA) Retreat, Renesse, the Netherlands*	2016	0,5
"Towards omitting sentinel lymph node biopsy after neoadjuvant systemic therapy in selected cN0 breast cancer patients" *American Society of Clinical Oncology (ASCO) Conference, Chicago, USA*	2017	0,5
"Breast conserving therapy after neoadjuvant systemic treatment in patients with cT3 breast cancer is feasible" *EBCC, Barcelona, Spain*	2018	0,5
"Towards Omitting sentinel lymph node biopsy after neoadjuvant systemic therapy in selected cN0 breast cancer patients" *Bossche Mamma Congres, Sint-Michielsgestel, the Netherlands*	2018	0,5
"Role of breast MRI in predicting pathologically negative nodes after neoadjuvant chemotherapy in cN0 breast cancer patients in the I-SPY2 trial" *Poster Discussion, SABCS, San Antonio, USA*	2018	0,5
"Towards omitting breast cancer surgery in patients with pathologic complete response after neoadjuvant systemic therapy: the MICRA trial" *SABCS, San Antonio, USA*	2018	0,5
"Breast conserving therapy after neoadjuvant systemic treatment in patients with cT3 breast cancer is feasible", *SABCS, USA*	2018	0,5

(Inter)national conferences attended

Conferences attended

Dutch Breast Cancer Course, Valkenburg, The Netherlands	2016	0,6
European Breast Cancer Conference (EBCC), Amsterdam, the Netherlands	2016	0,6
Nederlandse Vereniging voor Heelkunde (NVvH) Chirurgendagen, Veldhoven, the Netherlands	2016	0,6
Bossche Mamma Congres, Sint-Michielsgestel, the Netherlands	2016	0,6
European Society of Surgical Oncology (ESSO) Congress, Krakow, Poland	2016	0,6
Mammasymposium NKI-AVL, Amsterdam, the Netherlands	2016	0,3
Dutch Breast Surgeons Course, Amsterdam, the Netherlands	2017	0,6
The European CanCer Organisation (ECCO) Congress, Amsterdam, the Netherlands	2017	0,6
European Congress of Radiology (ECR), Vienna, Austria	2017	0,6
Nederlandse Vereniging voor Heelkunde (NVvH) Chirurgendagen, Veldhoven, the Netherlands	2017	0,6
American Society of Clinical Oncology (ASCO) Conference, Chicago, United States	2017	0,6
Mammasymposium NKI-AVL, Amsterdam, the Netherlands	2017	0,3
Mammacongres Harderwijk, Harderwijk, the Netherlands	2018	0,6
European Breast Cancer Conference (EBCC), Barcelona, Spain	2018	0,6
Bossche Mamma Congres, Sint-Michielsgestel, the Netherlands	2018	0,6
San Antonio Breast Cancer Conference (SABCS), San Antonio, United States	2018	0,6
Borstkanker Behandeling Beter (BBB) Congres, Rotterdam, The Netherlands	2020	0,3
San Antonio Breast Cancer Conference (SABCS), San Antonio, United States	2020	0,6

Meetings attended

Quarterly Mammatumoren werkgroep (MTWG), NKI-AVL, Amsterdam, the Netherlands	2015-2018	2
Weekly Multidisciplinair overleg (MDO), NKI-AVL, Amsterdam, the Netherlands	2015-2020	4
Monthly Section XI meeting, NKI-AVL, Amsterdam	2015-2018	0,9
EORTC Breast Cancer Group Meeting, Amsterdam, the Netherlands	2016	0,6
Borstkanker Onderzoek Groep Meeting, Utrecht, the Netherlands	2017-2018	0,6
Integraal Kankercentrum Nederland (IKNL) meeting, Leiden, the Netherlands	2018	0,3
Borstkanker Onderzoek Groep Meeting, Utrecht, the Netherlands	2017-2018	0,6
Weekly Breast Cancer Tumor Board, University of San Francisco Califonia (UCSF), United States	2018-2019	1
Monthly Breast Oncology Program, University of San Francisco Califonia (UCSF), United States	2018-2019	1

Research protocols written

Minimally Invasive Complete Response Assessment of the breast after neoadjuvant systemic therapy (MICRA trial)	2015-2016	8

Scientific grants written

Towards selective elimination of surgery of the breast and axilla in patients with pathologic complete response after primary systemic treatment *Koningin Wilhelmina Fonds Kankerbestrijding, not honored*	2016	10
Towards selective elimination of surgery of the breast and axilla in patients with pathologic complete response after primary systemic treatment *Department of Defense Breast Cancer Research Program (BCRP) for the Office of the Congressionally Directed Medical Research Programs (CDMRP), not honored*	2016	4

A

Towards omitting breast and axillary surgery in breast cancer patients who achieve a pathologic complete response after neoadjuvant systemic therapy *Heelkundige Oncologische Disciplines (HOD) startgeld, not honored*	2017	1
Towards optimal response prediction of the breast and axilla in patients treated with neoadjuvant systemic therapy *Koningin Wilhelmina Fonds Kankerbestrijding, not honored*	2017	10
Towards omitting breast surgery in patients with pathologic complete response after neoadjuvant systemic therapy: the MICRA trial (Minimally Invasive Complete Response Assessment) *Innovatiefonds Zorgverzekeraars (IFZ), honored (€287.423)*	2018	4

Teaching

Supervising		
R. Voorthuis (research intern)	2016-2017	2,6
F.N.E. Cuijpers (research intern)	2017	2

Parameters of esteem

Awards	
American Association for Cancer Research (AACR) Clinical Scholar Award, San Antonio Breast Cancer Conference	2018
American Association for Cancer Research (AACR) Clinical Scholar Award, San Antonio Breast Cancer Conference	2020

Travel grants	
Stichting Prof. Michael-van Vloten	2017
Rene Vogels Stichting	2017
Stichting De Drie Lichten	2017
Jo Kolk Studiefonds	2017
Stichting Nijbakker Morra	2017
Stichting Nijbakker Morra	2019

LIST OF PUBLICATIONS

Publications related to this thesis

van Loevezijn AA, **van der Noordaa MEM,** van Duijnhoven FH et al. Three-year follow up of de-escalated axillary treatment after neoadjuvant systemic therapy in clinically node-positive breast cancer: the MARI protocol. Breast Cancer Res Treat. 2022 May; 193(1):37-48.

Yau C*, Osdoit M*, **van der Noordaa MEM**, et al. Residual cancer burden after neoadjuvant chemotherapy and long-term survival outcomes in breast cancer: a multicenter pooled analysis of 5161 patients. Lancet Oncol. 2022 Jan; 23(1):149-160.

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Other publications

van Loevezijn AA, Stokkel MPM, Donswijk ML, van Werkhoven ED, **van der Noordaa MEM,** et al. [^{18}F]FDG-PET/CT in prone compared to supine position for optimal axillary staging and treatment in clinically node-positive breast cancer patients with neoadjuvant systemic therapy. EJNMMI Res. 2021 Aug 21;11(1):78.

Du L, Yau C, Brown-Swigart L, Gould R, Krings G, Hirst GL, Bedrosian I, Layman RM, Carter JM, Klein M, Venters S, Shad S, **van der Noordaa MEM** et al. Predicted sensitivity to endocrine therapy for stage II-III hormone receptor-positive and HER2-negative (HR+/HER2-) breast cancer before chemo-endocrine therapy. Ann Oncol. 2021 May;32(5):642-651.

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In preparation

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Hellingman D, **van der Noordaa MEM**, et al. Intratumoral heterogeneity on pretreatment 18F-FDG PET/CT is not associated with pathologic complete response to neoadjuvant systemic treatment in stage II/III triple-negative and HER2-positive breast cancer. Submitted

Cordeiro PG, Scott AM, **van der Noordaa MEM**, et al. Does the Baker Classification System, a Clinician-Reported Outcome Measure, correlate with the BREAST-Q, a Patient-Reported Outcome Measure? Submitted

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A

CURRICULUM VITAE

Marieke Emma Marguerite van der Noordaa was born on April 9th, 1989, in Amsterdam, the Netherlands. She graduated from Stedelijk Gymnasium Haarlem (grammar school) in 2007, after which she spent a year as a junior intern in Karapitiya Hospital in Sri Lanka and as a volunteer in Central America. In 2008, she started medical school at the University of Amsterdam (UvA-AMC) and developed an interest in oncology. Therefore, during her studies she worked on research projects at the Departments of Surgery and Radiology at the Netherlands Cancer Institute (NKI), and at the Department of Plastic and Reconstructive Surgery at Memorial Sloan Kettering Cancer Center in New York, USA. During the last year of her medical degree, she completed senior internships at the Departments of Medical Oncology and Surgical Oncology at NKI.

Marieke obtained her medical degree at UvA-AMC in 2016, after which she started working as a PhD candidate at the Department of Surgery at NKI under supervision of prof. dr. Marie-Jeanne Vrancken Peeters, prof. dr. Emiel Rutgers and dr. Frederieke van Duijnhoven. Her PhD research primarily focused on the de-escalation of locoregional treatment after neoadjuvant systemic therapy in breast cancer. She co-initiated and coordinated the MICRA trial, which investigates whether biopsies can replace surgery of the breast after neoadjuvant chemotherapy in patients with excellent response on MRI, to confirm pathological complete response. In addition, Marieke worked on several projects investigating de-escalation of regional therapy, including the MARI protocol for node-positive breast cancer patients and the foundation for the ASICS study for node-negative patients. In 2018, Marieke continued her research at the University of California, San Francisco (UCSF), for which she received multiple travel grants. At UCSF, she worked on several projects investigating the Residual Cancer Burden under the supervision of prof. dr. Laura van 't Veer and prof. dr. Laura Esserman. Marieke was awarded for her research twice with the Clinical Scholar Award from the American Association for Cancer Research.

During her PhD, Marieke provided medical care in a refugee camp in Lesbos, Greece. In 2020, she started working as a surgical resident not in training at NKI, after which she continued as a gynaecology resident not in training at Spaarne Gasthuis in Haarlem. Since 2022, Marieke is working as a gynaecology resident not in training at Onze Lieve Vrouwe Gasthuis in Amsterdam.

Marieke lives together with Ilan Nir and their son Eli in Bussum, the Netherlands. Marieke and Ilan are expecting their second son in June 2023.