Personalizing endocrine therapy for breast cancer using MRI

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(met een samenvatting in het Nederlands)

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Contents

CHAPTER 1

Introduction

Breast cancer

Breast cancer is the cancer with the highest incidence in women worldwide, including the Netherlands, and ranks first for most cancer-related deaths in the developing world and second in the developed world[1]. The Netherlands Comprehensive Cancer Organisation (IKNL) reported 14748 invasive breast cancer diagnoses in 2018 in the Netherlands[2]. The incidence of breast cancer has increased over the years, and is expected to increase by 46% (relative to 2018) by 2040[2,3]. This increase is partly due to an aging population and lifestyle changes, e.g. obesity is associated with post-menopausal breast cancer diagnosis[3], but also due to more sophisticated breast cancer screening strategies[4,5].

Together with screening and improved locoregional treatment (i.e., surgery and radiotherapy), adjuvant systemic therapy (AST) has led to a decrease in mortality of breast cancer patients: 10-year overall survival increased from 68% in 1991-2000 to 76% in 2001-2010[2,6]. There are different kinds of AST: endocrine, targeted, and chemotherapy. If, and what type of AST a patient receives depends on several clinicopathologic variables, such as age and tumor size, but also on the immunohistochemical (IHC) subtype of the tumor. The IHC subtype is especially important because it can be used as a predictive marker of therapy effectiveness[7]. It is classified through the expression of the estrogen- and the human epidermal growth factor-2 (HER2) receptor on the tumor. If a tumor expresses the estrogen receptor, i.e. is ER+, then endocrine therapy can be prescribed, similarly, if a tumor expresses HER2+ targeted therapy can be prescribed. Chemotherapy can be prescribed to all IHC subtypes.

ER+-breast cancer represents the largest proportion of the IHC subtypes, about 80% of all breast cancer are ER+[8], and many of these patients receive endocrine therapy. The indication for endocrine therapy as recommended by guidelines has increased over the years, and has included increasingly favorable prognostic profiles: 23% of patients diagnosed in 1990 in the Netherlands received endocrine therapy, which increased to 56% by 2012[9]. Although survival has increased over the years, there are now concerns about overtreatment[10]. A patient can be considered overtreated if she did not derive any survival benefit from AST, because she would have died despite AST, or, alternatively, because she would have also survived without AST. A patient who is overtreated is exposed to AST-related sided effects without any survival benefit. Side effects include sexual dysfunction, cognitive

and musculoskeletal problems[11–13]. A study performed in patients active in online breast cancer communities found that 91.2% experienced side-effects and that one-third of patients discontinued therapy[14]. Hence, it is important to consider the detrimental effects of AST as the considerable side-effects can be life-threatening and have a negative effect on the quality of life[11,13,15].

Guidelines can help treatment decisions by identifying patients at such high risk that AST is expected to improve survival significantly to justify the exposure to AST-related side-effects. However, such expectations are derived from clinical trials where results are aggregated over large groups of patients[16–18]. Breast cancer is a heterogeneous disease, and it is likely that personalized risk stratification is possible beyond the currently identified risk groups[19]. Indeed, gene expression assays, such as the MammaPrint and the Oncotype DX, can successfully identify patients who have a high clinical risk (based on the standard clinicopathologic variables) but a low genomic risk (based on the expression of certain high risk genes by the tumor)[6,7,19–21]. Large randomized clinical trials have shown that patients with high clinical risk, but low genomic risk, can be safely spared chemotherapy without concessions to survival benefit[6,7,22]. However, this is only pertinent for chemotherapy in ER+/HER2- patients, no clinically validated options exist for endocrine therapy in ER+ patients, even though they represent the largest proportion of patients and the largest subset of administered AST[10,23]. It has been hypothesized that risk stratification based on dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) of the breast can personalize endocrine therapy in these patients[24–27].

MRI of the Breast

MRI of the breast consists of different sequences that image different properties of the breast tumor. One the most important sequences of the breast MRI is the dynamic contrast-enhanced series: after intravenous injection of a contrast (based on gadolinium) the breast is imaged at specific time intervals[28]. This dynamic way of imaging provides information about the behavior of the tumor beyond the size and location[28]. An interesting imaging feature that has received attention recently is the enhancement of the healthy fibroglandular tissue after contrast injection on breast MRI. This parenchymal enhancement on MRI was found to be associated with future breast cancer risk[29–31], but also with tumor response (in

the other breast) after neoadjuvant therapy[32–38], and after adjuvant therapy[24,25,39,40]. Apparently, the healthy fibroglandular tissue contains information about tumor properties, the prognosis of patients, and maybe even therapy effectiveness[24–26].

Contralateral Parenchymal Enhancement

Specifically for ER+/HER2- breast-cancer, contralateral parenchymal enhancement (CPE), a quantitative measure of parenchymal enhancement of the contralateral breast on breast MRI, was found to be associated with survival and could potentially be used for risk stratification and personalization of endocrine therapy[24–26,41–43]. It is calculated by the ratio of enhancement in the 10% most enhancing voxels between the early post-contrast images (after approximately 90 s) and the late post-contrast images (after approximately 270 s) on the breast DCE MRI[24]. In previous research, high CPE was found to be associated with improved survival in ER+/HER2- breast cancer patients, and maybe endocrine therapy effectiveness[24,25]. These findings were validated in an independent cohort[25]. Additionally, CPE added complementary risk stratification independently from gene expression assays[43]. These findings led to the hypothesis that CPE could help personalize endocrine therapy in ER+/HER2- breast cancer patients. However, these results were from relatively small single-center cohorts with a relatively short follow-up time[24,25]. Especially in ER+/HER2- breast cancer it is important to have a long follow-up period[44]. Additionally, MRI acquisition varies between centers which influences CPE calculations[45]. To better reflect clinical reality it is important to include multiple centers with different MRI scanners to assess generalizability of CPE. Lastly, it is unknown why CPE is associated with improved prognosis in ER+/HER2- breast cancer. Uncovering the underlying biological mechanisms could potentially identify patients in whom further personalization with CPE is useful. This thesis sets out to research these aspects.

Thesis outline

The aim of this thesis was to investigate whether breast MRI, and specifically CPE, has the potential to play a role in the personalization of endocrine therapy in ER+/HER2- breast cancer.

In **Chapter 2** we start by giving an estimate of overtreatment with the current treatment strategies with data from the Netherlands and the United States of

America (USA). Estimates of overtreatment with AST were based on predictions from the PREDICT algorithm (a widely used online prognostic tool) based on actual prescribed systemic treatment in the Netherlands, and based on guideline-recommendations from Dutch and USA national guidelines.

In **Chapter 3** we present the results of the SELECT-study (Stromal enhancement on breast MRI as biomarker for survival with endocrine therapy). The SELECT-study is a large retrospective multicenter observational cohort study with approximately 1500 ER+/HER2- breast-cancer patients from 10 hospital in the Netherlands diagnosed between 2005 and 2010 and who underwent a pre-operative breast MRI. Survival status of these patients was updated in 2021 and CPE was calculated. The SELECT-study was designed to investigate whether CPE was associated with survival and to validate the previously observed association between CPE and survival.

Chapter 4 we investigated whether MRI can play a role in the treatment personalization of neoadjuvant endocrine therapy (therapy before surgery, [NET]). In **Chapter 4a** we investigate the behavior of CPE during NET, and relate it to the pre-operative endocrine prognostic index (PEPI). PEPI is an index that can be used to assess prognosis in patients after NET: patients with PEPI-1 have a good prognosis, whereas PEPI-2 and 3 have a relatively worse prognosis. In **Chapter 4b** we investigate whether conventional imaging features assessed by the radiologist, such as tumor size or the kinetic curve on MRI during NET, are able to predict PEPI (or prognosis).

In **Chapter 5** we aim to elucidate the biological mechanisms that underlie CPE and survival. Gene expression pathways in the tumor are related with CPE in patients from the MARGINS-study (Multimodality analysis and radiologic guidance in breast-conserving therapy). We hypothesize that CPE could represent the diseased breast before tumorigenesis or that the breast is secondarily affected by tumor-induced systemic effects. In both cases CPE might be associated with biological pathways expressed in the tumor that could also affect prognosis. Possible associations were validated in a large external independent cohort.

Lastly, in **Chapter 6** we summarize and discuss our findings, and conclude with future research directions.

2

CHAPTER

Overtreatment with adjuvant systemic therapy in early breast cancer patients

Population-based estimates of overtreatment with adjuvant systemic therapy in early breast cancer patients with data from the Netherlands and the USA

Ragusi, M. A. A., Van Der Velden, B. H. M., Van Maaren, M. C., Van Der Wall, E., Van Gils, C. H., Pijnappel, R. M., Gilhuijs, K. G. A. & Elias, S. G. Population-based Estimates of Overtreatment with Adjuvant Systemic Therapy in Early Breast-cancer Patients with Data from the Netherlands and the USA. Breast Cancer Res. Treat. 193, 161–173 (2022).

Abstract

Purpose: Although adjuvant systemic therapy (AST) helps increase breast cancer-specific survival (BCSS), there is a growing concern for overtreatment. By estimating the expected BCSS of AST using PREDICT, this study aims to quantify the number of patients treated with AST without benefit to provide estimates of overtreatment.

Methods: Data of all non-metastatic unilateral breast cancer patients diagnosed in 2015 were retrieved from cancer registries from The Netherlands and the USA. The PREDICT tool was used to estimate AST survival benefit. Overtreatment was defined as the proportion of patients that would have survived regardless of or died despite AST within 10 years. Three scenarios were evaluated: actual treatment, and recommendations by the Dutch or USA guidelines.

Results: 59.5% of Dutch patients were treated with AST. 6.4% (interquartile interval [IQI] = 2.5, 8.2%) was expected to survive at least 10 years due to AST, leaving 93.6% (IQI = 91.8, 97.5%) without AST benefit (overtreatment). The lowest expected amount of overtreatment was in the targeted and chemotherapy subgroup, with 86.5% (IQI = 83.4, 89.6%) overtreatment, and highest in the only endocrine treatment subgroup, with 96.7% (IQI = 96.0, 98.1%) overtreatment. Similar results were obtained using data from the USA, and guideline recommendations.

Conclusion: Based on PREDICT, AST prevents 10-year breast cancer death in 6.4% of the patients treated with AST. Consequently, AST yields no survival benefit to many treated patients. Especially improved personalization of endocrine therapy is relevant, as this therapy is widely used and is associated with the highest amount of overtreatment.

Introduction

Adjuvant systemic treatment (AST) has contributed to a reduction of breast cancer mortality over the past decades[16,17]. Whether a patient is recommended AST, and if so what type (endocrine, targeted, chemotherapy, or a combination) differs between countries but largely depends on several clinicopathological variables, including patient age, receptor status, tumor extent, tumor grade, and axillary tumor load. For example, the Dutch guidelines recommends AST when the absolute 10-year breast cancer specific survival (BCSS) is expected to increase by at least 3%[46]. Such BCSS-gain depends on clinicopathological variables and can be estimated for individual patients with tools such as PREDICT[47–49], which is endorsed by the Dutch breast cancer guidelines as well as the American Joint Committee on Cancer (AJCC)[49,50].

Over time, AST recommendations have expanded to include more favorable prognostic subgroups[9]. For example, only 23% of all breast cancer patients received endocrine therapy and 11% chemotherapy in 1990 in the Netherlands[9], which increased to 56% and 44%, respectively, by 2012[9]. Parallel to this trend, there is a growing concern about overtreatment.

Patients treated with AST but without benefit, because they would have survived breast cancer also without AST, or because they died from breast cancer despite AST, can be considered overtreated[51,52]. Such patients are unnecessarily exposed to the adverse effects of AST on health and quality of life[15]. Additionally, overtreatment also leads to unnecessary health care and societal costs.

Estimates of overtreatment can directly be derived from randomized controlled trials, but such studies often do not reflect everyday clinical practice with regard to patient mix and treatment standardization[53–55]. To address and substantiate the growing concern about AST overtreatment, there is, therefore, a need for population-based estimates of overtreatment associated with contemporary real-world AST prescribing practice. Such estimates are currently lacking.

In this study we aimed to estimate the amount of AST overtreatment, overall and separately for endocrine, targeted, and chemotherapy, on a population-level in real-world clinical care. For this we used population-based data from the Netherlands and the United States of America (USA) of breast cancer patients diagnosed in

2015. To obtain estimates of overtreatment, we projected individual BCSS-gain over a 10-year horizon using PREDICT, which we aggregated for all patients actually treated, or recommended to be treated with AST based on the Dutch or USA guidelines. Development and use of tools aimed at curbing overtreatment will be most relevant in breast cancer patients in whom the magnitude of overtreatment is particularly high.

Methods

Design

This study used real-world observational data from population-based cohorts of patients diagnosed with breast cancer in 2015 from 2 cancer registries: the Netherlands Cancer Registry (NCR) and the Surveillance, Epidemiology, and End Results (SEER) Program from the USA. In real-world observational data, estimates of overtreatment cannot be directly observed as it is impossible to distinguish whether a treated breast cancer patient survived because of AST or would also have survived without AST. Overtreatment estimates in the context of breast cancer survival using observational data can, however, be obtained by summarizing predictions of BCSS-gain by AST per patient. In this study we used PREDICT (version 2.0) to obtain such estimates of BCSS gain from AST[47–49]. PREDICT is an algorithm that uses several patient-specific clinicopathological variables to predict the absolute risk of dying from breast cancer over a 10-year horizon in the absence of AST, and then projects the therapeutic BCSS-gain of different AST subtypes as derived from randomized clinical trials to obtain an estimate of absolute individual BCSS-gain due to specific types of AST[47–49]. PREDICT performs well in many different prognostic subgroups and accurately projects absolute BCSS, adjusted for competing causes of death, in the presence and absence of administered AST[49,56–59].

In this study we address both overtreatment due to actual AST use as well as guideline-recommended AST use. Estimates of overtreatment due to actual AST use were based on patients registered by the NCR to have been treated with AST, which included type of treatment (i.e. endocrine, targeted, or chemotherapy, as mono- or combination therapy). As actual AST use is unavailable from SEER[60], we were unable to investigate actual AST use in the USA. To investigate overtreatment associated with guideline recommendations we applied both the Dutch (version 2.0)[46] and the National Comprehensive Cancer Network (NCCN) guidelines

(version 3.2015)[61] to both the Dutch and USA cohorts. Both guidelines were applied to both cohorts because the distribution of clinicopathological variables (i.e., the patient mix) may differ between countries (e.g. due to different breast screening strategies), which could lead to different expected BCSS-gain from AST on a population level.

Patient data

From the Dutch cohort we obtained all patient, tumor, and treatment characteristics of all female non-metastatic breast cancer patients diagnosed in 2015 (*N* = 15007). Patients who did not receive surgery (*N* = 1082), who received neoadjuvant treatment (*N* = 2926), or patients with bilateral tumors (*N* = 189) were excluded, leaving a total of 10810 patients for analysis. Similarly, from the USA cohort we obtained all patient, and tumor characteristics of all female non-metastatic breast cancer patients diagnosed in 2015 (*N* = 58429)[62]. Patients without data available from surgical pathology (*N* = 11214), who received neoadjuvant treatment (*N* = 481, based on pathological staging), or patients with bilateral tumors (*N* = 981) were also excluded, leaving a total of 45753 patients for analysis.

AST guidelines

We applied the 2012 Dutch guidelines (version 2.0, pertinent in 2015)[46] to both the Dutch and USA cohort. Similarly we applied the USA 2015 guidelines (version 2015.3)[61] to both the Dutch and USA cohort (Supplemental Materials 1 shows an overview of the differences between these guidelines, available online). Some adaptions and interpretations of these guidelines were necessary. First, we did not have the results of any possibly performed genomic assays available, and did, therefore, not take this into account. Second, when the guidelines were ambiguous, we applied the strictest recommendations. For instance, although the USA guidelines states to consider adjuvant endocrine therapy in a node-negative ER+/HER2- tumor of size $\leq$ 5 mm, we analyzed the data considering endocrine therapy to be not recommended in these patients.

Estimation of BCCS-gain and overtreatment from AST

PREDICT (version 2.0) estimates BCSS over a 10-year horizon from the different subtypes of AST based on several patient and tumor characteristics. PREDICT

takes the following characteristics as input: age, mode of detection, tumor size, tumor grade, number of positive lymph nodes, ER- and HER2-status, Ki-67 index and chemotherapy generation. Ki-67 index is not registered in the Dutch or the USA cohort and was always coded as unknown. A PREDICT script was created to calculate predicted 10-year BCSS-gain from each AST subtype. Additionally, the PREDICT script was adapted to calculate the area under the curve (AUC) of patient-specific predicted survival curves in the absence and presence of AST for the calculation of 10-year restricted mean survival time (RMST). RMST is the mean of the time to an event limited to some 'horizon' time (e.g. 10-years)[63]. It equals the AUC of the survival curve to that point in time[63]. The increased RMST due to AST can be interpreted as the added average survival time (or time to event) due to AST within these 10 years (for further explanation see Figure 1)[63,64].

To estimate the amount and distribution of expected overtreatment, we calculated the 10-year BCSS-gain, numbers needed to treat (NNT), and RMST (total and per patient) from AST based on actual treatment as registered in the Netherlands and the recommended treatment based on the Dutch and USA guidelines in both the Netherlands and USA. We defined overtreatment as the proportion of patients who would have survived without AST or died despite AST until the 10-year mark (Figure 1). Overtreatment per patient was calculated by adding the probability that this patient would have survived regardless of AST (the orange section in Figure 1) or died despite AST (the red section in Figure 1) at the 10-year mark. The patient-specific BCSS-gain was calculated by adding the BCSS-gain from the individual subtypes of AST that was received by or recommended to a patient, e.g., if a patient received both endocrine and chemotherapy the total BCSS-gain was calculated as the BCSS-gain from endocrine therapy plus chemotherapy (the green section in Figure 1). The numbers needed to treat (NNT) was calculated as the reciprocal of the total BCSS-gain (i.e., 1 / BCSS-gain). To calculate the population-based distribution of overtreatment and BCSS-gain, these estimates were aggregated over treatment groups (endocrine, targeted, and chemotherapy). Treatment-specific BCSS-gain was aggregated for all received or recommended AST because treatment decisions are based on total BCSS-gain, i.e., the Dutch guidelines recommend (combination) AST when the total BCSS-gain is ≥3%[46]. To quantify the number of patients experiencing low predicted BCSS-gain, we set a threshold of <3% total BCSS-gain from AST[46].

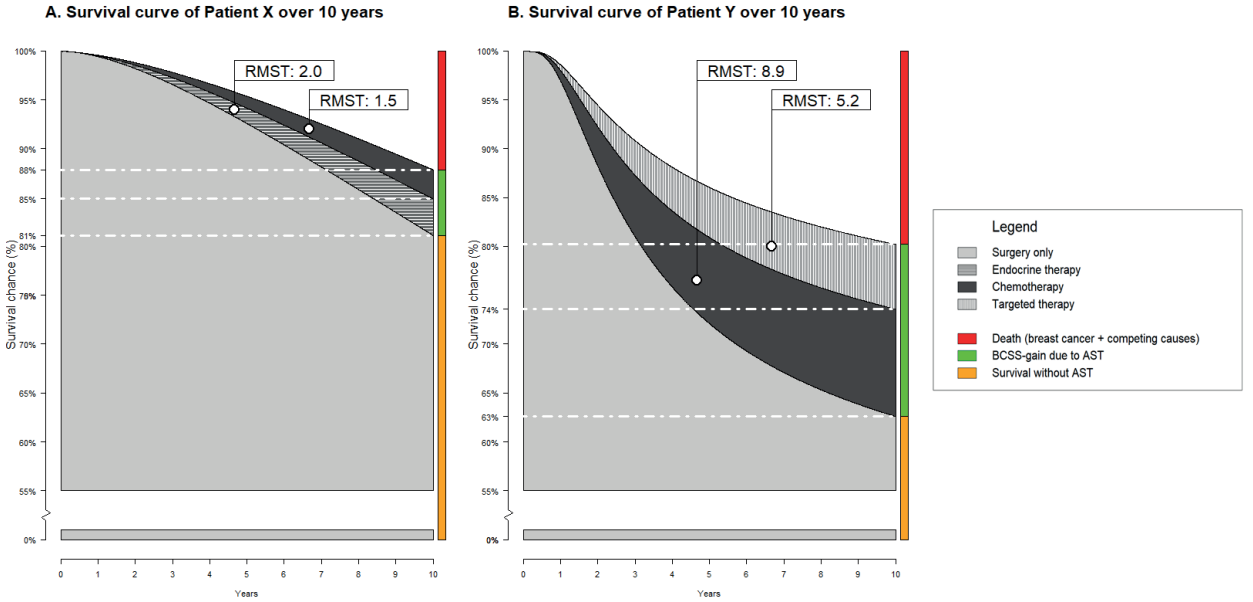

Figure 1. Two predicted 10-year survival curves of hypothetical patient X (**A**) and patient Y (**B**) estimated by the PREDICT algorithm with and without AST.

A. The left survival curve (patient X) represents a 57-year old patient with a grade 2 ER+/HER2- tumor with a size of 29 mm and no positive lymph nodes (pT2N0). Without AST this patient is expected to have an 81% chance at surviving for at least 10 years. After treatment with adjuvant endocrine therapy this would increase to 85% (an additional 4%), and with adjuvant chemotherapy this would further increase to 88% (an additional 3%), for a total expected increase in 10-year BCSS of 7% for the combination treatment. Overtreatment is calculated as the proportion of patients that would have survived for at least 10 years regardless of AST or died despite AST, e.g.: if 100 Patient X's were to be treated then we would expect 7 patients to have survived longer due to AST, whilst 81 patients would have survived regardless of AST and 12 patients would have died despite AST, therefore, 93 patients are overtreated. The expected survival gain expressed in the additional months of survival over a 10 year horizon, i.e. the RMST, would be 2 months for endocrine therapy alone, and 1.5 months for chemotherapy alone, amounting to an additional 3.5 months of survival within the first 10 years. Without AST this patient would have an estimated 110.4 months (9.2 years) of survival over a 10 year horizon. **B.** The right survival curve (patient Y) represents a 38-year old patient with a grade 3 ER-/HER2+ tumor with a size of 18 mm and 1 positive lymph node. Patient Y has a 63% chance of at least 10-year survival (in the absence of treatment with AST), this would increase by 11% with chemotherapy (to 74% 10-year survival), and additional 6% when treated with targeted therapy (to a total of 80% survival), for a total expected increase in 10-year BCSS of 17% for the combination treatment. Similar to the example described in A, this would lead to an overtreatment of 83% of patients. The RMST would increase by 8.9 months when treated with chemotherapy and by 5.2 months when treated with targeted therapy, amounting to a total of 14.2 months of additional survival within the first 10-years. Without AST this patient would have an estimated 91.3 months (7.6 years) of survival over a 10 year horizon. RMST = restricted mean survival time, BCSS = breast cancer-specific survival, AST = adjuvant systemic treatment, ER = estrogen receptor, HER2 = human epidermal growth factor receptor-2.

Statistical analysis

Missing variables of interest were multiply-imputed[65]. The number of imputed datasets was based on the percentage of rows with a missing variable of interest (20% in the Dutch cohort, and 25% in the USA cohort). Multiply-imputed estimates were aggregated using Rubin's Rules[66]. Estimates of (aggregated) overtreatment are reported as the mean, whereas BCSS-gain, NNT, and RMST are reported as median with their corresponding interquartile interval (IQI). Statistical analyses were performed using R version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria) and the multiple imputation was performed using the 'mice' (version 3.8.0)[67] package available in R.

Results

Table 1 shows the distribution of clinicopathological variables at diagnosis for both the Netherlands (*N* = 10810) and the USA (*N* = 45753). The median patient age was 63 years (IQI = 53, 71) in both cohorts. Overall, baseline clinicopathological variables were similar between the Netherlands and the USA. The frequency of actual AST distribution in the Netherlands, and AST recommendations based on the Dutch and USA guidelines is shown in Figure 2. Overall, Dutch early breast cancer patients received less chemotherapy than indicated based on the guidelines, particularly because a large proportion of patients with an indication for both endocrine therapy and chemotherapy, were actually treated with monoendocrine therapy. Compared to the Dutch guidelines, The USA recommends chemotherapy and endocrine therapy to a larger proportion of patients.

Overtreatment estimates of AST using actual prescribed treatment in the Netherlands

Table 2 shows the expected population-level 10-year overtreatment and survival benefit of each of the actually administered AST subtypes and regimens in the Netherlands. Overall, a total of 6431 patients (59.5%) received any type of AST in the Dutch cohort in 2015. AST (any combination) is expected to save 409 patients (6.4%) from dying of breast cancer within 10 years. The remaining 6022 patients (93.6%) are expected to be unaffected, i.e., overtreated, because 4509 patients (70.1%) are expected to survive also in absence of AST, and 1513 patients (23.5%) are expected to die from breast cancer or other causes despite AST. The median estimated 10-year absolute BCSS-gain in those treated with AST is 4.7% (IQI = 2.5,

8.2%), equivalent to an NNT of 21.4 (IQI = 12.1, 40.5) for patients who received any combination of AST (Table 2). The aggregated amount of expected increased survival time within the first 10-years due to AST (i.e., total RMST) was 2105.5 years for the entire Dutch population treated with AST, or 3.9 months (IQI = 1.3, 5.2) per patient.

Table 1. Characteristics of all female patients surgically treated for unilateral non-metastatic breast cancer without neoadjuvant therapy in 2015 in NL and USA

	NL (*N* = 10810)	**USA (*N* = 45753)**
Age (years)		
Median (IQI)	63 (53, 71)	63 (53, 71)
≤39	279 (3%)	1443 (3%)
40-49	1289 (12%)	6163 (13%)
50-74	7679 (71%)	30052 (66%)
75-84	1282 (12%)	6346 (14%)
≥85	281 (3%)	1749 (4%)
Tumor size (mm)		
Median (IQI)	15 (10, 22)	15 (9, 23)
<=5	758 (7%)	5006 (11%)
6-10	2139 (20%)	9329 (20%)
11-20	4866 (45%)	17463 (38%)
21-50	2740 (25%)	12203 (27%)
>50	308 (3%)	1751 (4%)
Number of Positive lymph nodes		
0	7945 (73%)	33331 (73%)
1-3	2438 (23%)	9890 (22%)
3-9	271 (3%)	1773 (4%)
≥10	156 (1%)	759 (2%)
Tumor grade		
1	3006 (28%)	12674 (28%)
2	5262 (49%)	21528 (47%)
3	2542 (24%)	11551 (25%)
IHC-subtype		
ER+/HER2+	721 (7%)	4014 (9%)
ER+/HER2-	8754 (81%)	36142 (79%)
ER-/HER2+	329 (3%)	1555 (3%)
TN	1006 (9%)	4042 (9%)

Unless otherwise specified, data are number of patients, with percentages between parentheses. Data are after multiple imputation. NL = Netherlands, USA = United States of America, IQI = interquartile interval, IHC = immunohistochemical, ER = estrogen receptor, HER = human epidermal growth factor-2, TN = triple-negative.

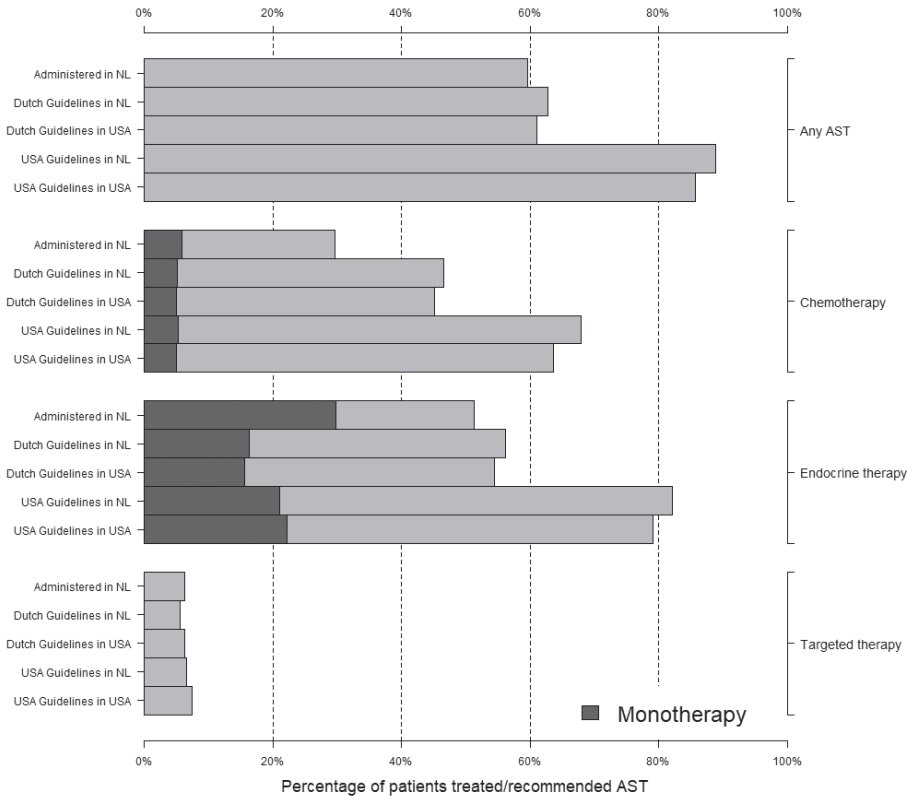

Figure 2. The distribution of administered and recommended AST, overall and according to subtype, for all surgically treated unilateral non-metastatic breast cancer patients in the Netherlands (NL) and the USA in 2015. Recommendations are based on the 2015 Dutch and USA (NCCN) guidelines. Patients who are treated with monotherapy (a single type of AST) are also indicated. AST = adjuvant systemic therapy, NCCN = National Comprehensive Cancer Network.

A relatively large proportion of patients (who were recommended endocrine and chemotherapy, but only received endocrine therapy) received a different AST regimen compared to the guideline recommendations (*N* = 1606, Figure 2). The median age of this subgroup was higher compared to the subgroup of patients who did receive endocrine and chemotherapy: 62 (IQI = 44, 70) versus 54 (IQI = 37, 68). The expected overtreatment was 97.2% (IQI = 97.0, 98.2%) based on the treatment they received (monoendocrine therapy) as opposed to an expected overtreatment of 95.0% (IQI = 94.6, 96.8%) based on the treatment they were recommended (endocrine and chemotherapy).

Patients who were treated with monoendocrine therapy were expected to experience a high probability of overtreatment and low BCSS-gain. Treatment with monoendocrine therapy of 3213 (29.7% of all breast cancer patients) resulted in an expected overtreatment of 96.7%. Figure 3 shows the distribution of 10-year BCSS-gain for the different treatment regimens based on actual treatment but also based on Dutch and USA guideline treatment recommendations.

Overtreatment estimates AST based on guideline recommendations in the Netherlands and the USA

Table 3 shows the expected population-level overtreatment and 10-year survival benefit of each of the recommended AST subtypes and regimens in Dutch patients based on Dutch and USA guidelines. Overtreatment was expected to be higher when based on USA guidelines compared to Dutch guidelines: 94.5% vs 93.1% of patients were overtreated in the any AST subgroup. The distribution of expected survival benefit of the different AST regimens based on Dutch and USA guidelines is shown in Figure 3. Overall, the USA recommended endocrine and chemotherapy to a larger number of patients (with a more favorable prognostic profile), resulting in lower survival benefit for these patients. Similarly, these analyses were applied to the patients from the USA (Supplemental materials 2 and 3, available online)

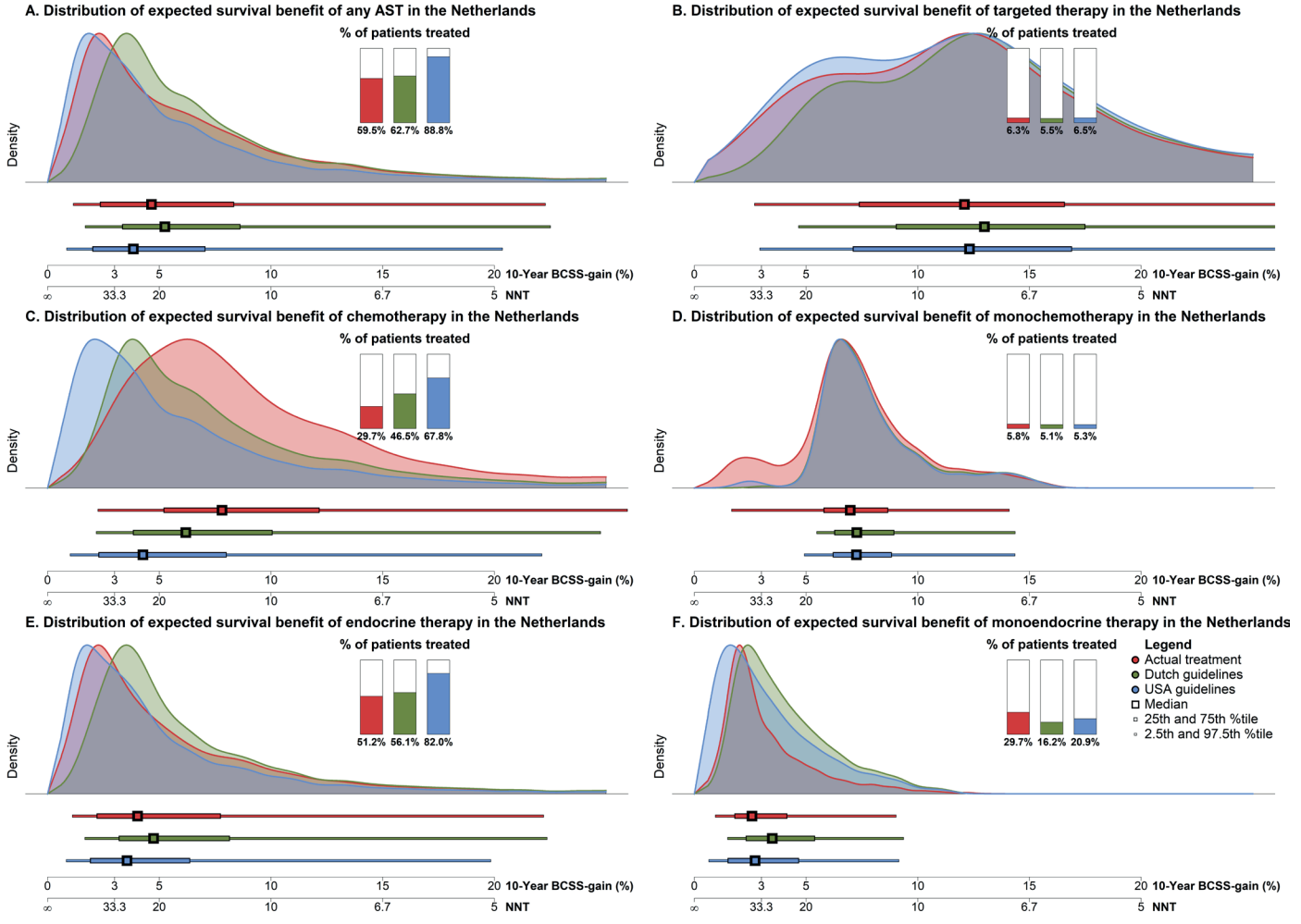
A. Distribution of expected survival benefit of any AST in the Netherlands
% of patients treated
59.5% 62.7% 88.8%
Density
0 3 5 10 15 20 10-Year BCSS-gain (%)
∞ 33.3 20 10 6.7 5 NNT
B. Distribution of expected survival benefit of targeted therapy in the Netherlands
% of patients treated
6.3% 5.5% 6.5%
Density
0 3 5 10 15 20 10-Year BCSS-gain (%)
∞ 33.3 20 10 6.7 5 NNT
C. Distribution of expected survival benefit of chemotherapy in the Netherlands
% of patients treated
29.7% 46.5% 67.8%
Density
0 3 5 10 15 20 10-Year BCSS-gain (%)
∞ 33.3 20 10 6.7 5 NNT
D. Distribution of expected survival benefit of monochemotherapy in the Netherlands
% of patients treated
5.8% 5.1% 5.3%
Density
0 3 5 10 15 20 10-Year BCSS-gain (%)
∞ 33.3 20 10 6.7 5 NNT
E. Distribution of expected survival benefit of endocrine therapy in the Netherlands
% of patients treated
51.2% 56.1% 82.0%
Density
0 3 5 10 15 20 10-Year BCSS-gain (%)
∞ 33.3 20 10 6.7 5 NNT
F. Distribution of expected survival benefit of monoendocrine therapy in the Netherlands
% of patients treated
29.7% 16.2% 20.9%
Density
0 3 5 10 15 20 10-Year BCSS-gain (%)
∞ 33.3 20 10 6.7 5 NNT
Legend
Actual treatment
Dutch guidelines
USA guidelines
Median
25th and 75th %tile
2.5th and 97.5th %tile

Figure 3. The distribution of expected 10-year BCSS-gain and NNT for all patients in the Netherlands who received or were recommended any AST (**A**), who were treated with targeted therapy (**B**), chemotherapy (**C**), monochemotherapy (**D**), endocrine (**E**) and monoendocrine therapy (**F**). For the overall AST subgroups (i.e. **A**, **B**, **C**, and **E**) the total BCSS-gain is aggregated from each received or recommended AST, e.g. chemotherapy (**C**) shows the distribution of total BCSS-gain of the entire registered or recommended AST regimen (including targeted and/or endocrine therapy) of patients that received chemotherapy (including monochemotherapy). Although the proportion of patients treated with endocrine therapy based on actual registered treatment and recommendations based on the Dutch guidelines is similar, the average survival benefit based on the Dutch guidelines is higher. This is due to the fact that a large proportion of patients that are recommended endocrine and chemotherapy, were actually treated with monoendocrine therapy, leading to a lower expected increased survival benefit (but also less treatment). Note that only 1 patient was registered with monotargeted therapy and guidelines do not recommend treatment with monotargeted therapy, therefore, the distribution is unavailable. AST = adjuvant systemic treatment, BCSS = breast cancer-specific survival, NNT = number needed to treat, USA = United States of America.

Table 2. Expected survival benefit of administered AST regimens in female patients surgically treated for unilateral non-metastatic breast cancer without neoadjuvant therapy in 2015 in the Netherlands (n=10810).

AST regimen	Number of patients treated (% of total)	Median Expected 10-year BCSS-gain (IQI)	Expected number of patient outcomes over a 10-year horizon			Expected NNT to prevent one breast cancer death in 10 years (IQI)	Total increased RMST in years from AST within 10 years	Median increased RMST in months from AST within 10 years per patient (IQI)	Number of treated patients with <3% 10-year BCSS-gain (% of treated)
			Benefit from AST	No benefit from AST, i.e., overtreatment					
			Survived due to AST (%)	Survived not due to AST (%)	Died despite AST (%)				
Any*	6431 (59.5%)	4.7 (2.5, 8.2)	409 (6.4%)	4509 (70.1%)	1513 (23.5%)	21.4 (12.1, 40.5)	2105.5	3.9 (1.3, 5.2)	2104 (32.7%)
Chemotherapy									
Monotherapy	627 (5.8%)	7.0 (5.9, 8.6)	45 (7.3%)	428 (68.2%)	154 (24.5%)	14.3 (11.7, 16.9)	297.1	5.7 (4.4, 6.7)	52 (8.3%)
In combination**	2577 (23.8%)	8.3 (5.1, 13.0)	257 (10.0%)	1896 (73.6%)	424 (16.4%)	12.0 (7.7, 19.5)	1288.1	6.0 (2.7, 7.6)	130 (5.0%)
Contribution to combination***	2577 (23.8%)	3.7 (2.2, 6.1)	119 (4.6%)	1896 (73.6%)	562 (21.8%)	27.3 (16.5, 45.1)	600.2	2.8 (1.1, 3.3)	1025 (39.8%)
Endocrine therapy									
Monotherapy	3213 (29.7%)	2.6 (1.9, 4.0)	105 (3.3%)	2177 (67.7%)	931 (29.0%)	38.7 (24.7, 52.4)	514.8	1.9 (1.0, 2.2)	1918 (59.7%)
In combination**	2326 (21.5%)	7.9 (4.9, 12.2)	223 (9.6%)	1740 (74.8%)	363 (15.6%)	12.6 (8.1, 20.1)	1062.0	5.5 (2.6, 6.7)	124 (5.3%)
Contribution to combination***	2326 (21.5%)	4.3 (2.7, 6.3)	114 (4.9%)	1740 (74.8%)	472 (20.3%)	23.3 (15.8, 37.2)	553.0	2.9 (1.4, 3.5)	724 (31.1%)
Targeted therapy**									
In combination**	678 (6.3%)	12.1 (7.5, 16.5)	88 (12.9%)	462 (68.2%)	128 (18.9%)	8.2 (6.0, 13.2)	483.7	8.5 (4.0, 11.1)	21 (3.1%)
Contribution to combination***	678 (6.3%)	2.9 (1.6, 4.9)	25 (3.7%)	462 (68.2%)	191 (28.1%)	34.1 (20.2, 60.5)	140.3	2.5 (0.9, 3.6)	344 (50.7%)
Specific combinations									
Endocrine, targeted, and chemotherapy	408 (3.8%)	10.9 (6.8, 16.4)	52 (12.7%)	295 (72.4%)	61 (15.0%)	9.1 (6.1, 14.5)	246.7	7.2 (3.5, 9.1)	10 (2.5%)
Endocrine and chemotherapy	1907 (17.6%)	7.4 (4.7, 11.2)	171 (8.9%)	1438 (75.4%)	298 (15.6%)	13.5 (8.9, 21.1)	809.9	5.1 (2.4, 6.1)	111 (5.8%)
Targeted and chemotherapy	259 (2.4%)	13.1 (10.4, 16.6)	35 (13.5%)	160 (61.9%)	64 (24.6%)	7.6 (6, 9.6)	231.5	6.5 (1.6, 10.3)	10 (3.9%)

Data are given as median (IQI), unless specified otherwise. *Endocrine, targeted, and/or chemotherapy. **Total BCSS-gain from all AST in the combination therapy (monotherapy is not included). ***Contribution of AST-subtype to the combination therapy. ****Only 1 patient was treated with monotargeted therapy, therefore, this row is omitted. AST = adjuvant systemic therapy, BCSS = breast-cancer specific survival, NNT = number needed to treat, RMST = restricted mean survival time.

Table 3. Expected survival benefit of recommended AST regimens based on Dutch and USA guidelines in female patients surgically treated for unilateral non-metastatic breast cancer without neoadjuvant therapy in 2015 in the Netherlands (n=10810).

AST regimen	Number of patients treated (% of total)	Median Expected 10-year BCSS-gain (IQI)	Expected number of patient outcomes over a 10-year horizon			Expected NNT to prevent one breast cancer death in 10 years (IQI)	Total increased RMST in years from AST within 10 years (years)	Median increased RMST in months from AST within 10 years per patient (IQI)	Number of treated patients with <3% 10-year BCSS-gain (% of treated)
			Benefit from AST	No benefit from AST, i.e., overtreatment					
			Survived due to AST (%)	Survived not due to AST (%)	Died despite AST (%)				
Any*									
NL	6782 (62.7%)	5.3 (3.5, 8.5)	470 (6.9%)	4678 (69.0%)	1634 (24.1%)	19.0 (11.7, 28.9)	2378.7	4.2 (1.8, 5.3)	1155 (17.0%)
USA	9595 (88.8%)	3.8 (2.1, 6.9)	526 (5.5%)	6990 (72.8%)	2079 (21.7%)	26.0 (14.4, 47.0)	2622.1	3.3 (1.1, 4.2)	3697 (38.5%)
Chemotherapy									
Monotherapy									
NL	554 (5.1%)	7.3 (6.4, 8.8)	44 (8.0%)	370 (66.7)	140 (25.3%)	13.8 (11.3, 15.7)	299.9	6.5 (5.0, 7.0)	0 (0.0%)
USA	568 (5.3%)	7.3 (6.3, 8.7)	45 (7.9%)	381 (67.0%)	143 (25.1%)	13.8 (11.5, 15.8)	302.0	6.4 (4.9, 6.9)	10 (1.8%)
In combination**									
NL	4471 (41.4%)	5.7 (3.8, 10.2)	354 (7.9%)	3444 (77.0%)	673 (15.1%)	17.5 (9.8, 26.5)	1705.1	4.6 (1.9, 5.5)	456 (10.2%)
USA	6757 (62.5%)	4.0 (2.2, 7.7)	405 (6.0%)	5415 (80.1%)	937 (13.9%)	25.3 (13, 44.4)	1926.9	3.4 (1.1, 4.1)	2452 (36.3%)
Contribution to combination***									
NL	4471 (41.4%)	2.5 (1.7, 4.4)	160 (3.6%)	3444 (77.0%)	867 (19.4%)	40.2 (22.7, 60.1)	772.9	2.1 (0.8, 2.3)	2639 (59.0%)
USA	6757 (62.5%)	1.7 (1.0, 3.3)	182 (2.7%)	5415 (80.1%)	1160 (17.2%)	57.7 (30.1, 101.4)	868.7	1.5 (0.5, 1.7)	4876 (72.2%)
Endocrine therapy									
Monotherapy									
NL	1751 (16.2%)	3.5 (2.4, 5.3)	71 (4.1%)	861 (49.1%)	819 (46.8%)	28.6 (18.9, 41.2)	373.8	2.6 (1.3, 3.1)	695 (39.7%)
USA	2264 (20.9%)	2.7 (1.6, 4.6)	76 (3.4%)	1190 (52.6%)	998 (44.1%)	36.7 (21.8, 62.1)	393.2	2.1 (0.8, 2.6)	1236 (54.6%)
In combination**									
NL	4312 (39.9%)	5.5 (3.7, 9.5)	328 (7.6%)	3351 (77.7%)	633 (14.7%)	18.1 (10.5, 26.8)	1526.1	4.2 (1.9, 5.1)	456 (10.6%)
USA	6595 (61.0%)	3.9 (2.2, 7.1)	378 (5.7%)	5321 (80.7%)	896 (13.6%)	25.8 (14, 45.2)	1741.4	3.2 (1.1, 3.8)	2452 (37.2%)
Contribution to combination***									
NL	4312 (39.9%)	3.0 (2.1, 5)	171 (4.0%)	3351 (77.7%)	790 (18.3%)	33.5 (19.8, 48.1)	807.4	2.2 (1.1, 2.7)	2167 (50.3%)

AST regimen	Number of patients treated (% of total)	Median Expected 10-year BCSS-gain (IQI)	Expected number of patient outcomes over a 10-year horizon			Expected NNT to prevent one breast cancer death in 10 years (IQI)	Total increased RMST in years from AST within 10 years (years)	Median increased RMST in months from AST within 10 years per patient (IQI)	Number of treated patients with <3% 10-year BCSS-gain (% of treated)
			Benefit from AST	No benefit from AST, i.e., overtreatment					
			Survived due to AST (%)	Survived not due to AST (%)	Died despite AST (%)				
USA	6595 (61.0%)	2.1 (1.2, 3.8)	198 (3.0%)	5321 (80.7%)	1076 (16.3%)	46.6 (25.9, 80.5)	925.4	1.7 (0.6, 2.7)	4356 (66.1%)
Targeted therapy**									
In combination**									
NL	592 (5.5%)	13.0 (9.1, 17.4)	84 (14.2%)	406 (68.5%)	102 (17.3%)	7.7 (5.7, 10.9)	455.9	9.2 (4.9, 11.7)	3 (0.5%)
USA	698 (6.5%)	12.0 (6.4, 16.5)	92 (13.1%)	489 (70.1%)	117 (16.8%)	7.2 (4.5, 10)	490.5	8.4 (3.8, 11.1)	18 (2.6%)
Contribution to combination***									
NL	592 (5.5%)	3.0 (1.8, 5.1)	22 (3.8%)	406 (68.5%)	164 (27.7%)	33.6 (19.7, 54.4)	124.8	2.5 (0.9, 3.7)	297 (50.2%)
USA	698 (6.5%)	2.6 (1.4, 4.9)	24 (3.5%)	489 (70.1%)	185 (26.4%)	38.9 (20.5, 69.3)	132.9	2.3 (0.7, 3.5)	395 (56.6%)
Specific combinations****									
Endocrine, targeted, and chemotherapy									
NL	434 (4.0%)	11.5 (7.4, 16.9)	58 (13.4%)	313 (72.2%)	63 (14.5%)	8.7 (5.9, 13.4)	276.8	7.6 (3.9, 9.2)	3 (0.7%)
USA	535 (4.9%)	10.5 (6.2, 15.1)	64 (12.0%)	395 (73.8%)	76 (14.2%)	9.5 (6.6, 16)	305.0	6.8 (3.2, 8.2)	18 (3.4%)
Endocrine and chemotherapy									
NL	3874 (35.8%)	5.0 (3.6, 8.6)	269 (6.9%)	3035 (78.3%)	570 (14.7%)	19.9 (11.5, 27.7)	1249.2	3.9 (1.9, 4.6)	453 (11.7%)
USA	6055 (56.0%)	3.6 (2.1, 6.3)	313 (5.2%)	493 (81.3%)	819 (13.5%)	27.6 (15.8, 47.4)	1436.4	2.8 (1.1, 3.3)	2434 (40.2%)
Targeted and chemotherapy									
NL	156 (1.4%)	15.1 (13.1, 18.4)	26 (16.5%)	91 (58.4%)	39 (25.0%)	6.6 (5.4, 7.6)	179.0	13.6 (10.3, 15.2)	0 (0.0%)
USA	161 (1.5%)	15.5 (13.2, 18.5)	27 (16.6%)	93 (57.9%)	41 (25.6%)	6.4 (5.4, 7.6)	185.5	13.6 (10.4, 15.2)	0 (0.0%)

Data are given as median (IQI), unless specified otherwise. *Endocrine, targeted, and/or chemotherapy. **Total BCSS-gain from all AST in the combination therapy (monotherapy is not included). ***Contribution of AST-subtype to the combination therapy. ****No patients are recommended monotargeted therapy or the combination of endocrine and targeted therapy according to the guidelines, therefore, these rows are omitted. AST = adjuvant systemic therapy, NL = Netherlands, USA = United States of America, BCSS = breast-cancer specific survival, NNT = number needed to treat, RMST = restricted mean survival time.

Discussion

In this study we estimated the amount and distribution of expected overtreatment of administered and recommended AST in unilateral early breast cancer patients with real world data from 2 national cancer registries. Actual treatment with any AST in the Netherlands is expected to save 6.4% of patients within 10 years (or an NNT of 21.4), whereas the remaining 93.6% of patients is expected to be overtreated. The largest amount of expected overtreatment was in the subgroup of patients who were treated with monoendocrine therapy: 96.7%. Overtreatment based on Dutch and USA guideline recommendations was also highest in the subgroup of monoendocrine therapy, respectively: 95.9% and 96.6%. A large proportion of patients treated with monoendocrine therapy in the Netherlands were actually also recommended chemotherapy. This may have led to an overestimation in overtreatment of the monoendocrine subgroup, and an underestimation in expected overtreatment of the endocrine and chemotherapy subgroup.

Our population-based AST survival-gain estimates from AST differ from previously reported survival-gain estimates based on randomized trial results, for example: the Early Breast Cancer Trialists' Collaboration Group (EBCTCG) reported that 7.9% in patients aged <50 years (or an NNT of 12.7) benefit from chemotherapy within 10 years, and 2.9% in patients aged 50-69 years (or an NNT of 34.5)[18], whilst our population-based estimates show that 7.3% of patients (or an NNT of 14.3) treated with monochemotherapy were expected to benefit from AST treatment, and 4.6% of patients (or an NNT of 27.3) who were treated with a combination of AST including chemotherapy were expected to benefit. Similarly, the EBCTCG report a 7.9% BCSS-gain after 5 years of tamoxifen (NNT is 12.7)[18], whilst our population-based estimates show that 3.3% (NNT is 38.5) were expected to benefit from monoendocrine therapy, and 4.9% (NNT is 23.3) were expected to benefit from an AST regimen including endocrine therapy. Although our estimates of overtreatment appear to be high, they largely agree with what can be expected from the randomized clinical trial results.

The issue of overtreatment has become increasingly recognized and efforts have been made to identify patients for whom AST can safely be omitted. Genomic assays, such as the 21-gene recurrence score[7] and the 70-gene signature[6,20], have become a popular method to identify patients where chemotherapy can safely be omitted, particularly in ER+/HER2- breast cancer[7,21,23,68]. However, de-escalation tools for endocrine therapy are less available[23,69], even though approximately half of all newly diagnosed early breast cancer patients receive endocrine therapy.

One reason why a higher overtreatment may be accepted in this subset of patients might be due to the fact that the adverse effects of endocrine therapy are generally regarded as less severe compared to targeted and chemotherapy[15]. However, patients are administered endocrine therapy for a long period of 5 to 10 years with side effects such as sexual dysfunction, cognitive and musculoskeletal problems that have a negative impact on the quality of life[11–13]. Therefore, also advancements in the personalization of endocrine therapy are valuable.

This study has several limitations. First, we did not obtain information regarding the use of genomic assays for both the Dutch and USA cohort, and was assumed to be unknown. This will have affected the analyses where treatment recommendations were based on guidelines, particularly for the USA guidelines (Supplemental Materials 1, available online), and will have led to an overestimation of the amount of expected overtreatment from chemotherapy in ER+/HER2- breast cancer patients. However, even if available, we could not incorporate genomic risk in our estimation of expected BCSS-gain, as genomic risk is not included in the PREDICT model (e.g. PREDICT will overestimate BCSS in patients with high clinical but low genomic risk). Second, we applied the strictest interpretation of the guidelines which will have resulted in an underestimation of the overall amount of overtreatment, because these lenient recommendations generally apply to patients with favorable prognosis in whom BCSS-gain from AST is low. Third, our estimations are based on patient data and national guidelines from 2015, however, in 2020 both the Dutch and the NCCN guidelines have updated their AST recommendations. The Dutch guidelines in particular have de-escalated chemotherapy recommendations in ER+/HER2- breast cancer compared to the 2015 guidelines (based on 2020 guidelines; Supplemental Materials 4 shows the analyses using the new Dutch 2020 guidelines, available online). No major updates were introduced for endocrine or targeted therapy. Registry data from 2015 was used as complete data from 2020, including administered treatment, was not available at time of the data request and no significant differences were expected in the distribution of clinicopathological variables between 2015 and 2020. Fourth, the estimations of survival and AST-specific 10-year BCSS-gain were calculated with the PREDICT algorithm. The use of expected survival benefit is necessary, as survival benefit from specific AST-subtypes cannot directly be observed on a patient level from real-world clinical observational data. Therefore, the validity of our estimates depends on the validity of the PREDICT algorithm. PREDICT is validated in several independent cohorts[49,56,58], including a Dutch cohort[59], where it performed well, although PREDICT slightly underestimated

survival in ER- and high-risk patients (T3, and grade 3), and overestimated survival in old patients (≥75 years)[59]. Additionally, it should be used with caution in patients aged <40 years[70]. Still, PREDICT is endorsed by the Dutch guidelines and AJCC[50] to support clinical decision making, and small under- and overestimations of survival are accepted. In that sense, the information we present in this study is also the information available to clinicians to support their clinical decision-making. Although, in 2015 the online prognostication most used was Adjuvant! Online (which has since been offline), which may have led to small differences in prognosis prediction compared to PREDICT[70]. Although genomic assays and prognostic tools have improved personal risk stratification, it remains difficult to predict recurrence in individual patients. Additionally, the PREDICT algorithm was developed and primarily validated in Western populations[47–49,56–59,70,71], and their might be variation in competing risk among women from the age (for instance due to differences in region), which might further affect personal risk stratification. However, a validation study performed in Malaysia showed that PREDICT performed relatively well[72]. Fifth, we have estimated overtreatment distributions based on the survival over a 10-year horizon with BCSS- and RMST-gain. AST is expected to increase survival beyond this 10-year horizon, and patient-level measures such as risk of side effects, therapy adherence and effect on quality of life, but also societal-level measures such as cost-benefit analyses of the treatment should, ideally, also be taken into account[1]. Additionally, prevention of non-life threatening recurrences due to AST that could also affect health care costs and quality of life are also not taken into account. The results should be interpreted with caution, and taken as estimates. Our findings do not recommend a change in treatment guidelines, but highlight the need for tools to allow for further treatment selection in certain subgroups of breast cancer patients.

To conclude, the percentage of expected overtreatment in patients treated with combination AST and monochemotherapy was relatively high but in the range that can be expected from randomized clinical trial results. However, expected overtreatment in patients treated with monoendocrine therapy was high. Comparable results were observed when estimating survival benefit based on Dutch and USA guideline recommendations, however, as the USA guidelines recommended AST to a larger number of patients (with more prognostically favorable profiles), overtreatment was higher. De-escalation tools to curb overtreatment of endocrine therapy are especially relevant, as this subgroup represents the largest portion of breast cancer patients treated with AST.

3

CHAPTER

Personalization of adjuvant endocrine therapy in early breast cancer patients with breast MRI

Contralateral Parenchymal Enhancement on MRI is associated with long-term survival in breast cancer patients: outcomes of the SELECT-study

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Abstract

Background: A number of single-center studies found that high contralateral parenchymal enhancement (CPE) on breast MRI is associated with improved long-term survival of patients with estrogen receptor-positive (ER+) human epidermal growth factor-negative (HER2-) breast cancer. Due to varying sample size, population characteristics and follow-up time, consensus of the association is currently lacking.

Purpose: To confirm that CPE is associated with long-term survival in a large multicenter retrospective cohort and to investigate if CPE is associated with endocrine therapy effectiveness.

Materials & Methods: This multicenter observational cohort included patients who underwent MRIs with unilateral ER+/HER2- breast cancer of size ≤ 5 cm and ≤ 3 positive lymph nodes in 2005-2010. Overall survival (OS), recurrence-free survival (RFS), and distant-recurrence free survival (DRFS) were collected. Multivariable Cox proportional hazards regression was performed to investigate if CPE was associated with prognosis and with endocrine therapy effectiveness. Kaplan-Meier analysis was performed to investigate differences in absolute risk after 10 years, stratified to CPE tertiles.

Results: 1432 patients were included from 10 centers. CPE was independently significantly associated with OS with a hazard ratio (HR) of 1.2 (95% CI = 1.0, 1.4; *P* = .047), but CPE was not associated with RFS (HR = 1.1; *P* = .162) or DRFS (HR = 1.1; *P* = .190). CPE was not associated with endocrine therapy effectiveness in OS (P = .430), RFS (P = .945), or DRFS (P = .925). Differences in absolute OS after 10 years stratified to CPE tertiles were: 88.5% (95% CI = 88.1%, 89.1%) in tertile 1 (lowest CPE), 85.8% (95% CI = 85.2%, 86.3%) in tertile 2, and 85.9% (95% CI = 85.4%, 86.4%) in tertile 3 (highest CPE).

Conclusion: High CPE was associated with decreased OS in ER+/HER2- breast cancer patients, but was not associated with RFS, DRFS or endocrine therapy effectiveness.

Introduction

Treatment of early breast cancer typically consists of surgery, on indication followed by radiotherapy and/or adjuvant systemic therapy (AST) in order to optimize local and regional control. Although the use of AST has reduced mortality and recurrence rates in breast cancer patients over the last decades[16,18,73], it is also associated with adverse side-effects that negatively impact quality of life[15]. One subtype of AST is endocrine therapy, which is exclusively prescribed to patients with estrogen receptor-positive (ER+) breast cancer. Endocrine therapy is a cornerstone in the treatment of ER+-breast cancer, however, patients are at risk of side-effects such as fatigue, sexual dysfunction, cognitive and musculoskeletal complaints[11–13].

There is growing concern about overtreatment with AST (including endocrine therapy)[10,51,74], as increasingly more patients with a more favorable prognosis are prescribed AST[9]. The likely benefits of omitting (or extending) treatment need to outweigh the potential harm, and personalization tools can aid in the clinical decision making. However, there are currently no clinically validated personalization tools for endocrine therapy beyond the expression of the ER[23], and there is an unmet need to tailor endocrine therapy to individual patients.

A number of single-center observational studies found that perfusion of the parenchymal breast tissue, derived from preoperative dynamic contrast-enhanced (DCE) MRI, is associated with long-term survival of patients with ER+ breast cancer, and may be predictive of endocrine therapy efficacy[24–26]. Results are conflicting, however, as in a cohort of Asian women this association between parenchymal enhancement and long-term outcome was not reproduced[27]. Thus far, all studies investigating parenchymal enhancement as a prognostic (or predictive) biomarker were single-center studies with often a relatively short follow-up time[24,25,27]. Hence, there is currently no consensus on the association between parenchymal enhancement and patient outcome.

The aim of this study was to investigate in a large multicenter retrospective cohort of patients with unilateral early ER+/HER2- breast cancer whether parenchymal enhancement on MRI is associated with long-term survival independent of standard clinicopathological prognostic factors and, secondly, whether parenchymal enhancement is related to endocrine therapy effectiveness.

Materials & Methods

Study Design

The SELECT-study (stromal enhancement on breast MRI as biomarker for survival with endocrine therapy) is a retrospective multicenter observational cohort study which included unilateral ER+/HER2- breast cancer patients diagnosed between 2005 and 2010 in 10 Dutch hospitals and who had undergone a preoperative MRI. At the study design phase, a-priori power analyses showed that we needed to include 215 events (approximately 1500 patients) for sufficient statistical power (based on the hazard ratios [HR] found in the previous studies[24,25]). Survival outcomes, in addition to standard clinicopathologic and treatment data, were collected between April and October 2020. Survival analysis was performed to investigate if parenchymal enhancement was associated with long-term patient survival, and secondly, whether parenchymal enhancement was associated with endocrine therapy effectiveness.

Patients

The study was performed with a waiver from the Institutional Review Board of the University Medical Center Utrecht. The inclusion criteria were: unilateral ER+/HER2- breast cancer patients with a tumor size of ≤ 5 cm and ≤ 3 positive lymph nodes, and who had undergone a preoperative MRI (Figure 1). Whether a preoperative MRI was performed, was at the discretion of the multidisciplinary team at each hospital as per standard clinical care at that time.

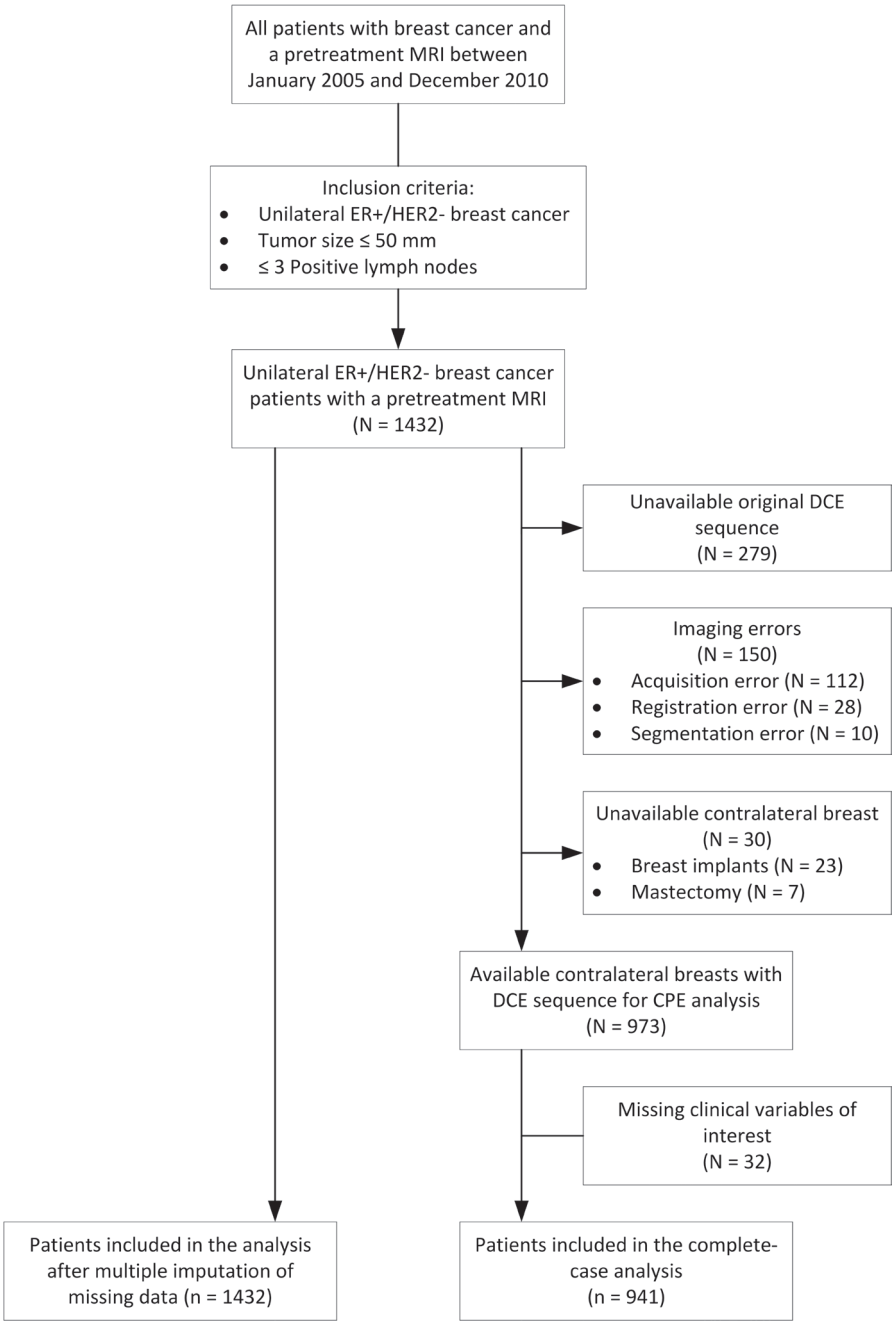


Figure 1. Overview of patient inclusion. Missing MRI or clinicopathological data were multiply-imputed. ER = estrogen receptor, HER = human epidermal growth factor-2, DCE = dynamic contrast-enhanced, CPE = contralateral parenchymal enhancement.

Clinicopathologic data and survival outcomes

Lists of patients who underwent a preoperative MRI at the participating hospitals were linked to the Dutch Cancer Registry (NKR) and Pathology Registry (PALGA)[75] to obtain clinicopathologic and follow-up data. Patient data were shared between the NKR, PALGA, participating hospitals, and the researchers through a Trusted Third Party using pseudonymization to ensure that no patient-identifying data was received by the researchers. Clinicopathological data pertaining to tumor characteristics (i.e., tumor size, tumor grade and number of positive lymph nodes) was based on the surgical tumor specimen (i.e. pathological staging). A tumors was deemed ER-positive if >10% of nuclei stained positive for ER. Standard patient outcomes were used as defined by Hudis et al[76]: overall survival (OS), recurrence-free survival (RFS), and distant recurrence-free survival (DRFS).

Magnetic resonance imaging

DCE MRI was performed on a 1.5-T or a 3.0-T scanner from either Philips, Siemens, or General Electric (GE), although 1 patient was scanned on a 1.0-T MRI (Panorama HFO, Philips). Table 1 shows an overview of the different imaging parameters used in the different hospitals. Flip angle ranged between 10º and 25º, repetition times between 3.9 ms and 17.3 ms, and echo times between 1.1 ms and 4.8 ms. Different types of contrast agents were used: Gadovist (Bayer), Magnevist (Bayer), Dotarem (Guerbet), Prohance (BRACCO), and Omniscan (GE).

Table 1. Overview of MRI model and acquisition parameters

Site	N	Field strength	Manufacturer & model	Flip angle (º)	Repetition time (ms)	Echo time (ms)	In plane resolution (mm)	Slice thickness (mm)	Early post-contrast timing (s)	Difference between early and late contrast timing (s)	Contrast agent
1	1	1	Philips, HFO	12	7.3	3.6	1.1	1.1	127	216	Gadovist
	90	1.5	Philips, Achieva	10	4.4 - 4.7	2.2 - 2.3	0.6 - 0.7	1.2 - 1.7	62	275	Gadovist
	16	1.5	Philips, Intera	10	4.5 - 4.7	2.2 - 2.3	0.6 - 0.7	1.2 - 1.6	82	285	Gadovist
	116	3	Philips, Achieva	10	3.0 - 3.5	1.1 - 1.3	0.8 - 1.0	1.0 - 1.4	82	227	Gadovist
2	1	1.5	Philips, Intera	15	4.1	2.1	0.6	1.3	59	252	Dotarem
	230	1.5	Philips, Intera	17	3.9 - 4.2	1.9 - 2.1	0.6	1.3-1.4	64	203	Dotarem
	2	1.5	Philips, Intera	25	17.3 - 18.6	9.2	0.7	3.5	56	178	Dotarem
	4	1.5	Philips, Achieva	10	5.1 - 5.3	2.6	0.7	1.5	86	230	Dotarem
	2	1.5	Philips, Achieva	17	4.0	1.9	0.6	1.3	98	204	Dotarem
3	14	1.5	Philips, Intera	10	5.1 - 7.3	2.5 - 4.6	0.7	1.2 - 1.5	75	286	Prohance
	22	1.5	Philips, Intera	12	8.2 - 8.3	4.6	0.8	1.0 - 1.2	86	232	Prohance
	2	1.5	Philips, Intera	20	8.0	3.9 - 4.0	0.7	2.0 - 2.1	140	249	Prohance
	7	3	Philips, Achieva	10	4.7 - 5.8	2.4 - 2.9	0.6 - 1.4	1.0 - 1.2	85	247	Prohance
	10	3	Philips, Achieva	12	3.9	2.3	0.8	1.0	70	263	Prohance
4	62	1.5	Siemens, Avanto	10	4.2	1.2	0.8 - 0.9	0.9	86	253	Dotarem / Omniscan
	41	1.5	Siemens, Symphony	20	9.5	4.8	0.6 - 0.7	1.5 - 3.0	101	273	Dotarem / Omniscan
5	12	1.5	Philips, Intera	10	7.4 - 7.7	3.4 - 3.5	1.0	1.0	109	288	Gadovist
	11	1.5	Philips, Intera	25	7.9 - 8.2	3.5 - 4.0	0.7 - 0.8	4.0 - 5.0	113	279	Gadovist

Site	N	Field strength	Manufacturer & model	Flip angle (º)	Repetition time (ms)	Echo time (ms)	In plane resolution (mm)	Slice thickness (mm)	Early post-contrast timing (s)	Difference between early and late contrast timing (s)	Contrast agent
6	21	1.5	Siemens, Espree	10	4.3	1.3	0.8	0.9	93	220	Magnevist
	5	1.5	Philips, Intera	10	5.1 - 7.4	2.6 - 3.7	0.7 - 1.0	1 - 1.2	111	228	Magnevist
7	178	1.5	Siemens, Avanto	10	4.2	1.2	0.8	0.9	95	247	Dotarem
	1	1.5	Siemens, Avanto	12	10.4	4.6	0.7	0.7	92	249	Dotarem
	25	1.5	GE, Genesis Signa	10	7.8 - 8.3	4.2	0.7	1.5 - 2.7	79	276	Dotarem
8	7	1.5	Siemens, Avanto	10	4.2	1.2	0.8	0.9	94	257	Prohance
9	25	1.5	Siemens, Avanto	10	4.2	1.3	0.9	1	105	207	Dotarem
	6	1.5	Siemens, Avanto	20	7.5	4.0	0.6	1.5	99	274	Dotarem
	9	1.5	Siemens, Avanto	25	7.5	4.0	1.2	1.5	99	274	Dotarem
	2	1.5	Siemens, Symphony	25	7.5	4.0	1.2	1.5	102	289	Dotarem
10	51	1.5	Siemens, Avanto	10	5.2	2.4	0.9 - 1.1	1.5	88	232	Prohance

GE = General Electric.

Image processing to quantify parenchymal enhancement

Parenchymal enhancement was defined and quantified according to the previously reported methods[24], i.e. using contralateral parenchymal enhancement (CPE). In short, to calculate CPE, field inhomogeneities were corrected[77], and the fibroglandular tissue of the contralateral breast was segmented from T1-weighted images. In the original study, segmentations were performed only on non-fat suppressed images from a single institution[24]. To account for the fact that non-fat suppressed images were unavailable in several institutions in the current study and to account for differences in MRI acquisition parameters, 2 additions were implemented: to segment the fibroglandular tissue in fat-suppressed images, a deep-learning based segmentation model was developed by training an Attention-gated U-Net[78]. Furthermore MRIs were harmonized to account for differences in flip angle and repetition time between different MRI acquisitions[45].

CPE was calculated using the following equation applied to the region of interest defined by the fibroglandular tissue segmentation in the contralateral breast: $(S_{late} - S_{early}) / S_{early}$, where S_{early} and S_{late} represent the signal intensities of the corresponding voxels in the early and late enhancement images, respectively[24–26]. Conform the original definition of CPE, the early enhancement images were selected to be those closest to 90 s after contrast injection and the late enhancement images to be closest to 270 s after the early image (Table 1). To account for patient motion between early and late enhancement, deformable image registration was performed[79]. Lastly, the top-10% most enhancing voxels, according to the previously defined equation above, were averaged to calculate CPE.

Image processing was implemented using Python (version 3.7.6; Python Software Foundation) and MeVisLab (version 3.0.2, MeVis Medical Solutions AG).

Multiple imputation

Missing data of interest, i.e. CPE and clinicopathological variables (Figure 1), were multiply imputed based on substantive model compatible fully conditional specification[80]. The number of imputations was based on the percentage of cases with missing values (34%)[81], and we used 50 iterations. We included all the variables of interest, outcome variables, as well as derived variables such as interaction terms and spline functions[82]. Results of the imputations were checked

by exploring the imputed values and investigating the convergence over iterations between imputation sets[83].

Survival analysis

Standard descriptive statistics were used to describe the overall population, and subgroups based on CPE tertiles. The association between CPE and the different survival outcomes (OS, RFS, and DRFS) were investigated with a multivariable Cox proportional hazards regression, including the standard clinicopathologic predictors: age, tumor size, tumor grade, axillary load and systemic treatment (endocrine and/or chemotherapy). Based on its known non-linear relation with patient outcome, age was modeled using a restricted cubic spline with 4 knots[84]. Additionally, survival stratified to CPE tertiles were visualized using Kaplan-Meier plots from which 10-year absolute survival differences between CPE tertiles were derived. The potential association between CPE and long-term survival was determined by testing whether the addition of CPE to the model containing the standard clinicopathologic variables improved model fit using the multivariate Wald-test, for each of the survival outcomes[80,85,86]. Similarly, to investigate whether CPE was associated with endocrine therapy effectiveness, we tested whether the multivariable model improved after addition of the interaction term between CPE and endocrine therapy[80,85,86].

Statistical analysis

CPE was standardized such that 1 unit increase in CPE represents 1 standard deviation increase over the range of CPE. Correlation between CPE and age was based on Pearson's correlation coefficient. Statistical analyses were performed using R version 4.0.2 (R Foundation for Statistical Computing) with the 'smcfcs' (version 1.4.2)[80] and the 'rms' (version 6.0.1) available in R. Coefficient estimates are reported with their corresponding 95% confidence interval (CI). A two-tailed $P < .05$ was considered to represent statistical significance.

Results

Table 2 shows the baseline characteristics for the patient cohort ($N = 1432$), as well as those stratified according to CPE tertiles. Overall median age was 53.5 years (interquartile interval [IQI] = 47, 63). Tumor size, tumor grade, and axillary load were similar between the CPE tertiles. The correlation between CPE and age was

-0.43 (95% CI = -0.47, -0.37, $P < .001$). Consequently, patients with a high CPE (and low age) received more adjuvant systemic therapy, as the indications for AST are broader in younger women.

Table 2. Overview of baseline characteristics for all patients and stratified to CPE tertiles

	All patients (*N* = 1432)	**CPE Tertile 1 (*N* = 324)**	**CPE Tertile 2 (*N* = 325)**	**CPE Tertile 3 (*N* = 324)**
Age (years)				
Median (IQI)	53.5 (47, 63)	58 (51, 65.2)	53 (48, 63)	50 (45, 58)
Tumor size (mm)				
Median (IQI)	15 (11, 21)	15 (11, 22)	15 (12, 22)	15 (10.8, 21)
Tumor grade				
1 (%)	496 (36.2%)	128 (40.3%)	100 (32.2%)	107 (34.3%)
2 (%)	649 (47.3%)	153 (48.1%)	151 (48.6%)	148 (47.4%)
3 (%)	226 (16.5%)	37 (11.6%)	60 (19.3%)	57 (18.3%)
Unknown	61	6	14	12
Number of positive lymph nodes (%)				
0 (%)	945 (66%)	217 (67%)	213 (65.5%)	197 (60.8%)
1 (%)	308 (21.5%)	66 (20.4%)	74 (22.8%)	79 (24.4%)
2 (%)	109 (7.6%)	24 (7.4%)	26 (8%)	31 (9.6%)
3 (%)	70 (4.9%)	17 (5.2%)	12 (3.7%)	17 (5.2%)
Systemic treatment				
No AST (%)	469 (32.8%)	120 (37%)	85 (26.2%)	91 (28.1%)
Only chemotherapy (%)	42 (2.9%)	8 (2.5%)	9 (2.8%)	7 (2.2%)
Only endocrine therapy (%)	324 (22.6%)	79 (24.4%)	74 (22.8%)	69 (21.3%)
Endocrine and chemotherapy (%)	597 (41.7%)	117 (36.1%)	157 (48.3%)	157 (48.5%)
CPE				
Median (IQI)	0.6 (0.4, 0.7)	0.4 (0.3, 0.4)	0.6 (0.5, 0.6)	0.8 (0.7, 0.9)
Unknown	459	0	0	0
Overall survival				
Event (%)	220 (15.4%)	40 (12.3%)	57 (17.5%)	58 (17.9%)
Median follow-up in years (IQI)	10.3 (9.5, 11.5)	10.1 (9.5, 10.9)	10.0 (9.4, 10.9)	10.3 (9.4, 11.4)
Recurrence-free survival				
Event (%)	292 (20.4%)	60 (18.5%)	68 (20.9%)	74 (22.8%)
Median follow-up in years (IQI)	9.1 (6.7, 10.1)	9.0 (6.7, 9.9)	9.0 (6.9, 10.0)	9.2 (6.2, 10.2)
Distant recurrence-free survival				
Event (%)	261 (18.2%)	54 (16.7%)	62 (19.1%)	67 (20.7%)
Median follow-up in years (IQI)	10.2 (9.4, 11.4)	10.0 (9.4, 10.9)	10.0 (9.3, 10.8)	10.2 (9.3, 11.3)

Data are number of patients with percentages between parentheses unless otherwise specified. Note that due to unavailability of CPE for a number of patients (*N* = 458) not all patients are included in the overview stratified to the CPE tertiles. CPE = contralateral parenchymal enhancement, IQI = interquartile interval, AST = adjuvant systemic treatment.

There were 220 OS events at a median follow-up of 10.3 years (IQI = 9.5, 11.5), 292 RFS events at a median follow-up of 9.1 years (IQI = 6.7, 10.1), and 261 DRFS events at a median follow-up of 10.2 years (IQI = 9.4, 11.4; Table 2). Figure 2 shows the survival curves stratified to the CPE tertiles. Absolute differences in the survival outcome after 10 years for OS was 88.5% (95% CI = 88.1%, 89.1%) in tertile 1 (lowest CPE), 85.8% (95% CI = 85.2%, 86.3%) in tertile 2, and 85.9% (95% CI = 85.4%, 86.4%) in tertile 3 (highest CPE). For RFS this was 77.7% (95% CI = 76.9%, 78.5%) in tertile 1, 78.1% (95% CI = 77.4%, 78.9%) in tertile 2, and 76.3% (95% CI = 75.5%, 77.0%) in tertile 3. Lastly, for DRFS this was 84.7% (95% CI = 84.1%, 85.3%) in tertile 1, 83.5% (95% CI =82.9%, 81.8%) in tertile 2, and 81.8% (95% CI = 81.2%, 82.4%) in tertile 3.

Table 3 shows the hazard ratios (HR) of the standard clinicopathologic variables and CPE. Notably, the estimated HR of adjuvant endocrine therapy (or chemotherapy) was not found to be associated with any of the survival outcomes (Table 3). CPE (on a standardized scale) was significantly associated with OS with an HR of 1.2 (95% CI = 1.0, 1.4; *P* = .047), but was not associated with RFS; HR of 1.1 (95% CI = 1.0, 1.3; *P* = .162), or DRFS; HR of 1.1 (95% CI = 1.0, 1.3; *P* = .190) for DRFS. CPE was not associated with endocrine therapy effectiveness (P = .362) in OS, in RFS (P = .945), or DRFS (P = .925). Complete case analysis (*N* = 941) showed comparable results (Supplemental Materials 1, available online).

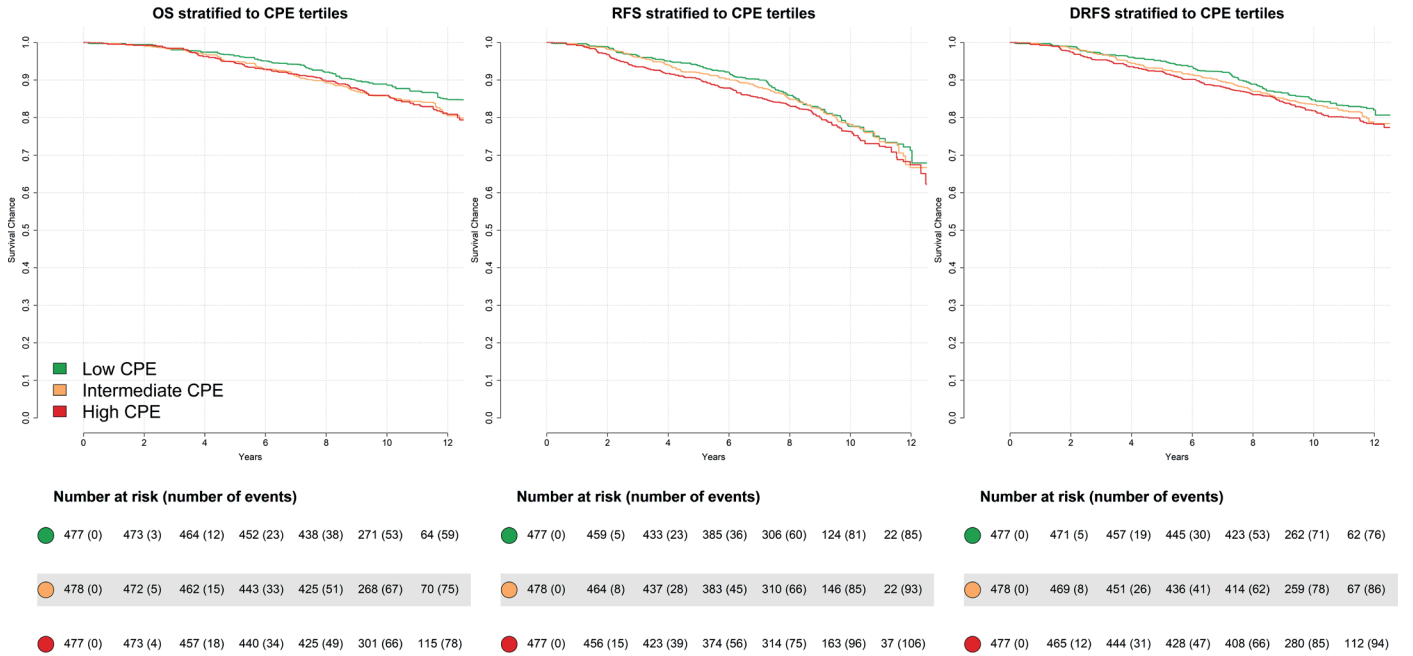


Figure 2. Survival curves for all 3 outcomes (OS, RFS, and DRFS) stratified to CPE tertiles. Note that the overall number of event for each outcome was less than the total number of outcomes as follow-up was restricted to 12 years (maximum follow-up of 90% of the patients). OS = overall survival, RFS = recurrence-free survival, DRFS = distant recurrence-free survival, CPE = contralateral parenchymal enhancement.

Table 3. Multivariable survival estimates for the different survival outcomes (OS, RFS, DRFS)

	HR for OS (95% CI)	P	HR for RFS (95% CI)	P	HR for DRFS (95% CI)	P
Tumor size (mm)	1.02 (1.002, 1.033)	.024	0.974 (0.933, 1.017)	<.001	1.021 (1.007, 1.035)	.003
Tumor grade 1	Ref		Ref		Ref	
Tumor grade 2	0.89 (0.643, 1.233)	.482	0.9 (0.681, 1.189)	.457	0.914 (0.679, 1.231)	.553
Tumor grade 3	1.634 (1.101, 2.425)	.015	1.398 (0.985, 1.983)	.06	1.473 (1.022, 2.123)	.038
Number of positive lymph nodes	1.161 (0.988, 1.365)	.070	1.146 (0.994, 1.32)	.06	1.209 (1.046, 1.398)	.011
Chemotherapy	1.014 (0.672, 1.531)	.947	0.827 (0.585, 1.171)	.284	1.018 (0.701, 1.478)	.924
Endocrine therapy	1.168 (0.744, 1.53)	.724	1.043 (0.765, 1.423)	.788	1.093 (0.78, 1.531)	.605
CPE	1.168 (1.002, 1.36)	.047	1.105 (0.96, 1.272)	.162	1.109 (0.949, 1.295)	.190
Number of events (N)	220		292		261	

Numbers are HR estimates with 95% CI between parentheses, unless stated otherwise. OS = overall survival, RFS = recurrence-free survival, DRFS = distant recurrence-free survival, HR = hazard ratio, CI = confidence interval.

Discussion

This large retrospective multicenter observational cohort study showed that high CPE on preoperative DCE MRI was significantly associated with decreased long-term OS in unilateral ER+/HER2- breast cancer patients after correction for standard clinicopathologic variables. CPE was not associated with RFS or DRFS. The direction of the association was opposite from that what was previously observed in patient treated with adjuvant endocrine treatment[24,25], but it was consistent with a more recent study investigating prognosis after neoadjuvant endocrine treatment[26]. Additionally, we found no indication that CPE is associated with endocrine therapy effectiveness.

Parenchymal enhancement has been investigated as a predictor of outcome in breast cancer[24,25,88–92,27,34–37,39,40,87]. However, there is considerable heterogeneity in the definition of parenchymal enhancement, i.e., qualitative assessment with background parenchymal enhancement (BPE) as codified by the Breast Imaging Reporting and Data System[34,40,87–89,91,92], or quantitative assessment according to different definitions of "quantitative" parenchymal enhancement (e.g., CPE)[24–27,35–37,90]. Additionally, several different outcome measures have been used, including: genomic assay results[92], pathologic complete response[34,35,88,91], and long-term outcome[24,25,27,39,40,87]. Lastly, there were differences in patient study population. This heterogeneity has led to partially conflicting results: in some studies high BPE

was associated with poor outcome[26,37,39,88], while in other studies it was associated with improved outcome[24,25,35,36,92], and yet in other studies not associated with outcome at all[27,91]. In the current study, we aimed to investigate a previously defined quantitative measure of parenchymal enhancement (CPE) in a large patient population (early ER+/HER2- breast cancer patients) with a long follow-up. Three studies have specifically investigated CPE in a similar patient population, of which 2 observed that high CPE was associated with improved survival[24,25], and one performed in an Asian population did not find an association[27]. Our observations indicate that a high CPE was associated with worse overall survival.

There are several differences between previous studies investigating CPE and the current study (the SELECT-study) that may have led to these differences in results. Firstly, differences in patient inclusion: the original studies (in which high CPE was associated with improved prognosis) consecutively included patients based on eligibility for breast-conserving surgery[24,25]. In the study of Shin et al. (in which CPE was not associated with survival) only patients with negative lymph node disease were included[27]. Notably, all patients in their study were treated with endocrine therapy[27]. This resulted in differences in patient and tumor characteristics compared to the patient cohort in the SELECT-study, e.g., in treatment regimens and axillary load. Secondly, studies were performed in different time periods: the study performed in the Netherlands by van der Velden et al. included patients primarily diagnosed in the early 2000's (2000-2008), whereas the SELECT-study included patients who were primarily diagnosed in the late 2000's (2005-2010). Several changes have taken place during this time period: aromatase inhibitors (AI) were introduced for post-menopausal women[93], taxanes were added to the chemotherapy regimen[46,94], and in 2008 guideline recommendations for endocrine therapy were extended in the Netherlands[46]. Different effects of AIs and taxanes on parenchymal enhancement have been reported compared to Tamoxifen and non-taxane chemotherapy[32,95]. Additionally, another study, performed in the time period 2013-2017 in patients undergoing neoadjuvant endocrine therapy, confirmed a positive association between high pretreatment CPE and poor outcome[26]. Lastly, the SELECT-study included more patients (1432) with a longer follow-up (10-15 years). It is likely that the differences in association between CPE and survival between the SELECT- and the other studies can be attributed to (a combination of) these factors.

Endocrine therapy was not observed to be associated with any survival outcome after multivariable correction in our observational data. It is well-established that endocrine therapy is associated with decreased rate of recurrence and is a cornerstone in the treatment of ER+-breast cancer[17,44,96]. The fact that endocrine therapy was not observed to be associated with decreased rate of recurrence suggests that the subgroups of patients receiving endocrine therapy were somehow dissimilar from the subgroup not receiving endocrine therapy. These differences could not be captured by our multivariable analysis which complicates the analysis of association between CPE and endocrine therapy effectiveness. In other words, if the true association between survival and treatment with endocrine therapy could not be estimated from our observational data, it is possible that estimation of the interaction between endocrine therapy and CPE could also have been affected. A similar issue was encountered in the development of the online prognostic tool PREDICT, where the hazard ratio of endocrine therapy could not be adequately determined from observational data[48].

This study has several strong points. We included a large number of patients from multiple centers based on a sample size analysis. We used state of the art techniques to be able to pool data from these 10 centers with different MRI acquisitions[45]. Our estimates include the remaining inter-center variability, and reflect the clinical reality leading to realistic expectations for clinical implementation. We have long-term follow-up of early ER+/HER2- breast cancer patients, other studies investigating parenchymal enhancement and survival generally have a more limited follow-up period[37,39,40].

This study also has several limitations. We were unable to accurately estimate the effect of endocrine therapy on survival after multivariable adjustment, due to this we were also unable to reliably estimate a possible association between endocrine therapy efficacy and CPE. The observed association between CPE and long-term survival are opposite from that previously reported. Although there are several reasons that can explain the opposing results, additional research is needed to investigate the role of CPE as a personalization tool before taking the next step in clinical implementation, i.e., prospective trials. Another limitation is that there was a relatively large fraction of missing data, which could have introduced increased variability and decreased statistical power. However, missing data was multiply-imputed and complete data analysis showed comparable results. CPE is a single

computer-extracted feature and the results could improve if multiple features were investigated with radiomics or artificial intelligence, for example. This is a future research direction, and is out of the scope of this study.

In this large multicenter retrospective study we have shown that CPE on MRI was associated with decreased long-term overall survival in unilateral early ER+/HER2- breast cancer patients. CPE was not associated with recurrence-free survival, distant recurrence-free survival or endocrine therapy effectiveness. Additional research is needed to explore the potential role of CPE or breast MRI as a personalization tool in ER+/HER2- breast cancer.

4

CHAPTER

Tumor response monitoring with breast MRI during neoadjuvant endocrine therapy in breast cancer patients

Chapter 4a: Contralateral parenchymal enhancement on breast MRI before and during neoadjuvant endocrine therapy in relation to the preoperative endocrine prognostic index[1]

Chapter 4b: Prognostic value of breast MRI characteristics before and during neoadjuvant endocrine therapy in patients with ER+/HER2- breast cancer[2]

[1]*Ragusi, M. A. A., Loo, C. E., van der Velden, B. H. M., Wesseling, J., Linn, S. C., Beets-Tan, R. G., Elias, S. G. & Gilhuijs, K. G. A. Contralateral parenchymal enhancement on breast MRI before and during neoadjuvant endocrine therapy in relation to the preoperative endocrine prognostic index. Eur. Radiol. 30, 6740–6748 (2020).*

[2]*Ragusi, M. A. A., Winter-Warnars, G. A., Wesseling, J., Linn, S. C., Beets-Tan, R. G., van der Velden, B.H., Elias, S. G., Gilhuijs, K. G. & Loo, C. E. Prognostic value of breast MRI characteristics before and during neoadjuvant endocrine therapy in patients with ER+/HER2- breast cancer. Br. J. Radiol. 94, (2021).*

CHAPTER 4A

Abstract

Objectives: To investigate whether contralateral parenchymal enhancement (CPE) on MRI during neoadjuvant endocrine therapy (NET) is associated with the preoperative endocrine prognostic index (PEPI) of ER+/HER2- breast cancer.

Methods: This retrospective observational cohort study included 40 unilateral ER+/HER2- breast cancer patients treated with NET. Patients received NET for 6 to 9 months with MRI response monitoring after 3 and/or 6 months. PEPI was used as endpoint. PEPI is based on surgery-derived pathology (pT- and pN-stage, Ki-67, and ER-status) and stratifies patients in 3 groups with distinct prognoses. Mixed effects and ROC analysis were performed to investigate whether CPE was associated with PEPI and to assess discriminatory ability.

Results: The median patient age was 61 (interquartile interval: 52, 69). Twelve patients had PEPI-1 (good prognosis), 15 PEPI-2 (intermediate), and 13 PEPI-3 (poor). High pretreatment CPE was associated with PEPI-3: pretreatment CPE was 39.4% higher on average (95% CI = 1.3, 91.9%; *P* = .047) compared with PEPI-1. CPE decreased after 3 months in PEPI-2 and PEPI-3. The average reduction was 24.4% (95% CI = 2.6, 41.3%; *P* = .032) in PEPI-2 and 29.2% (95% CI = 7.8, 45.6%; *P* = .011) in PEPI-3 compared with baseline. Change in CPE was predictive of PEPI-1 vs PEPI-2+3 (AUC = 0.77; 95% CI = 0.57, 0.96).

Conclusions: CPE during NET is associated with PEPI-group in ER+/HER2- breast cancer: a high pretreatment CPE and a decrease in CPE during NET were associated with a poor prognosis after NET on the basis of PEPI.

Introduction

A positive estrogen receptor (ER) in breast cancer determines if patients should receive endocrine treatment. However, not all patients with ER+ breast cancer benefit from endocrine treatment: 40 - 50% relapse after adjuvant endocrine therapy[97] and 50-70% show a clinical response after neoadjuvant endocrine therapy (NET)[97–99]. A more accurate prediction whether endocrine treatment will be effective would benefit these patients, and allow for better selection and personalization of endocrine treatment.

Early prediction of NET efficacy could be used to personalize the course of treatment, i.e., expedite surgery or switch to neoadjuvant chemotherapy (NAC) in poor responders.

Typically, response monitoring during neoadjuvant therapy is performed with imaging. Magnetic resonance imaging (MRI) of the breast is the most accurate and recommended modality[100,101]. Several MRI features have been identified as predictors of tumor response during NAC[102–107]. However, research regarding response monitoring in NET is limited[89,108].

A potential predictor of endocrine treatment efficacy is contralateral parenchymal enhancement (CPE). CPE is a quantitative measure of the relative late parenchymal enhancement of the healthy breast on MRI[24,25], and differs from background parenchymal enhancement (BPE), which is a qualitative measure of early parenchymal enhancement. CPE is calculated as the mean of the top-10% relatively most enhancing voxels. A high CPE was shown to be associated with improved survival in unilateral ER+ human epidermal growth factor 2 receptor-negative (HER2-) breast cancer patients after adjuvant endocrine therapy[24,25]. If CPE is also associated with NET efficacy, it could be used to personalize the course of NET in breast cancer patients.

It is hypothesized that the contralateral breast represents the diseased breast before tumorigenesis[24], or may represent systemic (inflammatory) effects induced by the tumor[109]. CPE represents the highest delayed enhancement in healthy fibroglandular tissue. CPE might be affected by hormonal activity, as parenchymal enhancement varies during the menstrual cycle[110]. The underlying biological reasons for the observed association between CPE and survival after

endocrine treatment is unknown, but was demonstrated in 2 independent studies [24,25]. Investigating the behavior of CPE during NET might not only provide a tool for the personalization of NET but could also provide insights into the underlying biological mechanisms.

Pathologic complete response (pCR) after neoadjuvant treatment is a controversial surrogate endpoint of prognosis in ER+/HER2- breast cancer[111,112]. pCR is poorly associated with prognosis in ER+/HER2-, and rate of pCR is low in both NAC and NET (about 7.5%, and <10% respectively)[111–113]. To understand how tumor response after NET is related to prognosis, the preoperative endocrine prognostic index (PEPI) was developed[114]. PEPI is derived from the surgical excision specimen after NET, and is based on pT- and pN-stage, Ki-67 index, and ER-status. PEPI stratifies patients in 3 groups with distinct prognoses: PEPI-1 has the most favorable prognosis, whereas PEPI-3 has the poorest prognosis. PEPI can be used to personalize treatment after NET: patients with PEPI-1 have such a favorable prognosis that adjuvant endocrine monotherapy could suffice, whereas appropriate adjuvant treatment should be considered for PEPI-2 and PEPI-3 patients[114,115]. PEPI was validated in the IMPACT trial[114], and the ACOSOG Z1031 trial[115].

In this study, we present a retrospective observational cohort study of patients with invasive unilateral ER+/HER2- breast cancer treated with NET. The aim was to determine whether pretreatment CPE or changes in CPE during treatment are associated with prognosis (on the basis of PEPI) after NET.

Materials and methods

Patient cohort and treatment

This retrospective explorative observational cohort study was approved by the institutional review board of the Antoni van Leeuwenhoek Hospital and the requirement for informed consent was waived. All female patients with pathologically proven unilateral ER+/HER2- breast cancer diagnosed between January 2013 and December 2017 and eligible for NET according to the hospital's institutional guidelines were included (N = 44). Additionally, the contralateral healthy breast did not contain any additional lesions (benign or malignant); a healthy breast is required for the calculation of CPE. The guidelines for NET are as follows: if breast-conserving surgery (BCS) cannot be performed or to reduce

risk of irradicality at surgery (e.g., in the case of an invasive lobular carcinoma) for strongly ER+ (≥50%) / HER2- tumors, NET is recommended for a duration of 6 to 9 months. Additionally, there should be no indication for NAC: the tumor is ≤30 mm and there is ≤1 suspicious lymph node in combination with a low risk Mammaprint 70-gene signature, or if there is excess comorbidity. This is decided during a multidisciplinary meeting. NET consisted of tamoxifen in premenopausal patients and aromatase inhibitors (AI) in postmenopausal patients. Clinical response is assessed after 3 and 6 months with ultrasound or MRI. If the tumor is stable or progressive, surgery is performed or the endocrine treatment is switched; otherwise, the duration of NET is completed.

MR imaging

MR images were acquired on a 1.5-T or 3-T imaging unit (Achieva, Philips) using a dedicated 4-, 7-, or 16-element SENSE breast coil (Philips). First, an unenhanced T1-weighted sequence with fat suppression was performed. Following intravenous injection of gadolinium-containing contrast (0.1 mmol/kg, Dotarem, Guerbet), dynamic contrast series were obtained with early timing 90 s post-contrast injection and late timing 360 s post-contrast injection. One of 2 sets of imaging parameters were used: acquisition time 60 s or 70 s, ratio of repetition time/echo time 3.7/1.9 or 4.3/1.8, flip angle 10°, voxel sizes 0.618 x 0.618 x 1.150 mm^3 or 0.885 x 0.885 x 0.900 mm^3, and a field of view of 400 mm. For 9 patients the pretreatment MRI was performed in a referring hospital. Details of the imaging parameters are provided in the Supplement Materials 1 (available online).

Contralateral parenchymal enhancement

MRIs were processed using a previously reported method[24,25]. Image processing was implemented using Python version 3.7 (Python Software Foundation) with the SimpleITK (version 1.2.0) library[116]. In short, field inhomogeneity was corrected. The breast area was segmented on pre-contrast non-fat-suppressed T1-weighted images and parenchymal tissue was segmented using fuzzy-C means clustering. Early and late post-contrast series were registered to the pre-contrast series to compensate for patient motion. Images with uncorrectable motion artifacts were excluded (N = 2). Relative parenchymal enhancement was calculated at each voxel within the healthy parenchymal tissue by subtracting the early parenchymal

enhancement from the late parenchymal enhancement, and dividing this by the early parenchymal enhancement: $(S_{late} - S_{early}) / S_{early}$, where S represents the signal intensity at the corresponding time point. CPE is calculated as the mean of the top-10% most relatively enhancing voxels, and is a measure of the relative late parenchymal enhancement. CPE is a dimensionless number and can be compared within and between patients.

Endpoint

PEPI was used as a surrogate endpoint of prognosis[114,115]. PEPI is derived from the surgical excision specimen and is based on the following characteristics: pT- and pN-stage, Ki-67 index, and ER-status[114]. Risk points are assigned based on these 4 characteristics. The total risk score (on a scale of 0 - 12) stratifies the patient in 1 of 3 prognostic groups: groups 1 (0 points), 2 (1-3 points), and 3 (≥4 points). Patients with unavailable PEPI-score due to insufficient tumor material in the surgical excision specimen were excluded (n=2). Additionally, the pCR results are provided. pCR was defined as the absence of invasive disease (ypT0/is N0)[117]. Pathologic partial or non-response was based on reduction of tumor cellularity using the Pinder classification[118].

Statistical methods

Standard descriptive statistics were used to describe the study population. Pretreatment CPE tertile values were used to split patients in 3 patient groups for baseline characteristics (baseline characteristics split according to PEPI-group is provided in the Supplement Materials 2, available online). Descriptive statistics are reported as median (interquartile interval [IQI]). A multivariable linear mixed model (LMM) was fit to investigate whether pretreatment CPE or changes in CPE over time are associated with PEPI-group. An LMM is a statistically efficient method to analyze repeated measurements within a patient[119]. In the multivariable analysis, CPE was modeled as a function of time (both categorically at 0, 3, and 6 months and continuously), PEPI-group and the interaction between PEPI-group and time. An interaction between PEPI-group and time allows a possible change of CPE over time to differ between PEPI-groups. CPE was adjusted for baseline differences in age, and type of NET regimen. The differences in pretreatment CPE and changes in CPE during NET between the PEPI-groups can be derived from the same model. To

account for repeated measurements, we included random intercepts for patients. CPE was log-transformed to improve model fit. Nested models were compared using maximum likelihood estimation. Effect estimates were based on restricted maximum likelihood with Satterthwaite's approximations to the degrees of freedom.

Univariable and multivariable logistic regressions were performed to set up models to assess the discriminatory ability of pretreatment CPE and change in CPE (slope). To assess discriminatory ability between PEPI-1 and PEPI-2+3, and between PEPI-1+2 and PEPI-3, the area under the curve (AUC) was calculated using the receiver operating characteristic (ROC) analysis. The ROC analyses were assessed by comparing the underlying logistic regression models using the likelihood ratio test.

Statistical analyses were performed using R version 3.4.4 (R Foundation for Statistical Computing) and the LMM was fit using the 'lme4' (version 1.1.21)[120] and 'lmerTest' (version 3.1.0)[121] packages available in R. Coefficient estimates are reported with their corresponding 95% confidence intervals (CI). A two-tailed $P < .05$ was considered to represent statistical significance. The study is reported following the STROBE guidelines[122].

Results

Patient, tumor and treatment characteristics are summarized in Table 1. Forty patients were included and 81 CPE measurements were available for analysis. The median patient age was 61 years (IQI = 52, 69). Characteristics between these baseline groups were balanced for age, tumor histology, cN-stage, ER-percentage, and pretreatment Ki-67 index (Table 1). Some unbalance was noted in the cT-stage and tumor grade: the group with high baseline CPE (third tertile) showed relatively more prognostic favorable characteristics compared to the groups with lower baseline CPE (e.g., more T1c and grade 1). Premenopausal patients seem overrepresented in the second tertile group, which is reflected in the distribution of NET regimen: more patients in this group received tamoxifen. There was a difference in CPE of +28.5% (95% CI = -48.6, 65.6%, $P = .358$) in premenopausal patients compared with postmenopausal patients.

Table 1. Patient, tumor and treatment characteristics of the entire cohort and according to pretreatment CPE tertile values

Characteristics	Overall (*N* = 40)	Baseline CPE tertile 1 (*N* = 13)	Baseline CPE tertile 2 (*N* = 12)	Baseline CPE tertile 3 (*N* = 13)
CPE				
Median (range)	0.29 (0.16, 0.80)	0.21 (0.16, 0.26)	0.30 (0.27, 0.37)	0.48 (0.39, 0.80)
Age (years)				
Median (IQI)	61 (52, 69)	61 (54, 70)	63 (48, 69)	62 (52, 69)
Menopausal status				
Premenopausal	10 (25.0%)	2 (15.4%)	5 (41.7%)	3 (23.1%)
Tumor size on pretreatment MRI (mm)				
Median (IQI)	28 (26-41)	30 (25-41)	28 (27-29)	36 (27-49)
cT-stage				
1c	8 (20.0%)	1 (7.7%)	2 (16.7%)	5 (38.5%)
2	24 (60.0%)	9 (69.2%)	8 (66.7%)	5 (38.5%)
3	6 (15.0%)	2 (15.4%)	1 (8.3%)	3 (23.1%)
4b	2 (5.0%)	1 (0%)	1 (8.3%)	0 (0%)
cN-stage				
Negative	30 (75.0%)	11 (84.6%)	7 (58.3%)	10 (76.9%)
Positive	10 (25.0%)	2 (15.4%)	5 (41.7%)	3 (23.1%)
Tumor grade				
1	6 (15.4%)	0 (0%)	2 (16.7%)	4 (33.3%)
2	27 (69.2%)	9 (69.2%)	9 (75.0%)	7 (58.3%)
3	6 (15.4%)	4 (30.8%)	1 (8.3%)	1 (8.3%)
Unknown	1	0	0	1
Tumor histology				
IDC	24 (60.0%)	7 (53.8%)	8 (66.7%)	8 (61.5%)
ILC	12 (30.0%)	4 (30.8%)	2 (16.7%)	5 (38.5%)
Other	4 (10.0%)	2 (15.4%)	2 (16.7%)	0 (0%)
ER-percentage				
Median (IQI)	100 (95, 100)	100 (90, 100)	100 (100, 100)	100 (95, 100)
Ki-67				
Pretreatment (IQI)	10 (5, 16.3)	10 (5, 20)	11.3 (10, 16.3)	7.5 (2, 10)
Posttreatment (IQI)	5 (1, 5)	2 (1, 10)	3 (1, 5)	5 (1, 5)
NET duration (months)				
Median (IQI)	7.2 (6.6, 8.0)	7.0 (6.6, 7.6)	7.5 (6.7, 8.7)	7.2 (6.6, 8.7)
Type of NET				
Combination	5 (12.5%)	0 (0%)	2 (16.7%)	3 (23.1%)
Aromatase inhibitor	23 (57.5%)	10 (76.9%)	5 (41.7%)	7 (53.8%)
Tamoxifen	12 (30.0%)	3 (23.1%)	5 (41.7%)	3 (23.1%)

Unless otherwise specified, data are number of patients, with percentages in parentheses. The discrepancy in overall and grouped total patient numbers is due to unavailability of baseline CPE for 2 patients. CPE = contralateral parenchymal enhancement, IQI = interquartile interval, IDC = invasive ductal carcinoma, ILC = invasive lobular carcinoma, ER = estrogen receptor, NET = neoadjuvant endocrine therapy.

Six patients (15%, 6/40) had progressive disease at 3 months of follow-up: 1 patient switched treatment regimen (tamoxifen to AI), and in 5 patients surgery was expedited. The remaining 34 patients were considered (partial) responders at 3 month follow-up and completed the full duration of NET. The median duration of NET was 7.2 months (IQI = 6.6 to 8.0). After NET, 12 patients had a good prognosis (PEPI-1), 15 patients had an intermediate prognosis (PEPI-2), and 13 patients had a poor prognosis (PEPI-3). For the 6 patients who were clinically considered to be non-responders after 3 months, the distribution of PEPI-scores was 1 patient with PEPI-1 (the patient who switched regimen), 2 patients with PEPI-2, and 3 patients with PEPI-3. One patient (2.5%) showed a pCR at surgical pathology, and 5 patients (12.5%) showed no pathologic response. The remaining 34 patients (85%) showed a partial pathologic response after NET (Supplemental Materials 2, available online). The 5 patients who showed no pathologic response related to the PEPI-2 or PEPI-3 group.

Pretreatment CPE and PEPI-group

In the multivariable analysis, pretreatment CPE was on average higher in the group with a poor prognosis after NET (PEPI-3), independent of age and type of NET by 39.4% (95% CI = 1.3, 91.9%; *P* = .047, Table 2). An average difference of +11.4% (95% CI = -17.5, 50.4%; *P* = .474) was observed in PEPI-2 (intermediate prognosis).

Change in CPE over Time and PEPI-group

Change in CPE over time during NET was significantly different between the PEPI-groups ($P_{interaction}$ = .004). In the multivariable analysis, CPE increased over time in patients with a good prognosis (PEPI-1) and decreased in patients with a poor prognosis (PEPI-2 and PEPI-3), independent of age and type of NET. In the model with time modeled categorically, most change in CPE occurred during the first 3 months of NET: CPE increased by 27.6% on average (95% CI = -0.1, 62.9%; *P* = .051) in PEPI-1 compared with baseline, decreased by 24.4% (95% CI = 2.8, 41.3%; *P* = .032) in PEPI-2, and decreased by 29.2% (95% CI = 7.8, 45.6%; *P* = .011) in PEPI-3 (Table 2).

Table 2. Multivariable estimates of differences in CPE according to PEPI-group in time

Variables	%-change in CPE	*P* value
Baseline CPE		
PEPI-1	REF	
PEPI-2	11.4 (-17.5, 50.4)	.474
PEPI-3	39.4 (1.3, 91.9)	.047
Change in CPE for PEPI-1 over time		
Baseline	REF	
After 3 months of NET	27.6(-0.1, 62.9)	.051
After 6 months of NET	29.4 (0.0, 67.4)	.050
Per month*	4.6 (0.3, 9.0)	.042
Change in CPE for PEPI-2 over time		
Baseline	REF	
After 3 months of NET	-24.4 (-41.3, -2.6)	.032
After 6 months of NET	-12.8 (-30.7, 9.6)	.232
Per month*	-2.7 (-6.4, 1.4)	.172
Change in CPE for PEPI-3 over time		
Baseline	REF	
After 3 months of NET	-29.2 (-45.6, -7.8)	.011
After 6 months of NET	-23.7 (-46.6, 9.1)	.135
Per month*	-6.0 (-11.6, 0.1)	.052

Data in parentheses are 95% confidence intervals. Estimates for %-change in CPE with the corresponding PEPI-group as reference group (e.g. change after 3 months in PEPI-3 is -29.2% relative to baseline CPE of PEPI-3). The interaction term (i.e., change in CPE over time dependent on PEPI-group) significantly improved the model (*P* = .004). Results from the model with time as a linear variable are marked with a '*'. Estimates were adjusted for age and type of NET. REF = reference group, CPE = contralateral parenchymal enhancement, PEPI = preoperative endocrine prognostic index, NET = neoadjuvant endocrine therapy.

A representative example is shown in Figure 2. CPE increased by 29.4% on average (95% CI = 0.0, 67.4%; *P* = .050) relative to baseline in PEPI-1 after 6 months. An average difference of -12.8% (95% CI = -9.6, 30.7; *P* = .232) was observed in PEPI-2 and -23.7% (95% CI = -9.1, 46.6%; *P* = .135) in PEPI-3 (Figure 3). In the multivariable analysis with time modeled linearly, CPE increased on average in PEPI-1 by 4.6% (95% CI = 0.3, 9.0 %; *P* = .042) each month, whereas an average difference of -2.7% (95% CI = -1.4, 6.4; *P* = .172) and -6.0% (95% CI = 0.1, 11.6 %; *P* = .052) was observed in PEPI-2 and PEPI-3, respectively, independent of age and type of NET.

Ability of pre- and during-treatment CPE to discriminate between PEPI-groups

Twenty-nine patients were available for ROC analysis to discriminate between PEPI-groups using pretreatment CPE and change in CPE during treatment. Pretreatment CPE was not able to discriminate between the PEPI-groups: the AUC

to distinguish between PEPI-1 and PEPI-2+3 was 0.65 (95% CI = 0.43, 0.87), and 0.67 (95% CI = 0.43, 0.90) to distinguish between PEPI-1+2 and PEPI-3. However, change in CPE was able to discriminate between the PEPI-groups: the AUC to distinguish between PEPI-1 and PEPI-2+3 was 0.77 (95 % CI = 0.57, 0.96), and 0.77 (95% CI = 0.54, 0.99) for PEPI-1+2 vs PEPI-3. Differences in pretreatment CPE were not useful in discriminating between the different PEPI-groups as the AUCs based on both pretreatment CPE and change in CPE during treatment were comparable with the AUCs based solely on the change in CPE: the AUC based on pretreatment and change in CPE was 0.77 (95% CI = 0.59, 0.94; *P* = .307) for PEPI-1 vs PEPI-2+3 and for PEPI-1+2 vs PEPI-3 0.81 (95 % CI = 0.63, 0.96; *P* = .325).

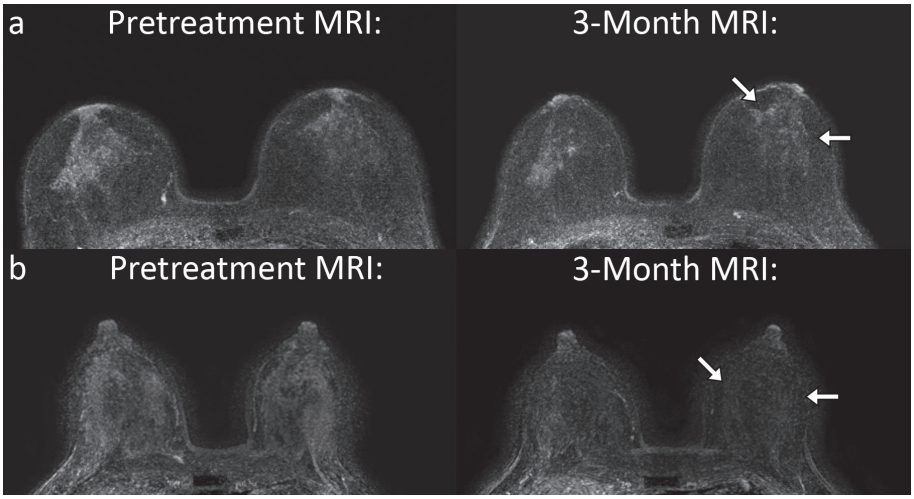


Figure 1. Pretreatment and 3 month follow-up maximum intensity projection images (slab = 25) of the subtraction of the late and early post-contrast series. The top row (**a**) shows the images of a 65-year old patient with a T2N0M0 lobular carcinoma in the right breast. Note the persistence of parenchymal enhancement after 3 months of neoadjuvant endocrine therapy on the subtraction images of the late and early post-contrast series (arrows). The tumor was PEPI-1 (good prognosis) at surgical pathology. The bottom row (**b**) shows the images of a 45-year old patient with a T1cN1M0 ductal carcinoma in the right breast. Note the decrease in parenchymal enhancement after 3 months of neoadjuvant endocrine therapy on the subtraction images of the late and early post-contrast series (arrows). The tumor ended up being PEPI-3 (poor prognosis) at surgical pathology.

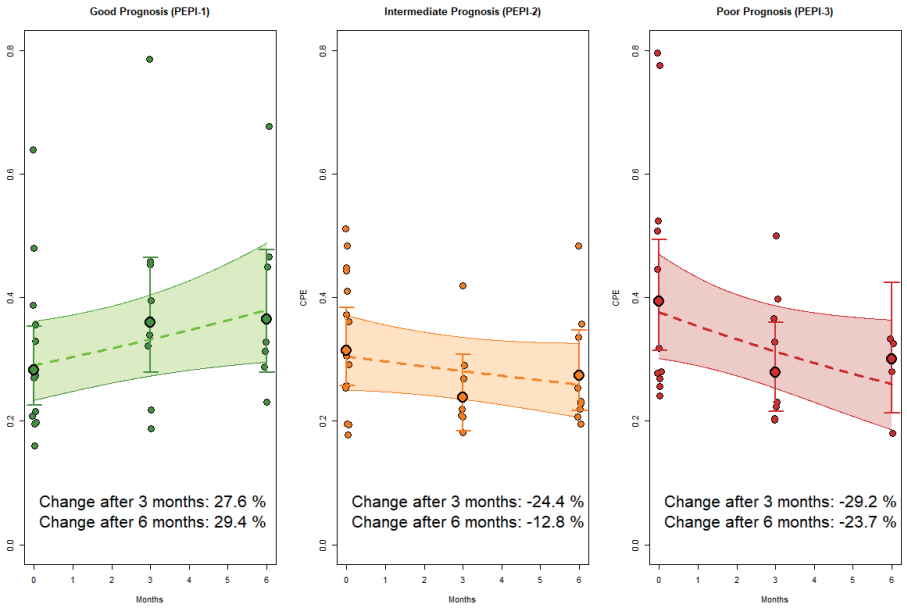


Figure 2. Overview of the change in CPE over time for the different PEPI-groups during neoadjuvant endocrine therapy at different time-points: pretreatment (0 months), after 3 months, and after 6 months and per month. Individual CPE values are shown as dots. Modeled CPE is shown over time, with time modeled categorically (points with the 95% CI as whiskers) and with time modeled linearly (dashed line with and the shaded areas as the 95% CI). CPE increased over time in patients with a good prognosis after NET (PEPI-1), whereas it decreased over time in patients with an intermediate or poor prognosis after NET (PEPI-2 and PEPI-3).

Discussion

In this retrospective single-center observational cohort study, we showed that pretreatment CPE, a quantitative measure of relative late parenchymal enhancement on MRI, and change in CPE during NET were associated with PEPI-group in the post-treatment surgical specimen: a high pretreatment CPE and a decrease in CPE during NET were associated with a higher PEPI-group (poor prognosis).

Research regarding response imaging during NET is limited. Our results are in agreement with the findings of Hilal et al., who found that high pretreatment BPE, classified according the BI-RADS lexicon, was associated with non-responders after NET[89]. In the NAC setting, BPE has been linked to several treatment outcomes[102]: a high BPE before start of NAC was associated with worse recurrence-free survival (RFS)[123], while a decrease in BPE during NAC was associated with pCR[124–126].

While a decrease in parenchymal enhancement on MRI during NAC is reported to be associated with pCR, in our study, a decrease in CPE was associated with an unfavorable prognosis after NET. Perhaps one would expect parenchymal enhancement to decrease in patients with effective endocrine treatment due to depressed hormonal activity, as BPE is increased during physiological hormonal activity[127] or during hormone replacement therapy[128,129]. BPE was associated with increased microvessel density[130]: persistent or increased parenchymal enhancement during NET might reflect increased perfusion and better drug delivery. CPE was not associated with percent staining of ER or progesterone receptor on immunohistochemistry, nor with genomic ER-pathway activity in the tumor[25,42]. A different explanation for these opposing effects between the different neoadjuvant therapies might be due to different immunohistochemical subtypes of breast cancer. It is known that breast cancer is a heterogeneous disease with different prognoses, treatment, and imaging characteristics, especially in ER+/HER2- breast cancer[131]. Differences in tumor biology and treatment mechanisms (cytotoxic chemotherapy vs antiproliferative endocrine therapy) could have had different systemic effects on the fibroglandular tissue, which could lead to differences in the behavior of parenchymal enhancement. Without a clear understanding of the biological basis of parenchymal enhancement and treatment efficacy, and the (dis)similarity between BPE and CPE, it is difficult to provide an explanation for these opposing findings between NAC and NET.

Although the changes in parenchymal enhancement are counterintuitive in the context of chemotherapy, a high CPE was previously associated with a favorable prognosis after adjuvant endocrine therapy[24,25]. In our study, an increase of CPE is associated with a favorable prognosis after NET. In that sense, a high CPE after NET was also associated with a favorable prognosis (PEPI-1).

Remarkably, high pretreatment CPE was related to a poor prognosis (PEPI-3) at final pathology, whereas high CPE was previously shown to be related with improved overall and invasive disease-free survival after adjuvant endocrine therapy[24,25]. The exact reason for this finding is unknown, although the difference might simply be due to different end points. Additionally, pretreatment CPE alone was not useful in distinguishing between the different PEPI-groups at final pathology.

PEPI was used as a surrogate endpoint of prognosis because pCR and change in tumor size are poorly associated with prognosis in ER+/HER2- breast cancer[111,112].

Specifically for ER+/HER2- breast cancer, change in tumor size during NAC is a poor predictor of response and a poorly reproducible surrogate endpoint of survival[132,133]. Change in tumor size during NAC yielded a non-significant AUC for the prediction of pCR in 1 study[134], and was not associated with survival after NAC in another study[131]. Additionally, clinical response during NET was not associated with survival [114]. In our study, change in CPE during NET was associated with prognosis (on the basis of PEPI), and performed similarly to other mid-treatment predictors of tumor response in ER+/HER2- breast cancer after NAC: change in CPE discriminated PEPI with an AUC of 0.77, and change in apparent diffusion coefficient discriminated pCR with an AUC of 0.76[107]. To our knowledge, CPE is the first quantitative imaging feature that was observed to be associated with prognosis at final pathology after NET.

Our results support the hypothesis that the healthy breast contains information about endocrine treatment success for patients with unilateral ER+/HER2- breast cancer. CPE was reported to stratify patients within high-risk groups based on genomic assays (70-gene signature and 21-gene recurrence score)[43]. These results suggest that CPE contains prognostic information independent of these genomic assays, and could potentially be used to further personalize treatment.

The main limitation of this study is its relatively small size, which is reflected in the wide CIs of the estimates, and limits the power to detect small effects. To account for the small population size we took full advantage of the statistical efficiency of a linear mixed model for the repeated measurements analysis, and the association between CPE and prognosis after NET was strong enough to reach the a priori defined significance threshold of $<.05$. The association between survival and CPE was previously shown to reproduce between different MRI vendors and small differences in imaging parameters[25]. For 9 patients, the pretreatment MRI was performed in the referring hospital on a different MRI vendor which could have led to variability in the CPE measurements. However, the flip angle and repetition time, being the imaging parameters with the most influence on intensity[135], were similar over the entire cohort. Despite the differences in parameters, CPE was observed to be significantly associated with PEPI. Additionally, exclusion of the 9 referred patients did not influence the results. Although there is currently no consensus on the optimal duration of NET, recent clinical studies treat patients for up to 24 weeks (about 6 months)[113], as there is evidence that maximum tumor

response may be reached after 6 to 7 months of NET[136]. In this study, patients received NET for a median duration of 7.2 months. The findings should be validated in a larger cohort to assess the discriminatory ability of CPE during NET. Lastly, an important step for the implementation of quantitative measurements of parenchymal enhancement is the development of software for use in clinical practice.

In conclusion, pretreatment and changes in contralateral parenchymal enhancement during neoadjuvant endocrine treatment were associated with PEPI-group in unilateral ER+/HER2- breast cancer patients: a high pretreatment CPE and a decrease in CPE during NET were associated with a poor prognosis after NET on the basis of PEPI. Future research will focus on the potential of CPE to assess endocrine treatment effectiveness.

CHAPTER 4B

Abstract:

Objectives: To investigate whether BIRADS MRI characteristics before or during neoadjuvant endocrine therapy (NET) are associated with the preoperative endocrine prognostic index (PEPI) in ER+/HER2- breast cancer patients.

Methods: This retrospective observational cohort study included 35 ER+/HER2- patients with 38 tumors (3 bilateral cases) treated with NET. The pretreatment and midtreatment (after 3 months) MRIs were evaluated by 2 breast radiologists for BIRADS imaging characteristics, shrinkage pattern, and radiologic response. PEPI was used as endpoint. PEPI is based on the post-treatment surgical specimen's pT- and pN-stage, Ki-67, and ER-status. Tumors were assigned PEPI-1 (good prognosis) or PEPI-2/3 (poor prognosis). We investigated whether pretreatment and midtreatment BIRADS characteristics were associated with PEPI.

Results: Median patient age was 65 years (interquartile interval [IQI]: 53, 70). Seventeen tumors (44.7%) were associated with good prognosis (PEPI-1), and 21 tumors (55.3%) with poor prognosis (PEPI-2/3). A larger reduction in tumor size after 3 months of NET was significantly associated with PEPI; 10 mm (IQI = 5, 13.5) in PEPI-1 tumors vs 4.5 mm (IQI = 3, 7; *P* = .045) in PEPI-2/3 tumors. Other BIRADS characteristics, shrinkage pattern or radiologic response were not associated with PEPI.

Conclusions: Only a larger reduction in tumor size on MRI after 3 months of NET was associated with PEPI-1 (good prognosis) in ER+/HER2- breast cancer patients.

Introduction

Neoadjuvant treatment for patients with estrogen receptor-positive (ER+) human epidermal growth factor receptor 2-negative (HER2-) breast cancer includes neoadjuvant endocrine therapy (NET) and neoadjuvant chemotherapy (NAC). NET leads to similar rates of breast-conserving surgery (BCS) and pathologic response rates compared to NAC in strong ER+ breast cancer patients[113]. However, NET has the advantage of being less toxic compared to NAC[113].

About 50-70% of patients show a clinical response during NET[97]. In order to identify patients who will benefit from NET, it is important to monitor the tumor during treatment to allow for therapy adjustment, e.g. expediting surgery or switching treatment regimen. Response monitoring during neoadjuvant treatment is mostly done using MRI because it is the most sensitive modality to assess tumor response[100]. Many studies have identified MRI characteristics during NAC that are associated with tumor response and prognosis[104,107,131,137], whereas studies investigating MRI during NET are limited[89,138].

The performance of MRI to predict response after NAC differs among the different immunohistochemical subtypes[104,131]. Especially predicting response in ER+/HER2- breast cancer has proven to be difficult[104,131]. For example, change in tumor size on MRI during NAC was associated with response in triple negative (TN) and HER2+ breast cancer, but was not associated with response or prognosis in ER+ breast cancer[131,134]. Changes in apparent diffusion coefficient (ADC)[107], and tumor shrinkage pattern during NAC, however, did show an association with tumor response in ER+/HER2- breast cancer[137].

Pathologic complete response (pCR) is typically used as surrogate endpoint of survival in neoadjuvant studies. However, pCR might not be suited for ER+ breast cancer, because the rate of pCR is low (about 10%), and is poorly associated with prognosis[111,112]. This might also explain the relatively poor performance of MRI to predict response in ER+/HER2- breast cancer. The preoperative endocrine prognostic index (PEPI) was developed as a surrogate endpoint of survival for ER+/HER2- breast cancer after NET, and might better predict survival than pCR in this subset of patients. PEPI is derived from the histopathological evaluation after NET. Patients are stratified in 3 prognostic groups (PEPI-1, PEPI-2, and PEPI-3) based on pT- and pN-stage, the Ki-67 index, and ER-status[114,115]. PEPI-1 is associated with the best prognosis, and PEPI-3

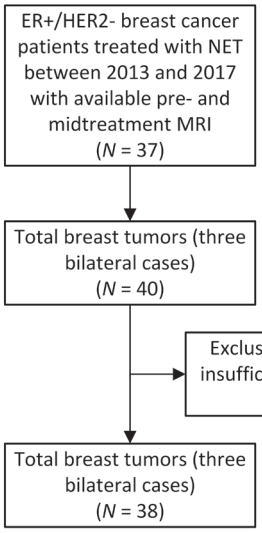
is associated with the poorest. Patients with a PEPI-1 after NET have such a favorable prognosis that adjuvant endocrine monotherapy might suffice, whereas patients with a PEPI-2 or PEPI-3 should be recommended adjuvant chemotherapy[114,115]. Prediction of PEPI before or during NET could allow for therapy adjustments in patients who are predicted to have a poor prognosis after NET (i.e. PEPI-2 or PEPI-3).

The aim of this study was to investigate whether MRI characteristics before and during NET were associated with PEPI after NET. We have focused on those characteristics that were previously associated with response or prognosis in NAC, namely: Breast Imaging Reporting and Data System (BIRADS) MRI characteristics, diffusion-weighted imaging (DWI) findings, and radiologic response.

Materials and methods

Patient cohort and treatment

This retrospective explorative observational cohort study was approved by the institutional review board of the Antoni van Leeuwenhoek Hospital and the requirement for informed consent was waived. All patients diagnosed with pathologically proven ER+/HER2- breast cancer treated with NET between January 2013 and December 2017 with available pretreatment and midtreatment (after 3 months) MRI were consecutively included (N = 37; Figure 1). Three patients had a bilateral tumor. In total 40 tumors were included in the study. NET was recommended to patients with strong ER+ (≥50%) / HER2- tumors where BCS could not be performed or to reduce the risk of involved surgical margins (e.g., in the case of an invasive lobular carcinoma [ILC]). Additionally, there should be no indication for NAC for these patients: the tumor is ≤30 mm and there is ≤1 suspicious lymph node in combination with a low risk Mammaprint 70-gene signature (Agendia). In case of excess comorbidity (e.g. in cases where NAC or primary surgery at that time is expected to put excessive strain on the patient), NET is also recommended. The decision for NET is made during a multidisciplinary meeting. Tamoxifen (for premenopausal patients), aromatase inhibitors (AI, for postmenopausal patients), or a sequential combination of both agents was recommended for a duration of 6 to 9 months. A breast tissue marker was placed before start of treatment for future localization of the tumor[104]. The midtreatment response MRI is performed after 3 months of NET: in case of unfavorable tumor response (i.e. stable or progressive disease), surgery is expedited or the endocrine therapy is switched.



Available imaging	Pretreatment	Midtreatment
Contrast-enhanced	38 (100%)	38 (100%)
Kinetic analysis	29 (76.3%)	36 (94.7%)
Diffusion-weighted	29 (76.3%)	34 (94.4%)

Figure 1. Flowchart of patient inclusion and availability of imaging sequences at the different timepoints. MRI = magnetic resonance imaging, ER = estrogen receptor, HER2 = human epidermal growth factor receptor-2.

MRI Technique

MRI was performed before start and after 3 months of NET and included axial DWI and dynamic contrast-enhanced (DCE) imaging with patients in prone position (Figure 1). MRI was performed on a 1.5-T or a 3-T imaging unit (Achieva, Philips) with a dedicated 7- or 16-element SENSE breast coil (Philips).

DWI was performed using *b* values of 0, and 800 sec/mm^2; *b* values of 0, and 1000 sec/mm^2*;* *b* values of 0, and 1200 sec/mm^2*;* or *b* values of 0, 150, and 1500 sec/mm^2. The following imaging parameters were used: ratio of repetition time/echo time 5500/71 or 7000/90, flip angle 90°, voxel sizes 0.90 x 0.90 x 5 mm^3 or 0.99 x 0.99 x 5 mm^3, and a field of view of 380 or 400 mm.

The DCE protocol consisted of an unenhanced 3-dimensional T1-weighted fast field echo sequence with fat suppression before intravenous injection of gadolinium-containing contrast (0.1 mmol/kg, Dotarem, Geurbet), followed by 5 consecutive series of dynamic post-contrast images at 60 s or 90 s intervals.

Two sets of imaging parameters were used: acquisition time 60 s or 90 s ratio of repetition time/echo time 4.3/1.8 or 3.7/1.9, flip angle 10°, voxel sizes 0.62 x 0.62 x 2.3 mm^3 or 0.89 x 0.89 x 1.8 mm^3, and a field of view of 400 mm (Supplemental Materials 1, available online). For 9 patients the pretreatment MRI was performed in the referring hospital.

MRI Evaluation

Two dedicated breast radiologists (C.L. and G.W., with 18 and 30 years of experience) retrospectively reviewed the pretreatment and midtreatment MRIs. The radiologists independently interpreted the images and were blinded to the pathologic outcome. Only information regarding the laterality was made available in the case of bilateral tumors. Disagreements were overcome by reviewing the images in consensus.

The morphologic and kinetic features were evaluated according to the BIRADS[139]. The largest tumor in the breast was considered the index lesion. The size of the tumor was measured as its largest diameter in 1 of the 3 planes (sagittal, coronal, or axial) during initial enhancement (60-90 s post-contrast) and late enhancement (360-450 s post-contrast). In the case of a bilateral tumor, the index tumor of each breast was assessed independently. Kinetic features of the lesions were evaluated using DynaCAD (Invivo, Philips). After 3 months the tumors were additionally evaluated on tumor shrinkage pattern, radiologic response, and the Response Evaluation Criteria in Solid Tumors (RECIST)[101]. The shrinkage pattern classification was adapted from Fukada et al.; complete response (no visible tumor), concentric shrinkage; reduction of the largest diameter with disappearance of non-mass enhancement (residual foci of < 5 mm were allowed), non-concentric shrinkage; if the shrinkage pattern couldn't be classified as concentric (e.g. decrease of intensity only, or diffuse decrease with non-mass enhancement), and stable or progressive growth (Figure 2)[137]. The radiologic response was classified as; complete response (absence of pathological enhancement), partial response (partial disappearance of enhancement), and no response (stable or progressive disease). Lastly, the RECIST response categories included: disappearance of enhancing tumor was classified as complete response, ≥30% decrease in tumor size (initial enhancement) was classified as partial response, ≥20% increase in tumor size (initial enhancement) or the appearance of new lesions was classified as progressive disease, and if

the shrinkage didn't qualify for partial nor progressive disease the response was classified as stable disease[101].

For the DWI assessment, the tumor was first identified on the DCE images and then localized on the DWI and the ADC maps. Both radiologists assessed the images for the presence of diffusion restriction in the tumor, which was defined as high signal intensity on the DWI combined with low signal intensity on the ADC maps.

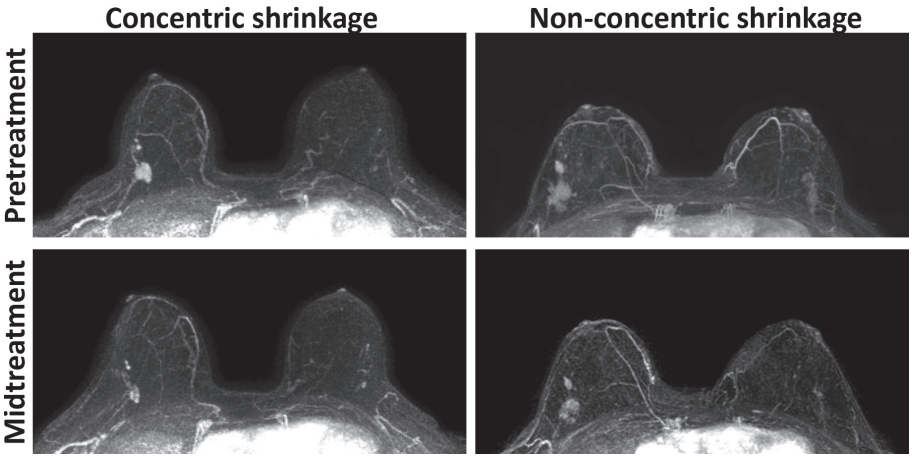


Figure 2. Examples of a concentric shrinkage pattern (**left column**) and a non-concentric shrinkage pattern (**right column**). The tumor in the right column shows a diffuse decrease after 3 months of NET (a non-concentric shrinkage pattern). This patient also showed segmental enhancement in the lateral upper quadrant of the left breast. This proved to be a complex sclerosing lesion at biopsy. The definitions of shrinkage pattern were adapted from Fukada et al.[137]**.** NET = neoadjuvant endocrine therapy.

Pathologic response assessment

PEPI was used as endpoint[114,115]. PEPI is derived from the surgical specimen after NET and is based on pT- and pN-stage, Ki-67, and ER-status. Tumors are assigned risk points (0-12) based on these characteristics. The risk points stratify patients in 1 of 3 prognostic groups: PEPI-1 (0 points), PEPI-2 (1-3 points), and PEPI-3 (4 or more points) with distinct prognosis[115]. It is proposed that patients with PEPI-1 have such a favorable prognosis after NET that monotherapy with adjuvant endocrine therapy can suffice after surgery, whereas adjuvant chemotherapy should be considered for PEPI-2 and PEPI-3[114,115]. As both PEPI-2 and PEPI-3 should be considered for adjuvant chemotherapy, the a priori decision to analyze PEPI-1 vs PEPI-2/3 was made, a method that was also adopted by a recent publication

on the validation of PEPI[115]. Two patients were excluded due to insufficient tumor material in the surgical specimen to assess PEPI (Figure 1).

Statistical analysis

Summary statistics are reported as median (interquartile interval [IQI]). The inter-rater agreement for categorical variables was calculated using Cohen's kappa. For continuous variables the mean difference with limits of agreement, based on Bland-Altman analysis, and the intraclass correlation coefficient (ICC; two-way random-effects, absolute agreement, single rater) were calculated with 95% confidence intervals (95% CI)[140]. Cohen's kappa was interpreted as: < 0, poor agreement; 0.01-0.20, slight agreement; 0.21-0.40, fair agreement; 0.41-0.60, moderate agreement; 0.61-0.80, substantial agreement; 0.81-1, almost perfect agreement[141]; and the ICC was interpreted as: < 0.5, poor reliability; 0.5-0.75, moderate reliability; 0.75-0.9, good reliability; > 0.9, excellent reliability[140]. The results after the consensus readings were used to investigate whether BIRADS characteristics on MRI before and after 3 months of NET were associated with the PEPI-groups. Statistical differences for categorical variables were calculated using Fisher's exact test, the Mann-Whitney U test for unpaired continuous variables, and the Wilcoxon signed-rank test for paired continuous variables. Statistical analyses were performed using R version 4.0.2 (R Foundation for Statistical Computing, Vienna). A two-tailed $P < .05$ was considered to represent statistical significance.

Results

Table 1 summarizes patient, tumor, and treatment characteristics. The pretreatment and midtreatment MRI of 35 patients and 38 tumors (3 bilateral cases) were evaluated. The median age at diagnosis was 65 years (IQI = 53, 70). Clinical stage was mostly stage I (26.3%) or II (60.5%), there was 1 clinical stage 0 (ductal carcinoma in situ in a bilateral case) and 4 cases of clinical stage III (10.5%). Pretreatment Ki-67 was similar between the PEPI-groups. Patients received NET for a median duration of 7.4 months (IQI = 6.6, 7.9), and BCS could be performed in 31 patients (81.6%). At histopathological evaluation 17 tumors (44.7%) were associated with a good prognosis, or PEPI-1, whereas 21 patients (55.3%) were associated with a relatively poor prognosis, or PEPI-2/3.

Table 1. Patient, treatment and tumor characteristics

	All tumors (*N* = 38)	PEPI-1 (*N* = 17) Good prognosis	PEPI-2/3 (*N* = 21) Poor prognosis
Age (years)			
Median (IQI)	65 (53, 70)	66.5 (54, 71)	60 (49.5, 69.5)
Laterality			
Unilateral	32 (84.2%)	13 (76.5%)	19 (90.5%)
Bilateral	6 (15.8%)	4 (23.5)	2 (9.5%)
Tumor histology			
DCIS	1 (2.6%)	1 (5.9%)	0 (0%)
IDC	22 (57.9%)	11 (64.7%)	11 (52.4%)
ILC	11 (28.9%)	3 (17.6%)	8 (38.1%)
Mixed IDC/ILC	4 (10.5%)	2 (11.8%)	2 (9.5%)
Clinical stage			
0	1 (2.6%)	1 (5.9%)	0 (0%)
I	10 (26.3%)	8 (47.1%)	2 (9.5%)
II	23 (60.5%)	7 (41.2%)	16 (76.2%)
III	4 (10.5%)	1 (5.9%)	3 (14.3%)
Tumor grade			
1	7 (18.9%)	5 (31.2%)	2 (9.5%)
2	24 (64.9%)	7 (43.8%)	17 (81%)
3	6 (16.2%)	4 (25%)	2 (9.5%)
Unknown	1	1	0
ER-percentage (IQI)			
Median (IQI)	100 (97.5, 100)	100 (100, 100)	100 (95, 100)
PR-percentage (IQI)			
Median (IQI)	80 (25, 92.5)	70 (45, 97.5)	80 (3, 90)
Ki-67 (%)			
Pretreatment (IQI)	10 (5, 20)	11.3 (3, 20)	10 (5, 16.3)
Posttreatment (IQI)	2 (1, 5)	1 (1, 2)	5 (1, 10)
Duration of NET (months)			
Median (IQI)	7.4 (6.6, 7.9)	7.6 (6.8, 8.6)	7.0 (6, 7.7)
Therapy			
AI	26 (68.4%)	12 (70.6%)	14 (66.7%)
Tamoxifen	8 (21.1%)	2 (11.8%)	6 (28.6%)
Combination	4 (10.5%)	3 (17.6%)	1 (4.8%)
Surgery			
BCS	31 (81.6%)	15 (88.2%)	16 (76.2%)
No BCS	7 (18.4%)	2 (11.8%)	5 (23.8%)

Unless otherwise specified data are number of tumors, with percentages in parentheses. PEPI = preoperative endocrine prognostic index, IQI = interquartile interval, DCIS = ductal carcinoma in situ, IDC = invasive ductal carcinoma, ILC = invasive lobular carcinoma, ER = estrogen receptor, PR = progesterone receptor, NET = neoadjuvant endocrine therapy, AI = aromatase inhibitor, BCS = breast conserving surgery.

Inter-rater agreement

The inter-rater agreement for the BIRADS characteristics are summarized in Table 2. Most BIRADS characteristics show fair to moderate agreement, although the inter-rater agreement of the subclassifications for (non)mass shape and enhancement characteristics were poor. The mean inter-rater difference in pretreatment tumor size was -3.68 mm with limits of agreement between -27.7 mm and 20.3 mm, similarly, the mean difference in midtreatment tumor size was 0.3 mm with limits of agreement between -22.5 mm and 23.0 mm (Figure 3). Large disagreements in tumor size were in cases when the radiologists disagreed about the focality of the tumor (i.e. the index lesion in a unifocal versus a multifocal tumor), or in the case of non-mass enhancement. The inter-rater agreement for tumor size at early enhancement was moderate with an ICC of 0.68 (95% CI: 0.50, 0.80; P <.001) for pretreatment tumor size, and 0.70 (95% CI: 0.53, 0.81; P < .001) for midtreatment tumor size.

Table 2. Inter-rater agreement for BIRADS characteristics, DWI, shrinkage pattern, and radiologic response of pretreatment and midtreatment MRI during NET

	Inter-rater agreement	
	Pretreatment	**Midtreatment**
Fibroglandular tissue	0.482 (0.260, 0.705)	0.440 (0.208, 0.672)
Background parenchymal enhancement	0.681 (0.502, 0.859)	0.298 (0.030, 0.566)
Presence of mass	0.713 (0.459, 0.968)	0.684 (0.458, 0.911)
Mass – Shape	0.090 (-0.077, 0.257)	0.095 (-0.086, 0.276)
Mass – Margin	0.292 (-0.063, 0.646)	0.486 (0.085, 0.886)
Mass – Internal enhancement	0.193 (0.029, 0.358)	0.289 (0.041, 0.538)
Presence of non-mass enhancement	0.612 (0.357, 0.867)	0.469 (0.189, 0.750)
Non-mass – Distribution	-0.236 (-0.427, -0.045)	0.158 (-0.124, 0.440)
Non-mass – Internal enhancement	0.441 (-0.034, 0.916)	0
Kinetics – Early enhancement	0.482 (-0.110, 1.000)	0.519 (0.294, 0.744)
Kinetics – Late enhancement	0.482 (0.120., 0.844)	0.449 (0.204, 0.694)
Presence of diffusion restriction	0.889 (0.676, 1.000)	0.422 (0.139, 0.705)
Shrinkage pattern		0.517 (0.308, 0.725)
Radiologic response		0.670 (0.428, 0.912)

Data are Cohen's kappa (95% CI). DWI = diffusion weighted-imaging, NET = neoadjuvant endocrine therapy, 95% CI = 95% confidence interval.

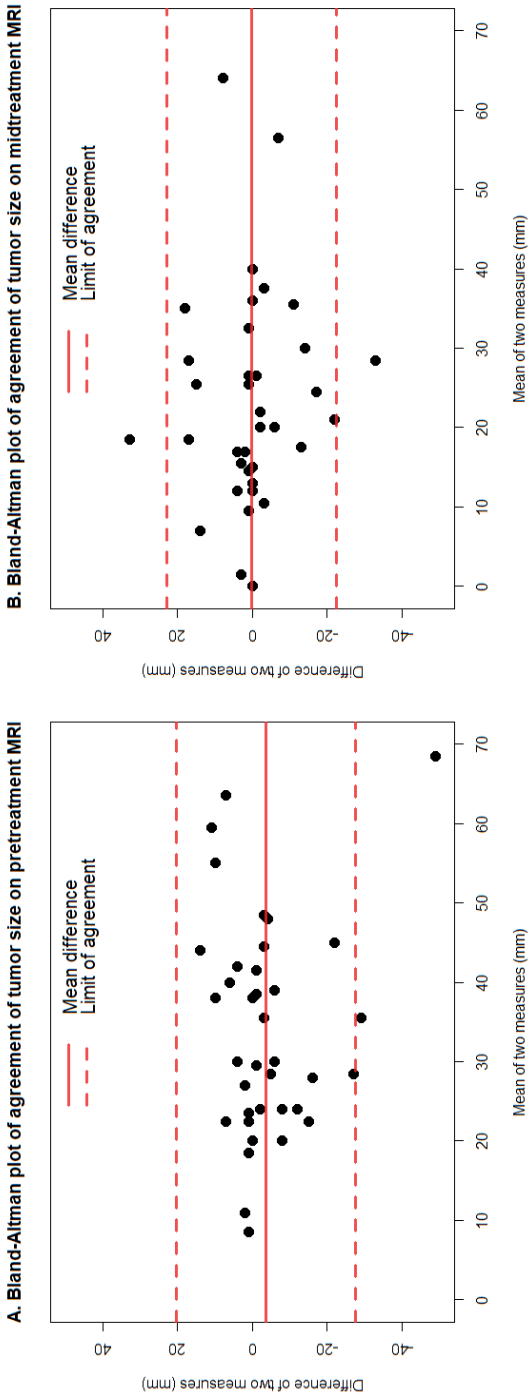


Figure 3. Two Bland-Altman plots showing the interrater agreement of pretreatment tumor size (**A**) and midtreatment tumor size (**B**) in mm at initial enhancement on MRI. MRI = magnetic resonance imaging.

Associations between BIRADS characteristics and PEPI-groups

Tumor size at initial or late enhancement on pretreatment imaging was not significantly different between the PEPI-groups (*P* = .803 and *P* = .162) nor after 3 months of NET (*P* = .953 and *P* = .517). The change in tumor size at initial enhancement, after 3 months of treatment, decreased in both PEPI-groups. However, a larger reduction in tumor size was observed in tumors that ended up being a PEPI-1 (good prognosis) at histopathological evaluation. Tumor size decreased on average in PEPI-1 by 10 mm (IQI = 5, 13.5) compared to an average decrease of 4.5 mm (IQI = 3, 7; *P* = .045; Figure 4) in PEPI-2/3. No other BIRADS characteristics of the pretreatment MRI or the midtreatment MRI were significantly associated with PEPI (Supplemental Materials 2, available online). Background parenchymal enhancement (BPE) decreased in all patients, but was not associated with PEPI (*P* = .770). Lastly, shrinkage pattern (*P* = .578), radiologic response (*P* = .483), and RECIST (*P* = .790) were also not associated with PEPI (Table 3). All 3 patients with a complete radiologic response were diagnosed with an ILC. Two of these patients with a radiologic complete response had a PEPI-2/3 (poor prognosis) at histopathological evaluation and in both patients BCS could not be performed. These patients had involved surgical margins at pathology after attempting BCS, and underwent a mastectomy afterwards. Two examples of the pretreatment and midtreatment MRIs are shown in figure 5 and 6.

Association between DWI and PEPI-groups

Pretreatment DWI was available for 29 tumors, and midtreatment DWI for 34 tumors. There was no significant difference between the presence of diffusion restriction assessed qualitatively on pretreatment imaging (*P* = .622) nor at the midtreatment imaging (*P* = .314) between the PEPI-groups (Supplemental Materials 2, available online).

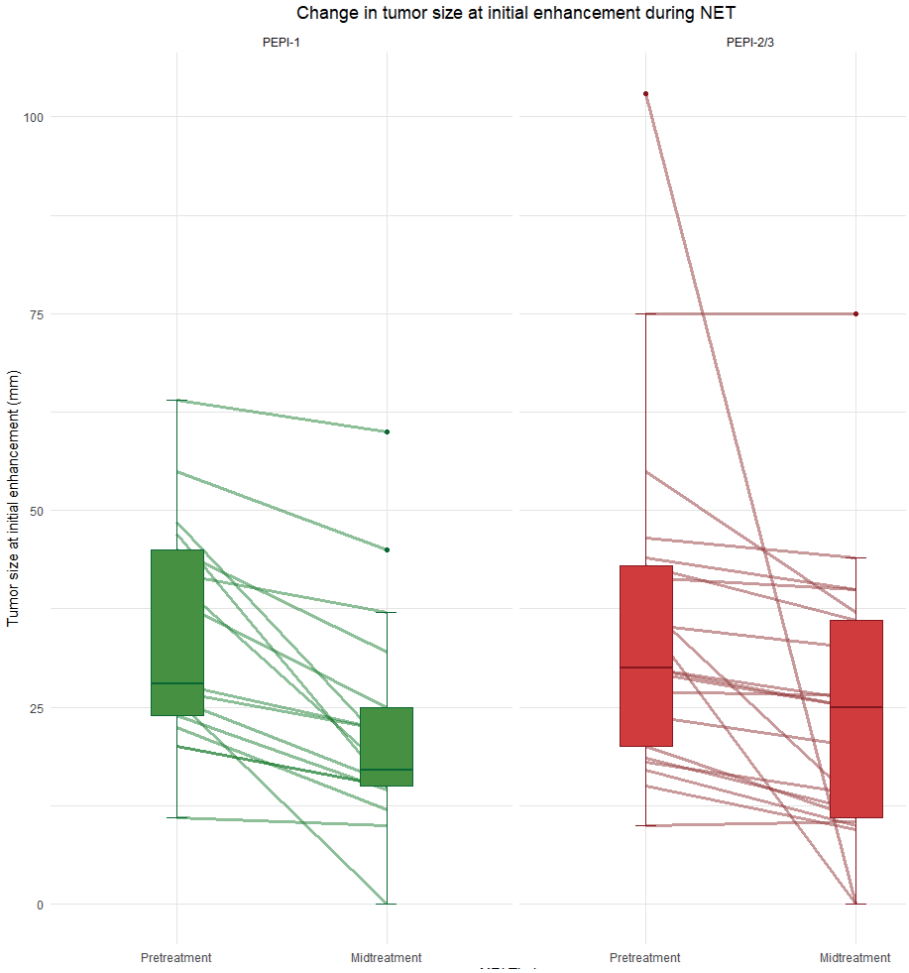


Figure 4. Tumor size at initial enhancement before start of NET and after 3 months of NET. Change in tumor size was associated with PEPI after NET (*P* = .045). However, tumor size decreased on average in both PEPI-groups: it decreased by 10 mm (IQI = 5, 13.5) in PEPI-1 (good prognosis) vs 4.5 mm (IQI = 3, 7) in PEPI-2/3 (poor prognosis). NET = neoadjuvant endocrine therapy, PEPI = preoperative endocrine prognostic index, IQI = interquartile interval.

Table 3. Shrinkage pattern and radiologic response at midtreatment MRI during NET

	PEPI-1 (*N* = 17) Good prognosis	**PEPI-2/3 (*N* = 21) Poor prognosis**	***P***
Shrinkage pattern			
Complete response	1 (5.9%)	2 (9.5%)	.578
Concentric	8 (47.1%)	6 (28.6%)	
Non-concentric	6 (35.3%)	7 (33.3%)	
No shrinkage	2 (11.8%)	6 (28.6%)	
Radiologic response			
Complete response	1 (5.9%)	2 (9.5%)	.483
Partial response	14 (82.4%)	13 (61.9%)	
No response	2 (11.8%)	6 (28.6%)	
RECIST			
Complete response	1 (5.9%)	2 (9.5%)	.790
Partial response	7 (41.2%)	6 (28.6%)	
Stable disease	9 (52.9%)	13 (61.9%)	
Progressive disease	0	0	

Shrinkage pattern and radiologic response at midtreatment MRI during NET. NET = neoadjuvant endocrine therapy, PEPI = preoperative endocrine prognostic index, RECIST = Response Evaluation Criteria in Solid Tumors.

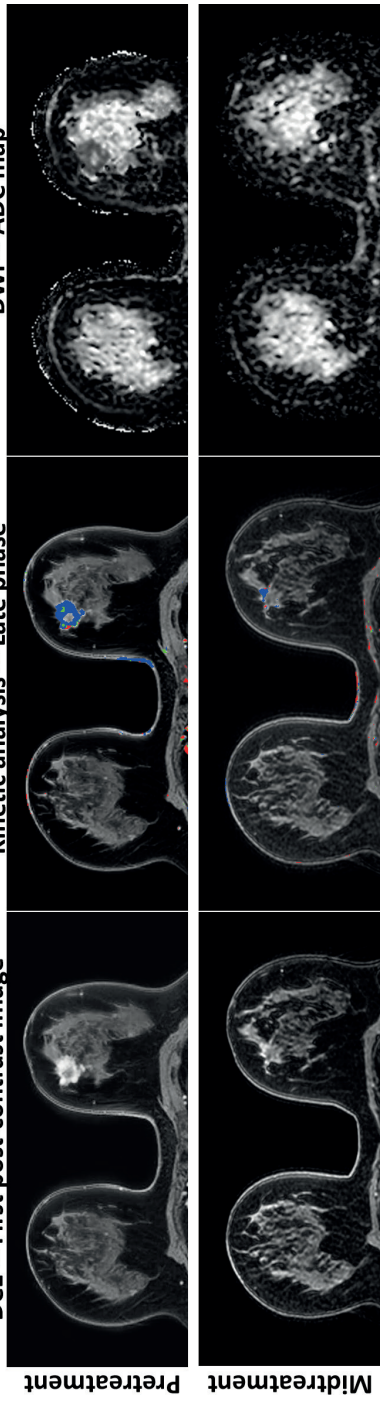


Figure 5. The images of a 68-year old patient with a T1N0 IDC (grade 2, ER 100%, PR 60%) of the left breast. On the pretreatment images (**top row**) a unifocal mass enhancing lesion with rim enhancement of 20 mm is visible. In the kinetic analysis (**middle row**) only a minimal part of the lesion shows wash-out (red), the vast majority of the tumor shows cumulative contrast enhancement (blue). The ADC map (**right column**) shows diffusion restriction in the rim of the lesion. After 3 months of AI the size of the mass decreased to 15 mm (largest diameter). Enhancement and diffusion restriction are still present but significantly reduced. This patient was considered a radiologic partial responder. At histopathological evaluation, the specimen was assigned a PEPI-1 (good prognosis). DCE = dynamic contrast-enhanced, DWI = diffusion weighted-imaging, ADC = apparent diffusion coefficient, IDC = invasive ductal carcinoma, ER = estrogen receptor, PR = progesterone receptor, AI = aromatase inhibitor, PEPI = preoperative endocrine prognostic index.

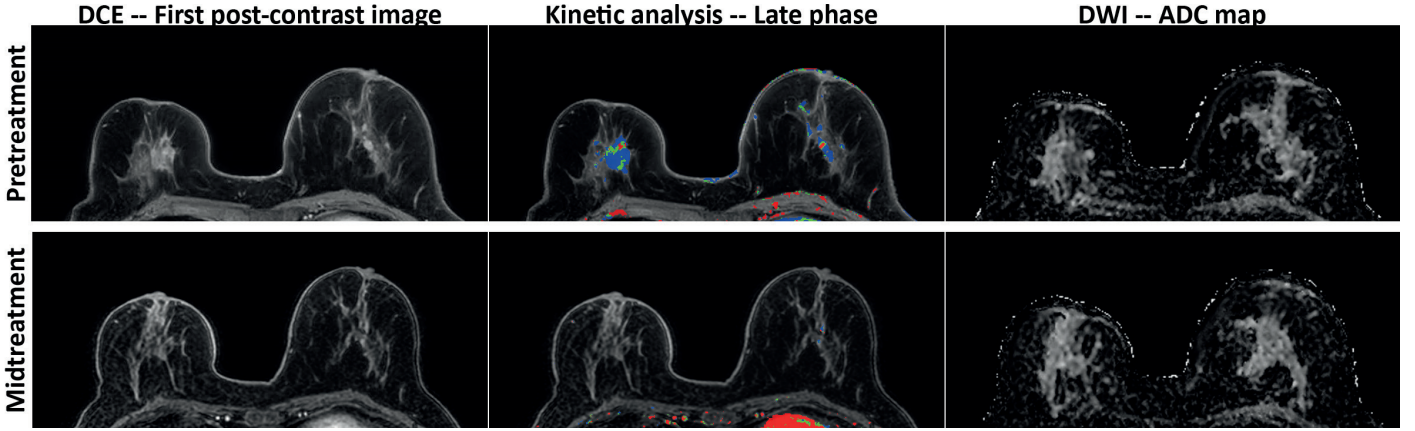


Figure 6. The images of a 71-year old patient with a bilateral tumor. The right breast showed a T2N0 ILC (grade 2, ER 100%, PR 5%), and the left breast showed a DCIS (TisN0). The kinetic analysis showed some plateau and wash-out sections in both lesions. Diffusion restriction in the right lesion was noted. After 3 months of AI, the right lesion (ILC) showed no enhancement on the DCE and no diffusion restriction. The left lesion decreased in size, however, some sections of the lesion still showed cumulative enhancement on the kinetic analysis (shown in blue). The right lesion was considered a radiologic complete response (no pathological enhancement). However, at histological evaluation an invasive component of 25 mm was found in the surgical specimen. The lesion was assigned a PEPI-2/3 (poor prognosis). The left lesion was considered a partial responder after 3 months of NET, and was assigned a PEPI-1 (good prognosis) at histological evaluation (based on pathologic complete response). DCE = dynamic contrast-enhanced, DWI = diffusion weighted-imaging, ADC = apparent diffusion coefficient, ILC = invasive lobular carcinoma, ER = estrogen receptor, PR = progesterone receptor, DCIS = ductal carcinoma in situ, AI = aromatase inhibitor, PEPI = preoperative endocrine prognostic index.

Discussion

In this study, we investigated whether pretreatment or midtreatment BIRADS characteristics, kinetic, and DWI findings on MRI were associated with prognosis (on the basis of PEPI) after NET in ER+/HER2- breast cancer patients. We found that only a larger reduction of tumor size after 3 months of NET was more strongly associated with PEPI-1 (good prognosis) than with PEPI-2/3 (poor prognosis) in our patient cohort, although tumor size measurements suffered from large inter-rater variability, especially in case of multifocal masses or nonmass enhancement.

Research on the use of MRI during NET is limited. For NAC, however, several characteristics and changes on MRI associated with response or prognosis have been identified in ER+/HER2- tumors, for example: a concentric shrinkage pattern was associated with improved survival[137]. In our study, shrinkage pattern was not associated with prognosis on the basis of PEPI after NET. On the other hand, changes in tumor size at initial and late enhancement were previously not associated with response in ER+/HER2- tumors during NAC[131], but a larger reduction in tumor size was associated with PEPI-1 (good prognosis) in this study. In our study, BPE decreased in all patients, a known effect of endocrine therapy[95], but was not associated with PEPI. However, a low pretreatment BPE was previously reported to be associated with a reduction in tumor size after NET[89]. Additionally, changes in contralateral parenchymal enhancement, a quantitative measure of the delayed enhancement of healthy breast tissue, during NET were predictive of PEPI[26]. Lastly, Reis et al., have reported a high correlation between residual disease size on MRI and pathology after NET and recommend the use of MRI for response monitoring during NET. Similar to our study, however, several patients (7 out of 35) were discordantly classified as complete responders on MRI with residual disease at pathology[138].

As NET is increasingly recommended as an alternative for NAC in ER+/HER2- breast cancer patients[142], it is important to identify accurate pretreatment or midtreatment methods to determine whether NET will be effective to allow for therapy adjustments in patients who are unlikely to experience benefit. As we report in this study, it is likely that MRI characteristics associated with a favorable prognosis after NAC are not necessarily associated with a favorable prognosis after NET. This could be due to differences in tumor biology (high proliferation versus low proliferation) or differences in treatment mechanisms (cytotoxic versus anti-

proliferative). Additionally, differences in findings compared to NAC studies could also be attributed to the differences in endpoints (pCR versus PEPI).

Although pCR is typically used as a surrogate endpoint in neoadjuvant breast cancer studies, it is poorly associated with prognosis in ER+/HER2- breast cancer[111,112]. Therefore, PEPI might be a more suitable surrogate endpoint for ER+/HER2- patients after NET, as PEPI stratifies patients in groups with distinct prognoses, and was validated in independent cohorts[114,115].

A larger reduction of tumor size was associated with improved prognosis after NET (PEPI-1), however, tumor size decreased on average in both PEPI-groups during treatment. Additionally, although the tumors were measured by experienced radiologists, measurements suffered from large inter-rater variability. Although the limits of agreement included clinically meaningful thresholds (±20 mm), this was mostly due to disagreement of the index tumor (in case of multifocal masses) and in tumors with nonmass enhancement. The agreement in radiologic response was substantial between the radiologists. Remarkably, 3 patients showed a radiologic complete response, 2 of whom had a poor prognosis (PEPI-2/3) at histopathological evaluation, a similar observation made by Reis et al[138]. All 3 patients were diagnosed with an ILC, which are known to grow diffusely without significant desmoplastic reaction (i.e., show nonmass enhancement), and are often ill-defined on imaging[143,144]. Response assessment based solely on changes in tumor size should be done with care, especially in the case of ILC. Automatic quantitative analysis tools could aid the radiologists in response assessment during NET, and also decrease interrater variability.

Our study has some limitations. Firstly, this exploratory study was retrospective, with a relatively small and heterogeneous cohort of 35 patients (38 tumors), which limits the power to detect small effects. However, for a NET MRI study, this is a large sample. Secondly, NET is a relatively new treatment option and the patient selection is not as clear-cut compared to NAC, which leads to a heterogeneous cohort treated with NET for varying reasons (e.g. strong ER+ tumors versus excess comorbidity). Additionally, there are no guidelines for response evaluation during NET: the patient cohort might be the result of selection bias, where difficult to image tumors were evaluated with MRI as opposed to ultrasound. This could also explain the large interrater variability. Thirdly, differences in tumor response and change in BPE exist between AI and tamoxifen[95,113], however, due to small sample size we

could not further stratify the patient cohort into different treatment groups. Lastly, tumor ADC at DWI was reported to be associated with tumor response after NAC and survival in general[107,145], however, due to the different b-value pairs used during the midtreatment imaging resulting in variability of ADC measurements[146,147], we could not perform a quantitative ADC analysis. The results should be interpreted with this perspective in mind and should certainly be validated in a larger cohort.

In conclusion, larger reduction of tumor size after 3 months of NET was significantly associated with PEPI-1 (good prognosis) at histopathological evaluation. No other investigated breast MRI characteristics were associated with PEPI. Response monitoring based only on change in tumor size should, however, be done with care, because tumor size also decreased on average in patients with PEPI-2/3 (poor prognosis). Particularly, in the case of an ILC, multifocal tumor or non-mass enhancement, size measurements on MRI suffers from inter-rater variability. MRI characteristics previously reported to be associated with prognosis after NAC in literature were not associated with prognosis after NET in the current study. Radiologists must be aware that response evaluation on MRI differ between NET and NAC.

5

CHAPTER

Biological mechanisms underlying the association between parenchymal enhancement on MRI and survival in breast cancer patients

Contralateral parenchymal enhancement on MRI is associated with tumor proteasome pathway gene expression and overall survival of early ER+/HER2- breast cancer patients

Ragusi, M. A. A., Bismeijer, T., van der Velden, B. H. M., Loo, C. E., Canisius, S., Wesseling, J., Wessels, L. F. A., Elias, S. G. & Gilhuijs, K. G. A. Contralateral parenchymal enhancement on MRI is associated with tumor proteasome pathway gene expression and overall survival of early ER+/HER2-breast cancer patients. Breast 60, 230–237 (2021).

Abstract

Purpose: To assess whether contralateral parenchymal enhancement (CPE) on MRI is associated with gene expression pathways in ER+/HER2- breast cancer, and if so, whether such pathways are related to survival.

Methods: Preoperative breast MRIs were analyzed of early ER+/HER2- breast cancer patients eligible for breast-conserving surgery included in a prospective observational cohort study (MARGINS). The contralateral parenchyma was segmented and CPE was calculated as the average of the top-10% delayed enhancement. Total tumor RNA sequencing was performed and gene set enrichment analysis was used to reveal gene expression pathways associated with CPE (N = 226) and related to overall survival (OS) and invasive disease-free survival (IDFS) in multivariable survival analysis. The latter was also done for the METABRIC cohort (N = 1355).

Results: CPE was most strongly correlated with proteasome pathways (normalized enrichment statistic = 2.04, false discovery rate = .11). Patients with high CPE showed lower tumor proteasome gene expression. Proteasome gene expression had a hazard ratio (HR) of 1.40 (95% CI = 0.89, 2.16; P = .143) for OS in the MARGINS cohort and 1.53 (95% CI = 1.08, 2.14; P = .017) for IDFS, in METABRIC proteasome gene expression had an HR of 1.09 (95% CI = 1.01, 1.18; P = .020) for OS and 1.10 (95% CI = 1.02, 1.18; P = .012) for IDFS.

Conclusion: CPE was negatively correlated with tumor proteasome gene expression in early ER+/HER2- breast cancer patients. Low tumor proteasome gene expression was associated with improved survival in the METABRIC data.

Introduction

Adjuvant systemic treatment (AST), such as endocrine, targeted, and chemotherapy, has improved the survival of breast cancer patients over the past decades[18]. Nonetheless, a substantial number of patients is overtreated with AST. Endocrine therapy can be administered to estrogen receptor-positive (ER+) breast cancer. However, besides the estrogen receptor (ER), no clinically validated options are available to support decisions to select endocrine therapy[148], despite the fact that ER+ breast cancer is the most frequently occurring breast cancer subtype and endocrine therapy constitutes the largest fraction of AST administered.

A tool under investigation to personalize endocrine therapy in patients with unilateral ER+ human epidermal growth factor 2-negative (HER2-) breast cancer is contralateral parenchymal enhancement (CPE) on dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI). CPE is a measure of the delayed contrast enhancement in the contralateral parenchymal breast tissue. CPE was previously associated with survival in ER+/HER2- breast cancer, but not in other breast cancer subtypes[24–26]. CPE was not associated with ER-percentage or with genomic ER-pathway activity of the tumor[42]. The biological mechanisms linking CPE to tumor biology, therefore, remain unknown.

The prognostic information that CPE contains, independent from routinely available clinicopathological variables (e.g. tumor size, axillary load), and genomic signatures[43], might be explained by the biological pathways expressed in the tumor. Background parenchymal enhancement (BPE; a qualitative measure of parenchymal enhancement) on MRI is a well-known independent risk factor for the development of breast cancer[29–31] and it may be an important indicator of the type of tumor that develops: high BPE was more strongly associated with invasive breast cancer as opposed to ductal carcinoma in-situ (DCIS)[29]. BPE was also associated with immunohistochemical subtype of the tumor, lymphovascular invasion, and tumor grade[149–151]. It has also been reported that breast cancer has local effects on tissue surrounding the tumor[152,153] as well as systemically on (distant) non-tumorous tissue[154,155], even before metastasis occur[156], and that these changes are associated with prognosis[156–158]. For example, enhancement of contralateral parenchymal tissue was associated with the presence of breast cancer (in the contralateral breast)[159], and ipsilateral parenchymal enhancement was associated with various biological pathways expressed in the tumor[157,160]. Based on these findings, we hypothesize that CPE could represent the diseased breast before tumorigenesis[24], in which case CPE could be associated with

an environment that gives rise to a certain type of tumor, or that CPE is secondarily affected by tumor-induced systemic effects. In both cases CPE might be associated with biological pathways expressed in the tumor that could also affect prognosis.

The purpose of this study was to investigate whether CPE is associated with biological pathways in the tumor, and, if so, whether these CPE-associated biological pathways expressed in the tumor carry prognostic information.

Materials and methods

Study design

To reveal biological pathways in ER+/HER2-early breast cancer that are associated with CPE and to investigate whether these CPE-associated gene expression pathways are related to survival, we performed this study in 2 steps. First, we identified gene expression pathways that are associated with CPE from patients included in the MARGINS-study (Multimodality Analysis and Radiologic Guidance in Breast-conserving Therapy) where CPE was first described, i.e. the discovery cohort[24]. Second, the ability of these CPE-associated gene expression pathways to stratify survival was assessed, and externally verified in a publically available dataset (Molecular Taxonomy of Breast Cancer International Consortium [METABRIC][161], Figure 1).

Patient cohort

This is a re-analysis of data from patients with unilateral ER+/HER2- breast cancer obtained in the MARGINS-study performed between 2000 and 2008 at the Netherlands Cancer Institute. Institutional review board approval and written informed patient consent were obtained[24,162]. In MARGINS patients with proven breast cancer and eligible for breast-conserving surgery based on conventional imaging (ultrasound and/or mammography) and clinical assessment were consecutively included. These patients underwent an additional preoperative breast MRI. A total of 598 patients with breast cancer were included (Figure 2). For 384 patients the preoperative DCE MRI could be matched to tumor material from the surgical excision in the Netherlands Cancer Institute biobank, which yielded enough high-quality RNA for sequencing in 303 patients. Patients without ER+/HER2- breast cancer (*N* = 67), bilateral breast cancer (*N* = 7), DCIS (N = 1), and with failed image acquisition or registration (*N* = 3) were excluded. A total of 226 patients with a preoperative DCE MRI matched with high-quality tumor RNA were included in the analysis.

Figure 1. Overview of study design. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com. CPE = contralateral parenchymal enhancement, MRI = magnetic resonance imaging, RNA seq = ribonucleic acid sequencing, PCA = principle component analysis, METABRIC = Molecular Taxonomy of Breast Cancer International Consortium.

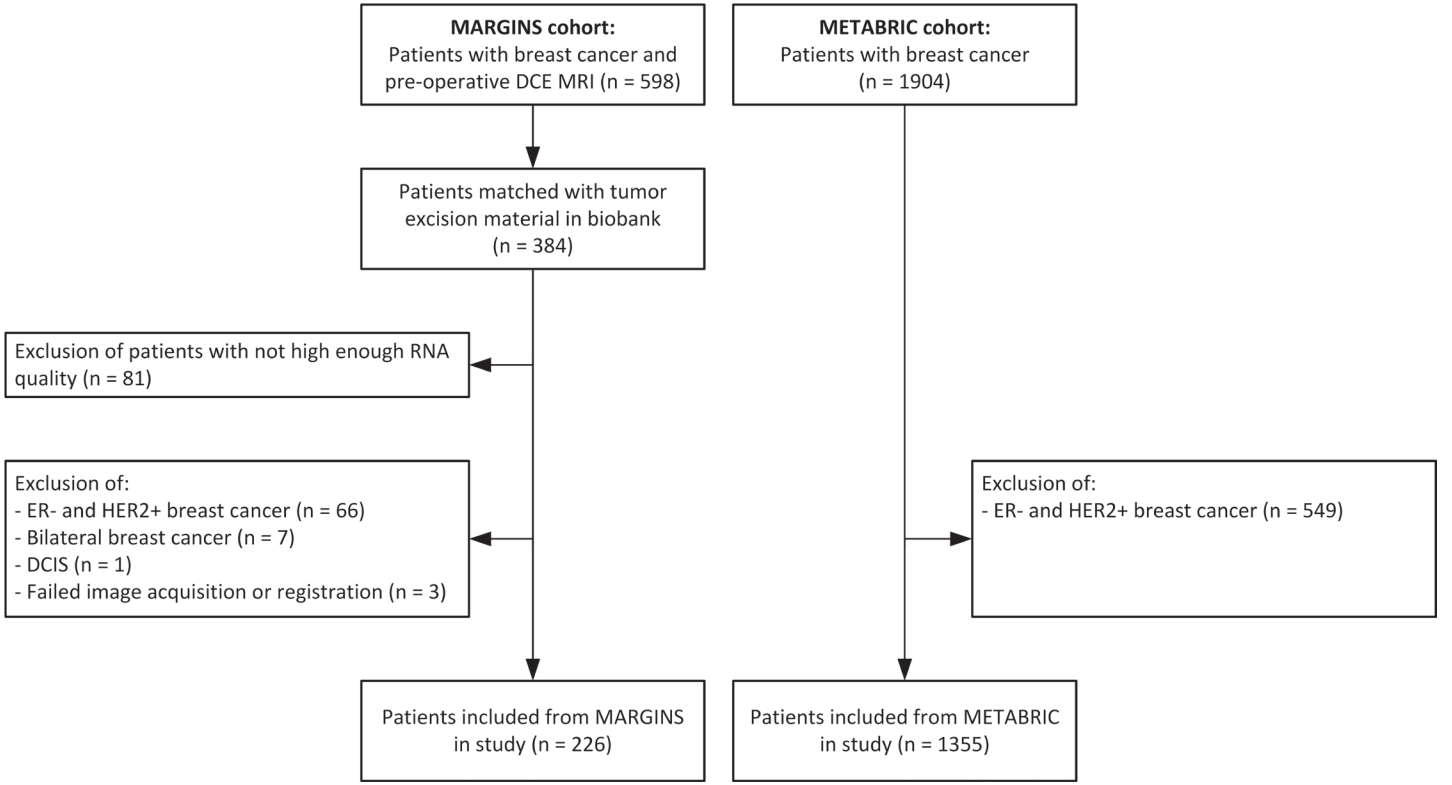


Figure 2. Patient inclusion chart. Low quality RNA was defined as < 30% tumor percentage or an RNA integrity number < 6. MARGINS = multimodality analysis and radiologic guidance in breast-conserving therapy study, DCE = dynamic contrast-enhanced, MRI = magnetic resonance imaging, ER = estrogen receptor, HER2 = human epidermal growth factor 2, DCIS = ductal carcinoma in situ, METABRIC = molecular taxonomy of breast cancer international consortium.

MR imaging

The MRIs were acquired by using a 1.5-T imaging unit (Magnetom, Siemens) with a dedicated four-channel double breast array coil (Siemens). The DCE-sequence consisted of an unenhanced coronal fast low-angle shot 3-dimensional T1-weighted image, followed by 4 consecutive contrast-enhanced series (90 s apart) after a bolus (14 mL) of a gadolinium-based contrast agent (0.1 mmol/kg, Prohance, BRACCO). The imaging parameters were: acquisition time 90 s, repetition time 8.1 ms, echo time 4.0 ms, a flip angle 20º, and voxel sizes 1.35 x 1.35 x 1.35 mm[324].

Contralateral parenchymal enhancement

Image processing and calculation of CPE are described elsewhere in detail[24]. Briefly, spatial variations in image intensity due to inhomogeneity of the magnetic field were corrected[77], the breast volume was segmented[163], as well as the fibroglandular tissue of the contralateral breast[164]. Post-contrast images were registered to the pre-contrast images using deformable image registration to reduce patient motion artifacts[165]. CPE is defined as the mean top-10% voxels in the contralateral fibroglandular tissue with the highest ratio of enhancement between the early (90 s post-contrast) and late (360 s post-contrast) image: $(S_{late} - S_{early}) / S_{early}$, where S denotes signal intensity[24]. CPE is a dimensionless number.

METABRIC Cohort

To externally validate a possible association between CPE-associated gene expression pathways and survival, gene expression data from the publicly available METABRIC cohort was used[161]. METABRIC contains clinical annotation and RNA profiles (*N* = 1904) derived from primary fresh frozen breast cancer specimens originating from patients from the United Kingdom and Canada (Figure 2). We selected all patients with ER+/HER2- breast cancer resulting in a total inclusion of 1355 patients participating in METABRIC with clinical, follow-up, and tumor gene expression data.

Gene expression

Gene expression in the MARGINS cohort was derived from whole transcriptome RNA sequencing, as described previously[43,162]. In short, the fresh-frozen tumor samples were collected from the biobank of the Netherlands Cancer Institute. Low tumor percentage (< 30%) or low RNA quality (RNA integrity number < 6; Bioanalyzer 2100, Agilent) samples were excluded (Figure 2). RNA sequencing of the samples

was performed using the HiSeq 2500 (Illumina) with single-end 65 base-pair reads. RNA sequencing reads were aligned with STAR 2.5.0a to the human genome (GENCODE 23) to quantify the RNA per gene[166]. Gene expression in the METABRIC cohort was measured using microarrays. Further details about the gene expression measurements in the METABRIC cohort are described elsewhere[161].

Gene expression pathway analysis

To identify gene expression pathways that are associated with CPE, we performed gene set enrichment analysis (GSEA)[167]. Firstly, CPE was regressed against all individual genes. The genes were ranked based on the strength of the association between the specific gene and CPE, quantified by the *t* statistic[168]. Based on this ranking, GSEA scored the enrichment of each gene set based on the ranking of the individual genes. To quantify the different associations between CPE and each gene set, GSEA calculated 3 additional scores: the normalized enrichment statistic (NES), the maximum enrichment statistic at (Max ES at), and the leading edge (LE). NES is the effect size of the gene set enrichment and can be compared between gene sets. A higher NES indicates a stronger association of CPE with that gene set. The Max ES at is the position in the ranked list at which the maximum enrichment occurred. The most relevant gene sets appear at the top or bottom of the list, i.e., have a high or low Max ES at. The leading edge is the proportion of genes in a gene set that contribute to the enrichment score. A high leading edge indicates that a large fraction of the gene set contributed to the enrichment[169]. Within the pathway analysis, differential expression on RNA sequencing data was performed using limma-voom[168]. Two gene set collections from the Molecular Signature Database (version 7.0) were used for the GSEA: c2.cgp, which contains experimentally derived gene sets (N = 3302); and c2.cp, which contains gene sets curated by domain experts (N = 2199). Together these 2 gene sets provide wide coverage of biological processes without being highly redundant[162]. Gene sets with a false discovery rate (FDR) < .25, the recommended threshold for the discovery of associated gene expression pathways[169], were considered significant and included in further analyses. Correlation of individual genes with CPE was measured with the Pearson's correlation coefficient.

Survival analysis

To investigate whether the CPE-associated gene expression pathways were associated with survival, we fit a multivariable Cox proportional hazards model

with Firth's penalized likelihood (due to the relatively low number of events) in the MARGINS cohort[170]. The endpoint was overall survival (OS) and invasive disease-free survival (IDFS) as defined by Hudis et al[76]. The survival models were adjusted for age, tumor size, tumor grade, axillary load, and AST (yes/no). The variables axillary load and AST are highly correlated, and were added as a construct variable (i.e. the combination of both variables in a single variable, e.g. positive lymph nodes and treated with AST, negative lymph nodes and not treated with AST, etc.). We decided not to impute missing data due to the low number of cases with missing values in both the MARGINS and METABRIC cohort (2% and 5% respectively)[171]. To deal with the high dimensionality of gene expression data, a principle component analysis (PCA) was performed on the scaled gene expression data of the specific gene set, and the first principal component (PC1) was treated as the variable representative of the biological pathway in the multivariable survival model[160,172]. To validate a possible association between discovered gene expression pathways associated with CPE and survival in an external dataset, we applied the PCA from the MARGINS data to the METABRIC data, and fitted a regular multivariable Cox proportional hazards model including the PC representative of the gene expression and adjusted for age, tumor size and grade, axillary load, and AST. To translate the PCA of the MARGINS data to the METABRIC data we linearly transformed the gene expression of each gene in METABRIC to have identical mean and variance as the corresponding gene in MARGINS, because MARGINS gene expression was derived from RNA-sequencing and gene expression in METABRIC was derived from microarrays. Lastly, to increase interpretability of CPE and PC1, we standardized both variables so that a 1 unit increase signifies an increase of 1 standard deviation (SD).

Statistical analyses were performed using R version 3.6.2 (R Foundation for Statistical Computing) with the 'limma' (version 3.42.2)[168], 'flexgsea' (version 1.3), and 'coxphf' (version 1.13)[170] packages available in R. Descriptive statistics are reported as median with the corresponding interquartile interval (IQI), and coefficient estimates are reported with their corresponding 95% confidence interval (CI). A two-tailed $P < .05$ was considered to represent statistical significance.

Results

Table 1 summarizes the patient, tumor, and treatment characteristics for both the MARGINS and METABRIC cohorts. Median patient age was 59 years (IQI = 50, 64) in MARGINS and 64 years (IQI = 53, 72) in METABRIC. Patients in MARGINS

underwent more breast-conserving surgery and consequently more often received radiotherapy. Additionally, the distribution of adjuvant systemic therapy (AST) differed between both cohorts: more patients were treated with only endocrine therapy in METABRIC, but less often with no AST or chemotherapy.

Table 1. Baseline patient, tumor, and treatment characteristics of ER+/HER2- breast cancer patients from the MARGINS and METABRIC cohorts

	MARGINS (*N* = 226)	**METABRIC (*N* = 1355)**
Age (years)		
median (IQI)	59 (50, 64)	64 (53, 72)
Tumor size (mm)		
median (IQI)	19 (14, 25)	22 (17, 30)
Unknown (N)	0	12
Tumor grade		
1	80 (36%)	159 (12%)
2	112 (50%)	651 (50%)
3	31 (14%)	484 (37%)
Unknown	3	61
Axillary load		
0	142 (63%)	745 (55%)
1-3	66 (29%)	418 (31%)
4 or more	16 (7%)	192 (14%)
Unknown	2	0
Adjuvant systemic therapy		
None	122 (54%)	366 (27%)
Only endocrine therapy	49 (22%)	859 (63%)
Only chemotherapy	1 (0%)	21 (2%)
Endocrine and chemotherapy	54 (24%)	109 (8%)
CPE		
median (range)	0.438 (0.105, 0.986)	
Cause of death		
Breast-cancer	11 (61%)	388 (49%)
Non breast-cancer	7 (39%)	398 (51%)
Unknown	0	1
Breast cancer recurrence		
Yes	22 (10%)	516 (38%)
No	204 (90%)	838 (62%)
Unknown	0	1

Values are numbers of patients with percentage between parentheses, unless otherwise specified. ER = estrogen receptor, HER2 = human epidermal growth factor 2, MARGINS = multimodality analysis and radiologic guidance in breast-conserving therapy study, METABRIC = Molecular Taxonomy of Breast Cancer International Consortium, CPE = contralateral parenchymal enhancement.

Pathway analysis

Figure 3 summarizes the 3 scores (NES, Max ES at, and LE) for all 78 biological pathways associated with CPE at FDR < .25. The pathway analyses showed that CPE is strongly associated with proteasome pathways. Most notably, CPE was most strongly associated with the KEGG_PROTEASOME pathway (NES = 2.04), with high specificity (LE; 93%). Supplemental materials 1 (available online) shows an overview of all gene sets with an FDR of < .25 and the associated enrichment scores. Analysis of individual genes in the KEGG_PROTEASOME pathway showed that the proteasome subunit beta 10 (PSMB10) gene had the strongest correlation with CPE: -0.389 (95% CI = -0.495, -0.273; *P* < .001). Figure 4 shows the 3 individual genes within the KEGG_PROTEASOME pathway that were most strongly correlated with CPE. Supplemental Materials 2 (available online) provides an overview for all genes in the KEGG_PROTEASOME pathway. All but one gene in the KEGG_PROTEASOME pathways were negatively correlated with CPE, i.e., patients with high CPE (favorable prognosis) had a lower tumor proteasome gene expression. Although other (non-proteasome) pathways were associated with CPE at FDR < .25, we focused on the proteasome pathways as only these pathways had both the strongest association with CPE (high NES) combined with a high proportion of genes contributing to the association with CPE (high LE; Figure 3 and Supplemental Materials 1, available online).

Principal component analysis

To investigate whether expression of the KEGG_PROTEASOME pathway is associated with survival, we first performed a PCA to condense the expression of the genes in this pathway into a single principal component to represent the KEGG_PROTEASOME pathway in the survival analysis. The first principal component explains 45% of the variance and has a correlation with CPE of -0.209 (95% CI = -0.33, -0.08, *P* = .002). The results of the PCA performed on the MARGINS RNA sequencing profiles were translated to the METABRIC data.

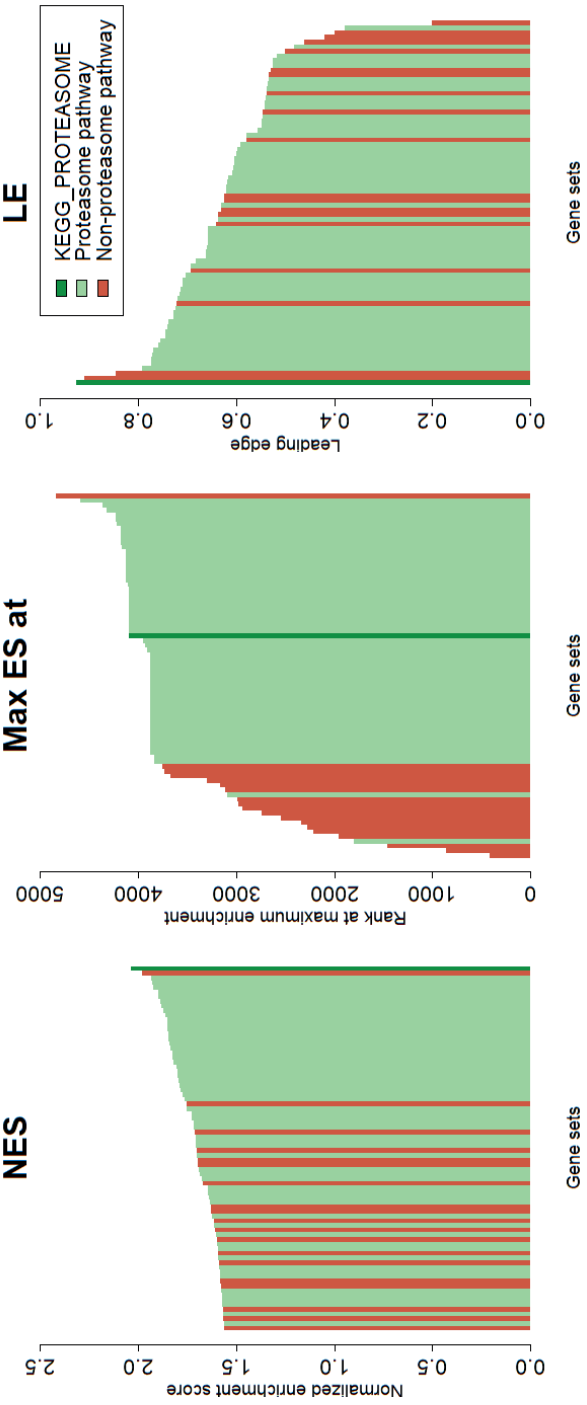


Figure 3. The results of the GSEA with the 3 association statistics of all 78 pathways with FDR < .25. CPE was most strongly associated with gene sets representing proteasome gene expression, and was most strongly associated with the KEGG_PROTEASOME pathway. NES = normalized enrichment statistic, Max ES at = maximum enrichment statistic at, LE = leading edge, GSEA = gene set enrichment analysis, FDR = false discovery rate, CPE = contralateral parenchymal enhancement.

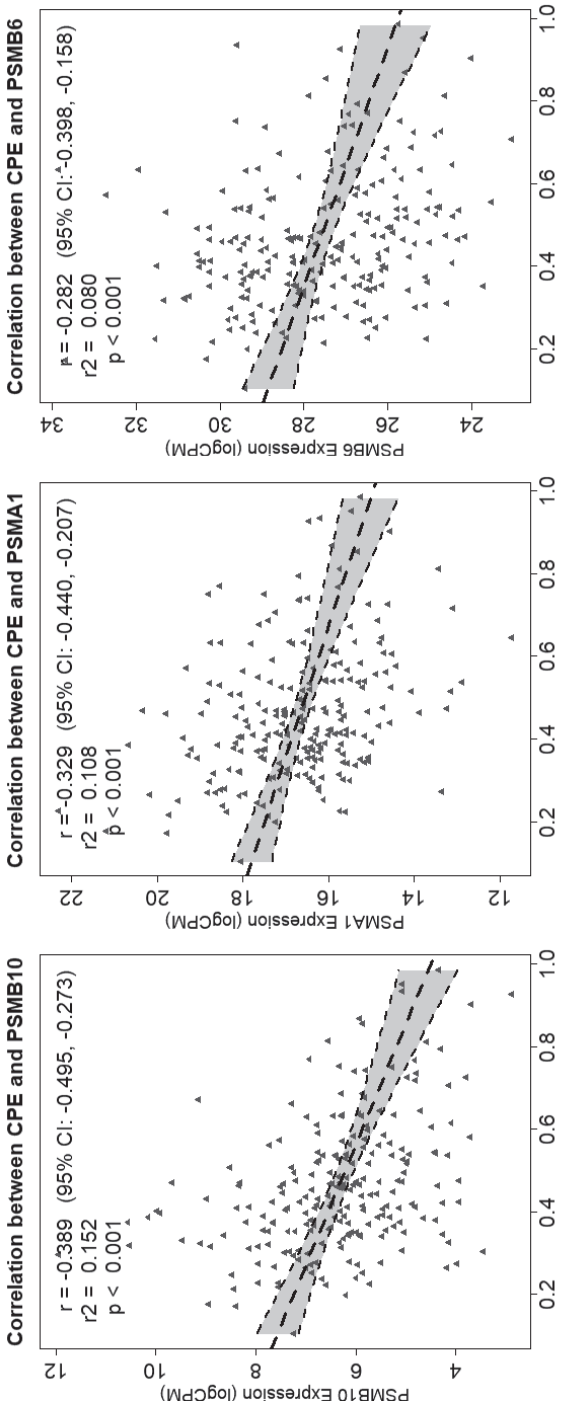


Figure 4. The 3 proteasome genes from the KEGG_PROTEASOME pathways that most strongly associate with CPE. CPE was negatively correlated with tumor proteasome gene expression, i.e. patients with a high CPE (good prognosis) had a lower proteasome gene expression on average. The regression line with 95% CI is indicated in grey. CPE = contralateral parenchymal enhancement, PSMB10 = proteasome subunit beta, PSMA1 = proteasome subunit alpha 1, PSMB5 = proteasome subunit beta 5, CI = confidence interval.

Survival analysis

The median follow-up was 86 months (IQI = 70, 109) with 18 OS events in MARGINS and 123 months (IQI = 73, 188) with 773 OS events in METABRIC. The median follow-up for IDFS was 84 months (IQI = 65, 107) with 30 events in MARGINS, and 108 months (IQI = 54, 170) with 804 events in METABRIC. The results of 3 multivariable survival models for OS in the MARGINS data adjusted for age, tumor size and grade, axillary load, and AST, are shown in Table 2: a model with only CPE, a model with only PC1 (representative of the proteasome pathway), and a model with both CPE and PC1. In the multivariable survival analysis with only CPE, CPE had a significant HR of 0.47 (95% CI = 0.23, 0.89; *P* = .017) per SD unit increase, i.e. patients with higher CPE have a more favorable prognosis. In the multivariable model with only PC1 (representative of tumor proteasome gene expression), PC1 had a non-significant HR of 1.40 (95% CI = 0.89, 2.16; *P* = .143) per SD unit increase. When modeling both CPE and PC1, the HR of CPE increased to 0.50 (95% CI = 0.24, 0.94; *P* = .030) and PC1 decreased to 1.26 (95 % CI = 0.80, 1.94, *P* = .310).

In the multivariable survival analysis of the METABRIC cohort, PC1 was significantly associated with survival with a HR of 1.09 (95% CI = 1.01, 1.18; *P* = .020). Table 3 shows the HR estimates of CPE and PC1 for IDFS, adjusted for age, tumor size and grade, axillary load, and AST. The associations between CPE and PC1 and IDFS were comparable to the associations found for OS: CPE had a HR of 0.69 (95% CI = 0.42, 1.06; *P* = .097) and PC1 had a HR of 1.53 (95% CI = 1.08, 2.41; *P* = .017). PC1 was significantly associated with IDFS with a HR of 1.10 (95% CI = 1.02, 1.18, *P* = .012) in the METABRIC cohort.

Table 2. Multivariable HR Estimates for OS for CPE and PC1 (representative of proteasome gene expression) in 3 models of the MARGINS cohort with only CPE, only PC1, and both CPE and PC1, and in one model of the METABRIC cohort with only PC1

	MARGINS (*N* = 221)						**METABRIC (*N* = 1283)**	
Variable	**Model with CPE**		**Model with PC1**		**Model with CPE and PC1**		**Model with PC1**	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Age (years)	1.08 (1.01, 1.14)	.015	1.09 (1.03, 1.15)	.001	1.08 (1.02, 1.14)	.012	1.05 (1.04, 1.06)	<.001
Tumor size (mm)	1.02 (0.98, 1.05)	.245	1.03 (0.99, 1.06)	.108	1.02 (0.99, 1.05)	.184	1.01 (1.01, 1.02)	<.001
Tumor grade 1	Ref		Ref		Ref		Ref	
Tumor grade 2	1.06 (0.29, 4.14)	.934	1.24 (0.34, 4.97)	.744	1.13 (0.30, 4.50)	.858	1.19 (0.91, 1.53)	.187
Tumor grade 3	1.70 (0.33, 8.66)	.518	1.31 (0.21, 7.42)	.766	1.34 (0.22, 7.46)	.740	1.39 (1.07, 1.81)	. 015
No AST with no positive lymph nodes	Ref		Ref		Ref		Ref	
AST with no positive lymph nodes	2.74 (0.60, 14.48)	.198	2.07 (0.43, 10.89)	.363	2.71 (0.58, 14.46)	.206	0.95 (0.77, 1.17)	.618
No AST with positive lymph nodes	5.19 (0.85, 25.80)	.071	3.87 (0.64, 19.19)	.129	4.78 (0.77, 24.20)	.087	1.73 (0.88, 3.39)	.111
AST with positive lymph nodes	1.04 (0.24, 4.68)	.953	0.96 (0.22, 4.42)	.962	1.04 (0.24, 4.76)	.958	1.31 (1.09, 1.58)	.004
CPE	0.47 (0.23, 0.89)	.017			0.50 (0.24, 0.94)	.030		
PC1			1.40 (0.89, 2.16)	.143	1.26 (0.80, 1.94)	.310	1.09 (1.01, 1.18)	.020

Data are HR estimates with 95% CI between parentheses. CPE and PC1 are standardized, i.e. a 1 unit increase is equal to a 1 standard deviation increase in the HR estimate. Data are corrected for age, tumor size and grade, axillary load, surgery, radiotherapy and AST. HR = hazard ratio, OS = overall survival, Ref = reference, CI = confidence interval, AST = adjuvant systemic treatment, CPE = contralateral parenchymal enhancement, PC1 = principal component 1.

Table 3. Multivariable HR Estimates for IDFS and DRFS for CPE and PC1 (representative of proteasome gene expression) in 3 models of the MARGINS cohort with only CPE, only PC1, and both CPE and PC1

	MARGINS (*N* = 221)						**METABRIC (*N* = 1283)**	
Variable	**Model with CPE**		**Model with PC1**		**Model with CPE and PC1**		**Model with PC1**	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	*P*
Age (years)	1.03 (0.99, 1.08)	.140	1.04 (1.00, 1.09)	.030	1.03 (0.99, 1.08)	.012	1.03 (1.02, 1.04)	<.001
Tumor size (mm)	1.03 (1.00, 1.05)	.070	1.03 (1.00, 1.05)	.033	1.02 (1.00, 1.05)	.184	1.02 (1.01, 1.02)	<.001
Tumor grade 1	Ref		Ref		Ref		Ref	
Tumor grade 2	1.48 (0.62, 3.73)	.377	1.65 (0.70, 4.23)	.270	1.61 (0.67, 4.11)	.858	1.21 (0.95, 1.54)	.127
Tumor grade 3	2.97 (0.88, 9.84)	.079	2.20 (0.58, 7.89)	.239	2.22 (0.59, 7.90)	.740	1.34 (1.04, 1.72)	.023
No AST with no positive lymph nodes	Ref		Ref		Ref		Ref	
AST with no positive lymph nodes	0.76 (0.22, 2.45)	.644	0.58 (0.16, 1.95)	.386	0.66 (0.18, 2.21)	.206	0.82 (0.67, 1.01)	.056
No AST with positive lymph nodes	2.93 (0.85, 8.60)	.084	2.45 (0.71, 7.18)	.144	2.59 (0.75, 7.63)	.087	1.20 (0.61, 2.36)	.587
AST with positive lymph nodes	0.55 (0.19, 1.55)	.261	0.51 (0.17, 1.47)	.216	0.54 (0.18, 1.54)	.958	1.16 (0.97, 1.39)	.098
CPE	0.69 (0.42, 1.06)	.097			0.78 (0.47, 1.21)	.030		
PC1			1.53 (1.08, 2.14)	.017	1.44 (1.07, 2.04)	.310	1.10 (1.02, 1.18)	.012

Data are HR estimates with 95% CI between parentheses. CPE and PC1 are standardized, i.e. a 1 unit increase is equal to a 1 standard deviation increase in the HR estimate. Data are corrected for age, tumor size and grade, axillary load, surgery, radiotherapy and AST. HR = hazard ratio, IDFS = invasive disease-free survival, Ref = reference, CI = confidence interval, AST = adjuvant systemic treatment, CPE = contralateral parenchymal enhancement, PC1 = principal component 1.

Discussion

CPE was most strongly associated with expression of the proteasome pathway in the tumor: high CPE (favorable prognosis) was associated with low proteasome gene expression in the MARGINS data. The association between tumor proteasome gene expression and survival was independently verified in the METABRIC data.

The proteasome is a protein complex that plays an essential role in the cellular protein homeostasis, regulating intracellular protein degradation, and is involved in processes such as apoptosis, cell cycle regulation, and angiogenesis[173–175]. Malignancies often exhibit increased proteasome activity to compensate for the aberrant protein synthesis and to maintain protein homeostasis[176]. Inhibition of the proteasome, e.g. through inhibition of nuclear factor-κB, will disrupt protein homeostasis and induce apoptosis in malignancies[175]. It has become a relatively novel target for cancer therapy[174,177–179]. Although proteasome inhibitors are currently approved for the treatment of multiple myeloma, and mantle-cell lymphoma, clinical efficacy with single-agent therapy is limited in solid tumors[175,180], including breast cancer[181–183]. Current efforts are aimed at combining proteasome inhibition with other therapeutic agents (i.e., endocrine therapy and chemotherapy)[180].

Increased proteasome activity is reported to be associated with poor prognosis in breast cancer[184,185]. Our results confirm these findings and suggest that CPE on MRI is associated with proteasome activity in the ER+/HER2- tumor. The proteasome plays an important role in the degradation and stability of the ER[186,187], and might play a role in acquired resistance against tamoxifen[188]. The role of the proteasome in ER turnover might explain why CPE was previously only associated with prognosis in ER+/HER2- breast cancer patients, although proteasome activity was also associated with prognosis in ER- breast cancer[157,184,185].

The proteasome pathway was previously associated with other features on MRI. Wu et al. observed that the proteasome pathway was significantly associated with imaging subtypes on breast MRI with distinct prognoses. These imaging subtypes were based on several quantitative imaging features, including ipsilateral parenchymal enhancement[157]. Quantitative analysis of the tumor and the ipsilateral parenchyma resulted in the identification of 2 imaging subtypes with minimal parenchymal enhancement and prominent parenchymal enhancement

in which the proteasome pathway was significantly associated[157]. Our current work focused on 1 imaging feature (CPE) and its association with gene expression, future work will include multiple imaging features based on radiomics or other artificial intelligence (AI).

This study has several limitations. First, we have not validated the association between CPE and the proteasome gene expression pathway. Publicly available gene expression data matched with MRI data are limited. The Cancer Genome Atlas offers a public gene expression dataset matched with MRIs of The Cancer Imaging Archive, however, the number of available ER+/HER2- breast cancer patients with the contralateral breast in the field of view is too small to achieve sufficient statistical power to validate the association. The current study should be considered hypothesis generating. Second, to facilitate the survival analysis the gene expression data was condensed into 1 PC to represent the pathway, which limits the interpretability and results in loss of information. Thirdly, patients received less endocrine therapy during the MARGINS-study period compared to the study period of the METABRIC cohort. This may have influenced the survival analysis. Nonetheless, the association between CPE and survival was validated in an external cohort from the United States of America, in which a large number of patients received endocrine therapy (93%)[25]. Another limitation of this study was that we were unable to investigate whether CPE and proteasome gene expression are associated with (contralateral) breast cancer risk, because we did not have data on contralateral breast occurrence in the MARGINS cohort.

To conclude, high CPE on DCE-MRI was associated with low tumor proteasome gene expression pathways in unilateral ER+/HER2- breast cancer patients. Low proteasome gene expression in the tumor was associated with improved survival.

CHAPTER 6

Summary and general discussion

Summary

Over the years improvements in locoregional and adjuvant systemic treatment (AST) of breast cancer has resulted in a decline of breast cancer mortality. Concurrently there has been an increase in the number of patients who are prescribed AST. Although AST has increased the survival of breast cancer patients on average, there are subgroups in which the additional survival benefit is relatively low and individual patients who do not experience any survival benefit. These patients are unnecessarily exposed to the (substantial) side-effects of AST. Treatment personalization is aimed at the identification of patients that are estimated to experience sufficient treatment benefit to justify the exposure to the treatment side-effects and costs.

Improvements have been made for the treatment personalization of chemotherapy with the introduction of genomic assays. However, for the largest subgroup of breast cancer patients, i.e., those with estrogen receptor-positive (ER+) human epidermal growth factor-negative (HER2-) cancer, and for the group receiving the most common AST subtype, i.e., endocrine therapy, there are currently no clinically validated personalization tools beyond the expression of ER on the tumor.

This thesis further investigated a biomarker on breast MRI that could help identify patients at high or low risk of breast cancer recurrence or mortality. This biomarker, contralateral parenchymal enhancement (CPE), was previously observed to be associated with survival. The aim of this thesis was to explore the potential of CPE on MRI to be used as a personalization tool for endocrine therapy in early ER+/HER2- breast cancer patients.

In **Chapter 2** we provide an overview of estimated overtreatment from AST according to actual prescribed treatment (and treatment guidelines from the Netherlands and the United States of America). In observational data, estimates of overtreatment cannot be directly observed as it is impossible to distinguish whether a treated breast cancer patient survived because of AST or would also have survived without AST. However, by using the PREDICT prognostic algorithm, we projected the estimated survival benefit for each individual AST subtype. Overtreatment was defined as the proportion of patients who were treated with AST and who would have survived regardless of AST or died despite AST within 10 years. In all Dutch patients diagnosed with breast cancer, approximately 60%

were treated with AST. The most prescribed AST is endocrine therapy (about 50%), followed by chemotherapy (30%) and targeted therapy (5%). The highest percentage estimated overtreatment was in the group of patients treated with endocrine therapy: 96.7% in patients treated with only endocrine therapy, and 90.4% in patients who were treated with endocrine therapy combined with any other AST. Comparably, a total of 92.7% of patients treated with only chemotherapy were estimated to be overtreated, and 90% of patients who were treated with chemotherapy combined with other ASTs. These estimates largely agree with what can be expected from randomized clinical trial results and highlight the fact AST yields no survival benefit to many treated patients. Especially improved personalization of endocrine therapy is relevant, as this therapy is widely used and is associated with a high fraction of overtreatment.

To further investigate the role of CPE to personalize endocrine therapy in early ER+/HER2- breast cancer patients we present the results of the SELECT-study (Stromal enhancement on breast MRI as biomarker for survival with endocrine therapy) in **Chapter 3**. In this large multicenter retrospective observational cohort with 1432 patients we observed that CPE was only associated with overall survival (OS), and not with recurrence-free survival or distant recurrence-free survival. Patients with a high CPE had a worse long-term OS, opposite to what was previously reported. Additionally, CPE was not associated with endocrine therapy effectiveness, although endocrine therapy itself was not associated with any of the survival outcomes either. Additional research is required before CPE can be clinically implemented as a personalization tool.

Patients can also be treated with endocrine therapy before surgery, this is called neoadjuvant endocrine therapy (NET). The aim of NET is primarily to shrink the tumor so that breast-conserving surgery as opposed to mastectomy (excision of all breast tissue) can be attained. The pre-operative endocrine prognostic index (PEPI) is based on pathologic assessment of the tumor after NET, and is especially developed to estimate prognosis and to personalize treatment after NET: a PEPI-1 is associated with such favorable prognosis that adjuvant treatment (after surgery) with only endocrine therapy can suffice, whereas PEPI-2 and 3 should be treated with endocrine and chemotherapy after surgery. In **Chapter 4** we investigate the usage of MRI during NET. In **Chapter 4a** we studied whether CPE was associated with PEPI. A high CPE before NET was associated with a high PEPI

(a worse prognosis) and a decrease in CPE during NET was associated with a low PEPI (a favorable prognosis). These results suggest a role for CPE for treatment assessment during NET. In **Chapter 4b** we investigated whether conventional imaging features (such as tumor size and kinetic curve on MRI) as assessed by radiologists were also associated with PEPI. We observed that there was a relatively large interrater-variability and that only the degree of tumor shrinkage was associated with PEPI. Tumor size decreased on average in PEPI-1 by 10 mm compared to an average decrease of 4.5 mm in PEPI-2 and 3. None of the other imaging features were associated with PEPI after NET, showing that additional features, such as CPE, could be helpful in the assessment of tumor response.

Lastly, it is unknown why CPE is associated with survival. A better understanding of the underlying biological mechanisms can improve patient selection where CPE could play a role in treatment personalization. CPE could represent the diseased breast before tumorigenesis, in which case CPE could be associated with an environment that gives rise to a certain type of tumor, or that CPE is secondarily affected by tumor-induced systemic effects. In both cases CPE might be associated with biological pathways expressed in the tumor that could also affect prognosis. In **Chapter 5** we aim to elucidate whether CPE is associated with gene expression pathways in the tumor, and if so, whether such pathways are related to survival. Firstly, we related the tumor gene expression pathways associated with CPE in the original MARGINS-study (Multimodality analysis and radiologic guidance in breast-conserving therapy study). Proteasome expression in the tumor was negatively associated with CPE, i.e., patients with a high CPE (and a good prognosis in the MARGINS-study) had a low proteasome expression in the tumor. A low tumor proteasome expression was independently associated with survival in the MARGINS-study. This association was validated in an independent dataset. These results suggest that the proteasome pathway may play a role in the association between CPE and survival.

In conclusion, a large amount of breast cancer patients are treated with endocrine therapy, and in a number of patients treatment can safely be omitted and exposure to the side-effects can be limited. However, there are currently no methods to identify these patients. This thesis investigated the usage of breast MRI, and in particular CPE, as a potential tool to personalize endocrine therapy. The results show that imaging features on MRI and CPE are associated with prognosis after

(neo)adjuvant endocrine therapy, however, the results are opposite as was previously observed. More research is required before personalization tools for endocrine therapy based on MRI can be clinically implemented for breast cancer patients.

General Discussion

The aim of this thesis was to further explore the potential role of a biomarker on breast MRI, contralateral parenchymal enhancement (CPE), as a potential tool to personalize endocrine therapy decisions in early estrogen receptor-positive (ER+) human epidermal growth factor-negative (HER2-) breast cancer patients. Patients are prescribed endocrine therapy for 5 years or even longer in high-risk breast-cancer, and many experience side-effects such as joint pain, cognitive issues and sexual dysfunction that have a negative impact on their quality of life[11–13]. This is also evidenced by the low adherence rates of endocrine therapy (23-28% non-adherence in trial populations[1]). Identification of patients in whom treatment with endocrine therapy can safely be omitted can spare unnecessary exposure to the side-effects of treatment.

Parenchymal enhancement on breast MRI

Parenchymal enhancement is increasingly being investigated as a predictor of outcome in breast cancer patients in both the adjuvant and neoadjuvant setting[24,25,88–92,27,34–37,39,40,87]. However, parenchymal enhancement is defined differently between studies, i.e., qualitative assessment by the radiologist[34,40,87–89,91,92], or quantitative assessment of parenchymal enhancement[24–27,35–37,90]. In addition, several different outcome measures have been used, including: genomic assay results[92], pathologic complete response[34,35,88,91], and long-term outcome[24,25,27,39,40,87]. Some studies observed that high parenchymal enhancement was associated with poor outcome[26,37,39,88], while in other studies it was associated with improved outcome[24,25,35,36,92], and yet in other studies not associated with outcome at all[27,91]. Due to these conflicting results, in part due to the heterogeneity between studies, and the absence of validation studies, risk assessment for survival based on parenchymal enhancement is currently not clinically implemented.

The results of the SELECT-study

In the SELECT-study (Stromal enhancement on breast MRI as biomarker for survival with endocrine therapy) we aimed to validate and explore the role of CPE on breast MRI for the personalization of endocrine therapy in early ER+/HER2- breast cancer patients, ultimately working towards clinical implementation. The study was designed to validate CPE, which was previously observed to be associated with

long-term survival in 2 independent studies[24,25], and to investigate whether CPE was predictive of endocrine therapy effectiveness. In the first study investigating CPE, a high CPE was associated with improved prognosis[24]. This finding was successfully validated in an independent study performed in a different hospital with a different MRI acquisition[25]. Although CPE was successfully validated in this independent cohort, the distribution of CPE between both cohorts differed and cut-off points for certain risk levels for death or recurrence could not be readily applied to both cohorts, probably in part due to differences in MRI acquisition protocols. Additionally, in the first study it was suggested that CPE could be associated with endocrine therapy effectiveness, however, this observation did not reach statistical significance. Lastly, the follow-up time of the studies was relatively short (median follow-up of 86 and 88 months), whilst ER+/HER2- breast cancer recurrences can occur a long time after diagnosis and long-term follow-up, especially for this type of patients, is important[44]. Therefore, the SELECT-study was designed. A large cohort of 1432 patients from 10 different hospitals in the Netherlands with 125 months median patient follow-up was collected and analyzed.

The results of this study showed that CPE was independently but negatively associated with overall survival, and not associated with recurrence-free survival or distant recurrence-free survival. Additionally, CPE was not associated with endocrine therapy effectiveness in any of the survival outcomes. Notably, endocrine therapy was also not associated with improved outcome in any of the survival outcomes.

Surprisingly, the results of the SELECT-study showed that a high CPE was associated with worse prognosis, opposite from that observed in the previous studies, where a high CPE was associated with improved prognosis. Several reasons could explain this opposing effect. Firstly, differences in patient inclusion. In the original studies patients were included based on eligibility for breast-conserving surgery only, whereas in the SELECT study, all patients with breast cancer size $\leq$ 5 cm and $\leq$ 3 positive lymph nodes were included[24,25]. These inclusion criteria roughly matched the distribution of breast cancer size and number or positive lymph nodes in the previous studies[24,25]. However, the previous studies were performed on a trial-basis or in the United States[24,25], which practically lead to all patients receiving a pre-operative MRI, whereas in the SELECT-study the pre-operative MRI was based

on the indication as set by the institutions multidisciplinary team. Secondly, the studies were performed in different time periods in the Netherlands. The original studies included patients from the early 2000's, whereas SELECT included patients from the late 2000's. Several changes have taken place between these time periods: aromatase inhibitors (AI) were introduced for post-menopausal women[93], taxanes were added to the chemotherapy regimen[46,94], and guideline recommendations for endocrine therapy were extended in 2008[46]. AIs and taxanes have a different effect on parenchymal enhancement compared to Tamoxifen and non-taxane chemotherapy[32,95], conversely, it could be that the effectiveness of the AST-subtype is (partly) affected by parenchymal enhancement. Although additional analyses have not conclusively been able to find a reason for these opposite effects, it is likely that the opposite directions of the association between CPE and survival can be attributed to (a combination of) these differences.

CPE was not associated with endocrine therapy effectiveness in any of the survival outcomes, although endocrine therapy itself was also not found to be associated with improved prognosis in our analyses. Endocrine therapy is a well-established biomarker associated with decreased rate of recurrence and is a cornerstone in the treatment of ER+-breast cancer[17,44,96]. In the development of the online prognostic tool PREDICT, a similar issue was encountered, their observational cohort data could also not estimate a plausible hazard ratio for endocrine therapy[48]. This suggests that in observational data, even after multivariable adjustment for confounders, subgroups of patients receiving endocrine therapy are somehow still dissimilar from subgroups not receiving endocrine therapy through unobserved or inadequately accounted confounders (i.e., residual confounding by indication). This complicates the analysis, if the true association between survival and treatment with endocrine therapy cannot be estimated, it is possible that estimation of the association between endocrine therapy and CPE can also not be adequately estimated.

The third goal of the SELECT-study was to investigate whether CPE harmonizes between different centers with different MRI acquisition protocols. Harmonization between different imaging protocols, especially on MRI, is a well-known issue[45,189]. Contrary to computed tomography, where the signal intensity of voxels is standardized (i.e. air has a value of -1000 Hounsfield units and water 0 Hounsfield units), the signal intensity of voxels in MRI depend on multiple acquisition

parameters and may also differ between vendors[45]. Therefore, CPE (and other computer-derived features) can differ between hospitals with different MRI vendors and acquisition protocols. As it is clinical reality that patients are scanned on different MRI scanners, it is important that risk stratification based on CPE does not depend on differences in MRI acquisition. In the SELECT-study we achieved this with a post-hoc harmonization model-based method, which could also be used in prospective studies[45].

Breast MRI for personalization during or after neoadjuvant endocrine therapy

To achieve breast-conserving surgery neoadjuvant endocrine therapy (NET) is a relatively new treatment option, however, only 50-70% show a clinical response after NET[97–99]. We studied whether CPE could help identify those patients, before or during NET, that have a good prognosis after treatment. A high CPE before NET or a decrease during NET was associated with poor prognosis. The pre-operative endocrine prognostic index (PEPI) was used as a surrogate endpoint of prognosis because pathologic complete response (the conventional surrogate endpoint in neoadjuvant studies) and change in tumor size are poorly associated with prognosis in ER+/HER2- breast cancer[111,112]. Specifically for ER+/HER2- breast cancer, change in tumor size during neoadjuvant chemotherapy (NAC) is a poor predictor of response and a poorly reproducible surrogate endpoint of survival[132,133]. Interestingly, in this study a high pretreatment CPE was also associated with poor prognosis (or high PEPI), similar as in the SELECT-study, and, therefore, also opposite from what was reported previously[24,25]. This could be due to differences in end points, but the same variables that could have reversed the association in the SELECT-study could have also affected this study population. The time period of inclusion was between 2013 and 2017 in the NET-study. The addition of CPE improved tumor response monitoring during NET and suggests a role for CPE, especially considering the fact that conventional imaging features on breast MRI could not improve response monitoring[26,190]. NET is becoming more established as a treatment option, even for young patients[191]. However, research regarding response monitoring during NET is scarce[89,132,138], and usually consists of small and retrospective studies (including ours). Although there is a lot more research regarding response monitoring during neoadjuvant chemotherapy, clinical implementation is still lagging[103,104,195–197,105,106,126,134,137,192–194].

Biological background of CPE

Although biological validation of biomarkers is not necessarily required, it can provide a more holistic model and biological relevance[189]. We investigated whether CPE correlated with gene expression pathways in the tumor in the contralateral breast. The hypothesis was that CPE could represent the diseased breast before tumorigenesis[24], in which case CPE could be associated with an environment that gives rise to a certain type of tumor, or that CPE is secondarily affected by tumor-induced systemic effects. In both cases, CPE might be associated with gene expression pathways in the tumor[41]. We observed that CPE was associated with proteasome expression[41], which is a protein complex that plays an essential role in the cellular protein homeostasis, regulating intracellular protein degradation, and is involved in processes such as apoptosis, cell cycle regulation, and angiogenesis[173–175]. Proteasome expression in the tumor was associated with survival, which was validated in an independent data set. Interestingly, the proteasome plays an important role in the degradation and stability of the ER[186,187], and might play a role in acquired resistance against tamoxifen[188], which might explain why CPE was only associated with survival in ER+/HER2- breast cancer patients in a time period during which patients were mostly treated with tamoxifen[24]. Uncovering the exact biological mechanisms underlying the association between survival and CPE will require additional research. Biological validation may help clinical implementation of biomarkers, in general, by increasing support for the biomarker and improved selection of patients, but it is still underemphasized in imaging biomarker research. A study found that only 28.6% found biological correlates to their radiomics signature[189].

Personalization of endocrine therapy with MRI

The results of the SELECT-study and the studies investigating prognostication with breast MRI during NET show that MRI can potentially be used for prognostication, however, additional research is required. CPE showed an opposite association with OS compared to the previous studies[24,25] , which is in line with the conflicting results on parenchymal enhancement in general[24,25,92,26,27,35–37,39,88,91]. For example Hattangadi et al.[35] observed that high signal enhancement ratio (SER), a measure of parenchymal enhancement, was associated with improved prognosis after NAC, whilst Kim et al.[39] observed that high SER was associated with decreased

prognosis after breast-conserving surgery of ductal carcinoma in situ. Similarly, a decrease in background parenchymal enhancement (BPE), a qualitative measure of parenchymal enhancement, during NAC was associated with tumor response by Oh et al.[34] but with a loss in tumor response by Rella et al.[91] Emphasizing the fact that the results suggest a potential role of parenchymal enhancement for risk prognostication and personalization of (endocrine) therapy but it is not yet ready for clinical implementation. Currently available tools for the selection of patients for (extended) endocrine are limited[23,198]. Several studies have examined immunohistochemical markers such as p27 expression (a gene involved in cellular proliferation) and Ki-67 index (a protein associated with increased cellular proliferation) as predictive factors for endocrine therapy success. P27 status might be predictive of tamoxifen effectiveness, however, it was not associated with prognosis of the patients[199]. Ki-67 was a prognostic marker, but was not associated with endocrine therapy effectiveness[200,201]. The evidence and reproducibility of both tests is insufficient to recommend clinical use[23]. One personalization tool that has been successfully clinically implemented for the personalization of chemotherapy, and is currently under investigation for personalization of endocrine therapy, are genomic assays[6,7,20,21]. These genomic assays have shown to be prognostic in ER+-disease but current (lack of) evidence does not (yet) support clinical implementation as a personalization tool for endocrine therapy. A current trial (LA LEAST; NCT03917082) is examining whether endocrine therapy duration can be reduced to 2 years in ER+/HER2- breast cancer patients aged 51 and above based on Prosigna scores (a genomic assay)[202]. Dose escalation is also a form treatment personalization and it will be interesting what the results will be. Similarly, personalization tools for NET are currently not clinically implemented. Tumor biopsy during NET with Ki-67 index measurements were associated with long-term outcome[203] and is used a tool to escalate NET to NAC in trials (ALTERNATE; NCT01953588)[113,204]. Options for (neoadjuvant) endocrine therapy personalization are lacking.

Future Perspectives

The contradicting results for CPE, but also for parenchymal enhancement in general, suggest that there may be a complex interplay between the MRI features, patient- and tumor characteristics, and outcome. This raises the question whether this complex interplay can be uncovered by a more in-depth analysis, based on

artificial intelligence (AI) for example. AI is an exciting field with current uses mainly in the field of diagnostic imaging (e.g. diagnosis of benign versus malignant breast tumors[205–207])[208,209], but is extending to find patterns that predict survival, for example in amyotrophic lateral sclerosis in MRI data[209,210]. In breast imaging, additional imaging features beyond only parenchymal enhancement, such as tumor-derived features, can also be considered with AI. Several studies have already observed that tumor-derived (radiomic) features are associated with survival and could potentially be used to guide treatment decisions[211–214]. Future research should be aimed at using AI to uncover the complex interplay between these imaging features (including parenchymal and tumor features), clinical characteristics, and outcome, and to ultimately develop a model that can help guide treatment decisions. It is essential to consider appropriate study designs because such models are at high risk of overfitting[215]. The generalizability of AI models to 'new' data (e.g. MRIs scanned with a different acquisition, or a slightly different patient population) is a well-known issue, and is partly the reason why clinical deployment of such models is limited[216]. The SELECT-cohort provides a good dataset for future research with more extensive research based on AI. The SELECT-data includes good quality data including patient and tumor characteristics, imaging from different MRIs with differences in MRI acquisition to reflect clinical reality, and multiple long-term survival endpoints. The data seem suitable for appropriate study designs involving AI. One issue the SELECT-study seemed to have, was the fact that there seemed to be confounding by indication (endocrine therapy was not associated with any of the survival outcomes), therefore, appropriate (prospective) validation should be performed of the potential models. It will be interesting to see what the results of these new models are. Hopefully, these models can reach clinical deployment and, ultimately, help patients and clinicians guide treatment decisions.

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B

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NEDERLANDSE SAMENVATTING

N

Nederlandse samenvatting

De behandeling van borstkanker

Borstkanker is de meest voorkomende kanker bij vrouwen en kent een hoge ziektelast. De overleving van borstkankerpatiënten is over de jaren heen verbeterd door de introductie van adjuvante systemische therapie, alhoewel deze behandeling ook ernstige bijwerkingen kent. Er zijn verschillende soorten systemische therapieën, afhankelijk van het type receptor welke de tumor uitdrukt kunnen deze gebruikt worden voor de behandeling van borstkanker. Indien de tumor oestrogeenreceptor uitdrukt dan is de tumor oestrogreenreceptor-positief (ER+), en kan er behandeld worden met anti-hormonale therapie, indien een tumor human epidermal growth factor receptor-2 uitdrukt (HER2+), dan kan er HER2-gerichte therapie worden gegeven. Chemotherapie kan in principe bij alle types borstkanker gegeven worden. De richtlijnen voor de behandeling met systemische therapie zijn steeds verder uitgebreid en patiënten met een steeds gunstigere prognose worden nu ook behandeling aanbevolen. We beginnen ons echter steeds vaker de vraag te stellen of er patiënten zijn die geen voordeel halen uit de systemische therapie. Patiënten die geen voordeel uit de behandeling halen, omdat ze niet zouden overlijden zonder systemische therapie of omdat ze overleden zouden zijn ondanks systemische therapie (kortom de behandeling heeft de overleving niet verbeterd), zijn overbehandeld. Overbehandeling is nadelig voor de patiënt, zij lopen immers wel risico op bijwerkingen maar halen geen voordeel uit de behandeling, maar ook op maatschappelijk niveau brengen de systemische behandeling en de behandeling van de bijwerkingen kosten met zich mee.

Recente ontwikkelingen hebben zich gericht op het identificeren van patiënten die zo'n laag risico hebben dat de borstkanker terugkomt, dat de systemische therapie veilig achterwege kan worden gelaten. Deze nieuwe ontwikkeling hebben zich vooral gericht op het terugbrengen van overbehandeling van chemotherapie, en met goed succes: het genetisch testen van de tumor op bepaalde hoog-risico genen kan patiënten identificeren die niet met chemotherapie hoeven te worden behandeld (met behulp van bijvoorbeeld de MammaPrint of de OncotypeDX). Deze genetische testen zijn nu beschikbaar en worden gebruikt door medisch oncologen om behandeling van chemotherapie te personaliseren. Echter, er zijn nog geen geavanceerde opties voor het personaliseren van anti-hormonale

therapie, terwijl dit de meest voorgeschreven systemische behandeling is voor borstkankerpatiënten. Anti-hormonale therapie wordt 5 jaar, en soms zelfs langer, voorgeschreven en patiënten kunnen o.a. de volgende bijwerkingen ervaren: overgangsklachten, botontkalking, depressieve klachten, en seksuele problemen. Een studie verricht onder borstkankerpatiënten op verschillende (online) fora liet zien dat ongeveer 90% van de patiënten bijwerkingen ervaart en dat ongeveer een derde stopt met de behandeling vanwege de bijwerkingen. Patiënten zullen baat hebben bij een gepersonaliseerde behandeling van anti-hormonale therapie bij borstkanker.

MRI van de borsten

Een vrij nieuwe manier om individuele risico-inschattingen van de tumor te maken is gebaseerd op eigenschappen van de tumor (en gezond weefsel) op de MRI. De MRI maakt gebruik van magnetische velden en niet-schadelijke radiogolven om de tumor in beeld te brengen, dit in tegenstelling tot de CT scan die gebruikt maakt van schadelijke ioniserende straling. Een MRI bestaat uit verschillende sequenties die verschillende eigenschappen van het weefsel in kaart kunnen brengen. Één van deze sequenties is de zogeheten dynamische sequentie: na intraveneuze toediening van contrast (gadolinium) worden er periodieke beelden gemaakt. De dynamische sequenties laten zien hoe een afwijking contrast opneemt en is eigenlijk een surrogaat voor hoe snel een afwijking bloed opneemt (en weer afgeeft). Zo kan de MRI verscheidene eigenschappen van de tumor of weefsel in beeld brengen: zoals bijv. de grootte van de tumor, of de aanwezigheid van lymfovasculaire invasie (m.b.v. de verschillende sequenties), maar ook naar functionele eigenschappen van de tumor zoals bijv. de snelheid van de tumoraankleuring (m.b.v. de dynamische sequenties). De MRI kan andere eigenschappen in beeld brengen t.o.v. genetische testen (alhoewel er wel bewijs is dat er correlaties zijn). Het relatief nieuwe idee is dat de MRI nieuwe eigenschappen van de tumor of het weefsel in beeld kan brengen die kunnen helpen bij de risico-stratificatie van patiënten en dus het personaliseren van anti-hormonale therapie.

Contralaterale parenchymale aankleuring

Een eigenschap op de MRI die mogelijk uitermate interessant is voor patiënten die met anti-hormonale therapie behandeld worden, is contralaterale parenchymale

aankleuring (CPE). CPE is een maat van aankleuring van het borstweefsel in de contralaterale borst. Mathematisch gezien is het de gemiddelde ratio van de 10% meest aankleurende borstweefselvoxels tussen de vroege dynamische opname (meestal de eerste opname na contrast) en de dynamische opname na 4-5 minuten. De aankleuring tussen de vroege dynamische opname en de late dynamische opname wordt ook wel de late aankleuring genoemd, dit in tegenstelling tot de aankleuring tussen de eerste (vóór contrast) en tweede (eerste na contrast) dynamische opnames die ook wel de vroege aankleuring wordt genoemd. CPE is dus een maat van de late aankleuring van het gezonde weefsel: een hoge CPE betekent dat er relatief veel vertraagde aankleuring plaatsvindt, terwijl een lage CPE juist een relatief lage vertraagde aankleuring vertegenwoordigt. CPE is interessant voor de personalisatie van anti-hormonale therapie omdat in de MARGINS-studie (Multimodality analysis and radiological guidance in breast conserving therapy) een associatie van CPE met overleving werd geobserveerd: patiënten met een hoge CPE hadden een gunstige prognose, en patiënten met een lage CPE hadden een ongunstige prognose. Deze bevinding is in een onafhankelijke patiëntencohort uit de VS gevalideerd. CPE kan potentieel hoog-risico en laag-risico patiënten van elkaar onderscheiden, onafhankelijk van de klinisch gangbare variabelen zoals bijv. leeftijd en tumorgrootte. Op basis van deze individuele risico-inschatting kunnen er mogelijk patiënten worden geïdentificeerd die zo'n laag risico hebben, dat anti-hormonale therapie veilig achterwege kan worden gelaten. De resultaten van deze twee onafhankelijke studies waren veelbelovend, echter waren het relatief kleine patiëntengroepen met een korte follow-up tijd (patiënten die met antihormonale therapie behandeld worden moeten een langere tijd gevolgd worden omdat zij een relatief lange tijd risico lopen op een borstkanker recidief). Daarnaast zijn de studies telkens binnen 1 centrum uitgevoerd. De manier waarop een MRI wordt gemaakt verschilt tussen centra en tussen de MRI-scanners, dit heeft ook invloed op de berekening van CPE. Het is belangrijk dat de individuele risico-inschatting op basis van CPE onafhankelijk is van het ziekenhuis waar de patiënt is gescand en het type MRI-scanner.

Doel proefschrift

Het doel van dit proefschrift is om verder uit te zoeken in hoeverre MRI, en met name CPE, een rol kan spelen bij het personaliseren van anti-hormonale therapie bij borstkankerpatiënten.

In **Hoofdstuk 2** beginnen we met een overzicht van schattingen van de overbehandeling van verschillende behandelstrategieën m.b.t. adjuvante systemische therapie in borstkankerpatiënten. Patiënten die geen voordeel uit behandeling halen, omdat ze zonder systemische therapie niet zouden overlijden of omdat ze overlijden ondanks systemische therapie, kunnen als overbehandeld worden bestempeld. Nieuwe personalisatie methodes zullen het effectiefst zijn in groepen die relatief weinig overlevingsvoordeel uit behandeling halen en uit een groot aandeel van de patiëntenpopulatie bestaat. Het doel van dit onderzoek was om deze groepen te identificeren. Het is moeilijk om te onderzoeken welke patiënten overbehandeld zijn, omdat het onmogelijk is om dezelfde patiënt wel én niet te behandelen. Er is echter een model, PREDICT, dat op basis van clinicopathologische variabelen (leeftijd, tumorgrootte, etc.) een inschatting kan maken van de overleving en de toegevoegde waarde van verschillende behandelingen (anti-hormonale, HER2-gerichte, en chemotherapie). Met PREDICT kunnen we wel ruwe schattingen geven van percentages overbehandeling, door de geschatte therapie-effectiviteit te middelen over de verschillende behandelingsregimes, dit hebben we gedaan met data uit Nederland en uit de VS. De resultaten lieten zien dat gericht onderzoek naar personalisatie van anti-hormonale therapie het meest effectief lijkt omdat deze patiënten een relatief lage geschatte behandeleffectiviteit hebben, en daarnaast ook het grootste aandeel van de patiënten vertegenwoordigd die behandeld wordt met adjuvante systemische therapie (ongeveer 50% van alle patiënten werd behandeld met anti-hormonale therapie, vs ongeveer 5% en 30% voor respectievelijk HER2-gerichte en chemotherapie).

In **Hoofdstuk 3** presenteren we de resultaten van de SELECT-studie (Stromal enhancement on breast MRI as biomarker for survival with endocrine therapy). In de SELECT-studie hebben we van een groot aantal ER+/HER2- borstkankerpatiënten (1432) uit 10 Nederlandse ziekenhuizen MRIs verzameld die gemaakt zijn tussen 2005-2010. In 2020 heeft het Intergraal Kankercentrum Nederland de overlevingsgegevens van deze patiënten geüpdate. CPE is in al die MRIs berekend en geanalyseerd met betrekking tot overleving. Verrassend genoeg was CPE geassocieerd met overleving, alleen in de omgekeerde richting dan dat het eerder is geobserveerd: een hoge CPE was juist geassocieerd met een slechte prognose. De reden achter deze omgekeerde associatie is onbekend, er zijn verschillende mogelijke redenen: ten eerste, de patiëntengroep tussen de SELECT-studie en

de originele studies was mogelijk anders. De originele studie, waar CPE voor het eerst beschreven was, was een prospectieve studie waarin vrijwel iedereen een MRI vóór de behandeling kreeg terwijl de SELECT-studie retrospectief was, en er afhankelijk van het multidisciplinaire team in het desbetreffende centrum er wel of niet een MRI werd gemaakt vóór de behandeling. Het kan zijn dat er toch verschillen zitten tussen deze twee patiëntenpopulatie waar we niet goed genoeg voor hebben kunnen corrigeren. Dit blijkt ook uit het feit dat anti-hormonale therapie geen beschermend effect leek te hebben in onze analyses, terwijl natuurlijk grote (gerandomiseerde) studies een duidelijk beschermend effect hebben aangetoond en anti-hormonale therapie een belangrijk onderdeel is van de behandeling van ER+-borstkanker. Ten tweede, de studies zijn verricht in 2 verschillende tijdsperiodes. De SELECT-studie heeft patiënten geïncludeerd tussen 2005-2010 en de originele studies patiënten tussen (ongeveer) 2000-2008. In 2008 zijn er veranderingen geweest in de richtlijnen en zijn er nieuwe medicijnen geïntroduceerd: aromataseremmers (anti-hormonale therapie) en taxanen (chemotherapie). We weten dat de aankleuring van borstweefsel anders reageert op deze medicijnen, het kan dus mogelijk ook een andere invloed hebben op de voorspelling van de overleving. De exacte reden achter deze omgekeerde associatie hebben we niet kunnen achterhalen, maar het is mogelijk dat (een combinatie) van deze factoren van invloed zijn geweest. Er moet meer onderzoek worden gedaan voordat CPE gebruikt kan worden als personalisatiemiddel voor anti-hormonale therapie in borstkanker.

In **Hoofdstuk 4** onderzoeken we de rol van MRI tijdens de behandeling met neoadjuvante endocriene (anti-hormonale) therapie (NET). Bij neoadjuvante therapie geef je de behandeling vóór de chirurgie, hierdoor geef je de tumor de kans om te krimpen waardoor het wellicht mogelijk wordt om borstsparende chirurgie te verrichten. Daarnaast kun je het gedrag van de tumor, omdat deze immers nog in de borst zit, onderzoeken door tijdens de behandeling MRIs te maken. In dit hoofdstuk hebben we op 2 manieren onderzocht of we tijdens de neoadjuvante therapie kunnen zien of we de prognose van een patiënt ná chirurgie kunnen voorspellen. In **Hoofdstuk 4a** hebben we onderzocht of we dit met CPE kunnen voorspellen, daarbij hebben we gekeken naar de CPE van voor de behandeling en de CPE tijdens de behandeling. Een hoge CPE vóór behandeling en een daling van CPE tijdens NET waren geassocieerd met een slechte prognose. In **Hoofdstuk 4b** hebben we gekeken of de radioloog de prognose tijdens NET kan

voorspellen a.d.h.v. kwalitatieve beeldkarakteristieken (zoals de radioloog zelf kan beoordelen). De resultaten lieten zien dat dat veel lastiger is in vergelijking met CPE: alleen verschil in tumorgrootte was voorspellend voor prognose na NET, en de verschillen waren erg klein. Voor het voorspellen van therapierespons na NET lijkt CPE een potentiële rol te kunnen spelen.

Ten slotte hebben we in **Hoofdstuk 5** onderzoek gedaan naar de biologische achtergrond van de relatie tussen CPE en overleving. Één van de hypotheses was dat het contralaterale borstweefsel een weerspiegeling is van het borstweefsel van de aangedane borst vóór de tumor, of dat de tumor systemische effecten heeft die ook invloed hebben op het contralaterale borstweefsel. In beide gevallen zijn er mogelijk bepaalde biologische processen in de tumor die geassocieerd zijn met CPE maar ook met overleving. Van de tumoren van patiënten uit de MARGINS-studie is de genexpressie bepaald. CPE was het sterkst geassocieerd met biologische processen die te maken hadden met proteasomen. Proteasomen zijn eiwitstructuren die een essentiële rol spelen binnen de cellulaire proteïne homeostase. De proteasomenexpressie was vervolgens ook geassocieerd met overleving en gevalideerd in een groot extern patiëntencohort. Het is mogelijk dat een deel van de associatie tussen CPE en overleving wordt verklaard door proteasomenexpressie in de tumor.

Concluderend, verbeterde personalisatie op het gebied van anti-hormonale therapie is noodzakelijk omdat dit de grootste groep borstkankerpatiënten betreft en er nog geen klinisch gevalideerde opties zijn. Dit proefschrift heeft onderzocht of de MRI, en met name de aankleuring van het contralaterale borstweefsel (CPE), een rol kan spelen bij deze personalisatie. CPE blijkt geassocieerd te zijn met overleving na (neo)adjuvante therapie, alhoewel de resultaten omgekeerd blijken te zijn t.o.v. eerdere observaties. Er moet meer onderzoek gedaan worden voordat de MRI gebruikt kan worden bij de personalisatie van anti-hormonale therapie. Gezien de omgekeerde relaties (binnen én buiten onze studies) lijkt er een complexe associatie te zijn tussen overleving en parenchymale aankleuring, waar wellicht geavanceerde beeldverwerkingsmethodes, zoals artificiële intelligentie, een rol kunnen spelen in het onthullen hiervan.

LIST OF PUBLICATIONS

List of publications

Peer-reviewed journal publications

Ragusi, M. A. A., Van Der Velden, B. H. M., C. Meeuwis, E. Tetteroo, E.G. Coerkamp, T.J.A. van Nijnatten, F.H. Jansen, E.J.M. Wolters – van der Ben, L. Jongen, A.F. van Raamt, M.D. Dorrius, J. Verloop, M.A. Viergever, R.M. Pijnappel, S.G. Elias & K.G.A. Gilhuijs. Contralateral Parenchymal Enhancement on MRI is associated with long-term survival in breast cancer patients: outcomes of the SELECT-study. *Submitted*.

Ragusi, M. A. A., Van Der Velden, B. H. M., Van Maaren, M. C., Van Der Wall, E., Van Gils, C. H., Pijnappel, R. M., Gilhuijs, K. G. A. & Elias, S. G. Population-based Estimates of Overtreatment with Adjuvant Systemic Therapy in Early Breast-cancer Patients with Data from the Netherlands and the USA. Breast Cancer Res. Treat. 193, 161–173 (2022).

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Ragusi, M. A. A., Loo, C. E., van der Velden, B. H. M., Wesseling, J., Linn, S. C., Beets-Tan, R. G., Elias, S. G. & Gilhuijs, K. G. A. Change in Contralateral Parenchymal Enhancement during Neoadjuvant Endocrine Treatment is Associated with Tumor Response in Unilateral ER+/HER2- Breast Cancer Patients. Radiological Society of Northern America (RSNA), 2019 (oral presentation).

National conference presentations

Ragusi, M. A. A., van der Meer, R. W., Joemai, R. M. S., van Schaik, J. & van Rijswijk, C. S. P. Beeldkwaliteit van CT met SEMAR reconstructie in patiënten na een complexe EVAR procedure. Radiologische Interventiedagen, 2018 (oral presentation).

D

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CURRICULUM VITAE

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Curriculum vitae

Max Ragusi was born on the 13th of March, 1991 in Peschiera del Garda, Italy. He moved to Utrecht in 2010 to start his Liberal Arts & Sciences study at the University College Utrecht. He specialized in Science with a minor in Economics and went on exchange to the University of California in Los Angeles in 2011. To pursue a career a medicine he started his Medicine Masters degree in Leiden in 2013 and gained an exceptional interest in radiology. He published his first article during his research internship at the Interventional Radiology department at the Leiden University Medical Center under the supervision of dr. Carla van Rijswijk. After his medical school graduation in 2018, interested in radiology and research, he started his PhD at the Image Sciences Institute under the supervision of dr. Kenneth Gilhuijs, dr. Sjoerd Elias, prof. dr. Ruud Pijnappel, and prof. dr. ir. Max Viergever. His research was aimed at personalizing endocrine therapy for breast cancer using MRI, the results of which are presented in this thesis. In July 2021 he started his radiology residency under the supervision of Monique Hobbelink, MD and prof. dr. Rutger Jan Nievelstein.