Prognostic factors for breast cancer recurrence

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Publication of this thesis was financially supported by:
Cancer Center University Medical Center Utrecht, Integraal Kanker Centrum Nederland, Novartis, Agendia, Wetenschapsstichting Diakonessenhuis.

ISBN 978-90-393-7054-4

Schilderij op cover: Bert Aalders
Layout: ProefschriftOntwerp.nl
Printed by: ProefschriftMaken.nl

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Prognostische factoren voor borstkanker recidief
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof. dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op
donderdag 27 september 2018 des middags te 2.30 uur

door

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geboren op 26 april 1988
te Ermelo

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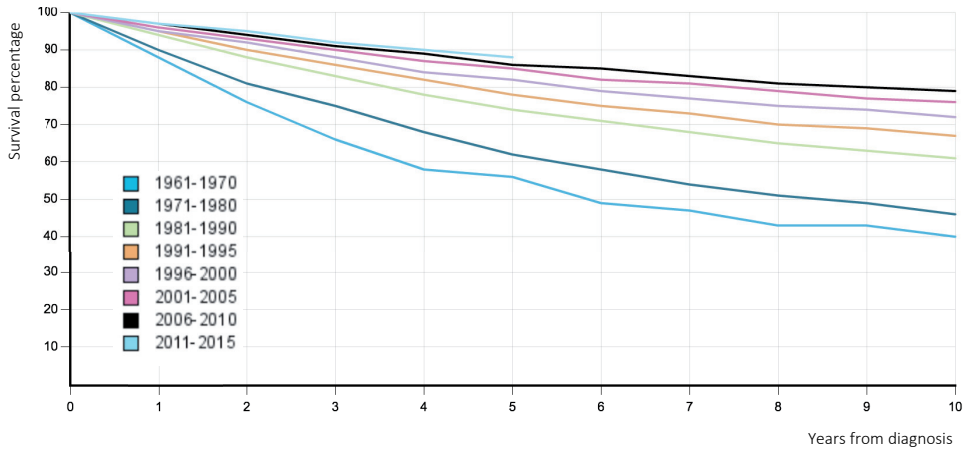
Table of contents

CHAPTER 1

Introduction and outline

Introduction and outline

Breast cancer is the most common type of cancer in women worldwide with an estimated yearly incidence of approximately 1.7 million[1]. In the Netherlands, currently over 14.000 women a year are diagnosed with invasive breast cancer[2]. Breast cancer incidence has increased steadily over the last decades. This can be attributed to the increasing life expectancy, late onset of first child birth, increasing prevalence of obesity and increased detection with the introduction of screening programs [3,4]. Fortunately, the prognosis of breast cancer patients is improving as well, with nowadays a 5-year survival rate of 88% (see figure below for Dutch survival data by year of diagnosis)[2,5].



Modern day breast cancer management is a multidisciplinary approach. Surgical resection forms the basis of primary treatment in most breast cancer patients, though the use of neoadjuvant treatment is increasing, potentially reducing the role of surgery in the future. The main aim of surgical resection, whether or not combined with radiotherapy, is locoregional tumor control.

In the 19th century, breast cancer was considered a purely local disease that was exclusively treated by surgery. The Halsted radical mastectomy described in 1894 consisted of the resection of the breast, pectoralis muscles and regional lymph nodes[6]. This surgical procedure with no consideration of the aesthetic consequences maintained the standard treatment of breast cancer for nearly a century. Over the course of the 20th century there were a number of initiatives exploring the use of more conservative surgery in order to avoid the mutilating consequences of the Halsted procedure. Insight in lymphatic anatomy contributed to modifications of the mastectomy technique while the deployment of radiotherapy catalyzed the development of less extensive surgery[7–11].

In the 1970s, three important randomized trials were started to establish the possibility and safety to reduce the extent of breast surgery[12–15]. The landmark studies from Veronesi et al. (Milan I) and Fisher et al. (NSABP B-06) paved the road for breast-conserving surgery supported by radiotherapy as the standard of care for primary tumor treatment that we know today[12–14]. The results of these trials were subsequently confirmed by the EORTC 10801 trial evaluating modified radical mastectomy versus breast conserving treatment (BCT).

After 20 years of follow-up of the Milan I, NSABP B-06 and EORTC 10801 trials, local recurrence rates were higher after BCT as compared to following mastectomy without significant differences in the occurrence of distant metastases and overall survival[13,15,16].

Radiotherapy to the breast after breast-conserving surgery is associated with reduction of the 10-year risk of breast cancer recurrence[17]. The addition of a boost on the original tumor bed further reduced the local recurrence rate, although without benefit on overall survival and potential negative effects on cosmetic outcome[18]. De-escalation of whole breast irradiation through the reduction of the number of fractions while increasing the daily dose (hypofractionation), has been proven safe and just as effective[19–21]. Accelerated partial breast irradiation (APBI), either by external beam or (intra-operative) brachytherapy, has been studied as an alternative to whole breast irradiation, providing the benefit of shorter treatment duration and more localized administration of radiotherapy sparing healthy surrounding tissue[22–24].

The indications for radiation therapy after mastectomy were recently updated with the recommendation to extend post-mastectomy irradiation to patients with tumors ≤5cm and 1-3 positive nodes in addition to the existing indication for patients with T3-4 tumors, positive resection margins or >3 positive lymph nodes[25–27].

In the late nineties, the sentinel lymph node biopsy (SNB) replaced axillary lymph node dissection (ALND) for lymphatic staging in clinically node negative patients, obviating the need for axillary dissection and reducing the morbidity of breast cancer surgery in patients without sentinel node metastases. The EORTC 10981-22023 AMAROS trial evaluated further de-escalation of regional treatment in patients with early-stage breast cancer and a positive sentinel lymph node, randomizing between completion ALND and axillary radiotherapy[28]. Although one third of women with a positive sentinel node had additional axillary metastases in their ALND resection specimen, the 5-year axillary recurrence rate was 0·43% (95% CI 0·00– 0·92) after ALND and only 1·19% (05% CI 0·31–2·08) after axillary radiotherapy after a median follow-up of 6 years. [29–31]. In addition, the 10-year results of the ACOSOG Z0011 phase 3 randomized trial in patients with sentinel node metastases demonstrated excellent regional control in patients who received no further axillary treatment and non-inferior overall survival[32].

The prognosis of breast cancer patients is readily improving while surgical treatment has become less invasive. Developments in systemic treatment in the context of increasing knowledge on tumor biology such as the identification of the importance of the hormone receptor and human epidermal growth factor receptor 2 (HER2) status and molecular subtype classification have an important role in the increasing survival rates[33–36]. At the same time, there is growing awareness that these same factors have an impact on locoregional control which for a long time had been considered the mere result of local treatment. The improved survival of breast cancer patients has increased the interest in the occurrence of breast cancer relapse and contributing factors.

Still, over 25% of women diagnosed with breast cancer will develop metastatic disease. Metastatic breast cancer, although treatable, is still associated with a dismal prognosis reflected by a 5-year survival rate of approximately 25%[37]. Obtaining more insight into factors associated with the risk and pattern of breast cancer recurrence is important to identify patients who should perhaps be treated more aggressively. At the same time, there may be subgroups of patients in whom the risk of disease recurrence is so low that current local or systemic treatment strategies may be safely scaled down.

Over the last few years, several gene expression profiles have been developed to better predict clinical outcome compared to standard assessment based on clinicopathological characteristics[38,39]. The prospective MINDACT study showed that the 70-gene signature MammaPrint® (70-GS) could accurately differentiate between patients with a low and high risk of distant metastases and death at 5 years, thereby providing valuable information for determining the potential benefit of adjuvant chemotherapy[40].

Outline of this thesis

In this thesis we aimed to evaluate breast cancer recurrence and factors that may be associated with disease relapse and overall survival, in order to further improve tailoring breast cancer management for maximum benefit with minimal morbidity.

In ***chapter 2*** of this thesis we evaluate the overall rates of local (LR) and regional (RR) breast cancer recurrence as well as the occurrence of contralateral breast cancer (CLC) in a relatively recent population of Dutch non-metastatic breast cancer patients treated for primary breast cancer with curative intent between 2003 and 2008. We also describe the trend in the rates of LR, RR and CLC during in this time and the influence of clinicopathological and treatment factors for breast cancer recurrence.

A personalized approach towards optimal breast cancer management can only be achieved through understanding of the biology of the disease. Therefore, in the next chapters we evaluate different factors that may be associated with the development of breast cancer recurrence and overall survival. There will likely be subgroups of patients in which the extent of treatment may be safely scaled down, while on the other hand there could be patients for whom there is a need for more extensive or new treatment strategies. Patient age has long been associated with aggressive breast cancer biology. Older studies reported that young age should be considered an independent risk factor for poor prognosis in patients with breast cancer. However, increasing knowledge on tumor biology, developments in systemic treatment strategies and improved outcome in the overall population raises the question whether young age is still associated with poor prognosis in the modern era. In ***chapter 3*** we assess the local and regional recurrence rates in young women aged <35 with primary non-metastatic breast cancer treated between 2003 and 2008. Additionally, we evaluate the association with tumor biology as expressed by tumor biomarker subtypes defined on the basis of hormone receptor and HER2 status in this group of very young breast cancer patients. In ***chapter 4*** we aim to further characterize primary breast cancer in young women, for this paper defined as <45 years of age. We describe the risk of breast cancer (BC) relapse according to the 70-gene signature (70-GS) result in relation to young age in early-stage BC patients enrolled in the MINDACT trial. Furthermore, we demonstrate the 5-year distant metastasis-free survival for the young population as compared to older patients.

There has long been discussion regarding the prognostic value of IM lymph nodes and their management given the risk of morbidity from either surgical or radiotherapy treatment as a result of their location. Therefore, in ***chapter 5*** we evaluate the clinical impact of tumor positive internal mammary (IM) lymph nodes on overall survival.
Multifocal disease has been associated with a higher tumor load. However, current guidelines recommend basing adjuvant systemic treatment decisions on characteristics of the largest lesion thus disregarding possible biological implications of having multifocal disease. ***Chapter 6*** describes the results of a substudy of the MINDACT trial addressing the value of performing the 70-gene signature MammaPrint® in multifocal breast cancer.

Chapter 7 and 8 focus on breast cancer recurrence in patients treated with chemotherapy in the neoadjuvant setting. Neoadjuvant systemic therapy is a well-established strategy for locally advanced disease with the aim of downstaging the tumor to enable more conservative surgery. However, in early stage breast cancer patients with small tumors systemic treatment is increasingly being administered before surgery as it allows for 'in vivo' monitoring of the efficacy of administered systemic treatment. The achievement of a pathologic complete response (pCR) has been associated with improved survival, mainly in hormone receptor negative breast

cancer[41]. For some patients, neoadjuvant treatment will result in (significant) downstaging without achievement of a complete pathologic response. This level of treatment effect could pertain important prognostic information that is lost by only classifying the response in pCR or not. Therefore, in ***chapter 7*** we assess the prognostic value of the neoadjuvant response index (NRI) as proposed by Rodenhuis et al.[42] for recurrence-free survival in a population-based cohort.

There is limited data on the association between achievement of a pCR and the pattern of metastatic spread. In ***chapter 8*** we evaluate whether the sites of first distant relapse differed between patients whose tumor achieved a pCR after neoadjuvant chemotherapy versus those who did not in the EORTC 10994/BIG-1-00 trial[43].

Finally, a discussion of the described results and literature is provided, addressing future perspectives regarding futher characterization of tumor biology and personalizing prognostic information with the aim of tailoring treatment to the individual patient.

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CHAPTER 2

Contemporary risks of local and regional recurrence and contralateral breast cancer in patients treated for primary breast cancer

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Eur J Cancer. 2016; 63: 118-126

Abstract

Introduction

Breast cancer treatment has evolved extensively over the past two decades with a shift towards less invasive local treatment and increased systemic treatment. The present study aimed to investigate the rates of local (LR) and regional (RR) recurrence and contralateral breast cancer (CBC), evaluating the influence of contributing factors.

Materials and methods

We selected all female patients operated for unilateral primary breast cancer (anyTN, M0) between 2003 and 2008 from the Netherlands Cancer Registry. The 5-year risks of developing LR, RR and CBC were estimated using Kaplan-Meier statistics. The influence of various patient, tumour and treatment characteristics was subsequently assessed in multivariable analyses.

Results

A total of 52,626 patients were identified. The rates of LR, RR and CBC were 2.7%, 1.5% and 2.9%, respectively. The rates of LR and RR decreased significantly over time in the period 2003–2008, from 3.2% to 2.4% for LR and 1.8 to 1.3% for RR, both becoming lower than the risk of CBC of 2.8%. Multivariable analysis showed that age, tumour size, lymph node involvement, tumour histologic type, grade and hormone receptor status were significant prognosticators for LR and RR, but not for CBC. A trend towards a beneficial effect of breast conserving surgery on LR and RR was seen, while systemic therapy proved to have a protective effect on all three end-points.

Conclusions

In breast cancer patients treated between 2003 and 2008 locoregional recurrence rates decreased and have ended up lower than the risk of developing CBC.

Introduction

Over the last decades, multimodality breast cancer treatment has changed extensively. Surgical procedures have become less invasive [1-4], while radiotherapy indications and techniques have changed. At the same time (neo-)adjuvant systemic treatment modalities have evolved, due to an expanded selection of patients for adjuvant treatment, targeted drugs (e.g. trastuzumab) [5], and effective combinations of treatments.

Despite less invasive local treatment, recent studies observed improved survival, which has resulted in a renewed interest in locoregional control [6-8]. Acceptable local recurrence (LR) rates were previously defined as a less than 5% risk within 5 years after initial treatment [9]. However, locoregional recurrence rates today may well have decreased below this norm and could have become low in such a way, that current follow-up and local treatment protocols should be redefined. On the other hand, there may still be groups of patients in whom the risk of LR requires a more aggressive treatment approach. Conceptually, for some patients a locoregional recurrence risk rate equalling the risk of contralateral breast cancer (CBC) may perhaps be regarded as the pursuable recurrence risk.

The present study aimed to investigate rates of developing a LR or regional (RR) recurrence and CBC, evaluating time trends and the influence of contributing patient and treatment factors over a time period in which local treatment had already become less invasive while systemic treatment options evolved.

Materials and methods

Study design and patients

A nationwide cohort study was conducted using data of the Netherlands Cancer Registry (NCR). The NCR is a national, population-based cancer registry containing information on patient, tumour and treatment characteristics. Trained personnel register data from patients' medical records. The Committee of Privacy of the NCR approved the use of data for this study.

All patients diagnosed with and operated for primary unilateral invasive breast cancer between January 1st 2003 and December 31st 2008, without distant metastases (DMs) at time of diagnosis, were selected from the NCR. Patients with in situ carcinomas were not included, nor patients with Paget's disease. Other exclusion criteria were neo-adjuvant treatment (n = 2889), macroscopic tumour residue after the final surgery of the primary tumour (n = 100) and incomplete follow-up data (e.g. no information since treatment for primary tumour, missing event

date; n = 128). Incomplete data on follow up were mainly applicable for the years 2007 and 2008 during which 47% (n = 43) of the hospitals provided follow-up data since data collection for those years was only performed on request.

The following patient and tumour characteristics were extracted; age, gender, histologic type (ductal, lobular, mixed ductal/lobular or other), pathologic tumour size, nodal involvement, Bloom-Richardson histologic grade, multifocality (yes/no), hormone receptor status (ER/PR) and human epidermal growth factor receptor 2 (HER2) status. The following information concerning treatment was extracted; type of surgery (breast conserving surgery [BCS] versus mastectomy), positive tumour margins after final surgery (yes/no, yes meaning microscopic tumour involvement defined by ink on tumour), axillary lymph node dissection (yes/no), radiotherapy (yes/no), chemotherapy (yes/no), hormone therapy (yes/no) and trastuzumab (yes/no). There were multiple national breast cancer guideline adjustments during the study period. Revisions to the content were mainly due to widening indications for adjuvant systemic treatment; first advising systemic treatment in patients with low-grade tumours >2 cm and subsequently also in T1C tumours of intermediate malignancy grade [10-14]. Standard assessment of HER2-status was implemented in the Netherlands mid-2005 [5], and treatment with trastuzumab was reimbursed starting 2006 [13]. Staging is recorded according to the TNM system of the Union for International Cancer Control and the American Joint Committee on Cancer current at time of diagnosis [15].

Five-year follow-up data on LR, RR, CBC, DM and vital status, were collected for all patients. The first event and any additional events occurring within 28d of the first event were included for analyses (e.g. in patients who presented with LR after which CBC was diagnosed during further examination within 28d, both events were included in the analyses). Vital status was obtained through linkage with the municipality registry and was complete until December 31st 2013.

Definitions

The primary end-points of the present study were LR, RR and CBC. LR was defined as any recurrence of invasive breast cancer or ductal carcinoma in situ (DCIS) in the ipsilateral breast or chest wall. Recurrence of breast cancer in the ipsilateral regional lymph nodes (e.g. axillary, infra/supraclavicular or in the internal mammary chain) was considered to be RR, and CBC invasive breast cancer in the contralateral breast. DMs were included as a secondary end-point to compare the previously reported decreasing trend in the occurrence of DM with the incidence of locoregional recurrence [16,17].

Statistical analysis

Distribution of baseline characteristics, including patient, tumour and treatment factors, is presented in percentages. Missing values (grade n = 3099 [6%], multifocality n = 8340 [16%], hormone receptor status n = 6028 [11%] and status of resection margin n = 1270 [2%]) were imputed. HER2-status was not imputed since HER2 receptor status was not routinely assessed until 2005. Missing values were imputed based on predictive distribution using an imputation model that included risk factors and outcome. This process was repeated multiple times to allow for imputation uncertainty, which resulted in 20 complete datasets. All analyses were performed on both the original dataset and the multiple imputation sets to check the stability of the results. The data presented in the article are based on the combined multiple imputed data.

Subsequently, clinicopathological characteristics of the included patients were assessed over time. Time- trends of different treatment modalities were evaluated using linear regression analyses.

Univariate overall 5-year rates for LR, RR and CBC were calculated by means of Kaplan-Meier estimates. Patients were censored at date of death (n = 2077) or date of last visit to the hospital (n = 4158). The prognostic significance of different independent clinicopathological and treatment factors on the 5-year risk of developing a LR, RR or CBC was assessed using multivariable Cox proportional hazards regression analyses. The prognostic factors that were evaluated in multivariable analysis were chosen on the basis of univariate analyses.

Statistical analysis was performed using Stata soft- ware (StataCorp. 2013. Release 13.1. College Station, TX: StataCorp LP). A P-value of less than 0.05 was considered statistically significant.

Results

Baseline characteristics

Patient and tumour characteristics as well as treatment information of the included patients (n = 52,626) are presented in Table 1. Mean age of included patients was 59 years (standard deviation ± 13.2).

Table 1. Clinicopathological characteristics of the 52,626 primary breast cancer patients diagnosed and operated between 2003 and 2008.

		Total	
		N	% (imputation)
Age	<35	1,005	2
	35-49	11,966	23
	50-69	26,893	51
	≥70	12,762	24
Gender	Male	316	1
	Female	52,310	99
Tumour histologic type	Ductal	42,291	80
	Lobular	5,630	11
	Ductal + lobular	2,232	4
	Other[a]	2,473	5
Pathologic tumour size	1A	1,973	4
	1B	7,609	14
	1C	22,403	43
	2	18,259	35
	3	1,443	3
	4	412	1
	X	527	1
Pathologic lymph node involvement	pN0	32,139	61
	pN1	14,000	27
	pN>1	5,727	11
	Unknown	760	1
Grade	Well differentiated	11,212	21 (23)
	Moderately differentiated	22,279	42 (45)
	Poorly differentiated	16,036	30 (32)
	Unknown	3,099	6 (-)
Multifocality	Yes	6,614	13 (15)
	No	37,672	72 (85)
	Unknown[b]	8,340	16 (-)
Hormone receptor status	Positive	38,443	73 (82)
	Negative	8,155	16 (18)
	Unknown[b]	6,028	11 (-)
Local treatment[c]	BCS with RTx	28,716	55
	BCS without RTx	789	2
	MAST with RTx	5,750	11
	MAST without RTx	17,371	33
Positive tumour margins	Yes[d]	2,016	4 (4)
	No	49,340	94 (96)
	Unknown	1,270	2 (-)
Axillary Lymph Node Dissection	Yes	25,429	48
	No	27,197	52
Chemotherapy	Yes	18,605	35
	No	34,021	65
Hormone therapy	Yes	22,783	43
	No	29,843	57
HER2 and trastuzumab	HER2 negative, no trastuzumab	27,755	53
	HER2 positive, no trastuzumab	2,240	4
	HER2 positive, trastuzumab	2,139	4
	Unknown[e]	20,492	39

Percentages may not add up to 100% due to rounding.

Records with missing values that were imputated: grade 3099 (6%), multifocality 8340 (16%), hormone receptor status 6028 (11%) positive tumour margins 1270 (2%).

[a] Histologic tumour type 'other': e.g. mucinous, medullary, metaplastic carcinoma.

[b] Category 'unknown' consists mostly of unknown due to missing in registration in the NCR in earlier years.

[c] Local treatment: final surgery combined with radiotherapy received. BCS = breast conserving surgery, MAST = mastectomy, RTx = radiotherapy, pN = nodal involvement.

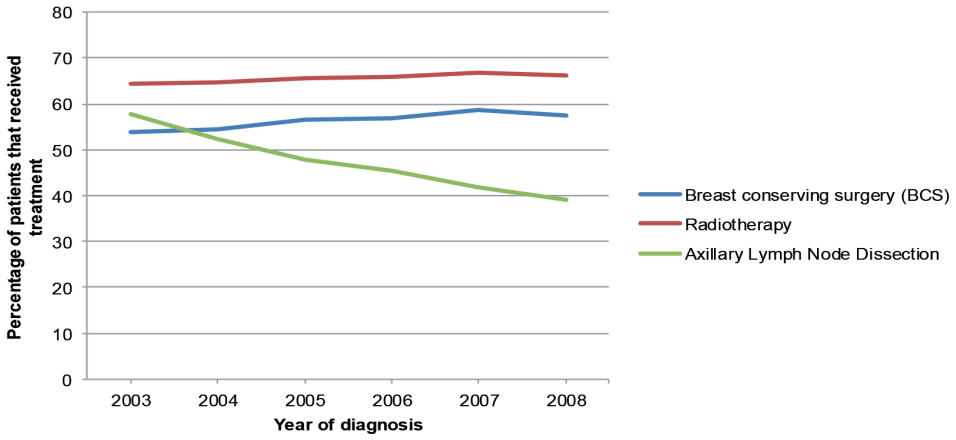
[d] Positive tumour margins after final surgery represents microscopic tumour depositions (defined by ink on tumour) without further surgery. Patients with macroscopic tumour residue after final surgery were excluded from the present study.

[e] Category 'unknown' consists mostly of unknown due to missing in earlier years since standard HER2 testing and treatment with trastuzumab were only routinely implemented starting September 2005.

Time trends

The distribution of clinicopathological factors remained stable during the study period. Between 2003 and 2008 the distribution of patients that underwent BCS did not change significantly. The proportion of patients with microscopically positive tumour margins after surgery decreased slightly during the study period, for both BCS (from 4.9% in 2003 to 3.7% in 2008, $p = 0.009$) and mastectomy (from 2.4% in 2003 to 2.0% in 2008, $p = 0.646$). Axillary lymph node dissection was per- formed gradually less frequent over time (from 58% in 2003 to 39% in 2008, $P < 0.001$, see Fig. 1A). The proportion of patients that received chemotherapy and hormonal treatment remained stable until the final year of the study period when an increase was observed for both systemic treatment options (additional 8% and 5% receiving CT and HT, respectively, see Fig. 1B). Standard treatment with trastuzumab in HER2+ disease was introduced in 2005 and generally applied since 2006. Approximately half of patients with HER2+ disease did not receive trastuzumab. This was mainly associated with increasing age, with 95% of HER2+ patients aged ≥70 years not receiving trastuzumab treatment ($P < 0.001$).

1A. Local treatment of breast and axilla



1B. Systemic treatment

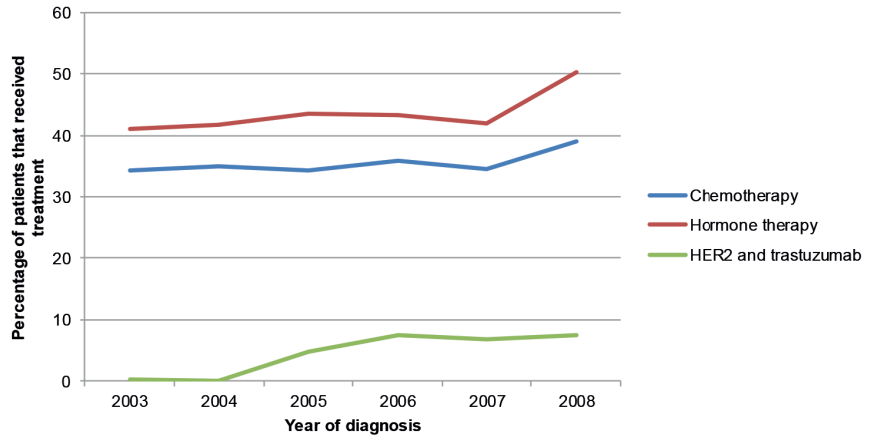


Figure 1. Time-trends of different treatment modalities applied in the 52,626 patients in the period 2003-2008.

Patient outcome

The overall 5-year risks of developing LR or RR, CBC and DM were 2.7%, 1.5%, 2.9% and 9.0%, respectively, and are shown for each year in Table 2. LR and DM occurred simultaneously (within 28d of first event) in 168 patients (13% of LRs) and in 217 patients for RR and DM (31% of RRs). The risks of LR and RR decreased significantly during the study period by approximately one-third, while at the same time a significant decrease in the risk of DM was observed. The decrease in locoregional recurrence rates particularly unfolded in the period 2003-2006 and stabilised from then on. The risk of developing CBC did not change over time (P = 0.56)

Table 2. Overall 5-year rates of local and regional recurrence, contralateral breast cancer diagnosed and operated between 2003 and 2008 over time.

	Local recurrence[a]		Regional recurrence		Contralateral breast cancer		Distant metastases	
	rate (%)	95% CI	rate (%)	95% CI	rate (%)	95% CI	rate (%)	95% CI
2003 (n=9,807)	3.2%	(2.8 – 3.6)	1.8%	(1.6 – 2.1)	3.1%	(2.8 – 3.5)	10.5%	(9.9 – 11.1)
2004 (n=10,025)	3.3%	(2.9 – 3.7)	1.9%	(1.6 – 2.2)	2.9%	(2.6 – 3.3)	10.4%	(9.8 – 11.1)
2005 (n=9,884)	2.6%	(2.2 – 2.9)	1.6%	(1.3 – 1.8)	2.6%	(2.3 – 3.0)	9.3%	(8.8 – 9.9)
2006 (n=10,195)	2.4%	(2.1 – 2.7)	1.2%	(1.0 – 1.4)	3.2%	(2.8 – 3.6)	8.3%	(7.7 – 8.8)
2007[b] (n=6,359)	2.3%	(1.9 – 2.7)	1.2%	(1.0 – 1.5)	2.8%	(2.4 – 3.2)	7.5%	(6.8 – 8.2)
2008[b] (n=6,356)	2.4%	(2.0 – 2.8)	1.3%	(1.1 – 1.7)	2.8%	(2.4 – 3.3)	7.1%	(6.5 – 7.8)
Overall *(n=52,626)*	*2.7%*	*(2.6 – 2.9)*	*1.5%*	*(1.4 – 1.6)*	*2.9%*	*(2.8 – 3.1)*	*9.0%*	*(8.8 – 9.3)*

CI, confidence interval.

Rates represent Kaplan-Meier estimates.

[a] Local recurrence (ipsilateral in-breast recurrence + new primary).

[b] Fewer patients were included in the years 2007-2008 compared to earlier years as some hospitals did not provide follow-up data for those years. These patients' data consisted of a heterogeneous group in which exclusion, based on their clinicopathological and recurrence data in previous years, should not have affected the representativeness of the included patients for the period 2007-2008.

Prognostic factors

Table 3 lists the absolute risks of LR, RR and CBC using Kaplan-Meier estimates and the hazard ratios (HRs) for the various patient categories from multi- variable analyses. The absolute risk of developing LR only exceeded the previously established consensus risk of 5% in patients with pT4 (LR-risk 8.8%) and hormone receptor negative tumours (LR-risk 5.3%) and in patients that underwent BCS without radiotherapy (LR- risk 10.6%, Table 3).

Table 3. The 5-year risk of LR, RR and CBC according to clinicopathological and treatment factors of the 52,626 breast cancer patients diagnosed and operated between 2003 and 2008.

		Local recurrence			Regional recurrence			Contralateral breast cancer		
		Univariate	Multivariable		Univariate	Multivariable		Univariate	Multivariable	
		%[a]	HR[b]	95% CI	%[a]	HR[b]	95% CI	%[a]	HR[b]	95% CI
Age										
<35	n= 1,005	3.5%	1.39	(0.96 – 2.02)	3.7%	**2.05**	**(1.41 – 2.98)**	2.5%	1.20	(0.77 – 1.87)
35-50	n=11,966	2.9%	**1.36**	**(1.18 – 1.57)**	1.8%	**1.36**	**(1.13 – 1.64)**	2.6%	1.03	(0.90 – 1.19)
50-70	n= 26,893	2.4%	Ref		1.3%	Ref		3.0%	Ref	
>70	n= 12,762	3.2%	0.94	(0.81 – 1.10)	1.4%	**0.60**	**(0.48 – 0.76)**	3.1%	0.89	(0.77 – 1.16)
Tumour histologic type										
Ductal	n= 42,291	2.8%	Ref		1.7%	Ref		2.8%	Ref	
Lobular	n= 5,630	2.5%	0.92	(0.76 – 1.12)	0.8%	**0.58**	**(0.41 – 0.81)**	3.4%	1.18	(1.00 – 1.40)
Ductal+lobular	n= 2,232	2.2%	0.84	(0.62 – 1.15)	0.8%	**0.56**	**(0.33 – 0.94)**	3.8%	**1.35**	**(1.07 – 1.71)**
Other	n= 2,473	2.1%	**0.69**	**(0.51 – 0.94)**	0.7%	**0.41**	**(0.25 – 0.68)**	3.4%	1.04	(0.82 – 1.33)
Pathologic tumour size										
1A	n= 1,973	2.2%	Ref		1.1%	Ref		4.3%	Ref	
1B	n= 7,609	2.2%	1.30	(0.91 – 1.85)	0.9%	1.43	(0.84 – 2.42)	3.5%	0.89	(0.68 – 1.15)
1C	n= 22,403	2.3%	**1.49**	**(1.07 – 2.08)**	1.2%	**2.09**	**(1.29 – 3.39)**	3.1%	0.95	(0.75 – 1.22)
2	n= 18,259	3.3%	**2.18**	**(1.56 – 3.06)**	2.1%	**3.23**	**(1.98 – 5.25)**	2.2%	0.90	(0.69 – 1.18)
3	n= 1,443	3.7%	**2.49**	**(1.58 – 3.92)**	2.9%	**4.34**	**(2.36 – 7.96)**	3.4%	1.32	(0.86 – 2.04)
4	n= 412	8.8%	**6.03**	**(3.66 – 9.95)**	5.2%	**6.47**	**(3.05 – 13.71)**	1.9%	0.74	(0.29 – 1.85)
X	n= 527	4.9%	**2.46**	**(1.47 – 4.12)**	2.1%	**2.46**	**(1.14 – 5.32)**	2.7%	0.80	(0.43 – 1.47)
Pathologic lymph node involvement										
N0	n= 32,139	2.4%	Ref		1.3%	Ref		3.4%	Ref	
N1	n= 14,000	2.8%	**1.85**	**(1.54 – 2.21)**	1.5%	**2.09**	**(1.63 – 2.69)**	2.0%	1.01	(0.83 – 1.22)
>N1	n= 5,727	4.4%	**4.02**	**(3.15 – 5.12)**	2.7%	**4.36**	**(3.09 – 6.14)**	2.2%	1.13	(0.83 – 1.54)
Unknown	n= 760	4.3%	1.19	(0.77 – 1.84)	3.2%	**2.12**	**(1.28 – 3.50)**	3.4%	0.96	(0.59 – 1.56)
Grade										
Well differentiated	n= 11,212	1.7%	Ref		0.5%	Ref		3.4%	Ref	
Moderately differentiated	n= 22,279	2.6%	**1.55**	**(1.29 – 1.85)**	1.1%	**2.00**	**(1.46 – 2.74)**	3.1%	1.09	(0.95 – 1.25)
Poorly differentiated	n= 16,036	3.5%	**1.92**	**(1.55 – 2.36)**	2.9%	**4.53**	**(3.23 – 6.34)**	2.3%	0.97	(0.80 – 1.18)
Multifocality										
No	n= 37,672	2.5%	Ref		1.4%	Ref		2.9%	Ref	
Yes	n= 6,614	3.4%	**1.30**	**(1.11 – 1.53)**	1,8%	1.25	(1.00 – 1.58)	3.5%	**1.29**	**(1.09 – 1.53)**
Hormone receptor status										
Negative	n= 8,155	5.3%	Ref		3.5%	Ref		3.3%	Ref	
Positive	n= 38,443	2.2%	**0.59**	**(0.50 – 0.71)**	1.1%	**0.58**	**(0.45 – 0.74)**	2.8%	1.04	(0.84 – 1.28)
Local treatment										
BCS with RTx	n= 28,716	2.0%	Ref		1.0%	Ref		2.8%	Ref	
BCS without RTx	n= 789	10.6%	**5.03**	**(3.83 – 6.61)**	3.5%	**3.21**	**(2.00 – 5.15)**	2.3%	0.91	(0.52 – 1.59)
MAST with RTx	n= 5,750	3.0%	**0.74**	**(0.58 – 0.94)**	2.2%	1.16	(0.86 – 1.58)	2.6%	**1.44**	**(1.10 – 1.88)**
MAST without RTx	n= 17,371	3.5%	**1.62**	**(1.41 – 1.85)**	2.2%	**2.33**	**(1.94 – 2.79)**	3.3%	**1.31**	**(1.15 – 1.49)**
Positive tumour margins										
No	n= 49,340	2.7%	Ref		1.5%	Ref		2.9%	Ref	
Yes	n= 2,016	3.6%	**1.42**	**(1.09 – 1.84)**	1.5%	1.06	(0.70 – 1.60)	2.9%	1.06	(0.79 – 1.43)

ALND										
No	n= 27,197	2.4%	Ref		1.4%	Ref		3.4%	Ref	
Yes	n= 25,429	3.1%	1.00	(0.85 – 1.18)	1.6%	**0.51**	**(0.40 – 0.65)**	2.4%	0.91	(0.78 – 1.06)
Chemotherapy										
No	n= 34,021	2.9%	Ref		1.4%	Ref		3.5%	Ref	
Yes	n= 18,605	2.4%	**0.45**	**(0.37 – 0.53)**	1.7%	**0.42**	**(0.33 – 0.52)**	1.9%	**0.66**	**(0.54 – 0.80)**
Hormone therapy										
No	n= 29,843	3.5%	Ref		1.8%	Ref		1.0%	Ref	
Yes	n= 22,783	1.7%	**0.37**	**(0.31 – 0.43)**	1.1%	**0.52**	**(0.41 – 0.66)**	1.6%	**0.41**	**(0.35 – 0.49)**
HER2 and trastuzumab										
HER2-, no trastuzumab	n= 27,755	2.4%	Ref		1.3%	Ref		3.0%	Ref	
HER2+, no trastuzumab	n= 2,240	4.2%	1.11	(0.87 – 1.41)	2.9%	1.14	(0.85 – 1.54)	2.6%	0.80	(0.59 – 1.08)
HER2+, trastuzumab	n= 2,139	2.0%	**0.56**	**(0.40 – 0.79)**	1.2%	**0.47**	**(0.31 – 0.73)**	1.7%	0.75	(0.51 – 1.08)
HER2 unknown	n= 20,492	3.1%	**0.73**	**(0.58 – 0.92)**	1.7%	**0.59**	**(0.44 – 0.80)**	3.0%	0.86	(0.69 – 1.08)

Significant hazard ratios and their respective CIs are in bold.

LR, local recurrence; RR, regional recurrence; CBC, contralateral breast cancer; HR, hazard ratio; CI, confidence surgery; MAST, mastectomy; RTx, radiotherapy; ALND, axillary lymph node dissection.

[a] Rates represent Kaplan-Meier estimates.

[b] Hazard ratio's assessed using multivariable Cox proportional hazards regression analyses.

Multivariable analyses showed the risks of LR and RR were inversely related with age, and increased with growing tumour size, hormone receptor negative tumours, higher histologic grade and extent of metastatic lymph node involvement. The risk of RR was also significantly higher for tumours with a ductal histologic type compared to the other histology types in multivariable analyses. Regarding treatment, risk reductions for both end-points were observed when radiotherapy was applied both after BCS and mastectomy. Patients treated with axillary lymph node dissection were shown to have a lower rate of RR. Chemotherapy and hormonal treatment were also associated with a decreased LR and RR risk. Treatment with trastuzumab in HER2þ patients conveyed a 50% risk reduction of both LR and RR compared to untreated HER2+ patients (Table 3). Clinicopathological characteristics at the time of the primary breast cancer (age, tumour size, lymph node status, grade and hormone receptor status) were not significant prognostic factors for developing CBC in multivariable analyses. Chemotherapy (HR 0.66; 95% CI 0.54-0.80) and hormonal therapy (HR 0.41; 95% CI 0.35-0.49) significantly reduced the risk of CBC.

Discussion

The 5-year LR and RR rates in patients treated for primary breast cancer between 2003 and 2008 in the Netherlands were very low: 2.7%, 1.5% and 2.9%, respectively, and generally lower than the risk of developing CBC. The rates of LR and RR decreased over the studies time period, while the risk of CBC remained stable.

The low and decreasing locoregional recurrence rates are similar to the rates reported by previous studies that only evaluated patients with early-stage disease [18,19]. The present study thus confirms the continuing decrease of local breast cancer relapses that accompanies the improved survival of breast cancer patients [7,20]. At the same time, the risk of CBC did not decrease significantly. Schaapveld et al [21] previously reported a 5-year cumulative incidence rate of 2.1% in pT3N1M0 patients treated in the Netherlands between 1989 and 2003, supporting our finding that the risk of developing CBC has remained relatively stable over time. Tumour size and extent of metastatic lymph node involvement and to a lesser degree age, subtype, grade and hormone receptor status proved to be contributing clinicopathological factors to the risk of developing LR and RR. The administration of radiotherapy and systemic therapy, were associated with a decreased risk of both LR and RR in the present study, in agreement with earlier studies [22]. Multivariable analyses showed comparable LR and RR risks for breast conserving therapy and mastectomy with a trend towards better outcome after breast conserving therapy (BCT). This finding contrasts with results of the landmark studies published on BCS in 2000-2002 after the introduction of BCS that observed significantly higher rates of LR after BCS when compared to mastectomy [2, 23,24].

The aforementioned characteristics of the primary tumour were not found to be predictive of the risk of CBC during follow up, suggesting that the incidence of CBC later on in life is not dependent on the tumour biology of the first breast tumour. Therefore, CBC may be considered a completely separate disease entity and will depend more on a patient's genetic predisposition [25,26]. The risk of CBC did reduce after chemotherapy and hormone therapy, consistent with previous reports [27-29].

The observed decreasing trends of LR and RR in the present study were accompanied by a similar decline in the risk of developing DM. This suggests that the improvement of locoregional control may, to a large extent, be attributable to developments in systemic treatment. While the proportions of patients that received adjuvant systemic treatment only increased in the last year of the study period, 2008, the introduction of trastuzumab in 2005 coincided with the decreasing locoregional recurrence rates in the whole study population. Treatment with trastuzumab in HER2+ disease indeed reduced the risk of LR and RR in the present study, as was also

reported by others [22,30,31]. Yet, the small proportion of patients receiving trastuzumab makes it unlikely that this is the only explanation for the overall observed improved locoregional control. Alternatively, although the amount of patients that received chemotherapy and hormonal treatment has remained stable for the vast majority of the studied period, both treatment modalities have evolved extensively in the last decade with the introduction of aromatase inhibitors [32] and taxane/anthracycline combination chemotherapy [33,34]. Moreover, the increasing knowledge on tumour biology will also have played a major role, especially in deciding optimal systemic treatment.

The absolute risk of developing an ipsilateral in- breast recurrence has become lower than the risk of developing CBC. The overall 5-year risk of LR was with 2.7% only half of the previously established limit of 5% within 5 years after treatment [9]. The absolute risk of developing LR only exceeded this consensus of 5% in patients with pT4 and hormone receptor negative tumours and in patients that underwent BCS without radiotherapy (LR-risk 10.6%).

Locoregional recurrence is a commonly used end- point in breast cancer studies. However, the definitions of locoregional, LR and RR are not always clear and may vary for different studies, thus limiting solid comparability of results [35,36]. The definitions for LR, RR and CBC used in the present study are consistent with the definitions established by consensus in the study of Moossdorff et al [37].

Other strengths of this study are the large number of analysed patients with complete 5-year follow-up data and the population based design resulting in generally applicable results not subjective to several types of bias, for example selection bias. Furthermore, trained registrars, using a standardised coding manual, collected the data resulting in comparable data from the different hospitals.

There are also some limitations to this study. Firstly, the NCR depends on the documentation in clinical records, which might have caused an underestimation of the number of recurrences than would be present in prospective studies. However, based on follow up protocols, patients visit the hospital regularly during the first 5 years after diagnosis. When no information was available, patients were censored at the date of their last visit. Secondly, data on biological tumour factors such as hormone and in particular HER2 receptor status were not available for all patients before 2005. Thirdly, in observational studies there is always the risk of confounding by indication that thus far has remained unsolvable, even with the use of multivariable analyses [38,39]. Some selection will have resulted from the exclusion of patients treated with neoadjuvant chemotherapy, as 50% of these patients had cT3 or cT4 tumours. Lastly, the NCR does not contain detailed information on radiotherapy and systemic treatment regimes, other than if

applied or not. Therefore, items such as radiotherapy target volumes or type of hormonal therapy could not be taken into account regarding their effect on the development of a LR or RR.

In conclusion, this large population based study showed that LR and RR rates decreased over time and are currently very low. For many patients the contemporary rates of LR and RR are lower than the risk of CBC. These low locoregional recurrence rates are the net result of better understanding of the differences in tumour biology and the evolution of systemic therapies against the background of previously optimised local treatment. Future adjustments should not merely aim at improvements through more intense treatment, but should also address the large number of patients for whom less intensive local treatment or follow-up strategies are achievable as the risk of developing a locoregional recurrence has become very low.

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CHAPTER 3

Contemporary locoregional recurrence rates in young patients with early-stage breast cancer

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J Clin Oncol. 2016; 20; 34(18): 2107-14

Abstract

Purpose

The aim of this study was to evaluate contemporary rates of local recurrence (LR) and regional recurrence (RR) in young patients with breast cancer in relation to tumor biology as expressed by biomarker subtypes.

Patients and Methods

Women <35 years of age who underwent surgery for primary unilateral invasive breast cancer between 2003 and 2008 were selected from the Netherlands Cancer Registry. Patients were categorized according to biomarker subtypes on the basis of hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2) status. The 5-year risks of developing LR and regional lymph node recurrence were estimated by using Kaplan-Meier statistics.

Results

A total of 1,000 patients were identified, of whom 59% had a known subtype: 39% HR-positive/HER2-negative; 17% HR-positive/HER2-positive; 10% HR-negative/HER2-positive; and 34% HR- negative/HER2-negative (triple negative). Overall 5-year LR and RR rates were 3.5% and 3.7%, respectively. A decreasing trend for both rates was observed over time and was accompanied by a significant decrease in the risk of distant metastases (DM). LR occurred in 4.2%, RR in 6.1%, and DM in 17.8% of patients in 2003, and in 3.2%, 4.4%, and 10.0%, respectively, in 2008. LR and RR rates varied with biomarker subtype. These differences were borderline significant when analyzed for the entire study period ($P = .056$ and $P = .014$, respectively) and leveled off after the introduction of trastuzumab after 2005 ($P = .24$ and $P = .42$, respectively). Patients with lymph node metastases at the time of diagnosis had an increased risk of RR. The type of surgery performed—breast-conserving or mastectomy—did not influence rates of LR and RR.

Conclusion

Overall, the rates of LR and RR in young patients with early-stage breast cancer were relatively low and varied by biomarker subtype.

Introduction

Of all patients with breast cancer in the Netherlands, 2% are younger than 35 years of age at the time of diagnosis[1]. Historically, young patients with breast cancer have had a poorer prognosis and a higher risk of locoregional recurrence (LRR)[2-4]. Younger patients with breast cancer seem to possess a more aggressive tumor biology compared with older women with breast cancer. This is reflected by the high proliferation and poor differentiation rates and by a higher frequency of hormone receptor (HR)–negative tumors[5]. In addition, unfavorable gene expression profiles are more frequently observed as well as the occurrence of pathogenic germ-line mutations, such as BRCA1 and BRCA2[6-9].

Previous studies observed significantly higher rates of local recurrence (LR) in young patients who underwent breast-conserving surgery (BCS) compared with older patients and those who underwent a mastectomy, even though overall survival did not differ[10-14]. Young age has been considered a justification for more aggressive surgical approaches to prevent LRs[10-14]. Until 2005, this was reflected in the Dutch Breast Cancer Guideline, which stated that young age (≤40 years) was an independent risk factor for LR after breast-conserving therapy[15]. During the last two decades, the occurrence of distant metastases(DM) has decreased[16,17] and the survival of patients with breast cancer has greatly improved. At the same time, LRR rates have also decreased[18]. Developments in systemic treatment, such as the introduction of trastuzumab[19,20], are considered to be the most important factor in both of these manifestations[10]. The evolution of radiotherapy techniques and regimes has also contributed to the decreasing rates of LR. Furthermore, the introduction of advanced computed tomography–based treatment planning has increased the precision of dose delivery considerably, leading to reduced toxicity[21].

The diminishing LRR rates in the overall population of patients with breast cancer and the acknowledgment of tumor biology and biomarker subtypes in relation to age has raised the question of whether the historically high risk of LRR has decreased in young women during a time in which systemic treatment has evolved, in particular, for the aggressive tumor types that frequently occur in young women. The aim of this study was to evaluate contemporary rates of LR and regional recurrence (RR) in young patients with breast cancer and the association with tumor biology as expressed by tumor biomarker subtypes.

Patients and methods

Patient data were selected from the population-based Netherlands Cancer Registry (NCR). The NCR contains data on patient and tumor characteristics and information regarding the applied treatment. On the basis of a notification from the Pathological Anatomy National Automated Archive, trained NCR personnel register the information directly from patients' medical records from all hospitals. The use of this data was approved by the NCR Committee of Privacy.

Patients selected were women between the ages of 20 and 35 years who were diagnosed with primary invasive, nonmetastatic breast cancer and underwent surgery between 2003 and 2008. Patients excluded from the study were those with a previous breast cancer diagnosis, with synchronous contralateral breast cancer, who received neoadjuvant treatment (n = 150), who lived or were treated outside the Netherlands, and those with incomplete follow-up data (eg, no information or missing event date). Only 43 hospitals (47%) provided follow-up data during 2007 and 2008 compared with all hospitals (n = 92) from 2003 to 2006.

Patient and tumor characteristics were collected from all patients. Tumor size and metastatic lymph node involvement were recorded according to the TNM system of the Union for International Cancer Control and the American Joint Committee on Cancer that was applicable at the time of diagnosis[22]. Estrogen receptor and progesterone receptor status were commonly available throughout the study period albeit fully available only from 2005. Standard assessment of human epidermal growth factor receptor 2 (HER2) status was implemented in the Netherlands in mid-2005.19 Biomarker subtypes were defined on the basis of HR and HER2 status and were categorized as HR-positive/HER2-negative, HR- positive/HER2-positive, HR-negative/HER2-positive, and HR-negative/ HER2-negative (triple negative [TN]).

Information was obtained regarding the type of surgery patients underwent—BCS or mastectomy—as defined by the last surgical procedure for the primary tumor. Positive tumor margins consisted of microscopic margin involvement after final surgery. Information regarding administered radiotherapy (yes or no), chemotherapy (yes or no), hormonal treatment (yes or no) and immunotherapy (yes or no) was obtained from the NCR, although detailed data on specific treatment regimens were not available. Five-year follow-up data for LR, RR, and DM, whichever occurred first, were collected for all patients in retrospect by NCR personnel. For all patients, vital status was ascertained through linkage with the municipal registry through to December 31, 2013.

Definitions of End Points

Follow-up commenced at the date of final surgery and ended with any type of recurrence (event), death (censored), or the date of last follow-up (censored). LR was defined as the occurrence of breast cancer or ductal carcinoma in situ in the ipsilateral breast or in the skin or subcutaneous tissue of the ipsilateral chest wall. RR consisted of breast cancer recurrence in the ipsilateral regional lymph nodes (eg, axillary, infra- or supra- clavicular or internal mammary nodes). DMs were used as end point to compare the previously reported downward trend in the occurrence of DM with the LRR end points.

Statistical Analyses

The distribution of clinicopathologic and treatment factors in the population of young patients with breast cancer was calculated and compared for the various biomarker subtypes by using $\chi 2$ tests. Subsequently, tumor characteristics in young patients were assessed over time. Time trends of different treatment modalities were evaluated by linear regression analyses.

Kaplan-Meier estimates were used to calculate univariate 5-year rates for LR and RR in the group of young patients with breast cancer. The trends of LR and RR and DM over time were evaluated by using linear regression analyses. DMs were included in this analysis to evaluate whether a similar trend could be observed between the occurrence of locoregional and distant breast cancer relapse over time. Subsequently, LR and RR rates were assessed according to biomarker subtypes for the entire study period and for the period that trastuzumab was reimbursed by insurers and routinely administered to patients (2005 to 2008). Within these groups, we assessed the association between the type of surgery and lymph node involvement with rates of LR and RR. Because the numbers of both LR and RR were low, reliable multivariable Cox proportional hazards regression analyses were not feasible. Therefore, all rates represent Kaplan-Meier estimates. The differences between groups were assessed by using log-rank tests.

STATA software version 13.1 (STATA, College Station, TX; Computing Resource Center, Santa Monica, CA) was used for all analyses. All statistical tests were two-sided, and $P < .05$ was considered statistically significant.

Table 1. Clinicopathological characteristics of surgically treated patients with primary breast cancer age <35 years diagnosed between January 1, 2003 and December 31, 2008 (n=1,000)

		No. of patients	%
Tumor histologic type*	Ductal	897	90
	Lobular	25	3
	Ductal and lobular	27	3
	Other*	51	5
pT	1A	36	4
	1B	87	9
	1C	379	38
	2	429	43
	3	47	5
	4	5	1
	X	17	2
pN	pN0	524	52
	pN1	311	31
	pN>1	162	16
	Unknown	3	0
Grade	Well differentiated	55	6
	Moderately differentiated	252	25
	Poorly differentiated	627	63
	Unknown	66	7
Multifocality	Yes	158	16
	No	667	67
	Unknown	175	18
HR status	Positive	489	49
	Negative	374	37
	Unknown	137	14
Biomarker subtype	HR-positive/HER2-negative	230	23
	HR-positive/HER2-positive	98	10
	HR-negative/HER2-positive	59	6
	TN	202	20
	Unknown†	411	41
Final surgery	Breast conserving	449	45
	Mastectomy	551	55
Positive tumor margins	Yes	36	4
	No	939	94
	Unknown	25	3
ALND	Yes	574	57
	No	426	43
Radiotherapy	Yes	629	63
	No	371	37
Chemotherapy	Yes	933	93
	No	67	7
Hormone therapy	Yes	480	48
	No	520	52
HER2 and trastuzumab	HER2-negative, no trastuzumab	452	45
	HER2-positive, no trastuzumab	35	4
	HER2-positive, trastuzumab	123	12
	Unknown	390	39

NOTE. Percentages may not add up to 100% as a result of rounding.

Abbreviations: ALND, axillary lymph node dissection; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; pN, pathological nodal status; pT, pathologic tumor size; TN, triple negative.

*Tumor histologic type other (eg, mucinous, medullary, metaplastic carcinoma).

†Unknown biomarker subtype category mainly reflects the earlier years when HER2 status was not determined.

Results

During the study period, 1,000 women with breast cancer, age < 35 years at the time of diagnosis, underwent surgery for primary breast cancer, constituting 2% of the total population of patients with breast cancer who underwent surgery in that period in the Netherlands (n = 52,310). The mean age of the group of young patients with breast cancer was 31 years (standard deviation ± 2.83 years). The youngest patient was 20 years old. Tumor and treatment characteristics of the group of young patients are presented in Table 1. Between 2003 and 2008, the distribution of most tumor characteristics did not vary significantly as tumor grade, HR status, and lymph node involvement remained stable (Appendix Table A1, online only).

There was a significant proportional shift of tumor size during the study period (P = .004): the proportion of T1c tumors increased, whereas the proportion of T2 tumors decreased (P ≤.001 using linear regression analyses). Overall, 95% of patients had early-stage breast cancer at pathology (stage I and II). The distribution of patients who underwent BCS remained stable over time. Axillary lymph node dissection was performed less often during the study period (from 69% in 2003 to 46% in 2008; P < .001). The proportion of patients receiving chemotherapy and hormonal therapy did not increase significantly over time (Fig 1). The proportion of patients receiving immunotherapy (trastuzumab) increased steeply after 2004 with the introduction of standard trastuzumab treatment in HER2-positive patients in 2005 (P < .001) and has been stable from 2006 (P = .346).

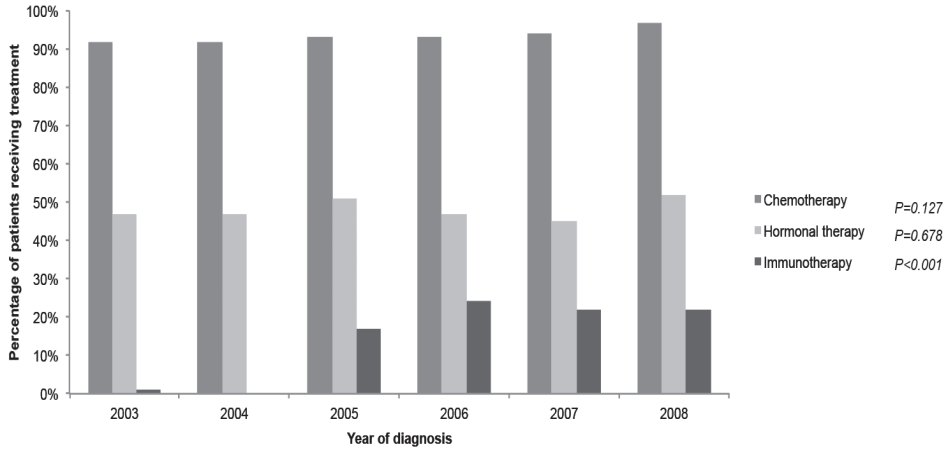

Figure 1. Time trends of systemic treatment modalities applied to 1,000 patients with breast cancer age < 35 years during the study period of 2003 to 2008. P values for time trends of different treatment modalities were evaluated by linear regression analyses. Immunotherapy constitutes treatment with trastuzumab.

Table 2. Distribution of the cliniopathologic characteristics of young patients with breast cancer age < 35 in relation to the various biomarker subtypes (n = 1,000)

	HR-Positive/ HER2- Negative		HR-Positive/ HER2- Positive		HR-Negative/ HER2- Positive		TN		Unknown		
	n= 230		n= 98		n= 59		n= 202		n= 411		
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	***P***
Tumor histologic type *											
Ductal	199	87%	91	93%	54	92%	178	88%	375	91%	*< .001*
Lobular	8	3%	2	2%	1	2%	1	1%	13	3%	
Ductal and lobular	17	7%	3	3%	2	3%	1	1%	4	1%	
Other	6	3%	2	2%	2	3%	22	11%	19	5%	
pT											
1A	13	6%	2	2%	5	8%	5	2%	11	3%	*.001*
1B	25	11%	8	8%	4	7%	15	7%	35	9%	
1C	115	50%	39	40%	20	34%	74	37%	131	32%	
2	66	29%	43	44%	25	42%	98	49%	197	48%	
3	9	4%	4	4%	3	5%	9	4%	22	5%	
4	1	0%	0	0%	0	0%	1	1%	3	1%	
X	1	0%	2	2%	2	3%	0	0%	12	3%	
pN											
pN0	125	54%	36	37%	19	32%	139	69%	205	50%	*< .001*
pN1	75	33%	41	42%	18	31%	38	19%	139	34%	
pN > 1	30	13%	21	21%	22	37%	25	12%	64	16%	
Unknown	-	-	-	-	-	-	-	-	3	1%	
Grade											
Well differentiated	25	11%	4	4%	2	3%	1	1%	23	6%	*< .001*
Moderately differentiated	101	44%	28	29%	14	24%	15	7%	94	23%	
Poorly differentiated	93	40%	64	64%	38	64%	174	86%	258	63%	
Unknown	11	5%	2	2%	5	8%	12	6%	36	9%	
Multifocality											
Yes	50	22%	22	22%	19	32%	22	11%	45	11%	*.001*
No	176	77%	71	72%	36	61%	174	86%	210	51%	
Unknown	4	2%	5	5%	4	7%	6	3%	156	38%	
Final surgery											
Breast conserving	107	47%	33	34%	18	31%	112	55%	179	44%	*.001*
Mastectomy	123	53%	65	66%	41	69%	90	45%	232	56%	
Positive tumor margins											
Yes	12	5%	1	1%	3	5%	7	3%	13	3%	*0.379*
No	213	93%	96	98%	55	93%	191	95%	384	93%	
Unknown	5	2%	1	1%	1	2%	4	2%	14	3%	
ALND											
Yes	130	57%	64	65%	47	80%	83	41%	250	61%	*< .001*
No	100	43%	34	35%	12	20%	119	59%	161	39%	
Radiotherapy											
Yes	143	62%	53	54%	34	58%	141	70%	258	63%	*.086*
No	87	38%	45	46%	25	42%	61	30%	153	37%	

Chemotherapy											
Yes	207	90%	97	99%	57	97%	198	98%	374	91%	< *.001*
No	23	10%	1	1%	2	3%	4	2%	37	9%	
Hormone therapy											
Yes	197	86%	83	85%	2	3%	4	2%	194	47%	< *.001*
No	33	14%	15	15%	57	97%	198	98%	217	53%	
Trastuzumab											
Yes	1	0%	80	82%	43	73%	1	1%	12	3%	< *.001*
No or unknown	229	100%	18	18%	16	27%	201	100%	399	97%	

NOTE. All data are given as No. of patients (%) unless otherwise noted. Percentages may not add up to 100% as a result of rounding. *P* values were assessed using $\chi 2$ test to compare the clinicopathologic characteristics with the various biomarker subtypes.

Abbreviations: ALND, axillary lymph node dissection; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; pN, pathological nodal status; pT, pathologic tumor size; TN, triple negative.

*Tumor histologic subtype other (eg, mucinous, medullary, metaplastic carcinoma).

Distributions of tumor characteristics by biomarker subtypes in the young age group are presented in Table 2. The unknown biomarker subtype category reflects the earlier years when HER2 status was not routinely determined; 96% of the patients in this unknown category were treated between 2003 and 2005. HR- positive/HER2-negative tumors were present in 23% of patients and were generally smaller and of a lower malignancy grade compared with other subtypes ($P < .001$). Patients with HER2- negative tumors presented less often with lymph node metastases than did patients with HER2-positive breast cancer. Mastectomy was performed more frequently in patients with HER2-positive tumors compared with patients with HER2-negative tumors (68% v 49%; $P < .001$). The proportion of patients receiving chemotherapy did not vary significantly between the different subtypes.

The overall 5-year rates for development of local and regional breast cancer recurrence were 3.5% and 3.7%, respectively (Table 3), and both rates showed a decreasing trend over time, although this was not significant. During the same period, the risk of DM decreased significantly in the entire cohort ($P = .040$). The 5-year LR rate in the unknown subtype group, mainly treated between 2003 and 2005, was 5.0% compared with 2.6% when the biomarker subtype was known ($P = .039$).

Table 3. Overall 5-Year local, regional, and distant recurrence rates over time in patients with breast cancer age < 35 years treated between 2003 and 2008 (n = 1,000)

		Local recurrence*		Regional recurrence†		Distant metastases†	
Year	No. of patients	No.	%	No.	%	No.	%
Overall	*1,000*	*31*	*3.5*	*33*	*3.7*	*131*	*13.9*
2003	213	8	4.2	11	6.1	36	17.8
2004	212	10	5.6	10	5.1	38	19.2
2005	182	3	2.0	5	3.1	25	14.6
2006	170	5	3.2	2	1.2	13	8.2
2007‡	117	2	2.1	1	0.9	9	8.1
2008‡	106	3	3.2	4	4.4	10	10.0

NOTE. Rates represent Kaplan-Meier estimates.

*Local recurrence (ipsilateral in-breast recurrence and new primary).

†$P < .05$ for trend in recurrence risk over time using linear regression analyses.

‡Only 43 of 92 hospitals were included in the years 2007 and 2008.

The risk of LR and RR varied with biomarker subtype. When the entire study period was considered, the differences between subtypes were borderline significant (P = .056 and P = .014 for LR and RR, respectively); however, when analyzed for the period after the introduction of trastuzumab (2005 to 2008), the observed differences leveled off (P = .24 and P = .42 for LR and RR, respectively; Figs. 2A and 2B). Patients with HR-negative/HER2- positive tumors displayed the highest rate of LR (5.6%), whereas patients with the TN subtype demonstrated the higher risk of RR (3.4%). Patients with HR-positive breast cancer had a 1% risk of LR, regardless of HER2 status, and similar low rates of RR were observed. In the 22 patients with HER2-positive status who were treated before 2005 and who did not receive trastuzumab, LRR was observed in four patients (18.2%; 1 LR and 3 RR). In the 136 patients with HER2-positive statues who were treated from 2005 onward, including the administration of trastuzumab, locoregional events were observed in five patients (3.3%; 3 LR and 2 RR).

The type of surgery did not influence the risk of LR. Overall, LR was 3.2% after BCS versus 3.8% after a mastectomy (P = .617). Lymph node involvement at the time of surgery increased the risk of RR in the total population (P = .035) as well as in all biomarker subtypes, albeit only significantly in the TN group (P = .04).

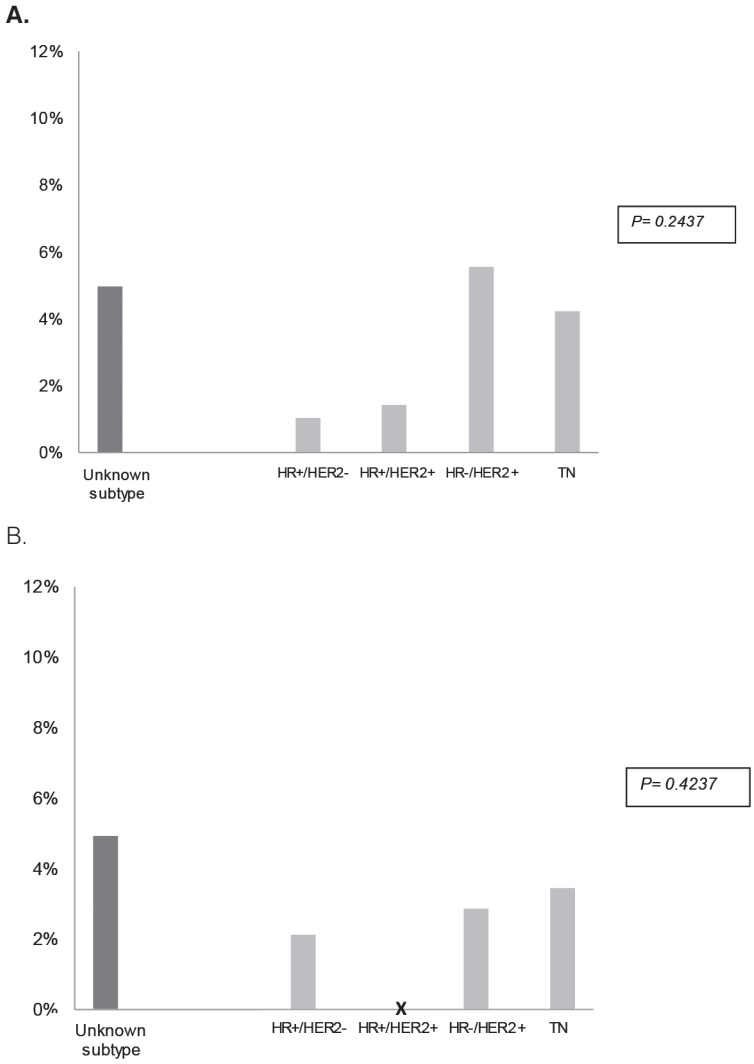

Figure 2. Five-year local recurrence (LR) and regional recurrence (RR) rates in 1,000 young patients with breast cancer (age < 35 years) surgically treated between 2003 and 2008 according to availability and kind of biomarker subtype on the basis of hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2) status. The rates for the different biomarker subtypes are displayed for the period of 2005 to 2008 of patients treated with trastuzumab for HER2-positive disease. (A) LR according to availability and kind of biomarker subtype. (B) RR according to availability and kind of biomarker subtype. Thirty-five HER2-positive patients who were treated before 2005 and/or who did not receive trastuzumab were excluded from analysis. Rates represent Kaplan-Meier estimates. P values for differences between subtypes used log-rank tests.

TN, triple negative; X, no patients had a RR.

Discussion

In the present population-based cohort study of young patients with breast cancer, we observed a decreasing trend in the rates of LR and RR. This improved outcome concurred with increased knowledge of tumor biology (ie, different biomarker subtypes) combined with developments in systemic treatment, such as the introduction of trastuzumab, improvements in diagnostic imaging, and radiotherapy techniques and schedules. Both the LR and RR rates varied with biomarker subtype. Although low rates of recurrence were observed in HR-positive tumors, regardless of HER2 status, these rates were higher in TN and HR-negative/ HER2-positive tumors.

The overall 5-year rates of developing LR and RR were 3.5% and 3.7%, respectively. These rates showed a decreasing trend over time, and are lower than previously reported. In patients with stage I and II breast cancer age $<$ 35 years treated between 1989 and 1996, a study by Bartelink et al[23] described a 5-year cumulative incidence of LR of approximately 9% after radical excision, followed by 50 Gy radiotherapy with a boost. A decade later, a study by Van der Sangen et al[10] reported a 5-year LR rate of 4.4% after mastectomy versus 8.3% after breast-conserving therapy in patients age $\leq$ 40 years with early-stage breast cancer who underwent surgery between 1988 and 2005. The data from the current study prove that the decreasing trend in the risk of locoregional breast cancer recurrence deducted from these previous studies continues, even when including higher stage disease.

Simultaneously, we observed a significant decrease in the occurrence of DM, also reported in previous studies[16,17], which is in line with reports that overall survival in patients with breast cancer has improved substantially in the last two decades[18,24].

Throughout the study period, more than 90% of patients received chemotherapy and more than 95% hormonal treatment in case of HR-positive disease. The proportion of patients who received chemotherapy increased only slightly during the study period; therefore, an increasing proportion of patients receiving systemic treatment in itself is not a likely explanation for the reduction of locoregional and distant recurrences observed in the current study. Improvements in systemic therapy and the use of targeted drugs, such as trastuzumab, may have played an important role. This is supported by the fact that rates of LR and RR were lower in patients with a known biomarker subtype compared with patients with an unknown subtype who mainly received treatment in earlier years when HER2 testing and treatment with trastuzumab was not routinely applied. Earlier studies from Kiess et al[25] and Lanning et al[26] also observed lower rates of LRR in patients treated with trastuzumab.

Previous studies have stated that young age should be considered an independent risk factor for poor prognosis in patients with breast cancer[27,28]; however, the results of this study demonstrate that young age itself does not imply an increased 5-year rate of LRR . In patients with HR-positive breast cancer, the overall 5-year LR rate was comparable to LR rates previously reported for older patients, regardless of HER2 status[29,30].

Biomarker subtype was a prognostic factor for both LR and RR, as has also been reported by others[31,32]. The rate of LR was highest in patients with HR-negative/HER2-positive tumors followed by patients with TN tumors, whereas TN tumors displayed the highest RR rate. In the current study, the unfavorable HR-negative/HER2-positive and TN subtypes constituted approximately one half of all cases in the young age category after HER2 typing became common practice. In HR- positive breast cancer, the LR and RR risks were $< 2\%$, whereas in the HR-negative subtypes, these rates were higher. The fact that the differences between subtype-specific recurrence rates decrease when taking into account only patients that were treated after trastuzumab was reimbursed emphasizes that some improvement has already been accomplished. The range of LRR rates within the population of young patients with breast cancer proves that generalization of this young group regarding treatment choice would be incorrect.

In the past, the high recurrence rates in young women have frequently led to the concept that mastectomy should be the preferred type of surgery in this patient population[14]. Older studies on LRR, in times that HER2 status was not addressed, showed striking differences in the risk of developing LR after BCS versus mastectomy in young patients with breast cancer[10] but with no influence on overall survival[33,34]. In the current study, the type of surgery did not significantly affect risk of LR and RR; this was true for the entire cohort and the biomarker subtype subsets of patients.

A 5-year follow-up period is possibly too short to draw firm conclusions on the basis of the presented recurrence rates, especially in these young women with breast cancer. Although patients with breast cancer experience relapse most frequently in the first 5 years after primary treatment, HR-positive tumors, in particular, can have a longer time to recurrence[35-38]. However, the LRR rates in this study were still substantially lower compared with previous reports[10,11]. Longer follow-up is required to demonstrate whether the decreasing trend of the current study will extend to the 10-year and even 20-year recurrence rates.

The major strengths of this study are the population-based design and large study population with complete 5-year follow-up data, making the results generally applicable. Furthermore, data were registered in the NCR by trained personnel using a standardized coding manual. The

definitions for LR and RR as used in this study are consistent with the definitions as established by consensus by Moossdorff et al[39].

This study also has important limitations. Fewer patients were included from 2007 and 2008 because some hospitals did not provide follow-up data for those years. These patients' data consisted of a heterogeneous group in which exclusion on the basis of clinicopathologic and recurrence data in previous years should not have affected the representativeness of the nationwide breast cancer population for the period of 2007 and 2008. Although we aimed to include all stages of nonmetastatic breast cancer for analysis, the vast majority of patients (95%) had early-stage breast cancer. Some selection resulted from the exclusion of patients treated with neoadjuvant chemotherapy as 50% of these patients had cT3 or cT4 tumors. The increased application of neoadjuvant treatment in smaller tumors could explain the observed proportional shift of tumor size over time. Data on biologic tumor factors, such as HR and, in particular, HER2 status, were limited before 2005 as they were not yet routinely assessed and central pathology review was not performed. In this study we stratified LRR rates according to biomarker subtype as defined by HR and HER2 expression. This classification, however, may not be as accurate as biomarker subtype classification on the basis of gene expression. In addition, information concerning specific radiotherapy and systemic therapy regimes, other than being administered or not, was not available. The administration of a boost dose could have played an important role in the reduction of LR. National guidelines at the time advised the routine administration of an additional radiotherapy boost to the primary tumor bed, and the Young Boost trial[40] was conducted in the Netherlands during the study period comparing a standard 16-Gy boost with a 26-Gy boost.
As a result of the observational study design, there is the possibility of confounding by indication, which remains unsolvable even after using multivariable analysis as there will always remain the possibility of unknown or unmeasured risk factors[41,42]. Because the number of recurrences was so low, multivariable analyses to correct for confounding and interaction were not feasible; results should be interpreted with this limitation in mind.

In conclusion, LRR rates in young patients with early-stage breast cancer decreased between 2003 and 2008, ending up relatively low. The higher recurrence rates in this young population were associated with the presence of more aggressive biomarker subtypes. Although longer follow-up is required, especially in these young women with breast cancer, the results of this study provide important insight into the LRR risks for this historically high-risk population.

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Appendix

Table A1. Tumor characteristics per incidence year for patients with breast cancer age < 35 years at the time of diagnosis (n = 1,000)

	2003		2004		2005		2006		2007*		2008*		
	n=213		n=212		n=182		n=170		n=117		n=106		***P***
Tumor histologic type†													
Ductal	193	91%	193	91%	157	86%	155	91%	104	89%	95	90%	*.278*
Lobular	8	4%	6	3%	5	3%	2	1%	3	3%	1	1%	
Ductal and lobular	4	2%	1	-	11	6%	4	2%	4	3%	3	3%	
Other	8	4%	12	6%	9	5%	9	5%	6	5%	7	7%	
pT													
1A	2	1%	8	4%	9	5%	8	5%	3	3%	6	6%	*.004*
1B	14	7%	16	8%	20	11%	16	9%	11	9%	10	9%	
1C	60	28%	75	35%	75	41%	67	39%	57	49%	45	42%	
2	113	53%	95	45%	68	37%	71	42%	42	36%	40	38%	
3	12	6%	12	6%	8	4%	7	4%	3	3%	5	5%	
4	4	2%	0	-	1	1%	0	-	0	-	0	-	
X	8	4%	6	3%	1	1%	1	1%	1	1%	0	-	
pN													
pN0	99	46%	103	49%	105	58%	89	52%	68	58%	60	57%	*.443*
pN1	71	33%	74	35%	47	26%	56	33%	33	28%	30	28%	
pN >1	42	20%	33	16%	30	16%	25	15%	16	14%	16	15%	
Unknown	1	0%	2	1%	0	-	0	-	0	-	0	-	
Grade													
Well differentiated	10	5%	10	5%	11	6%	12	7%	8	7%	4	4%	*.564*
Moderately differentiated	51	24%	48	23%	54	30%	37	22%	36	31%	26	25%	
Poorly differentiated	127	60%	143	67%	103	57%	111	65%	70	60%	73	69%	
Unknown	25	12%	11	5%	14	8%	10	6%	3	3%	3	3%	
Multifocality													
Yes	14	7%	30	14%	38	21%	26	15%	24	21%	26	25%	*.157*
No	99	46%	122	58%	135	74%	141	83%	91	78%	79	75%	
Unknown	100	47%	60	28%	9	5%	3	2%	2	2%	1	1%	
HR status													
Positive	76	36%	91	43%	110	60%	92	54%	61	52%	59	56%	*.776*
Negative	55	26%	68	32%	72	40%	77	45%	56	48%	46	43%	
Unknown	82	39%	53	25%	-	-	1	1%	-	-	1	1%	
Biomarker subtype													
HR-positive/HER2-negative	14	7%	0	-	67	37%	61	36%	45	38%	43	41%	*<.001*
HR-positive/HER2-positive	13	6%	0	-	29	16%	26	15%	15	13%	15	14%	*(2005-2008, .279)*
HR-negative/HER2-positive	9	4%	0	-	13	7%	15	9%	14	12%	8	8%	
TN	8	4%	2	1%	56	31%	57	34%	41	35%	38	36%	
Unknown	169	79%	210	99%	17	9%	11	6%	2	2%	2	25%	

NOTE. Data are given as No. (%) unless otherwise noted. Percentages may not add up to 100% as a result of rounding.
Abbreviations: HER2, human epidermal growth factor receptor 2; HR, hormone receptor; pN, pathological nodal status; pT, pathologic tumor size; TN, triple negative.
*Fewer patients were included in the years 2007 and 2008 compared with earlier years because some hospitals did not provide data for those years.
†Morphology other (eg, mucinous, medullary, metaplastic carcinoma).

CHAPTER 4

Young age and the risk of breast cancer recurrence as assessed by the 70-gene signature – an analysis from the EORTC 10041/ BIG 03-04 MINDACT trial

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Submitted

Abstract

Purpose

We aimed to evaluate the risk of breast cancer (BC) relapse according to the 70-gene signature (70-GS) result in relation to young age in early-stage BC patients enrolled in the MINDACT trial.

Patients and Methods

Patients enrolled in the MINDACT trial with available clinical (c), as per a modified version of Adjuvant!Online, and genomic (g), according to the 70-GS, risk assessments were categorized in three age groups; <45, 45-55 and >55 years. Clinicopathologic and genomic characteristics were compared for the different age groups and further split by corrected risk categories (cL/gL, cL/gH, cH/gL, cH/gH). Subsequently, the 5-year distant metastasis-free survival according to risk category was calculated.

Results

The study evaluated 1100 patients <45 (16%), 2272 aged 45-55 (34%) and 3321 patients >55 years of age (50%). The young age group had a higher frequency of lymph node involvement (25% vs. 22% and 19%), poorly differentiated tumors (42% vs. 26% and 27%), ER-negative tumors (20% vs. 11% and 11%) and triple negative immunohistochemistry subtype (16% vs. 9% and 8%). 61% of young patients were cH while the 70-GS assessed 48% gH. Overall, 31% were cL/gL (vs. 43% in other age groups), 9% cL/gH, 21% cH/gL and 40% cH/gH (vs. 24% and 25%). The 5-year DMFS was 94.1% (95% CI 92.4-95.4) in <45 age group, 95.3% (95% CI 94.2-96.1) in 45-55 and 94.9% (95% CI 94.0-95.6) in >55. For the young patients, 5-year DMFS was 98.3% for the cL/gL (96.0-99.3), 97.4% in cL/gH (90.0-99.4), 95.5% in cH/gL (91.6-97.7) and 89.2% in cH/gH (85.6-92.0). In the older two age groups, the 5-year DMFS rates were 97.8% (96.5.98.6) and 97.2% (96.2-98.0) for cL/gL, 93.9% (88.8-96.7) and 94.5% (91.0-96.7) for cL/gH, 94.5% (92.0-96.3) and 95.4% (93.5-96.8) for cH/gL and 92.0% (89.2-94.1) and 90.4% (88.0-92.4) for cH/gH, respectively. Numbers were too small to evaluate chemotherapy effect.

Conclusion

The use of the 70-GS reduces the proportion of patients characterized as high risk as compared to traditional clinical risk assessment (48% vs. 61%). Outcome was comparable for the age categories when patients were stratified for the clinical and genomic risk profiles.

Introduction

Although breast cancer incidence rises with age and median age at diagnosis is 62, breast cancer also remains the most common type of cancer in young women[1–3]. Approximately 10% of breast cancers are diagnosed before the age of 45 in developed countries[1–3]. Breast cancer in young women poses a therapeutic challenge, due to a generally higher recurrence and mortality rate[4, 5], and on the other hand treatment toxicity influencing fertility, pregnancy and overall quality of life[6, 7].

Young women are more likely to be diagnosed with breast cancers that possess more aggressive features as compared to their older counterparts, such as a higher frequency of lymph node involvement, high proliferation and poor differentiation rates, hormone receptor negative status, HER2 overexpression and a triple negative molecular subtype[4, 5, 8–10]. Hereditary predisposition and germline mutations such as BRCA1 and BRCA2 mutations are more often detected in the young population[5, 11–13]. Previous studies have suggested that young age itself is a poor prognostic factor[14–17]. Therefore, young women historically had a higher risk of being overtreated, both locally and systemic, as compared to older breast cancer patients presenting with similar disease characteristics purely based on age considerations.

Not all young women are at a high risk of disease recurrence[18]. Treatment decisions in breast cancer patients should be guided by the stage and biology of the disease, while bearing in mind a patients' age and other patient factors[19]. The development of gene expression signatures, such as the 21-gene and 70-gene signatures, allows further characterization of the tumor biology and improved prognostication of a patient with breast cancer[20–22]. The prospective MINDACT study showed that the 70-gene signature/MammaPrint® (70-GS) could accurately differentiate between patients with a low and high risk of distant metastases and death at 5 years, thereby providing valuable information for determining the potential benefit of adjuvant chemotherapy[23]. Currently, there is limited data available on the molecular landscape of breast cancer in young women[19].

The aim of this study was to evaluate the risk of breast cancer (BC) relapse according to the 70-gene signature (70-GS) result in relation to age, with the focus on young women aged <45 years, in early-stage BC patients enrolled in the MINDACT trial[23].

Methods

Study population

The EORTC 10041/BIG 03-04 MINDACT trial[23] enrolled patients with early-stage (cT1-2 or operable T3) and 0-3 lymph node positive breast cancer. For all patients, their risk of distant disease recurrence was assessed by using the 70-GS (genomic(g)) and per a modified version of Adjuvant!Online (clinical(c))[24]. The limit for the definition of clinical low-risk (cL) was pre-specified in the protocol as a 10-year breast cancer survival probability of >88% for ER+ disease without systemic therapy, and >92% for ER- breast cancer accounting for an average 4% absolute benefit of adjuvant endocrine treatment in ER+ disease[23]. Patients with cH/gH risk assessment received adjuvant chemotherapy (CT), while those with a cL/gL risk profile did not. Patients with discordant results for the two risk assessments were randomized to follow either the genomic or clinical risk for the decision regarding chemotherapy administration. Details on axillary surgery and applied radiotherapy schedules were not available.

This additional analysis evaluated all 6693 patients included in the MINDACT trial. Patients were divided in three age groups: <45 (young), 45-55 (peri-menopausal) and >55 (post-menopausal). Two additional exploratory subgroups of the young population were defined as women aged ≤35 and <40 years of age.

Objectives and end-points

The primary objective of this analysis was to compare the 5-year distant metastasis-free survival (DMFS) rate according to the four C/G combined risk groups (cL/gL, cL/gH, cH/gL, cH/gH). Secondary objectives included 1) describing the clinicopathological, genomic and treatment characteristics of the different age groups, 2) describing the compliance to protocol treatment for the different age and risk groups, 3) to estimate the 5-year rates of DMFS, distant-metastasis-free interval (DMFI), disease-free survival (DFS) and overall survival (OS) for the different age groups both overall and according to the assessed risk groups, and 4) comparing the 5-year outcome (DMFS, DMFI, DFS and OS) between the different age categories according to treatment randomized following either the clinical or genomic risk assessment for the discordant risk groups.

The primary endpoint DMFS was defined as the time until first distant metastatic recurrence or death from any cause, whichever occurred first. DMFI was defined as the time until distant metastasis or death due to disease progression or treatment toxicity. DFS was defined as the time until first disease progression (locoregional, distant relapse, ipsilateral or contralateral invasive breast cancer, ductal carcinoma in situ, or an invasive second primary cancer) or death from any cause. Finally, OS was defined as the time until death from any cause. Patients without

an event for the respective endpoints at cut-off date were censored at the date of last disease assessment.

Statistical analysis

The distribution of baseline clinicopathologic, genomic and treatment characteristics were compared by age category using descriptive statistics. Baseline characteristics within the young group (<45) were assessed using the alternative age cut-offs of ≤35 and <40 years to evaluate any differences. Compliance to protocol management was reviewed and described.

The 5-year DMFS, DMFI, DFS and OS rates were estimated with the non-parametric Kaplan Meier method by age group and further split by risk category. The 95% CIs were calculated based on asymptotic normality of log- log transformed survival estimates.
Analyses were only performed when the number of patients included was deemed sufficient for interpretation of any results. Interpretation of the results is based on the 95% confidence intervals; no formal statistical testing was performed. All analyses were carried out using SAS software, version 9.4 (SAS Institute).

Results

Patient population

Of the 6693 patients enrolled in the MINDACT trial, 1100 patients were <45 (16%) years of age, 2272 were aged 45-55 (34%) and 3321 patients >55 years of age (50%). Baseline clinicopathological characteristics of patients according to age group are presented in Table 1. The clinicopathologic, risk and treatment characteristics of the two additional exploratory subgroups of the young population (≤35 and <40 years of age) are displayed in Supplementary Table 1. There were 122 (1.8% of total) patients aged ≤35 and 416 patients (6.2%) <40 years of age.

Median age in the young (<45) age group was 41 (range 23-45). The young group had a higher frequency of lymph node involvement (25% vs. 22% and 19%), poor differentiation grade (42% vs. 26% and 27%), ER-negative tumors (20% vs. 11% and 11%) and triple negative immunohistochemistry subtype (16% vs. 9% and 8%) as compared to patients aged 45-55 and >55, respectively. Median tumor size was the same for the 3 age groups (17mm).

Table 1. Baseline patient, tumor (by local assessment) and treatment characteristics of included patients according to age category

	<45	45-55	>55	Total
	N (%)	N (%)	N (%)	N (%)
Age (median with range)	41 (23-45)	50 (45-55)	62 (55-71)	55 (23-71)
TUMOUR CHARACTERISTICS				
Histology				
Ductal	973 (88.5)	1885 (83.0)	2675 (80.5)	5533 (82.7)
Lobular	62 (5.6)	242 (10.7)	430 (12.9)	734 (11.0)
Mixed	30 (2.7)	71 (3.1)	107 (3.2)	208 (3.1)
Other	35 (3.2)	72 (3.2)	105 (3.2)	212 (3.2)
Missing	0 (0.0)	2 (0.1)	4 (0.1)	6 (0.1)
Tumor grade				
Well differentiated	173 (15.7)	548 (24.1)	726 (21.9)	1447 (21.6)
Moderately differentiated	457 (41.5)	1135 (50.0)	1695 (51.0)	3287 (49.1)
Poorly differentiated or undifferentiated	462 (42.0)	582 (25.6)	883 (26.6)	1927 (28.8)
Undefined	8 (0.7)	7 (0.3)	17 (0.5)	32 (0.5)
Pathological tumor size				
<= 1 cm	136 (12.4)	296 (13.0)	488 (14.7)	920 (13.7)
1 cm < . <= 2 cm	622 (56.5)	1315 (57.9)	1938 (58.4)	3875 (57.9)
2 cm < . <= 5 cm	321 (29.2)	630 (27.7)	868 (26.1)	1819 (27.2)
>5 cm	21 (1.9)	30 (1.3)	27 (0.8)	78 (1.2)
Missing	0 (0.0)	1 (0.0)	0 (0.0)	1 (0.0)
Lymph node status				
Node negative	831 (75.5)	1768 (77.8)	2689 (81.0)	5288 (79.0)
1 positive LN	185 (16.8)	329 (14.5)	428 (12.9)	942 (14.1)
2 positive LN	47 (4.3)	116 (5.1)	137 (4.1)	300 (4.5)
≥3 positive LNs	37 (3.4)	59 (2.6)	66 (2.0)	162 (2.4)
Missing	0 (0.0)	0 (0.0)	1 (0.0)	1 (0.0)
ER status				
Negative	216 (19.6)	247 (10.9)	361 (10.9)	824 (12.3)
Positive	884 (80.4)	2025 (89.1)	2960 (89.1)	5869 (87.7
PgR status				
Negative	275 (25.0)	417 (18.4)	855 (25.7)	1547 (23.1)
Positive	817 (74.3)	1839 (80.9)	2442 (73.5)	5098 (76.2)
Missing	8 (0.7)	16 (0.7)	24 (0.7)	48 (0.7)
HER2 status				
HER2 negative	972 (88.4)	2063 (90.8)	3008 (90.6)	6043 (90.3)
HER2 positive	126 (11.5)	206 (9.1)	306 (9.2)	638 (9.5)
Missing	2 (0.2)	3 (0,1)	7 (0.2)	12 (0.2)
Biomarker subtype				
HR+/HER2-	796 (72.4)	1869 (82.3)	2737 (82.4)	5402 (80.7)
HR+/HER2+	105 (9.5)	168 (7.4)	228 (6.9)	501 (7.5)
HR-/HER2+	21 (1.9)	38 (1.7)	78 (2.3)	137 (2.0)
HR-/HER2- (TN)	176 (16.0)	194 (8.5)	270 (8.1)	640 (9.6)
Missing	2 (0.2)	3 (0.1)	8 (0.2)	13 (0.2)

CLINICAL AND GENOMIC RISK					
Clinical risk (c)					
	Low risk	435 (39.5)	1171 (51.5)	1730 (52.1)	3336 (49.8)
	High	665 (60.5)	1101 (48.5)	1590 (47.9)	3356 (50.1)
	Missing[a]	0 (0.0)	0 (0.0)	1 (0.0)	1 (0.0)
Genomic risk (g)					
	Low risk	571 (51.9)	1531 (67.4)	2192 (66.0)	4294 (64.2)
	High	529 (48.1)	741 (32.6)	1128 (34.0)	2398 (35.8)
	Missing[a]	0 (0.0)	0 (0.0)	1 (0.0)	1 (0.0)
Risk group[b]					
	cL/gL	341 (31.0)	982 (43.2)	1421 (42.8)	2744 (41.0)
	cL/gH	94 (8.5)	189 (8.3)	309 (9.3)	592 (8.8)
	cH/gL	230 (20.9)	549 (24.2)	771 (23.2)	1550 (23.2)
	cH/gH	435 (39.5)	552 (24.3)	819 (24.7)	1806 (27.0)
	Missing	0 (0.0)	0 (0.0)	1 (0.0)	1 (0.0)
TREATMENT CHARACTERISTICS					
Type of surgery					
	Breast conserving surgery (BCS)	823 (74.8)	1829 (80.5)	2818 (84.9)	5470 (81.7)
	Mastectomy	277 (25.2)	443 (19.5)	503 (15.1)	1223 (18.3)
Radiotherapy					
	No	177 (16.1)	316 (13.9)	384 (11.6)	877 (13.1)
	Yes	903 (82.1)	1912 (84.2)	2893 (87.1)	5708 (85.3)
	Missing	20 (1.8)	44 (1.9)	44 (1.3)	108 (1.6)
Chemotherapy					
	No	435 (39.5)	1328 (58.5)	2075 (62.5)	3838 (57.3)
	Yes	657 (59.7)	934 (41.1)	1229 (37.0)	2820 (42.1)
	Missing	8 (0.7)	10 (0.4)	17 (0.5)	35 (0.5)
Endocrine treatment					
	No	263 (23.9)	459 (20.2)	677 (20.4)	1399 (20.9)
	Yes	818 (74.4)	1772 (78.0)	2584 (77.8)	5174 (77.3)
	Missing	19 (1.7)	41 (1.8)	60 (1.8)	120 (1.8)
Trastuzumab					
	No	989 (89.9)	2082 (91.6)	3066 (92.3)	6137 (91.7)
	Yes	93 (8.5)	147 (6.5)	187 (5.6)	427 (6.4)
	Missing	18 (1.6)	43 (1.9)	68 (2.0)	129 (1.9)

ER= estrogen receptor, PgR= progesterone receptor, HER2= Human epidermal growth factor receptor 2

[a]One patient had an unknown genomic risk due to sample error and was classified as cL/gL in subsequent analyses

[b]Risk categories: cL/gL (clinical and genomic low risk), cL/gH (clinical low/genomic high risk), cH/gL (clinical high/genomic low risk), cH/gH (clinical and genomic high risk)

The proportion of patients that underwent a mastectomy was highest in the young age group and lowest in patients >55 years of age; 25% in the <45 category, 20% in patients aged 45-55 and 15% in the >55 age group. Sixty percent (60%) of patients in the young age category received chemotherapy as compared to 41% and 37% in the older age categories, respectively. The proportion of patients receiving endocrine treatment was more or less the same across the three age groups (74% vs. 78% vs. 78%, respectively).

In the discordant risk groups, chemotherapy (CT) administration when randomized to no chemo occurred in 5% of young women as compared to 3% and 1% in the older age groups (Table 2). Reason for non-compliance was balanced between patient refusal and physician decision.

Table 2. Reasons for non-compliance when no CT was advised but CT was given per age category, in total patient population (n=6693)

	Age <45 N=57 (5%)	**Age 45-55 N=69 (3%)**	**Age >55 N=30 (1%)**	**Total N=156 (2%)**
Patient refusal	26 (45.6)	38 (55.1)	14 (46.7)	78 (50.0)
PI decision	25 (43.9)	22 (31.9)	11 (36.7)	58 (37.2)
Missing	5 (8.8)	7 (10.1)	3 (10.0)	15 (9.6)
Patient refusal + PI decision	1 (1.8)	2 (2.9)	2 (6.7)	5 (3.2)

PI= Principle Investigator

Risk assessment

The 70-gene signature classified 48% of young breast cancer patients at high risk (gH), while traditional clinicopathologic risk assessment by a modified Adjuvant!Online categorized 61% of young patients as high risk (cH), Table 1. Overall in the <45 age category, 31% were cL/gL, 9% cL/gH, 21% cH/gL and 40% cH/gH.

There was a higher prevalence of poor prognostic factors such as having tumors that were poorly differentiated, hormone receptor negative, HER2 positive and/or of a triple negative subtype in the group of young women with a high risk genomic signature (Supplementary Table 2).

There was a greater proportion of patients with a cH/gH risk profile in patients ≤35 (53.3%) and <40 (47.6%) as compared to when applying <45 as cut-off for categorizing young women (39.5%, Supplementary Table 1).

Distant metastasis-free survival (DMFS)

The 5-year DMFS was 94.1% (95% CI 92.4-95.4) in <45 age group, 95.3% (95% CI 94.2-96.1) in 45-55 and 94.9% (95% CI 94.0-95.6) in >55 (Figure 1).

Figure 2 shows the DMFS rates according to the different risk categories for the different risk groups. The 5-year DMFS rate for patients at cL/gL was 98.3% (95% CI 96.0-99.3) for the young patient group, 97.8% (95% CI 96.5.98.6) for patients aged 45-55 and 97.2% (95% CI 96.2-98.0) in patients >55 years of age. For patients at cL/gH risk, 5-year DMFS was 97.4% in CL/GH (95% CI 90.0-99.4) in the young group versus 94.5% (95% CI 92.0-96.3) and 95.4% (95% CI 93.5-96.8) in the older age groups. A cH/gL risk profile resulted in a 95.5% in CH/GL (95% CI 91.6-97.7) in the young, 94.5% (95% CI 92.0-96.3) for the middle and 95.4% (95% CI 93.5-96.8) for the oldest patient category. Finally, the 5-year rates with a cH/gH risk assessment were 89.2% in CH/GH (95% CI 85.6-92.0), 92.0% (95% CI 89.2-94.1) and 90.4% (95% CI 88.0-92.4) for the different age categories reported in increasing order.

Numbers were too small to evaluate chemotherapy effect. In the cH/gL risk category of young women, there were 3 DMFS events in the chemotherapy group versus 6 in the group that did not receive chemotherapy.

DMFS for patients ≤35 and <40 years of age are shown in Supplementary figure 1.

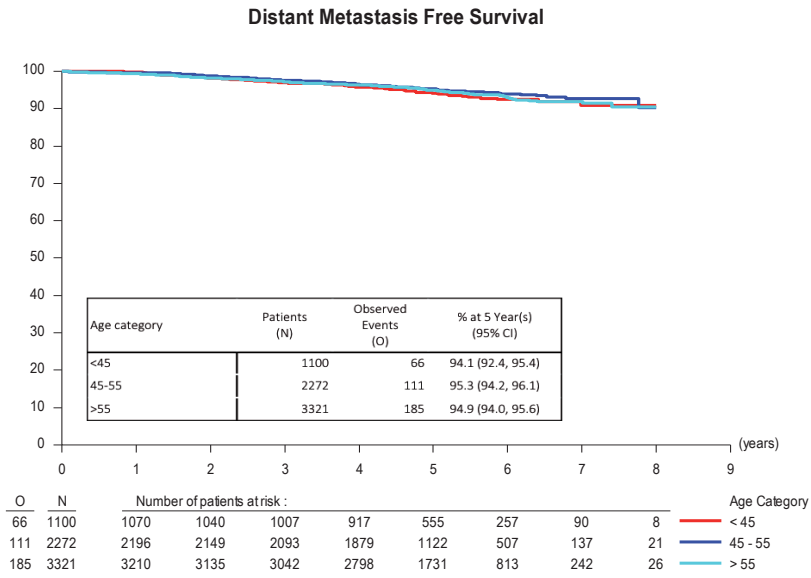

Figure 1. Distant metastasis-free survival according to age category

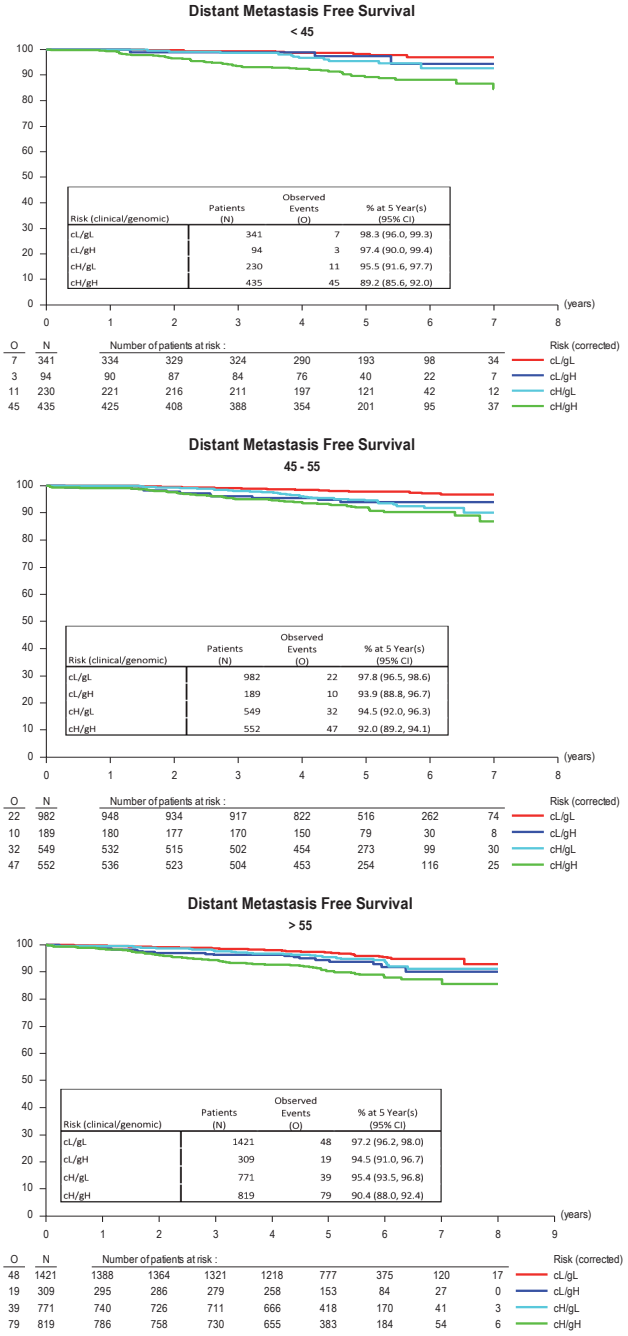

Figure 2. Distant metastasis-free survival according to risk category by age group (<45, 45-55, >55 years of age)

Table 3. Distant metastasis-free interval (DMFI), disease-free survival (DFS) and overall survival (OS) for the three age groups (<45, 45-55 and >55) further split by risk category (clinical/genomic)

	Distant metastasis-free interval (DMFI)		Disease-free survival (DFS)		Overall survival (OS)	
	Number of events	Rate (95% CI)	Number of events	Rate (95% CI)	Number of events	Rate (95% CI)
<45 (n=1100)	**62**	**94.5 (92.9, 95.8)**	**114**	**90.0 (87.9, 91.7)**	**31**	**97.2 (96.0, 98.1)**
cL/gL	7	98.3 (96.0, 99.3)	23	94.2 (90.9, 96.3)	0	(,)
cL/gH	3	97.4 (90.0, 99.4)	8	91.7 (81.7, 96.3)	1	98.9 (92.1, 99.8)
cH/gL	9	96.6 (92.9, 98.4)	19	92.0 (87.4, 94.9)	5	98.5 (95.4, 99.5)
cH/gH	43	89.9 (86.3, 92.5)	64	85.1 (81.0, 88.3)	25	94.0 (91.1, 95.9)
45-55 (n=2272)	**91**	**95.9 (95.0, 96.7)**	**202**	**91.2 (89.8, 92.4)**	**59**	**97.7 (96.9, 98.3)**
cL/gL	16	98.2 (97.0, 98.9)	66	93.4 (91.5, 94.9)	9	99.2 (98.2, 99.6)
cL/gH	7	95.5 (90.6, 97.9)	18	89.7 (83.8, 93.5)	5	97.8 (94.2, 99.2)
cH/gL	27	95.4 (93.1, 97.0)	48	91.2 (88.2, 93.4)	13	97.6 (95.6, 98.7)
cH/gH	41	92.6 (89.9, 94.6)	70	87.7 (84.4, 90.4)	32	95.3 (93.0, 96.9)
>55 (n=3321)	**138**	**96.1 (95.4, 96.8)**	**356**	**89.7 (88.5, 90.7)**	**118**	**96.7 (96.0, 97.3)**
cL/gL	25	98.7 (97.8, 99.2)	122	92.0 (90.4, 93.4)	38	97.6 (96.5, 98.3)
cL/gH	12	96.3 (93.2, 98.0)	32	90.2 (86.0, 93.2)	13	96.4 (93.5, 98.1)
cH/gL	33	96.3 (94.5, 97.5)	70	91.4 (88.9, 93.3)	21	97.4 (95.8, 98.3)
cH/gH	68	91.5 (89.1, 93.3)	132	83.7 (80.7, 86.3)	46	94.6 (92.6, 96.0)

cL/gL=clinical and genomic low risk, cL/gH=clinical low/genomic high risk, cH/gL=clinical high/genomic low risk, cH/gH=clinical and genomic high risk

Secondary outcomes: Distant metastasis-free interval (DMFI), disease-free survival (DFS) and overall survival (OS)

The other outcome measures were comparable for the different age groups and varied by risk category (clinical/genomic) as well (Table 3). The 5-year DMFI was 94.5% (95% CI 92.9-95.8) versus 95.9% (95% CI 95.0-96.7) versus 96.1% (95% CI 95.4-96.8) for patients aged <45, 45-55

and >55, respectively. The rates of DFS at 5 years were 90.0% (95% CI 87.9-91.7) in patients <45, 91.2% (95% CI 89.8-92.4) in patients aged 45-55 and 89.7% (95% CI 88.5-90.7). Finally, 5-year OS was 97.2% (95% CI 96.0-98.1), 97.7 (95% CI 96.9-98.3) and 96.7% (96.0-97.3), respectively.

Discussion

The results of this study show that although the frequencies of poor disease characteristics and a high-risk genomic signature were higher in the young (<45 years of age) patient category as compared to the two older age groups (aged 45-55 and >55), the 5-year DMFS was very good and comparable for the different age groups, both overall and for the different risk categories. Young breast cancer patients had a greater proportion of aggressive biological characteristics such as advanced disease, hormone receptor negative status, HER2 overexpression and a triple negative immunohistochemistry subtype. Not surprisingly, the percentage of patients categorized as being at high clinical risk was notably higher as compared to in the older age categories (61% versus 49% and 48%), reflected by the fact that 60% of young patients received chemotherapy. This is in support of other publications stating breast cancer diagnosed in young women has a more aggressive tumor biology[4,5,8–10].

The use of the 70-GS reduced the proportion of patients characterized as high-risk as compared to traditional clinical risk assessment in the young breast cancer patients from 61% to 48%; 35% of patients classified as cH had a low risk 70-GS result. There were more young patients classified as genomic high risk as compared to in the older age categories. This proportion increased when lowering the age cut-off. Nevertheless, more than half of patients (52%) aged <45 were classified at low genomic risk. This could imply that there is a window to reduce the extent of treatment in this patient group. Young women with breast cancer have a higher risk of being overtreated, although so far there is no evidence on whether more aggressive treatment has any impact on their survival[19].

The 5-year DMFS rate was very good and comparable for the different age groups, 94.1% (95% CI 92.4-95.4) in <45 age group, 95.3% (95% CI 94.2-96.1) in 45-55 and 94.9% (95% CI 94.0-95.6) in >55. The 5-year DMFI, DFS and OS were comparable across the three age groups as well. OS was 97.2% (95% CI 96.0-98.1), 97.7 (95% CI 96.9-98.3) and 96.7% (96.0-97.3), respectively. These results exceed those from previous reports and refute earlier publications claiming that age is an independent risk factor for breast cancer recurrence and mortality[5,14–17].

Outcome varied according to the four different risk categories to a similar extent for the different age groups. The 5-year DMFS rate the <45 age group was 98.3% (95% CI 96.0-99.3) in patients at cL/gL risk versus 89.2% in CH/GH (95% CI 85.6-92.0). There is no overlap in the confidence intervals, indicating that not all women are at high risk of recurrence. Furthermore, this suggests that the 70-GS is likely a reliable prognosticator in the young population as well. Unfortunately, numbers were too small to evaluate chemotherapy benefit in the discordant groups of young women. In the first report of the OncotypeDX trial on the HR+/HER2-, node-negative (N0) patients with a favorable 21-gene expression profile that received endocrine treatment, only 4%

of patients were ≤40 years of age[22]. Exploratory analyses were performed using ≤50 as the rather high cut-off for young age in this low-risk analysis as well as in the recently presented data from the intermediate arm that demonstrated no benefit of chemotherapy in addition to endocrine treatment for HR+/HER2-, N0 patients with an intermediate 21-gene assay result. In the age-analysis in the low-risk setting there was no difference, while in the intermediate risk group there was some benefit of chemotherapy in patients aged ≤50[25]. However, there are no details available yet on the further characteristics of these patients.

Non-compliance to allocated treatment when no chemotherapy was advised was very low (5%), although somewhat higher as compared to in the older age groups (3% and 1%, respectively). This would make it unlikely that the observed very good prognosis in our subset of young women would be based on over-treatment with chemotherapy, but rather that treatment allocation by risk assessment according to the MINDACT trial, designed to evaluate potential to de-escalate treatment by adding the 70-gene signature to the criteria for chemotherapy decision-making, was successful.

Chemotherapy has been shown to reduce the 10-year breast cancer mortality rate by at least 30%[26,27]. In patients at low risk for disease recurrence the absolute benefit will be much smaller. A large meta-analysis performed by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) evaluating proportional risk reductions from taxane-based or anthracycline-based chemotherapy regimens demonstrated age did not influence their effect[27]. Especially in young women, it is important to identify women in whom treatment may be scaled down to reduce long-term toxicity without impairing their outcome. Performing the 70-GS signature provides additional information concerning the prognosis for young early-stage BC patients categorized as clinical high risk. The results or our study add important new data to the limited available evidence on genomic expression in young BC patients[5,28]. More research investigating molecular landscape, for example using next-generation sequencing, and options to de-escalate treatment in this group[29].

For this substudy, we applied age 45 as cut-off age to define young women. This is a number applied in other reports as well[2,30], although there is also literature using 40 or even 35 to define the group of young women[4,14,18,30–33]. There is no strict definition to define this subgroup, but age 40 appears to be the most described[4,14,28,31,34,35]. The choice for the cut-off age of 45 was also based on the ability to have sufficient numbers for the analysis. We performed additional exploratory analysis within the subgroup of young women using ≤35 and <40 years of age to evaluate whether there were large differences regarding disease characteristics. We found that the prevalence of some features of aggressive tumor biology increased when lowering the age limit, specifically the proportion of poorly differentiated tumors, hormone receptor negative tumors and those of triple negative subtype. Patient number in the lower age groups were very small, but exploratory analyses demonstrate more or less comparable outcome for the different risk categories in the <40 population when compared to the <45 group which in turn proved comparable to the older age groups. However, due to the numbers this should be interpreted with caution.

In summary, the proportion of poor disease characteristics is higher in young breast cancer patients as compared to in older patients. The use of the 70-GS reduces the proportion of patients characterized as high risk as compared to traditional clinical risk assessment (48% vs. 61%). Outcome according to risk groups was comparable and very good among all three age categories. This study underscores that biology and not age determines the risk of breast cancer recurrence.

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Supplementary table 1. Patient, tumor (by local assessment), risk and treatment characteristics for young breast cancer patients using different age cut-offs (≤35, <40 and <45)

	≤35 (n=122)	<40 (n=416)	<45 (n=1100)
	N (%)	N (%)	N (%)
Age (median with range)	32 (23-35)	37 (23-40)	41 (23-45)
TUMOR CHARACTERISTICS			
Histology			
Ductal	113 (92.6)	382 (91.8)	973 (88.5)
Lobular	1 (0.8)	9 (2.2)	62 (5.6)
Mixed	1 (0.8)	10 (2.4)	30 (2.7)
Other	7 (5.7)	15 (3.6)	35 (3.2)
Tumor grade			
Well differentiated	15 (12.3)	54 (13.0)	173 (15.7)
Moderately differentiated	39 (32.0)	162 (28.9)	457 (41.5)
Poorly differentiated or undifferentiated	68 (55.7)	198 (47.6)	462 (42.0)
Undefined	0 (0.0)	2 (0.5)	8 (0.7)
Pathological tumor size			
<= 1 cm	19 (15.6)	60 (14.4)	136 (12.4)
1 cm < . <= 2 cm	67 (54.9(	235 (56.5)	622 (56.5)
2 cm < . <= 5 cm	33 (27.0)	114 (27.4)	321 (29.2)
>5 cm	3 (2.5)	7 (1.7)	21 (1.9)
Lymph node status			
Node negative	97 (79.5)	316 (76.0)	831 (75.5)
1 positive LN	15 (12.3)	67 (16.1)	185 (16.8)
2 positive LN	7 (5.7)	22 (5.3)	47 (4.3)
≥3 positive LNs	3 (2.5)	11 (2.6)	37 (3.4)
ER status			
Negative	36 (29.5)	113 (27.2)	216 (19.6)
Positive	86 (70.5)	303 (72.8)	884 (80.4)
PgR status			
Negative	47 (38.5)	140 (33.7)	275 (25.0)
Positive	72 (59.0)	272 (65.4)	817 (74.3)
Missing	3 (2.5)	4 (1.0)	8 (0.7)
HER2 status			
HER2 negative	105 (86.1)	359 (86.3)	972 (88.4)
HER2 positive	16 (13.1)	56 (13.5)	126 (11.5)
Missing	1 (0.8)	1 (0.2)	2 (0.2)
Biomarker subtype			
HR+/HER2-	76 (62.3)	263 (63.2)	796 (72.4)
HR+/HER2+	13 (10.7)	46 (11.1)	105 (9.5)
HR-/HER2+	3 (2.5)	10 (2.4)	21 (1.9)
HR-/HER2- (TN)	29 (23.8)	96 (23.1)	176 (16.0)
Missing	1 (0.8)	1 (0.2)	2 (0.2)

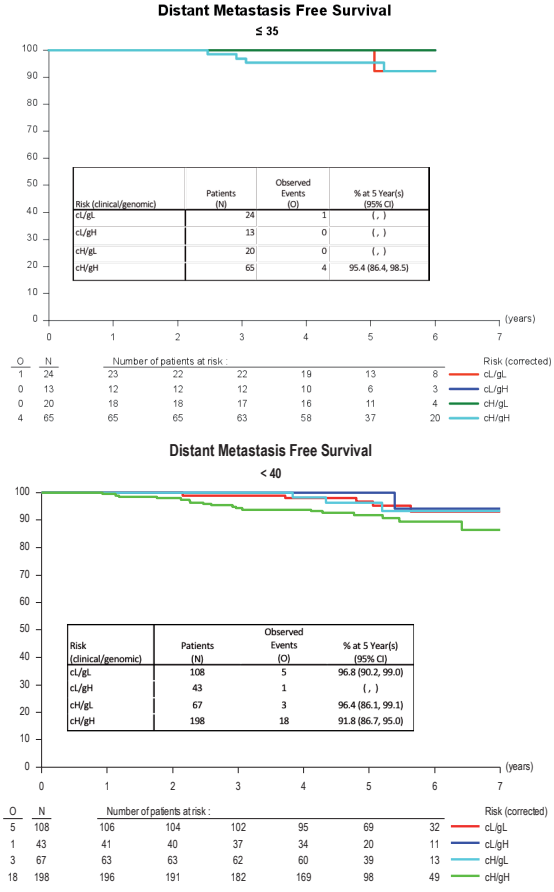
CLINICAL AND GENOMIC RISK			
Clinical risk (c)			
Low risk	37 (30.3)	151 (36.3)	435 (39.5)
High	85 (69.7)	265 (63.7)	665 (60.5)
Genomic risk (g)			
Low risk	44 (36.1)	175 (42.1)	571 (51.9)
High	78 (63.9)	241 (57.9)	529 (48.1)
Risk group[a]			
cL/gL	24 (19.7)	108 (26.0)	341 (31.0)
cL/gH	13 (10.7)	43 (10.3)	94 (8.5)
cH/gL	20 (16.4)	67 (16.1)	230 (20.9)
cH/gH	65 (53.3)	198 (47.6)	435 (39.5)
TREATMENT CHARACTERISTICS			
Type of surgery			
Breast conserving surgery (BCS)	76 (62.3)	293 (70.4)	823 (74.8)
Mastectomy	46 (37.7)	123 (29.6)	277 (25.2)
Radiotherapy			
No	32 (26.2)	82 (19.7)	177 (16.1)
Yes	87 (71.3)	327 (78.6)	903 (82.1)
Missing	3 (2.5)	7 (1.7)	20 (1.8)
Chemotherapy			
No	38 (31.1)	133 (32.0)	435 (39.5)
Yes	83 (68.0)	279 (67.1)	657 (59.7)
Missing	1 (0.8)	4 (1.0)	8 (0.7)
Endocrine treatment			
No	39 (32.0)	124 (29.8)	263 (23.9)
Yes	81 (66.4)	285 (68.5)	818 (74.4)
Missing	2 (1.6)	7 (1.7)	19 (1.7)
Trastuzumab			
No	106 (86.9)	366 (88.0)	989 (89.9)
Yes	15 (12.3)	45 (10.8)	93 (8.5)
Missing	1 (0.8)	5 (1.2)	18 (1.6)

ER= estrogen receptor, PgR= progesterone receptor, HER2= Human epidermal growth factor receptor 2
[a]Risk categories: cL/gL (clinical and genomic low risk), cL/gH (clinical low/genomic high risk), cH/gL (clinical high/genomic low risk), cH/gH (clinical and genomic high risk)

Supplementary Table 2. Baseline patient, tumor (by local assessment) and treatment characteristics of included patients by risk category based on clinical and genomic assessment in the group of patients aged <45

	cL/gL	cL/gH	cH/gL	cH/gH
	N (%)	N (%)	N (%)	N (%)
TUMOUR CHARACTERISTICS				
Histology				
Ductal	289 (84.8)	89 (94.7)	188 (81.7)	407 (93.6)
Lobular	27 (7.9)	1 (1.1)	24 (10.4)	10 (2.3)
Mixed	14 (4.1)	0 (0.0)	10 (4.3)	6 (1.4)
Other	11 (3.2)	4 (4.3)	8 (3.5)	12 (2.8)
Tumor grade				
Well differentiated	289 (84.8)	89 (94.7)	188 (81.7)	407 (93.6)
Moderately differentiated	27 (7.9)	1 (1.1)	24 (10.4)	10 (2.3)
Poorly differentiated or undifferentiated	14 (4.1)	0 (0.0)	10 (4.3)	6 (1.4)
Undefined	11 (3.2)	4 (4.3)	8 (3.5)	12 (2.8)
Pathological tumor size				
<= 1 cm	81 (23.8)	38 (40.4)	7 (3.0)	10 (2.3)
1 cm < . <= 2 cm	250 (73.3)	56 (59.6)	102 (44.3)	214 (49.2)
2 cm < . <= 5 cm	10 (2.9)	0 (0.0)	108 (47.0)	203 (46.7)
>5 cm	0 (0.0)	0 (0.0)	13 (5.7)	8 (1.8)
Missing	81 (23.8)	38 (40.4)	7 (3.0)	10 (2.3)
Lymph node status				
Node negative	310 (90.9)	89 (94.7)	122 (53.0)	310 (71.3)
1 positive LN	24 (7.0)	4 (4.3)	81 (35.2)	76 (17.5)
2 positive LN	4 (1.2)	0 (0.0)	14 (6.1)	29 (6.7)
≥3 positive LNs	3 (0.9)	1 (1.1)	13 (5.7)	20 (4.6)
Missing	310 (90.9)	89 (94.7)	122 (53.0)	310 (71.3)
ER status				
Negative	1 (0.3)	12 (12.8)	7 (3.0)	196 (45.1)
Positive	340 (99.7)	82 (87.2)	223 (97.0)	239 (54.9)
PgR status				
Negative	15 (4.4)	22 (23.4)	14 (6.1)	224 (51.5)
Positive	322 (94.4)	72 (76.6)	216 (93.9)	207 (47.6)
Missing	4 (1.2)	0 (0.0)	0 (0.0)	4 (0.9)
HER2 status				
HER2 negative	323 (94.7)	75 (79.8)	206 (89.6)	368 (84.6)
HER2 positive	17 (5.0)	18 (19.1)	24 (10.4)	67 (15.4)
Missing	1 (0.3)	1 (1.1)	0 (0.0)	0 (0.0)
Biomarker subtype				
HR+/HER2-	323 (94.7)	67 (71.3)	205 (89.1)	201 (46.2)
HR+/HER2+	17 (5.0)	15 (16.0)	21 (9.1)	52 (12.0)
HR-/HER2+	0 (0.0)	3 (3.2)	3 (1.3)	15 (3.4)
HR-/HER2- (TN)	0 (0.0)	8 (8.5)	1 (0.4)	167 (38.4)
Missing	1 (0.3)	1 (1.1)	0 (0.0)	0 (0.0)

ER= estrogen receptor, PgR= progesterone receptor, HER2= Human epidermal growth factor receptor 2
cL/gL=clinical and genomic low risk, cL/gH=clinical low/genomic high risk, cH/gL=clinical high/genomic low risk, cH/gH=clinical and genomic high risk



Supplementary figure 1. Distant metastasis-free survival for patient aged ≤35 and <40

CHAPTER 5

Prognostic Significance of Tumor-Positive Internal Mammary Sentinel Lymph Nodes in Breast Cancer: A Multicenter Cohort Study

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Ann Surg Oncol. 2015;22(13): 4254-62

Abstract

Introduction

The introduction of the sentinel lymph node biopsy (SLNB) in breast cancer has renewed interest in lymphatic drainage to the internal mammary (IM) nodes. The clinical impact of tumor positive IM nodes is not completely clear. This study evaluated the incidence and impact on overall survival of metastatic IM SLNs.

Methods

Between 1997 and 2010, 3685 patients underwent surgery including SLNB for primary breast cancer following an intratumoral or peritumoral radioactive-tracer injection. The presence of lymph node metastases was categorized according to the TNM-classification. Cumulative overall survival was estimated and the influence of metastases in the IM nodes and other factors was assessed by Cox-regression-analysis.

Results

In 754 patients (20.5 %) ipsilateral IM lymph nodes were visualized on preoperative lymphoscintigraphy, retrieval rate of IM SLNs was 81.0 %. IM metastases were detected in 130 patients (21.3 % of retrieved SLNs and 3.5 % of all patients respectively). The presence of IM metastases was associated with axillary metastases ($p < 0.001$). After a median follow-up of 61.2 months, 10.9 % of patients had died. In a multivariate analysis IM metastases did not have a significant effect on overall survival [HR] 1.20; CI: 0.73–1.98. In patients without axillary metastases ($n = 2398$), the presence of IM metastases ($n = 43$) was associated with worse survival [HR] 2.68; 95 % CI: 1.30–5.54.

Conclusion

Overall, the presence of IM metastases did not affect overall survival independent of other prognostic factors including axillary metastases. However, the small subgroup of patients who had IM metastases alone had worse outcome than patients without any regional lymph node metastases.

Introduction

Historically, internal mammary (IM) lymph node metastases were associated with an unfavorable prognosis in breast cancer patients[1,2]. This observation stems from the era when IM lymph nodes were dissected as part of an extended mastectomy. Today, IM lymph node dissection is not performed in breast cancer patients as it causes substantial morbidity and fails to contribute to locoregional control or overall survival (OS)[2].

Introduction of the sentinel lymph node biopsy (SLNB) in breast cancer patients offered the opportunity for a more targeted surgical approach to the IM chain. Depending on the method of radioactive tracer injection, drainage to the IM sentinel lymph node (SLN) is observed in 13–37 % of patients, among whom only 8–24 % have metastases[3].

Although the need to harvest these IM SLNs is controversial, it can be performed with minimal morbidity[4,5].

Observation of IM SLNs has renewed interest in the prognostic relevance of IM lymph node metastases. A number of studies have addressed the clinical impact of IM metastases in terms of additional treatment[4,6,7]. The present study adds to this knowledge with its evaluation of the prognostic impact of lymph node metastases in harvested IM SLNs.

Patients and methods

Between February 1997 and November 2010, a total of 4232 patients in three hospitals (Diakonessenhuis Utrecht/ Zeist (A), The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital (B), and Orbis Medical Centre, Sittard (C) underwent surgical treatment including SLNB for primary cT1-2N0 breast cancer. Data regarding the operative procedures were collected prospectively. Ultimately excluded were 12 men with in situ carcinoma (n = 121), patients with a history of previous breast cancer or other malignancies (n = 200 and n = 68, respectively), patients with a synchronous, contralateral breast cancer (53 patients, 106 tumors), and patients who had received neoadjuvant chemotherapy (n = 44). One patient was lost to follow-up immediately after the operation.

Lymphoscintigraphy and Surgery of SLNs

Lymphoscintigraphy protocols contained discrete differences but consistently included intratumoral or peritumoral injection of 99mTc nanocolloid. One hospital used a 1-day protocol and the other institutions a 2-day protocol. There were differences in the administered 99mTc

doses.4,6,8 Intraoperatively, a peritumoral injection of patent blue dye (Bleu patenté V; Laboratoire Guerbet, Aulnay-sous-Bois, France) was used for SLN identification. Visualization rates have been published previously for the three institutions (22, 22, and 20 % in hospitals A, B, and C, respectively)[4,6,8]. Axillary SLNs were retrieved first. When no axillary SLN was visualized on preoperative lymphoscintigraphy, the axilla was explored in search of an SLN containing the blue dye. Subsequently, we evaluated the patient for visually identified IM SLNs. A c-ray detection probe was used to guide a parasternal intercostal incision. Partial rib resection was not required to retrieve IM lymph nodes. In addition to retrocostal localization of an IM SLN, the impossibility of discerning radioactivity of the SLN from the background activity following intra- parenchymatous tracer injection was a main reason why IM SLNs could not be retrieved in these institutions.[4,6,8]

Pathology

The number of sections of a lymph node and distance between the cuts varied. In hospital A, bisected axillary SLNs were formalin-fixed and cut at five levels with intervals of 250 lm. Because IM SLNs were usually too small to bisect, they were processed as a whole and sectioned at five levels. In hospital B, bisected SLNs and IM SLNs were formalin-fixed, embedded in paraffin, and cut at a minimum of six levels at 50- to 150-lm intervals. In hospital C, the SLNs were formalin-fixed and bisected if large enough, with five cuts at 100-lm intervals. At all three hospitals, pathological evaluation of all SLNs consisted of hematoxylin-eosin and immunohistochemical cytokeratin-8 staining.

Primary tumor characteristics were also noted. Estrogen (ER) and progesterone (PR) receptor status and the Bloom– Richardson (BR) malignancy grade of the primary tumor were determined throughout the study period. Beginning in 2004, the HER2 receptor status was routinely assessed. The presence of metastases in axillary and IM lymph nodes and the number of involved metastatic lymph nodes were recorded. Lymph node status was classified according to the International Union Against Cancer TNM classification, 7th edition.[9]

Postoperative Treatment

Patients received adjuvant systemic therapy based on Dutch guidelines. These guidelines were adjusted several times during the study period, resulting in an increasing proportion of patients with node-negative disease that was a result of systemic therapy. Locoregional radiotherapy was indicated in patients with four or more metastatic axillary lymph nodes. In patients with IM metastases and none to three tumor-positive axillary lymph nodes, parasternal irradiation was advised.

Follow-Up

The last patient included in our study for hospital A was treated in November 2010, for hospital B in June 2006, and for hospital C in August 2010. Follow-up for hospital A was conducted until January 2011. The local databases of hospitals B and C were merged with The Netherlands Cancer Registry (NCR). This database contains information on patients' vital status through linkage with data of the municipal personal records database, which has complete information on all deceased and emigrated residents of The Netherlands. Vital status was complete up to February 1, 2010.

Statistical Analysis

Baseline characteristics between patients with and without IM lymph node metastases for relevant prognostic clinicopathological factors were compared using Fisher's exact tests for categorical data and Student's t tests or the Mann–Whitney U tests for continuous data (Table 1). We then used Cox proportional hazard analyses to assess the relation between IM metastases and OS. Follow-up started at the date of the operation and ended with death (event) or with the date of last follow-up (censored). We defined multiple Cox models by adjusting the possibly confounding effects of IM status for an increasing number of clinicopathological factors: model 1 was adjusted for age (continuous). Model 2 was additionally adjusted for year of diagnosis (continuous), tumor size (pT2 and pT3 versus pT1), Bloom–Richardson (BR) grade (grades 2 and 3 vs. 1), ER+ and/or PR+ (yes/no), HER2 status (±), and number of axillary lymph node metastases (continuous). Model 3 was additionally adjusted for type of surgery (mastectomy versus breast-conserving therapy) as well as adjuvant radiotherapy, trastuzumab treatment, hormonal treatment, and chemotherapy (yes/no for the latter four factors). As patients were treated in three hospitals, this clustering was taken into account in all models by including a random effect for each hospital using a frailty approach. Age and the number of axillary lymph node metastases were modeled using restricted cubic spline functions as they showed significant nonlinearity with OS [based on the likelihood ratio (LR) test compared to fully adjusted models with only the linear term]. The proportionality assumption was checked and found not violated by inspecting the Schoenfeld residuals for all variables. We performed subgroup analyses for patients with and without axillary metastases and for patients treated with mastectomy and with breast-conserving therapy. We then statistically tested for differential effects using interaction terms between IM status and the subgroups (LR tests). We also repeated the analyses considering tumor deposits <0.2 mm in IM SLNs (isolated tumor cells) as IM metastasis-negative.

Not all patients had complete data. HER2 status was not routinely determined before 2004, so it was not available for 27 % of patients. Other variables were complete for >98 % of cases. Missing values were multiply imputed,[10–12] and results were pooled.[13,14] Data were analyzed in R software, version 3.0.1 (R Tech Solutions, Kolkata, India). All reported p values were two-sided with a 5 % threshold for statistical significance.

Table 1. Baseline characteristics according to IM lymph node status of 3685 patients with cT1-2N0 breast cancer operated on in three Dutch hospitals between 1997 and 2010

Characteristics	IM-negative N=3555 (96%)	IM-positive N=130 (4%)	*P**
Patients			
Age at surgery (years), median (min-max)	58 (24-96)	50 (32-85)	<0.001**
<= 50 years	888 (25%)	66 (51%)	<0.001
> 50 years	2667 (75%)	64 (49%)	
Missing	0	0	
Tumors			
Axillary status			
Node negative	2355 (66%)	43 (33%)	<0.001
Node positive	1200 (34%)	87 (67%)	
Missing	0	0	
Tumor size			
pT1	2379 (67%)	76 (58%)	0.078
pT2	1121 (32%)	51 (39%)	
pT3	47 (1%)	3 (2%)	
Missing	8	0	
Tumor grade			
1	1222 (35%)	39 (31%)	0.58
2	1458 (41%)	54 (43%)	
3	836 (24%)	34 (27%)	
Missing	39	3	
Hormone receptor status			
Negative	572 (16%)	20 (16%)	0.9
Positive	2909 (84%)	108 (84%)	
Missing	74	2	
HER2 status			
Negative	2263 (87%)	85 (87%)	1.0
Positive	345 (13%)	13 (13%)	
Missing	947	32	
Treatment			
Surgical procedure			
Breast-conserving	2114 (59%)	74 (57%)	0.59
Mastectomy	1441 (41%)	56 (43%)	
Missing	0	0	
Radiotherapy			
No	1189 (34%)	17 (13%)	<0.001
Yes	2346 (66%)	112 (87%)	
Missing	20	1	
Adjuvant chemotherapy			
No	2356 (67%)	40 (31%)	<0.001
Yes	1172 (33%)	89 (69%)	
Missing	27	1	

Adjuvant hormonal therapy			
No	2152 (61%)	34 (27%)	<0.001
Yes	1372 (39%)	93 (73%)	
Missing	31	3	
Adjuvant trastuzumab			
No	3465 (97%)	125 (96%)	0.39
Yes	90 (3%)	5 (4%)	
Missing	0	0	

IM internal mammary

* Fisher's exact test, except for ** Mann–Whitney U test

Results

SLNs were visualized using lymphoscintigraphy in 3606 of the 3685 patients (98 %). In all, 2852 patients (79 %) had axillary SLNs, 703 (20 %) had axillary and IM SLNs and 51 (1.4 %) had only IM SLNs on lymphoscintigraphy. SLNs were retrieved in 3640 patients (99 %). Only axillary SLNs were removed from 3029 patients (83 %), axillary and IM SNLs were removed from 584 patients (16 %), and only IM SLNs were removed from 27 patients (0.7 %). The retrieval rate of IM SLNs was 81.0 %.

Pathology evaluation revealed axillary metastases in 1287 patients (35 %) and IM metastases in 130 patients (21.0 % of retrieved SLNs—3.5 % of all patients). Extrapolating the metastatic rate (21 %) to the 143 patients in whom IM SLNs were visualized but could not be retrieved implied an additional unidentified 30 patients with IM metastases and an expected overall percentage of metastatic IM SLNs in 4.3 % in all patients. Among the 130 patients with IM SLNs, 14 had isolated tumor cells in the IM SLN. Women with IM metastases were significantly more likely to be younger and more often had axillary lymph node involvement than patients without IM metastases (67 vs. 34 %; $p < 0.001$). In the group with IM metastases, only 43 patients had metastatic IM lymph nodes (Table 1). Patients with IM metastases were significantly more likely to have been exposed to radiotherapy and adjuvant hormonal or chemotherapy (89, 67, and 73 %, respectively; $p < 0.001$).

Table 2. Overall survival for 130 patients with cT1-2N0 breast cancer with IM metastases (from three Dutch hospitals operated on between 1997 and 2010) under various adjustment

	Unadjusted		**Age-adjusted**		**Clinicopath. adjusted, with HER2[a]**		**Full adjustment including adjuvant treatment**	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
IM positive	1.05 (0.64-1.71)	0.85	1.27 (0.78-2.08)	0.33	1.11 (0.68-1.83)	0.68	1.20 (0.73-1.98)	0.48
Age at diagnosis (nonlinear)[b]	---	---	---	<0.001	---	<0.001	---	<0.001
Year of diagnosis (per year)	---	---	---	---	0.95 (0.91-1.00)	0.035	0.96 (0.92-1.00)	0.08
No. of positive axillary lymph nodes (nonlinear)[b]	---	---	---	---	---	<0.001	---	<0.001
pT2	---	---	---	---	1.62 (1.30-2.02	<0.001	1.69 (1.34-2.12)	<0.001
pT3	---	---	---	---	2.51 (1.43-4.41)	0.001	2.69 (1.50-4.82)	0.001
Bloom Richardson grade 2	---	---	---	---	1.53 (1.15-2.03)	0.003	1.57 (1.18-2.09)	0.002
Bloom Richardson grade 3	---	---	---	---	1.87 (1.36-2.59)	<0.001	2.09 (1.50-2.91)	<0.001
ER- and/or PR- positive	---	---	---	---	0.56 (0.43-0.72)	<0.001	0.56 (0.41-0.77)	<0.001
HER2-positive	---	---	---	---	1.00 (0.72-1.39	0.99	1.03 (0.74-1.44)	0.85
Adjuvant chemotherapy	---	---	---	---	---	---	0.68 (0.51-0.90)	0.007
Adjuvant hormonal therapy	---	---	---	---	---	---	0.91 (0.69-1.21)	0.53
Adjuvant trastuzumab	---	---	---	---	---	---	0.44 (0.06-3.29)	0.43
Radiotherapy	---	---	---	---	---	---	0.92 (0.66-1.30)	0.65
Mastectomy	---	---	---	---	---	---	1.03 (0.74-1.42)	0.88

Clustering for different hospitals was adjusted by including a random effect for the hospital using a frailty approach

Clinicopath. clinicopathologically, *HR* hazard ratio, *CI* confidence interval, *ER* estrogen receptor, *PR* progesterone receptor

[a] Data on HER2 status were imputed in 27 % of patients

[b] Age and number of axillary lymph nodes were modeled using restricted cubic spline functions

Patient Outcomes

After a median follow-up of 61months (0.1– 163 months), 3264 women were still alive (88.6 %). Al- together, 403 patients (10.9 %) had died, and 18 (0.5 %) were lost to follow-up before February 1, 2010. Of the patients with IM metastases, 17 (13.1 %) had died.

After adjustment for age differences (model 1), patients with IM metastases had a 27 % higher risk of dying than patients without IM metastases, albeit the difference was not [hazard ratio (HR) 1.27, 95 % CI 0.78–2.08)]. This result remained after full adjustment for clinicopathological and treatment factors (HR 1.20, 95 % CI 0.73–1.98) (Table 2). Although considering the 14 patients with isolated tumor cells in IM SLNs as IM metastasis-negative led to a higher risk estimate (HR 1.30, 95 % CI 0.77–2.19; fully adjusted), it also was not statistically significant.

The relation between IM status and OS depended on the presence of axillary metastasis in our data (p for interaction <0.001). Among the patients without axillary metastases ($n = 2398$), 43 had IM metastases, and they had a higher risk of dying (HR 2.68, 95 % CI 1.30–5.54; $p = 0.008$; fully adjusted) than patients without IM metastases in this group. When axillary metastases were present, there was no relation of IM metastases with outcome for the 1200 IM-negative patients (HR 0.79, 95 % CI 0.40–1.57; $p = 0.51$; fully adjusted). There were eight deaths among the 87 IM-positive patients and 190 deaths.

The relations between other clinicopathological factors and OS are shown in Table 2. Especially tumor size and BR grade increased the risk of dying, whereas patients with a hormone receptor-positive tumor were at lower risk. The nonlinear relation between age and OS is shown in Fig. 1. The HR for dying increased steeply with each additional axillary metastasis up to two, after which the risk still increased but less strongly (Fig. 2). In comparison to the risk of axillary lymph node involvement, the absolute HR of IM metastases, albeit statistically nonsignificant, approximated the risk of less than one involved axillary metastasis.

Discussion

In this multicenter cohort of patients staged by SLNB using an intraparenchymal tracer injection, 36.0 % of the patients had metastases in the regional lymph nodes, and 3.5 % had metastases in the IM chain. In terms of OS, IM SLN metastases did not have a significant prognostic impact independent of other clinicopathological factors, including axillary metastases. The subgroup of patients without axillary metastases had a worse outcome than those with uninvolved regional lymph nodes.

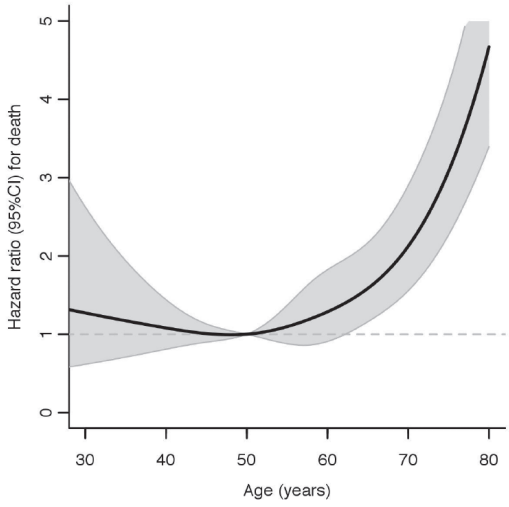

Figure 1. Overall survival for cT1-2N0 breast cancer patients according to age (continuous) on the basis of Cox proportional hazard analyses fully adjusted for clinicopathological factors—e.g., tumor size, Bloom–Richardson (BR) grade, receptor status—and adjuvant treatment.
CI confidence interval

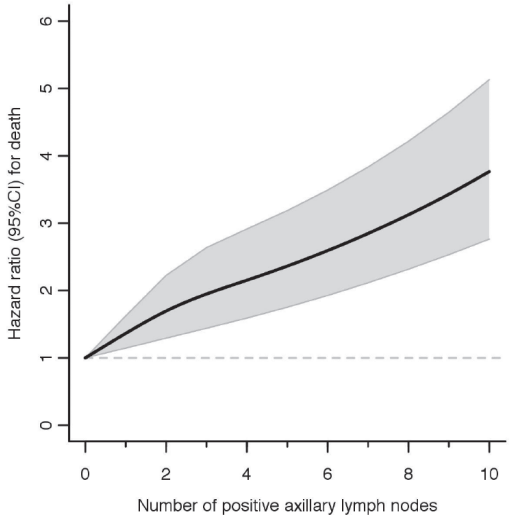

Figure 2. Overall survival for cT1-2N1-2 breast cancer patients according to the number of positive axillary lymph nodes on the basis of Cox proportional hazard analyses fully adjusted for clinicopathological factors (e.g., tumor size, BR grade, receptor status), and adjuvant treatment

The main strengths of this study are its multicenter approach and the relatively large cohort of patients with IM metastases. With more than 100 patients having IM lymph node metastases, the present study describes the largest cohort of patients with IM node metastases to date. SLNB procedures were comparable with respect to the use of an intraparenchymal nanocolloid injection at all three hospitals. It is well known that this technique is associated with a higher rate of visualizing IM SLNs,[15–17] which was the reason for pooling the data of these particular institutions in the first place. The long time frame during which we collected data on breast cancer patients also implied that there have been changes in confounding factors. Because the proportion of patients receiving adjuvant treatment increased during the study period owing to guideline changes over the years, we adjusted for the year of diagnosis as well as other potential confounders. Visualization and retrieval rates for IM SLNs have been reported previously. [16,18,19] The retrieval rate in the present study was 81 %, although not all IM metastases were likely identified as such. This potentially led to an underestimation of the true relation between IM metastases status and OS as these unrecognized IM metastases were misclassified as IM- negative. It is unlikely that this misclassification is related to the outcome (i.e., random misclassification).

Although we did not find that IM metastases has a statistically significant independent effect on OS in the present study, this finding is in contrast to earlier reports. IM metastases were considered a poor prognostic sign in earlier times. In a landmark study by Veronesi et al.[20] patients with metastases in the IM chain alone had a prognosis similar to that of patients with axillary metastases, and patients with both axillary and IM metastases had the poorest prognosis. A comprehensive review also showed that metastases in the IM lymph nodes added to the prognostic impact of the status of other regional lymph nodes.[21] Patients with IM lymph node metastases were then classified as pN3.[22] The gloomy prognosis associated with IM metastases in earlier times contrasts with the in- significant influence observed in the present study. After multivariate adjustment for systemic therapy, the relative risk of death associated with IM metastases was HR 1.20. Albeit not significant, in absolute terms it is comparable to a relative risk increase in the presence of one involved axillary node, as shown in Fig. 1. The adverse prognostic impact of IM metastases in patients who have uninvolved axillary lymph nodes is in line with findings from the aforementioned studies. All in all, metastases in IM SLNs are better regarded as ''just'' another regional lymph node than considering it as a staged category in itself. The SLNB offers a minimally invasive, targeted approach to determine IM lymph node status. Even though the present study did not show a significant prognostic influence of IM metastasis, the subgroup of patients without axillary metastasis but with IM metastasis did have a worse outcome than patients with uninvolved regional lymph nodes. Therefore, not addressing IM lymph node status could lead to understaging.

The introduction of systemic treatment is a potential confounder and has had a major impact on survival rates for all breast cancer stages since the time that IM node dissections were abandoned. In the present cohort of breast cancer patients, the 5-year OS was approximately 90 %, with half of the deceased patients having died from other causes. Although systemic therapy has influenced the ab- solute survival rates, it cannot be the sole explanation for the absence of a significant prognostic impact of IM metastases. We therefore tried to adjust for the use of systemic treatment. A certain degree of patient selection persisted, however, so full adjustment for confounding remains difficult to achieve. Our results should be interpreted from that perspective.

A likely explanation for the statistical and clinical prognostic irrelevance of IM metastases lies in the SLNB procedure itself. First, IM lymph nodes are smaller than axillary nodes and are thus unlikely to be detectable by means other than an SLNB procedure. Consequently, IM nodes retrieved by SLNB reflect a different selection than IM lymph nodes harvested during earlier times. In addition, the current pathological workup of SLNs reveals smaller tumor deposits. The 10 % of patients with IM lymph node involvement in the present study who had deposits <0.2 mm (isolated tumor cells) underscores this retrieval of smaller IM metastases during the SLNB era. Our study supports considering IM metastasis as a ''variety'' of regional lymph node involvement. Thus, the presence of IM metastases, in prognostic terms, equates to a single involved axillary node.

The aim of this study was to determine the impact of IM lymph node metastases on the prognosis of breast cancer patients. In this large cohort study IM metastases were found in a considerable proportion of patients, but we did not observe an overall impact of IM lymph node metastases on OS, independent of axillary metastases and other clinicopathological factors. Only 1 % of all of the patients who had IM metastases—but otherwise uninvolved regional lymph nodes—had significantly impaired prognosis. Then again, previous studies demonstrated that the detection of these IM node metastases altered nonsurgical treatment in a larger proportion of patients. Hence, we advise that SLNB of the IM nodes be performed for optimal staging of the breast cancer, at least in patients who will not undergo adjuvant systemic treatment based on the primary tumor's characteristics. Concomitantly, we consider parenchymatous tracer injection as the preferable technique for optimizing visualization of IM SLNs.

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Appendix

Lymphoscintigraphy Protocols

In hospital A, lymphoscintigraphy was performed on the day of surgery. Patients received a combination of peritumoral intraparenchymal and subcutaneous injections around and ventral of the tumor of an average dose of 77.6 MBq (spread 53–150 MBq) 99mTc-nanocolloid (Nanocoll, GE Health). The total volume was 0.6 mL nanocolloid in physiologic saline, given in 2–4 equal doses. In case of non-palpable breast tumors injections were guided by using a 7.5 MHz ultrasound probe (Aloka). After injection the area was massaged gently until the appearance of the SLN. Semi-dynamic images were performed at the initial visualization time of the lymphatic channel. Static images were obtained approximately 2 h after injection depending on the time of surgery. Semi-dynamic and static images were obtained during a 2 min imaging time on the Toshiba 901 HG single-head gamma camera, using low energy high resolution collimators between June 1999 and October 2005. Since November 2005 the images were performed on the Philips skylight dual head gamma camera, using low energy general purpose collimators. The images were performed with a 57Co flood source. A skin marker was placed on the projection of the SN using a handheld c-ray detection probe (Europrobe, PI Medical diagnostic equipment BV).

In hospital B a 2-day protocol was used. On the day before surgery, 99mTc-labeled nanocolloid (Nanocoll; Amersham Cygne, Eindhoven, The Netherlands) was injected into the lesion in a mean volume of 0.2 mL and a mean radioactivity dose of 114.9 MBq (3.1 mCi). In case of nonpalpable breast cancer, the intratumoral injection was guided by ultrasound or stereotaxis. Static imaging was performed at 30 min and 4 h after injection with simultaneous transmission scanning by using a cobalt-57 flood source to outline the body contour. Since July 1999, additional views were obtained after 2 h. Both anterior and lateral images were obtained by using a dual-head gamma camera (Vertex; ADAC, Milpitas, CA). The location of the node was marked on the skin with indelible ink. In patients with nonpalpable breast cancer, a localization procedure was performed after the last scintigraphic image, including placement of a catheter for intratumoral administration of patent blue dye.[6] In hospital C, the injection of 10 mCi (370 MBq) 99mTc-nanocolloid, the day before surgery in 3–4 depots around the tumor or in the breast parenchyma surrounding the cavity of a previous excisional biopsy. In case of non-palpable tumors, the radiocolloid tracer was injected within the relevant quadrant of the breast, without the use of ultrasound guidance. Lymphoscintigraphy was performed on the next day, after a period of 16–18 h following radiotracer injection, and shortly before surgery. Lymphoscintigraphic images were obtained in three standard positions: anterior, anterior oblique and lateral. The location of axillary and non-axillary SNs was marked on the skin. After induction of general anesthesia in the operating room, 10–15 min before the incision, 0.8–1.0 ml Patent Blue V (Laboratoire Guerbet, France) was injected intradermally above the tumor or alongside the scar of the excisional biopsy.[8]

CHAPTER 6

Characterisation of multifocal breast cancer using the 70-gene signature in clinical low-risk patients enrolled in the EORTC 10041/BIG 03-04 MINDACT trial

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Eur J Cancer. 2017; 79: 98-105

Abstract

Background

In multifocal breast cancer, guidelines recommend basing adjuvant systemic treatment decisions on characteristics of the largest lesion, disregarding multifocality as an independent prognosticator. We assessed the association between multifocal disease and both the 70-gene signature (70-GS), and distant metastasis-free survival (DMFS) in clinical low-risk breast cancer patients enrolled in the European Organisation for Research and Treatment of Cancer 10041/BIG 03-04 Microarray In Node-negative and 1 to 3 positive lymph node Disease may Avoid ChemoTherapy (MINDACT) trial.

Patients and methods

The analysed population consisted of enrolled patients in the MIND- ACT trial with clinical low-risk disease, defined by a modified Adjuvant! Online cut-off for the 10-year risk of recurrent disease or death. Eligibility criteria of MINDACT dictate that patients with multifocal disease could be included if the different lesions had similar pathological characteristics. The presence of multifocal disease was deducted from the case report form (CRF)-question for sum of diameter for all invasive tumour foci. Clinicopathological characteristics and gene expression of patients with unifocal and multifocal (largest lesion) disease were compared. Subsequently, the association between multifocal disease and the 70-GS was evaluated as well as the association between multifocality and 5-year DMFS.

Results

The study included 3090 clinical low-risk patients with unifocal and 238 patients with multifocal disease. Apart from a higher prevalence of lobular tumours (21.8% versus 10.8%, by local pathology), we did not observe differences in baseline characteristics between multifocal and unifocal tumours. Patients with multifocal tumours were more likely to be at high genomic risk as compared to patients with unifocal tumours (22.7% versus 17.3%, odds ratio [OR] 1.45, 95% confidence interval [CI] 1.02-2.07, P Z 0.038). We did not find a significant association between tumour focality and DMFS (97.1% for unifocal versus 96.9% for multifocal, hazard ratio [HR] = 1.55, 95% CI 0.68-3.46, P = 0.172), nor a signal for a potential interaction between the prognostic effect of the 70-GS and focality of the tumour regarding DMFS.

Conclusion

In the group of clinical low-risk MINDACT patients, multifocal tumours were more likely to have a high-risk 70-GS profile compared to unifocal tumours. We did not observe a significant interaction between multifocality and the 70-GS with respect to survival without distant metastasis in these patients.

Introduction

Multifocal breast cancer, generally defined as the presence of multiple invasive tumour foci in the same quadrant of the breast, has a wide-ranged incidence varying from 6% to 77%, depending on the definition and method of diagnosis [1-3]. Multifocal disease is more often seen in lobular carcinomas and has been associated with an increased incidence of lymph-node involvement, poor differentiation grade, HER2 positivity and lymphovascular invasion as compared to unifocal tumours [2,4-8]. Due to improvements in diagnostic imaging and increased use of magnetic resonance imaging (MRI), multifocal disease is diagnosed more often [2,9]. Nevertheless, the prognostic relevance of multifocality remains largely unclear [1].

Current guidelines recommend basing adjuvant systemic treatment (AST) decisions on pathological characteristics of the largest lesion, thus assuming that outcome in multifocal disease depends entirely on the prognostic features of this lesion and the extent of lymph-node involvement [10-12]. This approach might result in omission of AST in patients who are regarded as low-risk for disease recurrence based on clinicopathological assessment of their largest lesion. Furthermore, as multifocality has been suggested to be a sign of high tumour burden, which in turn has been associated with a greater tendency to metastasise, disregarding multifocality as an independent prognosticator may result in under treatment [4,13].

Over the last few years, several gene expression profiles have been developed to better predict clinical outcome compared to standard assessment based on clinicopathological characteristics [14]. The prospective MINDACT study showed that the 70-gene signature/ MammaPrint" (70-GS) could accurately differentiate between patients with a low and high risk of distant metastases and death at 5 years, thereby providing valuable information for determining the potential benefit of adjuvant chemotherapy [15].

The aim of the current study was to assess whether multifocal disease is associated with an increased rate of having a high genomic risk as assessed by the 70-GS in clinical low-risk patients enrolled in the European Organisation for Research and Treatment of Cancer (EORTC) 10041/BIG 03-04 MINDACT trial. In addition, we evaluated the association between tumour focality, the 70-GS result and distant metastasis-free survival (DMFS) to determine whether multifocality in clinical low-risk patients would be an argument for performing the 70-GS to better select patients for systemic treatment.

Methods

Study design and eligible patients

The EORTC 10041/BIG 03-04 MINDACT [15] trial (NCT00433589) enrolled women aged 18-70 years diagnosed with histologically proven unilateral primary early-stage (cT1-2 or operable T3) breast cancer with 0-3 positive lymph nodes, that had their risk of distant disease recurrence assessed by both the 70-GS (genomic) and a modified version of Adjuvant! Online (clinical) [16]. Patients with C-high/G-high risk assessment received adjuvant chemotherapy, while those with a C- low/G-low risk profile did not. Patients with discordant results for the two risk assessments were randomised to follow either the genomic or clinical risk for the decision regarding chemotherapy administration.

For this study, only patients with clinical low-risk disease and a known focality status were included. Clinical low-risk, as per the modified Adjuvant! Online, was defined as a 10-year breast cancer survival probability of >88% for oestrogen receptor (ER) positive disease without systemic therapy, and >92% for ER negative breast cancer accounting for an average 4% absolute benefit of adjuvant endocrine treatment in ER+ disease [15].

Patients with multifocal disease were eligible to be included in the MINDACT trial if the different tumour foci were of similar histopathology (histological subtype, grade, ER, progesterone receptor [PgR] and HER2 status). The genomic risk assessment had to be performed on the largest lesion. To select our population, the presence of multifocal disease was deduced from the mandatory baseline CRF-question for sum of diameter for all invasive tumour foci, a question that only needed to be answered in the presence of multifocal disease (Appendix A). In the case of multifocal breast cancer, clinicopathologic and genomic characteristics of the largest lesion were considered for analysis. Whenever the clinical or genomic risk changed after enrolment, e.g. due to incorrect reporting of lymph-node status or logistical errors, we used the corrected risk status [15].

Objectives and end-points

The primary objective of this substudy was to evaluate the association between multifocality and genomic risk result (70-gene signature) in clinical low-risk patients. Secondary objectives included (1) assessment of the association between routine clinicopathological characteristics (including age, stage, grade, ER, PgR, HER2, histology) and focality of the tumour in clinical low-risk patients, (2) evaluating the association between multifocal disease and 5-year DMFS, within the group of clinical low-risk patients and (3) to study a potential interaction between multifocality and 70-gene risk in relation to outcome (DMFS) in clinical low-risk patients. DMFS was defined as the time until first distant metastatic recurrence or death from any cause, whichever occurred

first. Patients without a DMFS event at cut-off date were censored at the date of last disease assessment.

Statistical analysis

We hypothesised that in clinical low-risk patients with multifocal disease the percentage of patients at high genomic risk according to the 70-GS would be higher than in patients with unifocal breast cancer; 15% versus 7% [17]. This would correspond to a relative risk increase of 114% and an absolute risk increase of 8%. With 3088 clinical low-risk unifocal tumours and 238 clinical low-risk multifocal tumours in the MINDACT population, there would be a 97% power to detect the hypothesised association at a significance level (alpha) of 5%.

The association between multifocality and genomic risk was evaluated using Fisher's exact test. This association was further examined using multivariate logistic regression adjusting for age, pathological tumour and nodal status, grade, hormone receptor (ER and PgR) and HER2 status, and histology as per local assessment. The distribution of baseline patient and tumour characteristics were compared by tumour focality and presented in percentages. Subsequently, the association between multifocality and DMFS was evaluated using multivariate Cox regression adjusting for the abovementioned clinicopathological factors as well as adjuvant chemotherapy and endocrine treatment. Patients with missing information for (one of) the considered variables were excluded from this analysis (n = 82). Following the primary results of MINDACT [15], the 70-GS was not included as a factor in the main model but a sensitivity analysis was conducted which adjusted for the prognostic effect of the 70-gene risk (as a potential confounder). Finally, Cox regression analyses were performed to assess a potential interaction be- tween multifocality and 70-GS result in relation to outcome (DMFS).

All analyses were performed using SAS software, version 9.4 (SAS Institute). A significance level of 5% was considered for all analyses.

Results

Patient population

Out of the 6693 patients enrolled in MINDACT, a total of 3328 patients had a clinical low-risk and known focality status and were included in this study. We excluded 9 patients with missing information on the focality of the tumour. Of the included patients, 238 (7%) were registered as having multifocal and 3090 (93%) unifocal breast cancer. Baseline clinicopathological characteristics of patients are presented in Table 1. Apart from a higher incidence of lobular tumours (21.8% for multifocal versus 10.8% for unifocal tumours), we did not observe any

Table 1. Baseline patient and tumour (by local assessment of largest lesion) characteristics of included patients according to focality of the tumour.

	Unifocal (N=3,090)	Multifocal (N=238)	Total (N=3,328)
	N (%)	N (%)	N (%)
Age (median with range)	56 (26 - 71)	53 (26 - 71)	56 (26-71)
Age (categories)			
< 30	10 (0.3)	1 (0.4)	11 (0.3)
30-<40	125 (4.0)	15 (6.3)	140 (4.2)
40-<50	747 (24.2)	73 (30.7)	820 (24.6)
50-<60	1,081 (35.0)	83 (34.9)	1,164 (35.0)
60 and more	1,127 (36.5)	66 (27.7)	1,193 (35.8)
Pathological tumour size			
<= 1 cm	788 (25.5)	64 (26.9)	852 (25.6)
1 cm < . <= 2 cm	2,179 (70.5)	164 (68.9)	2,343 (70.4)
2 cm < . <= 3 cm	123 (4.0)	10 (4.2)	133 (4.0)
Lymph-node status			
Node negative	2,916 (94.4)	223 (93.7)	3,139 (94.3)
1 positive LN	133 (4.3)	7 (2.9)	140 (4.2)
2 positive LN	24 (0.8)	5 (2.1)	29 (0.9)
3 positive LN	17 (0.6)	3 (1.3)	20 (0.6)
Tumour grade			
Well differentiated	1,250 (40.5)	81 (34.0)	1,331 (40.0)
Moderately differentiated	1,717 (55.6)	148 (62.2)	1,865 (56.0)
Poorly differentiated or undifferentiated	111 (3.6)	8 (3.4)	119 (3.6)
Undefined	12 (0.4)	1 (0.4)	13 (0.4)
ER status			
Negative	58 (1.9)	7 (2.9)	65 (2.0)
Positive	3,032 (98.1)	231 (97.1)	3,263 (98.0)
PgR status			
Negative	417 (13.5)	30 (12.6)	447 (13.4)
Positive	2,647 (85.7)	208 (87.4)	2,855 (85.8)
Missing	26 (0.8)	0 (0.0)	26 (0.8)
HER2 status			
HER2 negative	2,935 (95.0)	224 (94.1)	3,159 (94.9)
HER2 positive	148 (4.8)	13 (5.5)	161 (4.8)
Missing	7 (0.2)	1 (0.4)	10 (0.2)
Tumour histology			
Ductal	2,546 (82.4)	165 (69.3)	2,711 (81.5)
Lobular	333 (10.8)	52 (21.8)	385 (11.6)
Mixed	93 (3.0)	15 (6.3)	108 (3.2)
Other	115 (3.7)	6 (2.5)	121 (3.6)
Missing	3 (0.1)	0 (0.0)	3 (0.1)
Local treatment			
BCS alone	16 (0.5)	2 (0.8)	18 (0.5)
BCS + radiotherapy	2,754 (89.1)	119 (50.0)	2,873 (86.3)
BCS – radiotherapy unknown	34 (1.1)	1 (0.4)	35 (1.1)
Mastectomy	255 (8.3)	84 (35.3)	339 (10.2)
Mastectomy + radiotherapy	23 (0.7)	29 (12.2)	52 (1.6)
Mastectomy – radiotherapy unknown	8 (0.3)	3 (1.3)	11 (0.3)

Chemotherapy			
Yes	317 (10.3)	35 (14.7)	352 (10.6)
No	2,767 (89.5)	202 (84.9)	2,969 (89.2)
Missing	6 (0.2)	1 (0.4)	7 (0.2)
Endocrine treatment			
Yes	2,461 (79.6)	191 (80.3)	2,652 (79.7)
No	573 (18.5)	43 (18.1)	616 (18.5)
Missing	56 (1.8)	4 (1.7)	60 (1.8)
Trastuzumab treatment			
Yes	67 (2.2)	6 (2.5)	62 (1.9)
No	2,967 (96.0)	226 (95.0)	3,193 (95.9)
Missing	56 (1.8)	6 (2.5)	62 (1.9)

PgR, progesterone receptor; ER, oestrogen receptor; BCS, breast conserving surgery.

Percentages may not add up to 100% due to rounding.

differences in baseline characteristics between multifocal and unifocal breast tumours. The vast majority of patients (94%) had node negative (micrometastases of 0.2-2 mm were considered as pNþ and isolated tumour cells as pN0) and hormone receptor positive disease (98% ER+), while 5% were HER2+. Patients with multifocal disease were less often treated with breast-conserving surgery (51% versus 91%). Chemotherapy was administered in 10% of patients with unifocal and 15% of patients with multifocal tumours.

Primary end-point: multifocality and 70-gene signature

There was a significant association between the 70-GS result and focality of the tumour in the group of clinical low-risk patients (P = 0.043, Table 2). In patients with unifocal disease 17.3% were assigned to the genomic high-risk profile, while this was 22.7% in patients with multifocal tumours. This corresponds to an absolute increase by 5.4% and a relative increase of 31%, which is smaller than hypothesised. In multivariable regression analysis, adjusting for age, pathological tumour and nodal status, grade, ER status, PgR status, HER2 status and histology, multifocality remained significantly associated with a high-risk 70-GS profile (OR 1.45, 95% CI 1.02-2.07, P = 0.038).

Table 2. Association between focality and genomic risk as assessed by the 70-GS.

Genomic risk (corrected)	Unifocal (N=3090) N (%)	Multifocal (N=238) N (%)	Total (N=3328) N (%)	p-value[a]
Low risk	2,554 (82.7)	184 (77.3)	2,738 (82.3)	0.043
High risk	536 (17.3)	54 (22.7)	590 (17.7)	

[a]Fisher exact test for association

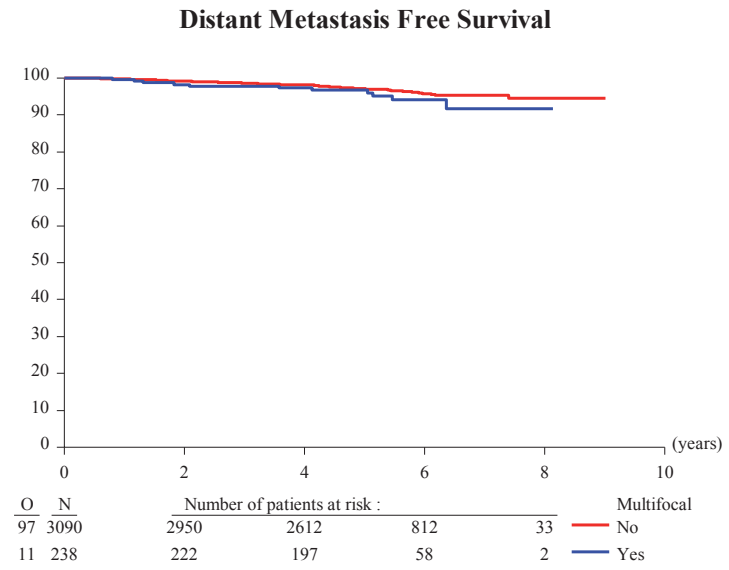

Figure 1. Distant metastasis-free survival according to focality of the tumour.

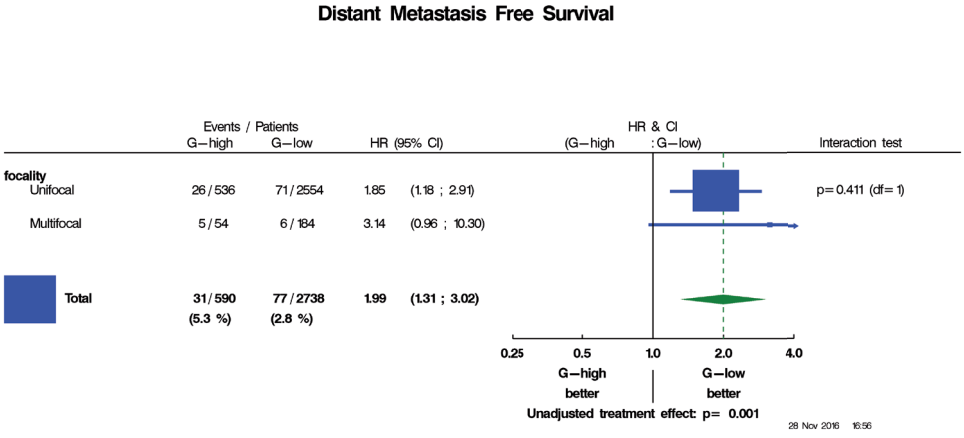

Figure 2. Forest plot exploring the prognostic effect of the 70-gene signature on distant metastasis-free survival by focality of the tumour.

Secondary end-points: outcome

The 5-year DMFS rate was 97.1% (95% CI 96.4-97.7) for patients with unifocal and 96.9% (95% CI 93.5-98.5) for patients with multifocal tumours (Fig. 1). We did not find a significant association between tumour focality and DMFS (HR Z 1.55, 95% CI 0.68-3. 56, P = 0.172). A similar result was found in the sensitivity analysis adjusting for the result of the 70-GS (HR 1.49, 95% CI 0.65-3.42, P = 0.217). Additionally, the Cox regression model did not demonstrate a signal for a potential interaction between the prognostic effect of the 70-GS and tumour focality regarding DMFS (P = 0.411; Fig. 2). We did observe a prognostic effect of the 70-GS on DMFS in both unifocal (HR = 1.85, 95% CI 1.18-2.91) and multifocal tumours (HR 3.14, 95% CI 0.96-10.30), although with 11 events in 238 patients the latter trend was not powered to be conclusive (Fig. 2)

Discussion

In this study, multifocal disease was independently associated with a high genomic risk according to the 70- GS in clinical low-risk patients, albeit smaller than hypothesised. This could have been a reflection of possible limitations in the current staging strategy for multifocal breast cancer, as current guidelines do not take into account the higher tumour burden that is generally attributed to multifocal tumours [4,11]. This study did not demonstrate a significant interaction be- tween the prognostic effect of the 70-GS and multifocality with respect to patients' outcome (DMFS). These results appear to be in accordance with the primary results of the MINDACT trial, indicating limited value of performing the 70-GS in clinical low-risk patients [15]. It should however be noted that the interaction analysis of the present study was not adequately powered to answer this question. The prognostic value of the 70-GS signature in multifocal breast cancer will need to be confirmed with more follow-up data.

To our knowledge, this is the first study evaluating the association between tumour focality and gene expression in a large population of early-stage breast cancer patients. We evaluated the clinical low-risk patients in order to determine whether performing the 70- GS in these patients would improve risk assessment and therefore could have clinical implications. In clinical high-risk patients the decision to give AST would already have been made based on the clinicopathological assessment of largest lesion so the presence of multifocality will not impact the AST decision.
Heterogeneity, not only inter-tumoral but also intra-tumoral, is one of the hallmarks of cancer, which complicates AST decisions [18]. It is generally believed that multifocal disease arises from one type of cancer cell resulting in different lesions with largely identical phenotypes, though evolution can occur during proliferation [3,19,20]. Previous research has shown multiple lesions in multifocal disease are largely concordant with respect to hormone receptor status [19,21], suggesting that consideration of only one lesion to determine hormonal treatment would be

safe. However, there were differences demonstrated between different foci regarding histologic tumour type, differentiation grade, HER2 status and p53 expression [19,20,22] while these characteristics are important when deciding on adjuvant chemotherapy. This heterogeneity in the case of multifocal disease could indicate different foci may display different genomic risks, raising the question whether multiple lesions should be assessed.

We observed no differences in clinicopathological characteristics between patients with multifocal and unifocal disease apart from the expected higher incidence of lobular tumours in the multifocal group. This is likely the consequence of only selecting the clinical low-risk patients, as patients with multifocal disease and poor clinical prognostic features, occurring more frequently in case of multifocality, would have been classified as clinical high-risk.

There was a significant association between the 70- GS result and focality of the tumour in the group of clinical low-risk patients. Overall, the outcome of this clinical low-risk population was excellent (5-year DMFS 97%) as was the case for the whole MINDACT population [15] making it difficult to identify clinical and statistically significant differences between groups. In this study, multifocality was not an independent prognosticator for DMFS in this clinical low-risk population. This is in accordance with previous reports, although studies on the association between multi-focality and outcome have contradictory results [1,3,23-26]. Weissenbacher et al. [13] performed a matched-pair analysis comparing patients with unifocal (n = 288) and multifocal/multicentric (n = 288) disease, demonstrating a significant increase in the occurrence of distant metastasis in the latter group (21.2% versus 12.5%, P = 0.004). Neri et al. [27] confirmed these results in 131 patients, also reporting that administration of adjuvant anthracyclines appeared to reduce this difference. In the largest series to date, including 1554 patients with multifocal breast cancer, multifocality was associated with a decreased breast cancer specific survival yet without an influence on overall survival [28].

The incidence of multifocal disease in this study was 7%, which is relatively low when compared other reports [1e3]. The rate was the same in the overall MINDACT population with 497 out of 6693 patients reported as multifocal (n = 28 with unknown focality status). The relatively low rate of multifocality in this study is likely the result of the eligibility criteria of the MINDACT trial. The MINDACT protocol dictated only patients with multifocal disease whose separate foci displayed similar histopathology could be included, meaning the subgroup of patients with more heterogeneity was missing. This could have led to an underestimation of the reported association between multifocal disease and DMFS ('confounding by indication'). Furthermore, in MINDACT, over 80% of patients underwent breast-conserving surgery which could have resulted in a lower detection rate of multifocality. Finally, we have no information about the use of MRI in the diagnostic process which might have impacted the detection rate.

A limitation of this study is the deduction of the presence of multifocal disease from the CRF-question for sum of diameter for all invasive tumour foci (Appendix A). Multifocal breast cancer is generally defined as the presence of more than one invasive tumour lesion in one quadrant of the breast. However, for this study we did not possess detailed information on location of the various lesions. This means both multifocal and multicentric (multiple lesions in different quadrants of the breast) disease could be included in the multifocal population evaluated in this study. Then again, this distinction is increasingly considered arbitrary as is reflected by the current tumour-node-metastasis (TNM) staging manual establishing the term 'multiple cancers' [11]. Furthermore, we only had access to the clinicopathologic characteristics and 70-GS result of the largest lesion in the case of multifocal disease.

In summary, while multifocality proved to be associated with an increased incidence of a high genomic risk as per the 70-GS, this study did not demonstrate a significant interaction between multifocality and the 70-GS with respect to survival without distant metastasis in patients regarded as clinical low-risk.

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Appendix A. CRF-question on baseline information form on which presence of multifocal disease was based to select patient population

Only for multifocal tumors ☐ mm [###] ☐ Unknown

Provide the sum of the diameters of all invasive tumor foci (including the largest one)

CHAPTER 7

Response to neoadjuvant chemotherapy as a predictor for breast cancer recurrence using the neoadjuvant response index: a population-based study

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Submitted

Abstract

Background

The objective of this study was to assess the value of the neoadjuvant response index (NRI), quantifying the level of response to neoadjuvant chemotherapy above achievement of a pathologic complete response (pCR), in a population-based cohort.

Patients and methods

All female patients, with invasive non-metastatic breast cancer, that received neoadjuvant chemotherapy between 2003-2008 were selected from the Netherlands Cancer Registry and evaluated for level of response using the NRI. The NRI is calculated by adding a breast response score to an axillary response score and dividing this score by the total achievable score in case of pCR. The NRI will be a number between 0 (no response) and 1 (pCR breast and axilla). The association between this score and 5-year recurrence-free survival (RFS) was evaluated in the overall population and according to biomarker subtype based on hormone receptor and Her2 status.

Results

The NRI was calculated for 1,793 women with a median age of 49 (range 43-57) years who were treated with NAC and subsequently underwent surgery in the period 2003-2008. The mean NRI for the overall population was 0.35. A total of 641 events occurred during a median follow-up of 4.6 years. There was a significant association between NRI and 5-year RFS ($p<0.0001$). The NRI had prognostic significance in all four biomarker subtypes, although most pronounced in the HR-/HER2+ subgroup. Adding the NRI to a multivariable model with age, clinical tumor size, clinical nodal status, histological type, grade, biomarker subtype and pCR, increases the prognostic value of the model with regards to 5-year RFS.

Conclusions

The extent of response to neoadjuvant chemotherapy as determined by the NRI provides additional prognostic information regarding 5-year RFS, on top of the achievement of pCR.

Introduction

Neoadjuvant chemotherapy (NAC) was introduced as a method to obtain reduction of the tumor to offer the possibility of more conservative surgery for locally advanced disease and is increasingly being applied in early stage breast cancer patients requiring chemotherapy[1,2]. Additionally, NAC allows for 'in-vivo' monitoring of the efficacy of systemic treatment[3].

The response to NAC in terms of achieving a pathologic complete response (pCR), specifically the absence of tumor at the postoperative pathology evaluation with the exception of isolated tumor cells, is considered an important prognostic factor for disease-free survival[4]. The proportion of women achieving a pCR in the neoadjuvant setting could be predictive of benefit in the adjuvant setting. Previous studies have shown a decrease in both local recurrences and distant metastases after pCR[5]. Considering the response to NAC as a binary variable results in loss of potentially important information about the extent of response that was achieved in case of non-pCR. This is especially relevant in patients with luminal A or luminal B/HER2 positive disease as the tumors of these patients rarely achieve a pCR while the prognosis of these women is usually superior to the prognosis of patients with tumors that are of the triple negative or HER2 positive subtype. In these patients pCR is not a suitable surrogate endpoint for long-term outcome[6]. Residual tumor burden after NAC has been reported as an alternative prognostic factor after NAC by Symmans et al.[7] However, this quantification does not implement information about the primary characteristics of the tumor. In 2009, Rodenhuis et al[8] described a neoadjuvant response index (NRI), a quantitative measure comparing the breast and axillary status before and after NAC[8]. This score was associated with differences in recurrence-free survival.

The objective of this study is to assess the prognostic value of the NRI as a continuous variable for recurrence-free survival in a large population-based cohort.

Materials and methods

Patients

All female patients with primary non-metastatic invasive breast cancer who received NAC between 2003-2008 and subsequently underwent surgery, were identified from the Netherlands Cancer Registry (NCR). The population-based NCR contains data on patient and tumor characteristics as well as information regarding the applied treatment. Based on a notification from the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA), trained NCR personnel register the information directly from patients' medical records in all hospitals. The use of data for this study was approved by the Privacy Review Board of the NCR.

Patients were excluded from this study in case of previous breast cancer, synchronous contralateral breast cancer (defined as a second primary breast cancer within three months of diagnosis), living or being treated outside the Netherlands, unknown date of final surgery for primary breast cancer or incomplete follow-up data (eg. no information, missing event date). For the years 2007-2008 follow-up data was available from 43 (47%) hospitals in contrast to all hospitals (n=92) for the period 2003-2006.

Tumor size and lymph node involvement were recorded according to the TNM system of the Union for International Cancer Control (UICC) and the American Joint Committee on Cancer (AJCC) applicable at time of diagnosis[9]. If the sentinel lymph node biopsy was performed before the start of NAC, the pathological outcome was included into the clinical lymph node status. Estrogen receptor (ER) and progesterone receptor (PR) status were largely available throughout the study period. Routine assessment of HER2 status was implemented in the Netherlands in 2005. The subtypes discussed here constitute biomarker subtypes on the basis of hormone receptor (HR) and HER2 status and were categorized as HR+/HER2-, HR+/HER2+, HR-/HER2+ and HR-/HER2- (TN=triple negative). The final surgical procedure for the primary tumor, breast-conserving surgery (BCS) or mastectomy, was included for the analysis. Information regarding administered radiotherapy (yes/no), endocrine treatment (yes/no) and immunotherapy (yes/no) was available from the NCR, although detailed information on the treatment regimens was not available. Five-year follow-up data regarding local and regional recurrence and distant metastases, whichever occurred first, were collected for all patients in retrospect by the NCR personnel. Vital status was obtained through linkage with the municipality registry and complete until December 31st 2013.

Neoadjuvant Response Index

The response to neoadjuvant chemotherapy was calculated using a modified version of the NRI previously described by Rodenhuis et al. This score is calculated by combining a breast response score and axillary response score before and after NAC.

The breast response score (BRS) consists of two parts; a score for the achievement of pCR and a score calculated by the change in T-stage from the moment of clinical to pathological staging. The definition of pCR was the absence of invasive tumor at pathological examination established by a pT0 stage in the NCR. A near-pCR indicated a residual tumor size of $<$0.5cm in size at pathology. A patient received 1 point for every decrease in T-stage with the exception of the step to T0. The achievement of pCR amounted to 2 points while achieving near-pCR earned 1 point. The axillary response score (ARS) was calculated by the change in N-stage between clinical and pathological evaluation, awarding 1 point for every decrease in stage. The NCR data contains information about the clinical nodal status, but not the basis for this staging (eg. palpability of the

nodes, imaging techniques, FNA or surgical staging), therefore the ARS calculated in this study represents a modified version of the originally described axillary scoring method by Rodenhuis et al [8]. The final NRI was determined by adding the BRS and ARS, and dividing this number by the sum of obtainable points. This implies that the NRI would always be a value between 0 and 1, with a 0 signifying an absent response and 1 a pCR.

Endpoints

Five-year recurrence-free survival (RFS) was defined as the time from final surgery after NAC to the first event. Events that were considered for the primary endpoint were local recurrence, regional recurrence, distant metastases and death due to any cause. Contralateral breast cancer or in situ disease were not included as an event. Patients alive without recurrence within 5 years were censored at the date of last follow-up.

Statistical analysis

The clinicopathological and treatment characteristics of the included patients were evaluated for the total population and according to biomarker subtype. The average NRI was calculated for the total population as well as for the different subtypes.

Association between response and RFS was analyzed using the Kaplan Meier method with log-rank test for categorical variables (eg. pCR) and univaria Cox proportional hazards for continuous variables (eg. NRI). The independent prognostic significance of the NRI for RFS was evaluated by multivariable linear regression analysis adjusting for covariates. Factors included in the model were chosen by backward elimination using univaria Cox regressions with a significance level of 0.10. To assess the prognostic value of the NRI in addition to the value of pCR, a Cox proportional hazards model was used with both pCR and NRI (full model A) as covariates and compared with a model with pCR alone using a likelihood ratio test (reduced model B (nested in A)). Both models were evaluated based on discrimination, meaning the ability to categorize patients in order of risk, by Harrell's C index. The relative quality of the models was calculated by the Akaike information criterion (AIC) and Bayesian information criterion (BIC). The relationship between the NRI and RFS was evaluated by linear prediction models.

Analyses were performed using Stata version 14.1 (Statacorp. 2016. Stata Statistical Software: College Station, TX: StataCorp LP).

Table 1. Clinicopathologic and treatment characteristics of 1,793 analyzed patients treated with neo-adjuvant chemotherapy between 2003-2008

		Total N=1,793		HR+/HER2- N=741		HR+/HER2+ N=180		HR-/HER2+ N=182		HR-/HER2- (TN) N=297		Unknown N=393	
		N	%	N	%	N	%	N	%	N	%	N	%
CLINICAL INFORMATION													
Age in years (median with IQR)			49 (43-57)		49 (44-56)		47 (42-57)		51 (43-60)		47 (40-56)		50 (42-58)
Age (categories)	<35	125	7.0%	31	4.2%	11	6.1%	11	6.0%	40	13.5%	32	8.1%
	35-50	828	46.2%	359	48.5%	98	54.4%	71	39.0%	136	45.8%	164	41.7%
	50-70	751	41.9%	337	45.5%	65	36.1%	78	42.9%	104	35.0%	167	42.5%
	>70	89	5.0%	14	1.9%	6	3.3%	22	12.1%	17	5.7%	30	7.6%
cT	0	3	0.2%	1	0.1%	0	0%	0	0%	2	0.7%	0	0%
	1	146	8.1%	65	8.8%	19	10.6%	6	3.3%	27	9.1%	29	7.4%
	2	664	37.0%	302	40.8%	69	38.3%	45	24.7%	110	37.0%	138	35.1%
	3	454	25.3%	183	24.7%	43	23.9%	58	31.9%	75	25.3%	95	24.2%
	4	526	29.3%	190	25.6%	49	27.2%	73	40.1%	83	28.0%	131	33.3%
cN	N0	588	32.8%	283	38.2%	36	20.0%	36	19.8%	99	33.3%	134	34.1%
	N1	1,060	59.1%	424	57.2%	129	71.7%	119	65.4%	166	55.9%	222	56.5%
	N2	67	3.7%	16	2.2%	4	2.2%	10	5.5%	15	5.1%	22	5.6%
	N3	78	4.4%	18	2.4%	11	6.1%	17	9.3%	17	5.7%	15	3.8%
PATHOLOGY INFORMATION													
Histological type	Ductal	1,482	82.7%	549	74.1%	164	91.1%	177	97.3%	270	90.9%	322	81.9%
	Lobular	200	11.2%	142	19.2%	9	5.0%	2	1.1%	8	2.7%	39	9.9%
	Ductal + lobular	64	3.6%	35	4.7%	5	2.8%	0	0%	5	1.7%	19	4.8%
	Other*	47	2.6%	15	2.0%	2	1.1%	3	1.7%	14	4.7%	13	3.3%
ypT	0	306	17.1%	51	6.9%	42	23.3%	57	31.3%	95	32.0%	61	15.5%
	1	577	32.2%	258	34.8%	66	36.7%	61	33.5%	75	25.3%	117	29.8%
	2	578	32.2%	281	37.9%	50	27.8%	39	21.4%	68	22.9%	140	35.6%
	3	207	11.5%	95	12.8%	14	7.8%	17	9.3%	29	9.8%	52	13.2%
	4	125	7.0%	56	7.6%	8	4.4%	8	4.4%	30	10.1%	23	5.9%
ypN	N0	599	33.4%	206	27.8%	56	31.1%	77	42.3%	131	44.1%	129	32.8%
	N1	615	34.3%	257	34.7%	72	40.0%	56	30.8%	96	32.3%	134	34.1%
	N2	376	21.0%	192	25.9%	25	13.9%	32	17.6%	34	11.5%	93	23.7%
	N3	203	11.3%	86	11.6%	27	15.0%	17	9.3%	36	12.1%	37	9.4%
Grade	Well differentiated	97	5.4%	72	9.7%	1	0.6%	1	0.6%	2	0.7%	21	5.3%
	Moderately differentiated	280	15.6%	134	18.1%	23	12.8%	20	11.0%	30	10.1%	73	18.6%
	Poorly differentiated	439	24.5%	98	13.2%	49	27.2%	55	30.2%	117	39.4%	120	30.5%

	Unknown	977	54.5%	437	59.0%	107	59.4%	106	58.2%	148	49.8%	179	45.6%
Multifocal disease	No	1,164	64.7%	487	65.7%	114	63.3%	124	68.1%	215	72.4%	224	57.0%
	Yes	412	22.9%	210	28.3%	55	30.6%	44	24.2%	50	16.8%	53	13.5%
	Unknown	217	12.4%	44	5.9%	11	6.1%	14	7.7%	32	10.8%	116	29.5%
Biomarker subtype	HR+/HER2-	741	41.3%										
	HR+/HER2+	180	10.0%										
	HR-/HER2+	182	10.2%										
	TN	297	16.6%										
	Unknown	393	21.9%										
INFORMATION REGARDING TREATMENT													
Surgery	BCS	392	21.9%	164	22.1%	46	25.6%	29	15.9%	76	25.6%	77	19.6%
	Mastectomy	1,401	78.1%	577	77.9%	134	74.4%	153	84.1%	221	74.4%	316	80.4%
ALND	Yes	1,604	89.5%	656	88.5%	164	91.1%	173	95.1%	258	86.9%	353	89.8%
	No	189	10.5%	85	11.5%	16	8.9%	9	5.0%	39	13.1%	40	10.2%
Radiotherapy	Yes	1,559	87.0%	649	87.6%	159	88.3%	156	85.7%	260	87.5%	335	85.2%
	No	234	13.1%	92	12.4%	21	11.7%	26	14.3%	37	12.5%	58	14.8%
Endocrine treatment	Yes	1,065	59.4%	674	91.0%	163	90.6%	8	4.4%	12	4.0%	208	52.9%
	No	728	40.6%	67	9.0%	17	9.4%	174	95.6%	285	96.0%	185	47.1%
HER2 and trastuzumab	HER2 negative, no trastuzumab	1,100	61.4%	741	100%	0	0%	0	0%	297	100%	62	15.8%
	HER2 positive, no trastuzumab	90	5.0%	0	0%	40	22.2%	50	27.5%	0	0%	0	0%
	HER2 positive, trastuzumab	274	15.3%	0	0%	140	77.8%	132	72.5%	0	0%	2	0.5%
	Unknown	329	18.4%	0	0%	0	0%	0	0%	0	0%	329	83.7%

Percentages may not add up to 100% due to rounding

Abbreviations: BCS= Breast-Conserving Surgery, ALND= Axillary Lymph Node Dissection, TN=triple negative

*Histologic subtype other (eg, mucinous, medullary, metaplastic carcinoma).

Results

Study population

We identified 2,213 women who were treated with NAC and subsequently underwent surgery in the period 2003-2008. The NRI could be calculated for 1,793 patients; for 310 patients the BRS could not be calculated because of missing information on the clinical (n=48) and/or pathological (n=275) tumor size, while ARS could not be determined for 134 patients due to missing data on the clinical (n=71) and/or pathological (n=64) nodal status.

Median age of the 1,793 patients was 49 years (IQR 43-57). Characteristics of the total patient population as well as according to biomarker subtype can be viewed in Table 1. The majority of patients had locally advanced disease, although we observed an increase in the proportion of patients with smaller tumors over time (35% cT1-2 in 2003 to 55% cT1-2 in 2008).

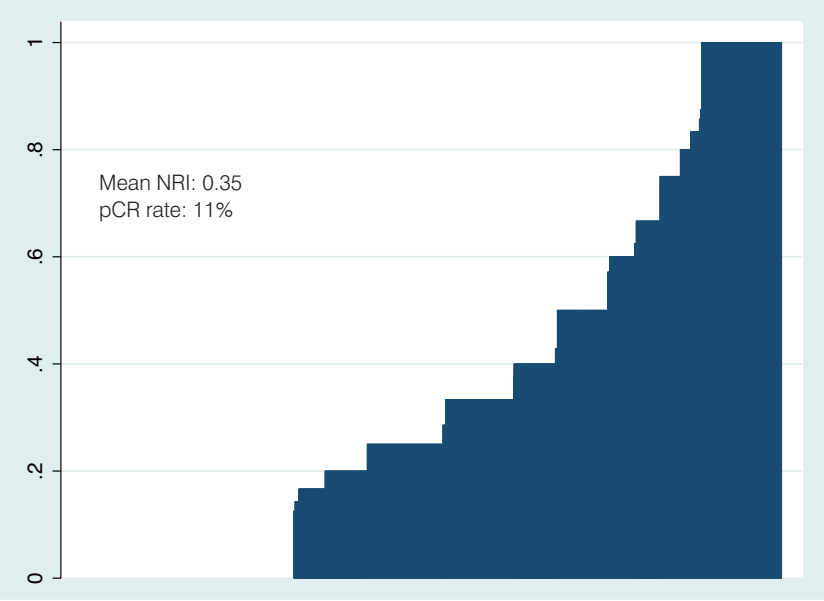


Figure 1. Waterfall plot of the distribution in NRI across the 1,793 patients

Neoadjuvant Response Index

The average NRI in our population-based cohort was 0.35 (median 0.25, range 0-1). The NRI was 0 for 543 patients (30%), indicating that these patients did not achieve any downstaging or even had progression of their disease. In contrast, 205 (11%) patients had a NRI of 1 indicating the achievement of a pCR in both the breast and axilla. The remaining patients (59%) had some degree of response (Figure 1).

Patients with HR+/HER2- tumors showed the least response to NAC (mean NRI 0.25, pCR rate 4%), while those with HR-/HER2+ had the highest NRI (mean 0.53) and the largest proportion of patients that achieved a pCR (23%; Figure 2).

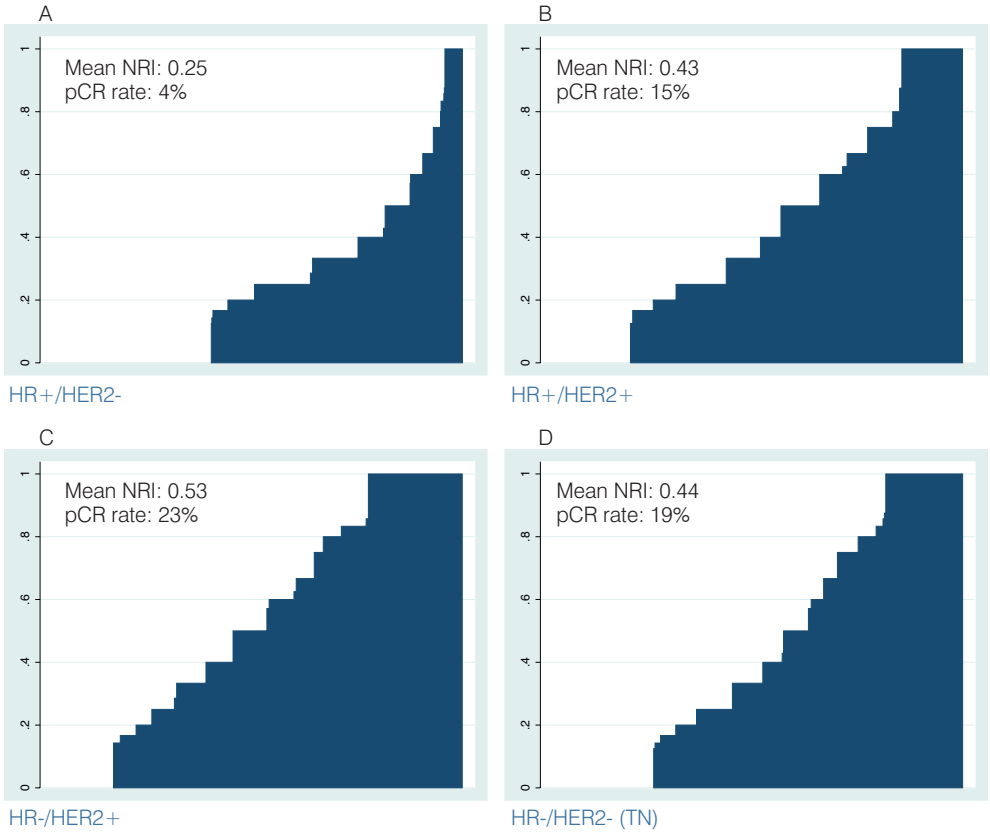


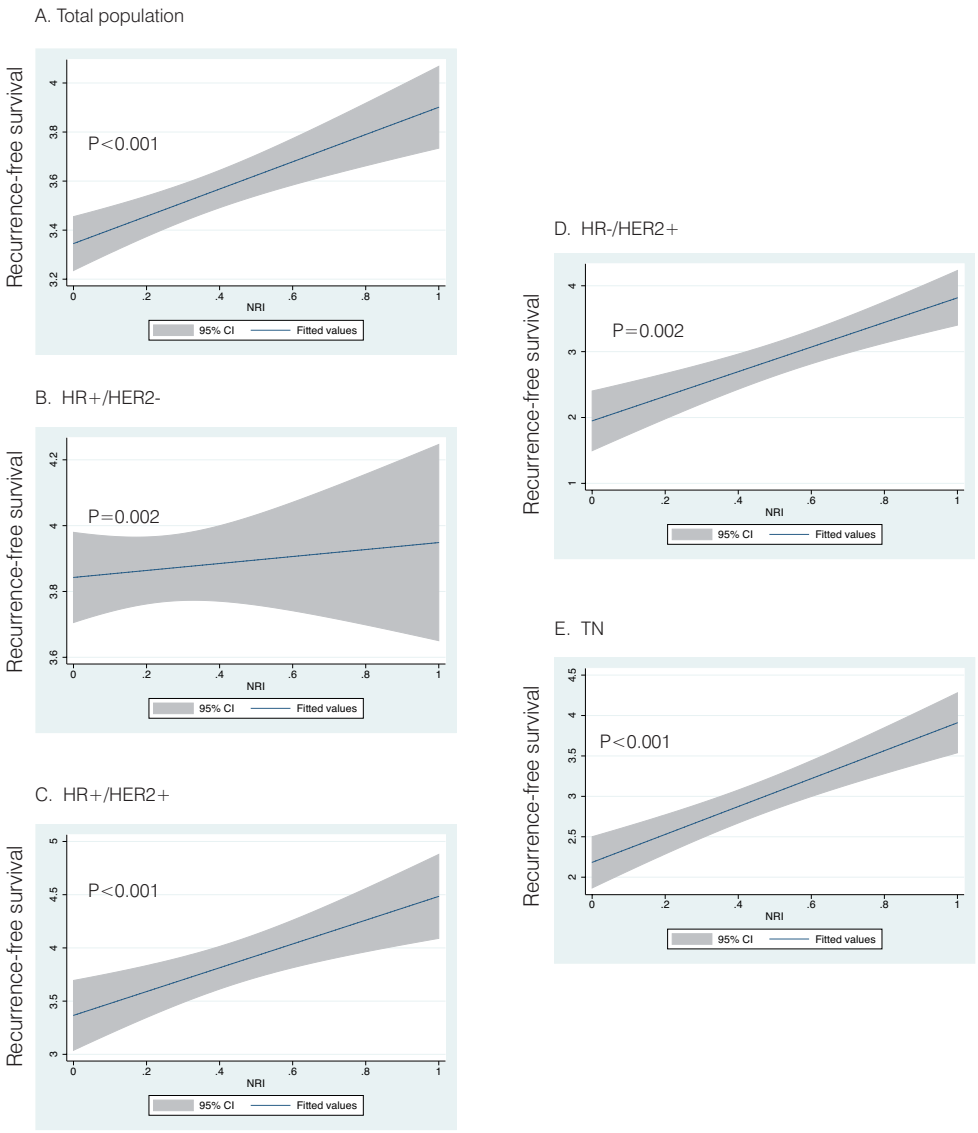
Figure 2. Waterfall plots of NRI according to biomarker subtype (a, b, c, d)

Association NRI and recurrence

A total of 641 events occurred during a median of 4.6 years; 107 local recurrences, 40 regional recurrences, 437 distant metastases and 57 deaths due to any cause. There was a statistically significant difference in 5-year RFS between patients that had achieved pCR versus those with non-pCR (84% vs. 56%, $p<0.001$).

In addition, the NRI was also significantly associated with RFS on a continuous scale (Table 2); a higher NRI was related to improved outcome in terms of 5-year RFS. In multivariable regression analyses adjusting for age, clinical tumor and nodal status, histology, subtype and pCR status, the NRI remained significantly associated with RFS.

The added prognostic value of the NRI in addition to considering pCR as a binary parameter was evaluated by comparing a multivariable model with both pCR and NRI as covariates with a model with pCR alone, showing an increase in the prognostic prediction in benefit of the model including the NRI (Harrell's C-index for both models, p-value of likelihood ratio <0.0001). The significant association between the NRI and RFS is graphically shown in Figure 3 (A-E) and is present for all biomarker subtypes. In contrast, although achievement of pCR was a significant prognosticator for the overall population, this was not the case when evaluated according to biomarker subtype with less discriminative ability regarding RFS in the HR+ subgroups (HR+/HER2- $p=0.107$, HR+/HER2+ $p=0.079$, HR-/HER2+ $p=0.001$ and TN (HR-/HER2-) $p=0.001$, not shown).



Y-axis displays recurrence-free survival, censored at 5 years

P-values calculated by multivariable Cox regression analysis

Figure 3. Association between NRI and recurrence-free survival

Table 2. Univariable and multivariable analysis of prognostic factors for recurrence-free survival

		Number of patients	Number of events	Univariable analyses		Multivariable models					
						pCR and NRI		NRI alone		pCR alone	
				HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Age (continuous)		1,793	641	1.02 (1.01-1.03)	<0.0001	1.01 (1.00-1.02)	0.037	1.01 (1.00-1.02)	0.037	1.01 (1.00-1.02)	0.004
cT	0	3	0	1.37e-19 (-)		1.81e-13 (-)	1	6.68e-14 (-)	1	3.03e-14	1
	1	146	30	REF		REF		REF		REF	
	2	664	177	1.34 (0.91-1.97)	<0.0001	1.57 (1.07-2.32)	0.022	1.58 (1.07-2.32)	0.022	1.49 (1.01-2.20)	0.044
	3	454	167	1.95 (1.32-2.87)		2.43 (1.64-3.60)	<0.0001	2.43 (1.64-3.60)	<0.0001	2.12 (1.43-3.14)	<0.0001
	4	526	267	3.13 (2.15-4.57)		3.65 (2.48-5.37)	<0.0001	3.65 (2.49-5.37)	<0.0001	2.93 (2.00-4.30)	<0.0001
cN	N0	588	136	REF		REF		REF		REF	
	N1	1,060	425	1.95 (1.61-2.37)	<0.0001	1.91 (1.57-2.32)	<0.0001	1.91 (1.57-2.32)	<0.0001	1.84 (1.51-2.23)	<0.0001
	N2	67	35	3.04 (2.10-4.41)		2.53 (1.73-3.69)	<0.0001	2.53 (1.73-3.69)	<0.0001	2.44 (1.67-3.56)	<0.0001
	N3	78	45	3.40 (2.43-4.76)		3.20 (2.26-4.54)	<0.0001	3.21 (2.26-4.54)	<0.0001	2.74 (1.93-3.88)	<0.0001
Histologic type	Ductal	1,482	554	REF		REF		REF		REF	
	Lobular	200	55	0.66 (0.50-0.88)	0.0058	0.77 (0.58-1.04)	0.086	0.77 (0.58-1.04)	0.085	0.85 (0.64-1.14)	0.288
	Ductal + lobular	64	22	0.85 (0.56-1.31)		0.85 (0.55-1.30)	0.448	0.84 (0.55-1.30)	0.445	0.97 (0.63-1.49)	0.884
	Other*	47	10	0.52 (0.28-0.98)		0.54 (0.29-1.01)	0.054	0.54 (0.29-1.01)	0.053	0.54 (0.29-1.02)	0.057
Grade	Well differentiated	97	16	REF		REF		REF		REF	
	Moderately differentiated	280	96	2.30 (1.36-3.91)	<0.001	1.89 (1.11-3.22)	0.019	1.89 (1.11-3.22)	0.019	1.90 (1.11-3.23)	0.018
	Poorly differentiated	439	223	4.08 (2.46-6.78)		2.76 (1.65-4.64)	<0.0001	2.76 (1.65-4.64)	<0.0001	2.70 (1.61-4.53)	<0.0001
	Unknown	977	306	2.09 (1.26-3.45)		1.98 (1.19-3.29)	0.008	1.98 (1.19-3.30)	0.008	1.81 (1.09-3.01)	0.022
Multifocal disease	No	1,164	405	REF							
	Yes	412	147	1.02 (0.84-1.23)	0.1352			Not included			
	Unknown	217	89	1.26 (1.00-1.59)							
Biomarker subtype	HR+/HER2-	741	208	REF		REF		REF		REF	
	HR+/HER2+	180	64	1.27 (0.96-1.68)		1.18 (0.88-1.57)	0.266	1.18 (0.88-1.57)	0.265	1.05 (0.79-1.40)	0.747
	HR-/HER2+	182	91	2.26 (1.77-2.90)	<0.0001	2.20 (1.68-2.87)	<0.0001	2.20 (1.68-2.87)	<0.0001	1.79 (1.38-2.32)	<0.0001
	TN	297	130	2.01 (1.61-2.50)		2.21 (1.75-2.80)	<0.0001	2.21 (1.75-2.80)	<0.0001	1.90 (1.51-1.54)	<0.0001
	Unknown	393	148	1.47 (1.19-1.82)		1.35 (1.09-1.68)	0.007	1.35 (1.09-1.67)	0.007	1.24 (1.00-1.54)	0.050
pCR	No	1,588	606	REF	<0.0001	REF				REF	
	Yes	205	35	0.40 (0.29-0.57)		0.97 (0.63-1.49)	0.885			0.37 (0.26-0.53)	<0.0001
NRI (continuous)		1,793	641	0.46 (0.35-0.59)	<0.0001	0.23 (0.16-0.34)	<0.0001	0.23 (0.17-0.31)	<0.0001		
Harrell's C-index						0.7200		0.7199		0.7037	
AIC						8934.164				8999.374	
BIC						9043.963				9103.683	

Due to the large amount of missing data, grade was not included in the multivariable model

Discussion

In this study, NRI as a continuous variable, representing the quantitative response to NAC, was significantly associated with 5-year RFS in the overall population. Its prognostic value was present across all four biomarker subtypes. The largest discriminative effect was seen in the hormonal receptor negative subgroups, similar to the prognostic implications of subtype-related pCR[5,6]. These results are in accordance with those presented in the original paper from Rodenhuis et al[8].

The mean NRI varied by biomarker subtype in benefit of the HR- subtypes, with the lowest NRI (0.25) in the group of patients with HR+/HER2- disease as compared to 0.53 in the HR-/HER2+ subgroup. These results are in accordance with those presented by Rodenhuis et al[8]. Especially in the HR+ patients, further quantification of the change in disease extent using the NRI could therefore be of prognostic value.

Patients with HR+ tumors are less likely to achieve a pCR than those with the more chemotherapy-sensitive HR- tumors. In this study, pCR rates were 4% and 15% in the HR+ subgroups (HR+/HER2- and HR+/HER2+ respectively) versus 23% and 19% in the HR- biomarker subtypes (HR-/HER2+ and TN respectively). The achievement of pCR has been considered a potential surrogate marker for survival and has even been adopted as an alternative primary endpoint for neoadjuvant clinical trials over the past years[1,10]. However, previous studies have shown that pCR correlates with survival in HR- tumors, but in HR+, particularly in luminal A or luminal B/HER2-positive disease, the low pCR rate is not related to a poor outcome and will thus not be a useful surrogate endpoint[6].

Major strengths of this study are the population-based design and large study population with complete 5-year follow-up which make the results generally applicable. Data were registered in the NCR by trained personnel, using a standardized coding manual. This study also has a number of limitations. Firstly, fewer patients were included in the years 2007-2008 due to the fact that for these years collection was only performed on request. These patient data consisted of a heterogeneous group in which exclusion, based on their clinicopathological and recurrence data in previous years, should not have affected the representativeness of the nationwide breast cancer population for the period 2007-2008. Data on certain biological tumor factors such as hormone and in particular HER2 receptor status were limited before 2005 as they were not yet routinely assessed and central pathology review was not performed. Furthermore, for over 50% of patients' data regarding tumor grade was missing. This is largely explained by the absence of evaluable tumor material after pCR and tumor degradation following NAC. Finally, in this study we stratified patients according to biomarker subtype, defined by hormone receptor and HER2

expression. This classification may not be as accurate as the biomarker subtype classification based on gene expression, although these biomarker subtypes have also been shown to provide prognostic information similar to the corresponding molecular subtypes[11].

In the present study, in situ carcinoma was categorized as no residual disease. This could have resulted in an overestimation of the recurrence risk after achieving a pCR. Von Minckwitz et al[6]. have stated that no invasive nor in situ residual disease in breast or lymph nodes best distinguishes patients favorable and unfavorable outcomes. However, in the CTNeoBC pooled analysis presence of carcinoma in situ did not have an influence on long-term outcome[5].

The NRI as applied in this study is a modified simpler version of the NRI presented by Rodenhuis et al[8]. This adaption was required due to the use of NCR data in which exact information regarding method of diagnosis (eg. ultrasound, MRI) and applied treatment other than administered (eg. type of chemotherapy) is missing. Palpability of lymph nodes, an important factor for both the clinical and pathological staging in the Rodenhuis et al[8]. publication, was not available and the sentinel node biopsy was not always performed before the start of NAC (n=167; 9% of patients). The presented NRI requires information that is generally available and is therefore an easily applicable instrument to determine the level of response to NAC.

Another measure to calculate efficacy of neoadjuvant treatment is residual cancer burden (RCB) described by Symmans et al.[7]. The RCB is based on the measurement and cellularity of residual disease and percentage of in situ disease as well as the number and diameter of involved lymph nodes at pathology. Although this measurement also includes more information than just achievement of pCR, the characteristics of the primary tumor before neoadjuvant treatment are not taken into account. On the other hand, the prognostic CPS+EG (Clinical + Pathological Stage, Estrogen receptor and Grade)[12,13] and the upgraded Neo-Bioscore (CPS+EG+HER2)[14] scoring systems do include primary tumor information, but also lack the stepwise quantification of tumor response that the NRI provides. Addition of more biological information such as grade and receptor status could further improve the tailored prognostic information of the NRI.

In conclusion, in this population-based study the NRI provided additional prognostic information beyond the achievement of pCR. The NRI is a useful and simple instrument to determine chemotherapy sensitivity of micrometastatic disease and could be applied to compare the efficacies of different chemotherapy regimens offering guidance in choice of any adjuvant treatment in patients with non-pCR.

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CHAPTER 8

Do patients whose tumor achieved a pathological response relapse at specific sites? A substudy of the EORTC 10994/BIG-1-00 trial

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Breast Cancer Res Treat. 2018; 169(3):497-505

Abstract

Purpose

To determine the sites of first distant relapse in patients with or without pCR following neoadjuvant chemotherapy in breast cancer patients enrolled in the EORTC 10994/BIG-1-00 trial.

Methods

We included patients enrolled in the EORTC 10994/BIG-1-00 trial who received at least one chemotherapy cycle before surgery and who had been diagnosed with a distant relapse. pCR was defined as no evidence of residual invasive cancer in the primary tumor and axillary lymph nodes with or without residual ductal carcinoma in situ. Site of first distant relapse was categorized as 'soft tissue,' 'visceral,' 'skeletal,' 'central nervous system (CNS),' and 'other.' The association between relapse site and achievement of pCR was assessed using multivariate logistic regression models for molecular subtypes classification and preceding locoregional recurrence.

Results

The study included 383 (21%) eligible patients out of the 1856 randomized, of whom 28 (7%) had achieved pCR. Median follow-up was 5.4 years. Achievement of pCR was associated with a trend towards a decreased presentation of skeletal metastases [21% (pCR) vs. 50% (non-pCR), OR 0.32, adjusted p value = 0.071] and an increase in the proportion of patients with CNS metastases as first distant relapse site (21% vs. 9%, OR 2.39, adjusted p value = 0.183). Patients with pCR were more likely to present with only one relapse location category when compared to non-pCR (86% vs. 69%).

Conclusion

Patients that achieved a pCR appeared less likely to present with skeletal metastases and more frequently presented with CNS metastases as first site of distant relapse, even after adjustment for molecular subtypes.

Introduction

Neoadjuvant systemic therapy is a well-established strategy for locally advanced disease with the aim of downstaging the tumor to enable more conservative surgery. Additionally, over the last few years, it is also increasingly applied in early-stage breast cancer patients requiring systemic treatment showing similar long-term outcome as compared to adjuvant administration [1–4]. The early administration of systemic therapy also permits 'in vivo' monitoring of the efficacy of administered systemic treatment [5].

The achievement of a pathologic complete response (pCR) after neoadjuvant treatment is associated with improved long- term survival and a decrease in both locoregional and distant metastases [5]. The pCR rate varies according to molecular subtype as does the association between pCR and long-term outcomes, with the strongest correlation for the more aggressive subtypes [5–7].

The widely accepted explanation for this association is that the biology of the primary tumor and the micrometastatic disease are similar, and respond equally to the systemic neoadjuvant therapy. However, at least 10% of patients whose tumor achieved a pCR develop a recurrence within 5 years and more than two-thirds of patients whose tumor did not achieve a pCR will not relapse [5]. In patients with triple-negative tumors, in whom neither adjuvant hormonal nor trastuzumab therapies are going to interfere with the association between pCR and outcome, 15–20% of patients whose tumor achieved a pCR develop a recurrence within 5 years and 50% of patients whose tumor did not achieve a pCR will not relapse [5, 6, 8]. Thus, although pCR is a good prognosticator for survival it is not perfect. Several series have reported differences in estrogen receptor (ER) or human epidermal growth receptor 2 (HER2) status between the primary tumor and distant metastases in up to 32 and 14.5%, respectively [9, 10]. The biology of the micrometastatic disease in distant sites is certainly more com- plex than the biology of the primary tumor [11]. One could hypothesize that the correlation between pCR and survival is excellent in some specific sites while it remains poor in other sites. The first obvious example would be central nervous system metastasis. To our knowledge, this question has never been addressed.

The aim of this study was to evaluate whether the sites of first distant relapse differed between patients whose tumor achieved a pCR after neoadjuvant chemotherapy versus those who did not in the EORTC 10994/BIG-1-00 trial [12].

Methods

Study design, eligibility, and treatment

The EORTC 10994/BIG 1-00 trial enrolled patients aged ≤ 70 years with large operable or locally advanced/ inflammatory breast cancer without evidence of distant metastases, who were candidates for neoadjuvant treatment. Patients were randomly assigned to receive either six cycles of anthracycline-based chemotherapy (FEC), or a taxane-based regimen, docetaxel for three cycles followed by epirubicin + docetaxel for three cycles (T-ET), all given prior to primary surgery. Subsequent locoregional treatment was determined according to guidelines described in the study protocol. Women with hormone receptor-positive tumors were recommended to receive adjuvant endocrine therapy for 5 years. Patients with HER2-positive tumors were allowed to enter adjuvant clinical trials assessing trastuzumab or to receive this treatment in the adjuvant setting when it became standard practice [12].

For this substudy, we selected a subgroup of patients based on the following criteria: (i) Patients eligible in the EORTC 10994/BIG 1-00 trial, (ii) patients who received at least one cycle of neoadjuvant chemotherapy and who underwent surgery, (iii) patients who had a known pathological response status, and (iv) patients diagnosed with a distant relapse after surgery of which the site was specified. Patients with T4d tumors or who received radiotherapy before surgery were excluded from the analysis.

Pathologic complete response was defined in this sub- study as no evidence of residual invasive cancer (or very few scattered tumor cells) in the primary tumor and axillary lymph nodes with or without residual ductal carcinoma in situ (DCIS). Information on Ki-67 was not collected within the main study. Therefore, tumor subtype classification was performed according to the simplified approach as proposed in the 2011 St. Gallen consensus, where Ki-67 is replaced by tumor grade [13] (Supplement A). Tumor histology, grade, ER, progesterone receptor (PgR), and HER2 status were based on local pathology assessment of the diagnostic biopsy.

Objectives and and-points

The primary objective of this study was to assess whether there are differences in the sites of first distant relapse between patients who achieved a pCR after neoadjuvant chemotherapy versus those who did not (non-pCR). Secondary objectives included (1) describing the differences in site of first distant relapse in the pCR and non-pCR groups by breast cancer subtype, (2) describing the clinicopathological characteristics of patients according to site of first distant relapse, (3) evaluating the effect of a preceding or concomitant locoregional recurrence (LR) on the occurrence of a specific site as first distant relapse, (4) studying the association between concomitant sites of first distant relapse.

We evaluated the first site of distant relapse as reported in the case report forms, i.e., 'soft tissue,' 'visceral,' 'skeletal,' 'CNS,' or 'other.' In case of multiple lesions, all concomitant lesions at first presentation were included. As part of the secondary objectives, invasive locoregional recurrences were considered if they occurred before or at the same time of the first distant relapse. Locoregional recurrences were ipsilateral invasive breast recurrences and regional recurrences (chest wall and regional lymph nodes: axillary, internal mammary, infra, and supraclavicular).

Statistical analysis

A statistical analysis plan was prospectively developed. The association between the occurrence of a specific site of first distant relapse and pCR status was evaluated using four multivariate logistic regression models, one for each site ('soft tissue,' 'visceral,' 'skeletal,' 'CNS') except 'other,' adjusting for intrinsic subtype and preceding locoregional recurrence (yes/no). Sites of first distant relapse classified as 'other' were not evaluated because the obtained estimation would have not been interpretable due to the heterogeneity of the corresponding sites. The association with pCR was assessed using the Wald Chi-square test with adjusted p values for multiple testing (Benjamini–Hochberg correction). A p value < 0.05 was considered statistically significant. Sensitivity analyses were subsequently performed, with univariate and multivariate models adjusting for age, clinical tumor and nodal status, histologic tumor type, subtype, and allocated chemotherapy regimen. Differences in site of first distant relapse between the molecular subtypes according to pCR- status, patient and tumor characteristics, and the presence of preceding locoregional recurrence per relapse site, and the occurrence of concomitant sites of first distant relapse were tabulated (no formal statistical testing was done due to the limited number of patients in the subgroups).

All statistical analyses were performed using SAS soft- ware version 9.4 (SAS Institute).

Results

Of the 1856 patients randomized in the trial, 383 patients diagnosed with a distant relapse were eligible for this substudy, of whom 28 (7%) were in the pCR-group and 355 (93%) in the non-pCR group. Reasons for ineligibility are shown in the consort diagram (Supplement B). The median follow-up was 5.4 years from date of randomization. Baseline patient and tumor characteristics according to pCR status are presented in Table 1. Median age of included patients was 49 years and most patients had clinically node-positive disease (271/383; 71%). Overall, visceral (197/383; 51%) and skeletal metastases (185/383; 48%) were the most common sites of first distant relapse (Table 2).

Table 1. Baseline characteristics of 383 included patients

	pCR N= 28		Non-pCR N= 355		Total N= 383	
	N	%	N	%	N	%
Median age at diagnosis	48		49		49	
Age at diagnosis						
≤40	7	25.0	83	23.4	90	23.5
41-50	9	32.1	123	34.6	132	34.5
51-70	12	42.9	149	42.0	161	42.0
Menopausal status						
Premenopausal	15	53.6	207	58.3	222	58.0
Postmenopausal	13	46.4	148	41.7	161	42.0
cT stage						
T1-2	11	39.3	126	35.5	137	35.8
T3	15	53.6	160	45.1	175	45.7
T4	2	7.1	69	19.4	71	18.5
cN stage						
N0	13	46.4	97	27.3	110	28.7
N1	13	46.4	224	63.1	237	61.9
N>1	2	7.1	32	9.0	34	8.9
Unknown	0	0.0	2	0.6	2	0.5
Tumor histology						
Ductal	26	92.9	291	82.0	317	82.8
Lobular	2	7.1	43	12.1	45	11.7
Other	0	0.0	18	5.1	18	4.7
Missing	0	0.0	3	0.8	3	0.8
Tumor grade						
I	0	0.0	14	3.9	14	3.7
II	12	42.9	153	43.1	165	43.1
III	16	57.1	130	36.6	146	38.1
Not assessed/unknown	0	0.0	58	16.4	58	15.1
Subtype						
Luminal A-like	3	10.7	82	23.1	85	22.2
Luminal B-like (HER2-negative)	1	3.6	42	11.8	43	11.2
Luminal B-like (HER2-positive)	6	21.4	64	18.0	70	18.3
HER2+, non-luminal-like	6	21.4	34	9.6	40	10.4
Triple negative	6	21.4	55	15.5	61	15.9
Unknown	6	21.4	78	22.0	84	21.9
Neoadjuvant chemotherapy regimen						
FEC	17	60.7	193	54.4	210	54.8
T-ET	11	39.3	162	45.6	173	45.2
Number of cycles						
1	0	0.0	1	0.3	1	0.3
2	0	0.0	3	0.8	3	0.8
3	0	0.0	5	1.4	5	1.3
4	0	0.0	5	1.4	5	1.3
5	1	3.6	4	1.1	5	1.3
6	27	96.4	337	94.9	364	95.0

Type of surgery						
BCS	15	53.6	100	28.2	115	30.0
Mastectomy	13	46.4	254	71.5	267	69.7
Unknown	0	0.0	1	0.3	1	0.3
Preceding locoregional recurrence						
No	21	75.0	294	82.8	315	82.2
Yes	7	25.0	61	17.2	68	17.8

BCS Breast-conserving surgery, *pCR* pathological complete response, *cT* clinical tumor, *cN* clinical lymph nodes, *FEC* 5-fluorouracil, epirubicin, and cyclophosphamide, *T-ET* docetaxel x3 –epirubicin + docetaxel x3
Percentages may not add up to 100% due to rounding

Table 2. Association between site of first distant relapse and pCR status after neoadjuvant chemotherapy

	pCR N=28	**non-pCR N=355**	**Total N=383**	**Univariate**	**Multivariate[a]**	
				pCR vs. non-pCR	**pCR vs. non-pCR**	
	N (%)	N (%)	N (%)	OR (95% CI)	OR (95% CI)	*Adj. P*-value
Soft tissue						
No	24 (86%)	312 (88%)	336 (88%)			
Yes	4 (14%)	43 (12%)	47 (12%)	1.21 (0.40-3.65)	0.94 (0.30-2.95)	.909
Visceral						
No	14 (50%)	172 (48%)	186 (49%)			
Yes	14 (50%)	183 (52%)	197 (51%)	0.94 (0.44-2.03)	0.80 (0.36-1.74)	.756
Skeletal						
No	22 (79%)	176 (50%)	198 (52%)			
Yes	6 (21%)	179 (50%)	185 (48%)	0.27 (0.11-0.68)	0.32 (0.12-0.82)	.071
CNS						
No	22 (79%)	323 (91%)	345 (90%)			
Yes	6 (21%)	32 (9%)	38 (10%)	2.75 (1.04-7.29)	2.39 (0.87-6.58)	.183
Other						
No	24 (86%)	292 (82%)	316 (83%)			
Yes	4 (14%)	63 (18%)	67 (17%)	0.77 (0.26-2.31)	0.73 (0.24-2.22)	NA[b]

pCR pathological complete response, *CNS* central nervous system
[a]Adjusted for subtype and preceding locoregional relapse (yes/no)
[b]The odds ratio for the 'other' category is displayed without the corresponding *p* value, bringing the number of *p* values included in the Benjamini–Hochberg correction to 4-tested values

Table 3. Site of first distant relapse according to pCR status per tumor subtype

	Lum-A		Lum-B (HER2-)		Lum-B (HER2+)		HER2+		Triple Negative		Unknown	
	pCR N=3	Non-pCR N=82	pCR N=1	Non-pCR N=42	pCR N=6	Non-pCR N=64	pCR N=6	Non-pCR N=34	pCR N=6	Non-pCR N=55	pCR N=6	Non-pCR N=78
Soft tissue												
N	0	4	0	4	1	10	1	6	1	8	1	11
%	0%	5%	0%	10%	17%	16%	17%	18%	17%	15%	17%	14%
Visceral												
N	1	36	1	14	3	38	4	19	4	33	1	43
%	33%	44%	100%	33%	50%	59%	67%	56%	66%	60%	17%	55%
Skeletal												
N	2	50	0	26	1	31	1	12	1	17	1	43
%	67%	61%	0%	62%	17%	48%	17%	35%	17%	31%	17%	55%
CNS												
N	0	2	0	2	2	3	1	5	1	8	2	12
%	0%	2%	0%	5%	33%	5%	17%	15%	17%	15%	33%	15%
Other												
N	0	12	0	7	1	10	0	7	1	9	2	18
%	0%	15%	0%	17%	17%	16%	0%	21%	17%	16%	33%	23%

CNS Central Nervous System

Percentages displayed for column

Patients could have presented with multiple sites of relapse then percentages are not cumulative

Association between pCR status and site of first distant relapse

Patients whose tumor achieved pCR were less likely to present with skeletal metastases as compared to non-pCR patients (6/28 (21%) versus 179/355 (50%) patients; OR 0.32, 95% CI 0.12–0.82; p = 0.071, Table 2). A similar trend was observed in all subtypes apart from Luminal A-like, though the numbers are very small (Table 3).

Conversely, the proportion of patients with CNS metastasis as first metastatic site was numerically higher in the pCR-group as compared to in the non-pCR group (6/28 (21%) versus 32/355 (9%) patients; OR 2.39, 95% CI 0.87–6.58; p = 0.183, Table 2). This difference was greatest in the HER2 + Luminal B-like subtype (Table 3). These differences in the incidence of skeletal and CNS metastases between patients whose tumor did or did not achieve pCR remained after further adjustment for age, histologic type, clinical node and tumor status, subtype, and received chemo- therapy regimen (data not shown).

For the remaining sites (soft tissue and visceral), we did not observe an association with pCR status (Table 2).

Clinicopathological characteristics of patients according to site of first distant relapse

Patients with soft tissue and CNS metastases were older and had higher grade tumors as compared to patients with visceral and skeletal metastases (Table 4). Patients presenting with skeletal metastasis more frequently had tumors with a lobular histology, a lower-grade (I and II), and a luminal-like subtype (17, 54, and 59%, respectively). Patients presenting with CNS metastases were predominantly clinically node positive (84% N + vs. 16% N0, Table 4). Furthermore, in these patients, we observed a higher rate of HER2 +/non- luminal and TN breast cancer.

Preceding locoregional recurrence

In 68 patients, the first distant relapse was preceded by or occurred concomitantly with a locoregional recurrence (Table 1). The proportion of patients with a prior LR was highest in those presenting with soft tissue metastases [18/47 (38%) versus 13% (CNS) to 19% (visceral) in the other groups (Table 4)]. We did not observe differences in site of first distant relapse according to pCR status for patients that did or did not have a preceding LR event (results not shown).

Table 4. Patient, tumor, and treatment characteristics according to site of first distant relapse

	Soft tissue N=47		Visceral N=197		Skeletal N=185		CNS N=38		Other N=67	
	N	%	N	%	N	%	N	%	N	%
Median age at diagnosis	50 years		48 years		48 years		53 years		49 years	
Age at diagnosis										
≤40	8	17.0	43	21.8	54	29.2	7	18.4	16	23.9
41-50	16	34.0	80	40.6	59	31.9	10	26.3	27	40.3
51-70	23	48.9	74	37.6	72	38.9	21	55.3	24	35.8
Menopausal status										
Premenopausal	24	51.1	121	61.4	112	60.5	19	50.0	39	58.2
Postmenopausal	23	48.9	76	38.6	73	39.5	19	50.0	28	41.8
cT stage										
T1-2	13	27.7	69	35.0	66	35.7	14	36.8	23	34.3
T3	18	38.3	93	47.2	85	45.9	16	42.1	33	49.3
T4	16	34.0	35	17.8	34	18.4	8	21.1	11	16.4
cN stage										
N0	17	36.2	64	32.5	50	27.0	6	15.8	18	26.9
N1	19	40.4	118	59.9	123	66.5	25	65.8	42	62.7
N>1	10	21.3	14	7.1	12	6.5	7	18.4	7	10.4
Unknown	1	2.1	1	0.5	0	0.0	0	0.0	0	0
Tumor histology										
Ductal	39	83.0	169	85.8	145	78.4	32	84.2	52	77.6
Lobular	3	6.4	16	8.1	32	17.3	4	10.5	12	17.9
Other	5	10.6	11	5.6	7	3.8	1	2.6	2	3.0
Missing	0	0.0	1	0.5	1	0.5	1	2.6	1	1.5
Tumor grade										
I	0	0.0	6	3.0	8	4.3	0	0.0	0	0.0
II	13	27.7	85	43.1	92	49.7	14	36.8	30	44.8
III	24	51.1	74	37.6	59	31.9	18	47.4	25	37.3
Not assessed/unknown	10	21.3	32	16.2	26	14.1	6	15.8	12	17.9
Subtype										
Luminal A-like	4	8.5	37	18.8	52	28.1	2	5.3	12	17.9
Luminal B-like (HER2-negative)	4	8.5	15	7.6	26	14.1	2	5.3	7	10.4
Luminal B-like (HER2-positive)	11	23.4	41	20.8	32	17.3	5	13.2	11	16.4
HER2+, non-luminal-like	7	14.9	23	11.7	13	7.0	6	15.8	7	10.4
Triple negative	9	19.1	37	18.8	18	9.7	9	23.7	10	14.9
Unknown	12	25.5	44	22.3	44	23.8	14	36.8	20	29.9

Neoadjuvant regimen										
FEC	28	59.6	101	51.3	112	60.5	21	55.3	34	50.7
T-ET	19	40.4	96	48.7	73	39.5	17	44.7	33	49.3
Type of surgery										
BCS	10	21.3	59	29.9	51	27.6	11	28.9	22	32.8
Mastectomy	37	78.7	137	69.5	133	71.9	27	71.1	45	67.2
Unknown	0	0.0	1	0.5	1	0.5	0	0.0	0	0.0
Preceding locoregional recurrence										
No	29	61.7	159	80.7	158	85.4	33	86.8	52	77.6
Yes	18	38.3	38	19.3	27	14.6	5	13.2	15	22.4

BCS breast-conserving surgery, *pCR* pathological complete response, *cT* clinical tumor, *cN* clinical lymph nodes, *CNS* central nervous system, *FEC* 5-fluorouracil, epirubicin and cyclophosphamide, *T-ET* docetaxel x3 → epirubicin + docetaxel x3
Percentages are displayed for columns. Percentages may not add up to 100% due to rounding

Association between pCR status and extent of metastatic disease

The proportion of patients who presented with more than one site of metastatic spread was numerically lower in the group of patients whose tumor achieved a pCR as compared to patients in the non-pCR group (4/28 (14%) versus 112/355 (32%) patients with > 1 relapse site, Table 5). Patients with soft tissue as first site of distant relapse most frequently presented with at least one other metastatic site [32/47 patients (68%)]. In 60% of patients with CNS as first site of distant relapse, the CNS was the only site of metastatic disease. Skeletal and visceral metastases were the most common combination in case of more than one site of relapse [70 out of 116 patients (60%) with > 1 metastatic site, Table 5].

Table 5. Occurrence of concomitant sites of first distant relapse

A. Number of concomitant sites of first distant relapse

	Number of concomitant sites of first distant relapse			
	One single site N= 267		More than 1 site N= 116	
	N	%	N	%
pCR status				
pCR	24	*85.7*	4	*14.3*
Non-pCR	243	*68.5*	112	*31.5*
Site of relapse				
Soft tissue	15	*31.9*	32	*68.1*
Visceral	103	*52.3*	94	*47.7*
Skeletal	96	*51.9*	89	*48.1*
CNS	23	*60.5*	15	*39.5*
Other	**30**	***44.8***	**37**	***55.2***

B. Distribution of concomitant sites of first distant relapse

Sites of first distant relapse		Patients with more than one relapse site category (N=116)									
		Soft tissue		Visceral		Skeletal		CNS		Other	
		Yes N=32	No N=84	Yes N=94	No N=22	Yes N=89	No N=27	Yes N=15	No N=101	Yes N=37	No N=79
Soft tissue	*N*			21	11	20	12	4	28	11	21
	%			*22.3%*	*50%*	*22.5%*	*44.4%*	*26.7%*	*27.7%*	*29.7%*	*26.6%*
Visceral	*N*					70	24	10	84	26	68
	%					*78.7%*	*88.9%*	*66.7%*	*83.2%*	*70.3%*	*86.1%*
Skeletal	*N*							7	82	21	68
	%							*46.7%*	*81.2%*	*56.8%*	*86.1%*
CNS	*N*									1	14
	%									*2.7%*	*17.7%*
Other	*N*										
	%										

pCR pathological complete response

In A, Percentages are displayed for rows. Number of patients by site of relapse for those with more than one sites of relapse are not cumulative

In B, Percentages displayed for columns and are not cumulative

Discussion

In this study, there was a numerical difference in presentation with CNS metastasis, with a higher incidence in the pCR group (Odds Ratio 2.39). Of note, CNS metastasis accounted for one-fifth of all first distant metastasis in the pCR group. This finding supports the concept of the brain being a sanctuary site where malignant cells are protected from anti-cancer therapeutics by the blood–brain barrier [14]. Patients with a non-luminal HER2 + or TN subtype have higher pCR rates as compared to patient with luminal subtypes [5] and have been shown to more frequently metastasize to the brain and viscera [15, 16]. Thus, an excess of CNS metastases in the pCR group might be expected in the non-luminal HER2 + and TN subtypes.

Furthermore, patients whose tumor achieved a pCR had a lower rate of skeletal metastasis as first site of distant relapse as compared to patients whose tumor did not achieve pCR (Odds Ratio 0.32). This observed difference could perhaps have been explained by a molecular subtype bias. Patients with a luminal subtype are less likely to achieve a pCR and are known to more frequently metastasize to skeletal tissue [17, 18].

We also evaluated the influence of a preceding LR, since such an event could have an influence on the subsequent distant metastatic spread as these patients might receive additional systemic treatment. Furthermore, the local recurrence itself could give rise to metastatic spread. We did not observe a statistically significant association between a prior LR and any site of first distant relapse after adjustment for pCR status and subtype. These results should be interpreted with caution since the number of LRs preceding distant relapses was relatively low.

The median follow-up of patients included in this study was 5.4 years. This time frame will have an influence on the distribution of relapse sites we observed. Previous studies have shown that patients with a shorter disease- free survival (DFS) more often present with visceral and CNS metastases [19]. The incidence of these metastatic sites reaches its peak in the second year of follow-up after which the incidence declines, whereas bone metastases can even occur later on. Furthermore, patients with a shorter DFS are more often of the TN subtype which is known to metastasize frequently to the visceral tissue as is also demonstrated in this study [8, 20, 21]. Longer follow-up is needed.

This study has strengths and limitations. A major strength of the study is that the population consists of patients from a large randomized trial with a total of 383 patients with events of interest. The main limitation is the small number of patients in the pCR group, which prohibits drawing firm conclusions from this study. This limitation is even more important when trying to analyze the results by molecular subtypes. It is well known that the metastatic behavior and

prognosis of breast cancer are dependent on tumor biology of the different intrinsic subtypes [22]. We attempted to adjust for possible molecular subtype bias by performing multivariate analyses.

In conclusion, there appear to be differences in the occurrence of tissue-specific sites of first distant relapse between patients that achieved pCR after neoadjuvant chemotherapy when compared to those that did not, even after adjustment for molecular subtypes. The trends observed in the present study need to be confirmed in a meta-analysis as well to establish the clinical implication on long-term prognosis.

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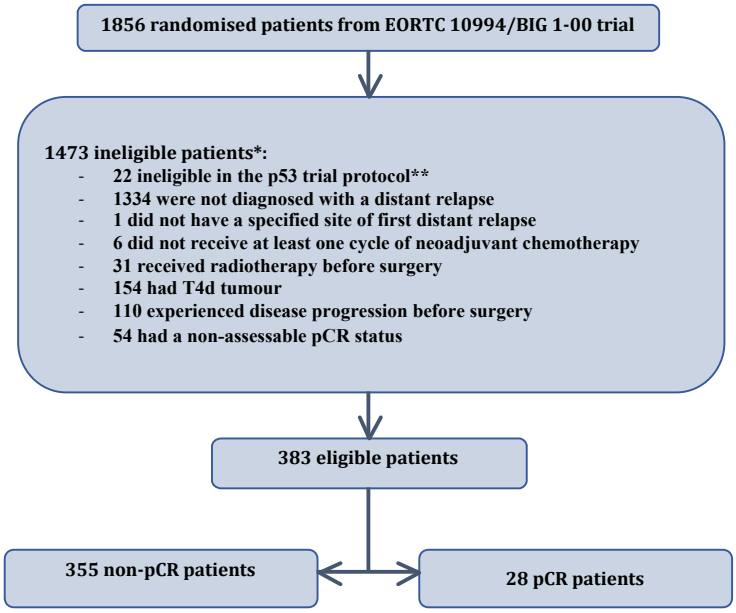
Supplement A: Simplified breast cancer subtype classification proposed by the 2011 St. Gallen consensus

Breast cancer subtype	ER status		PgR status	Her2 status	Tumor grade
Luminal-A like	ER+	*and/or*	PgR+	Her2-	Grade 1 or 2
Luminal-B like (HER2-)	ER+	*and/or*	PgR+	Her2-	Grade 3
Luminal-B like (HER2+)	ER+	*and/or*	PgR+	Her2+	Any
HER2+, non-luminal	ER-		PgR-	Her2+	Any
Triple Negative	ER-		PgR-	Her2-	Any

Supplement B: Consort diagram



* Patients can be ineligible for more than one reason.
** Reasons for ineligibility are listed in the original 10994 trial publication[12]

CHAPTER 9

General discussion and future perspectives

General discussion

The main focus of this thesis was to evaluate breast cancer recurrence and related prognostic factors to support further tailoring breast cancer treatment with the aim of improved outcome and reduced morbidity. Over the past decades, surgical management of breast cancer has become increasingly less aggressive. Nevertheless, local and regional recurrence rates are decreasing over time and have become very low. For the majority of patients, the local recurrence rate has become even lower than the risk of developing contralateral breast cancer (***chapter 2***). This very low risk of local disease relapse suggests that in a subset of patients, further de-escalation of local treatment is feasible.

Influence of clinicopathologic factors

The risk of recurrence is associated with several traditional clinicopathological parameters like age, tumor size, nodal involvement, grade, hormone receptor status and HER2 expression. These well-known factors were confirmed prognostic factors in our work as well. The improved outcome and increasing knowledge on tumor biology, raise the question whether these factors are independent prognosticators or related to one another within the context of more in-depth biological profiles.

Several studies have reported young age as an independent prognostic factor for poor outcome[1,2]. This has long resulted in more intensive treatment in this patient population, evidenced by higher rates of mastectomy and chemotherapy administration[3]. Young age at diagnosis is associated with unfavorable breast cancer biology[4–6]. This is reflected by higher frequencies of poor differentiation grade, metastatic lymph node involvement, hormone receptor negative disease, HER2 overexpression and a triple negative molecular subtype (***chapters 3 and 4***). Hereditary predisposition and germline BRCA 1 and BRCA2 mutations are more frequently encountered in young women with breast cancer as well[7].

In a Dutch population-based cohort of women aged <35, we demonstrated relatively low rates of local and regional recurrence. In addition, we observed differences in local recurrence rates when evaluated according to hormone receptor and HER2 derived biomarker subtype, with higher rates in the HR-/HER2+ and triple negative biomarker subtypes. These data suggest that not young age in itself, but the biology of the disease is indicative of the risk of recurrence. The more aggressive biology of breast cancer in young women is supported by the observation that a greater proportion of women aged <45 had a high risk 70-gene signature as compared to women aged 45-55 or >55 year of age (***chapter 4***). Still, the 5-year distant metastasis free survival rate was comparable for young breast cancer patients and their older counterparts.

As described above, young women are at a higher risk of being over treated on the basis of age considerations. It remains unclear whether more aggressive local and systemic therapy is required and beneficial for the prognosis of all young women, while the negative implications with regards to toxicity, fertility and psychosocial wellbeing are considerable. To this end, it is unfortunate that young women are underrepresented in studies evaluating molecular tools to personalize and possibly de-escalate systemic treatment[8,9].

The Tumor-Node-Metastasis staging system has been applied for over 60 years to determine a patient's risk and tailor treatment. Axillary lymph node status is an established prognostic factor for long-term outcome[10,11]. Following the introduction of screening programs, breast cancer is more often diagnosed in early stages. Consequently, the majority of patients diagnosed with breast cancer will have clinically node-negative disease. Sentinel lymph node biopsy (SNB) has become standard procedure for the nodal staging, confirming a negative nodal status in approximately 75% of patients[12,13].

In up to 20% of SNB procedures lymphatic drainage to the internal mammary (IM) lymph nodes is observed, yet these nodes are not routinely sampled[14]. Some reports have advocated routine SNB of the internal mammary chain for optimal staging, given the prognostic value of isolated internal mammary metastasis and effect on adjuvant systemic therapy and radiotherapy decision-making reported in earlier studies[15,16]. We evaluated the prognostic value of internal mammary lymph node metastasis and did not observe a statistically significant effect on overall survival when correcting for other prognostic factors such as axillary metastasis (***chapter 5***). Figure 2 in this chapter illustrates that the negative implications of tumor-positive axillary lymph nodes increase with the involvement of additional lymph nodes, with an estimated 25% risk of death increase per involved node. A positive IM node approximates the risk associated with one involved axillary lymph node.

Results from the EORTC 22922/10925 trial evaluating the effect of radiation to the internal mammary and medial supraclavicular lymph nodes in patients without confirmed IM involvement demonstrated small absolute differences in disease-free and overall survival[17]. The Dutch guideline recommends offering internal mammary irradiation when macrometastatic disease in the internal mammary chain has been confirmed through either pathology or imaging, although routine sampling of internal mammary nodes is not incorporated[3]. When drainage to the internal mammary chain is observed without further evidence of metastasis (for example when the internal mammary nodes have not been removed), radiotherapy can be considered. In that perspective, although SLNB of IM nodes has limited prognostic value, determining the IM status either through biopsy or PET-CT can enhance more selective treatment especially for patients with isolated IM metastases without axillary nodal involvement.

On the other hand, the value of performing routine axillary SNB is currently under debate, since the result of the SNB for most patients is no longer of essence in deciding on administration of adjuvant systemic therapy[18]. Furthermore, additional treatment in case of a positive node (axillary lymph node dissection or radiotherapy) does not result in improved survival[19,20]. The question whether some patients can safely be spared the sentinel lymph node biopsy is studied in the BOOG 2013-08 randomized trial [21].

At the start of the 21st century, an important next step in the characterization of breast cancer biology was taken with the identification by Perou et al. of four molecular subtypes with distinct gene expression patterns; luminal A, luminal B, HER2-enriched and basal-like[22,23]. These subtypes proved to be associated with patient outcome and treatment effect[23,24]. Luminal breast cancers benefit from endocrine treatment and are generally associated with favorable prognosis, although longer-term follow-up data have shown that Luminal B disease has a relatively high rate of late relapses. Hormone receptor positive tumors have been shown to have a continuing rate of recurrence even after 10 years of follow-up, therefore especially in these patients long-term follow-up is of importance[25,26]. HER2-enriched and basal-like tumors are associated with poorer prognosis and a higher sensitivity to chemotherapy[27].

For clinical practice, the molecular subtypes can be approximated by intrinsic or biomarker subtyping on the basis of routinely assessed histopathology data, specifically ER, PgR and HER2 status, and either grade or Ki67 to discern the Luminal-like subtypes A and B [28,29]. Although less accurate as compared to molecular subtyping, these surrogate subtypes provide important information that can be used to tailor treatment. In our studies with data from the Netherlands Cancer Registry, we applied an even further simplified subtype classification using only hormone receptor and HER2 status that still demonstrated discriminating prognostic value regarding local and regional recurrence (***chapter 3***).

Concomitant with the discovery of the molecular subtypes, several gene expression profiles (GEP) were developed [30,31]. GEPs evaluate the expression of a (type of GEP-specific) number of genes in tumor tissue, that are associated with different biological processes, such as proliferation and angiogenesis. The results of these tests provide prognostic information on the risk of disease recurrence and death[32] and could help select patients for adjuvant systemic therapy. The 70-gene signature MammaPrint® classifies patients at either low or high genomic risk, while the 21-gene recurrence score OncotypeDX has a low, intermediate and high risk score.

Recently, a number of prospective randomized trials have evaluated the value of performing a GEP to better select patients for adjuvant systemic therapy. In the MINDACT trial, 6693 early stage breast cancer patients with 0-3 positive lymph nodes had their risk of recurrence assessed on the basis of traditional clinicopathological factors (as per a modified Adjuvant!Online, including HER2 status) as well as by the 70-gene signature to evaluate whether the use of the 70-gene signature could be used to identify patients with early-stage breast cancer who might safely forgo chemotherapy. Subsequent chemotherapy administration depended on the risk assessment. When both strategies resulted in a low risk score, the patient did not receive chemotherapy. In patients at high risk according to both assessments chemotherapy was advised. Patients with discordant results were randomized to either follow the clinical or genomic risk assessment to determine chemotherapy prescription (high=chemo, low= no chemo). Compliance to protocol treatment was >95%. Primary results of the study showed that patients classified as clinical high risk but with a low genomic risk profile and who received no chemotherapy, had a 5-years DMFS rate of 95% and derived little benefit of chemotherapy. The TailorX trial included node-negative patients with hormone receptor positive, HER2 negative breast cancer who had their genomic risk assessed using the 21-gene recurrence score. Patients at low genomic risk received endocrine treatment, while patients at high genomic risk were treated with both endocrine treatment and chemotherapy. Patients at intermediate risk were randomized to endocrine treatment with or without chemotherapy. First results from the low risk arm demonstrated excellent disease-free survival after 3 years with endocrine treatment[9]. The results of the randomized intermediate arm demonstrated no benefit of adding chemotherapy to endocrine treatment[33]. These data are in line with the results of the MINDACT trial, demonstrating no added benefit from chemotherapy in clinical low risk disease.

In ***chapter 4*** we demonstrated that young breast cancer patients, historically known to have a higher rate of poor prognostic pathological features, were also more likely to be at high genomic risk according to the 70-gene signature as compared to older patients (***chapter 4***). Survival without distant metastasis (DMFS) was comparable between the different age categories (<45, 45-55, >55), overall as well as according to MINDACT risk assessment group. These results corroborate the knowledge that not age but biology determines prognosis, and that the 70-gene signature provides additional prognostic information in this complex patient population.

Breast cancer guidelines recommend basing adjuvant systemic therapy decisions in multifocal disease on the features of the largest lesion, disregarding the potential biological implication of having multifocal disease[34,35]. As the 70-gene signature provides additional prognostic information that can aid chemotherapy decisions, we evaluated the value of performing the 70-gene signature in patient with multifocal disease (***chapter 6***). Previous reports have observed that multifocality is associated with a higher tumor load and thus more aggressive tumor biology.

Omission of chemotherapy when classified as clinical low risk on the basis of the largest lesion may result in under treatment in multifocal breast cancer. In our study, multifocal disease was independently associated with a high genomic risk according to the 70-GS in clinical low-risk patients. We did not observe a significant interaction with distant metastasis-free survival. There was a trend towards poorer outcome in patients with multifocal disease. The interaction was likely not demonstrated due to inadequate power, given that our study was an unplanned additional analysis.

Apart from the value of GEPs in the decision process of administering systemic treatment, their application could also be of use in de-escalation of local treatment.

The influence of treatment modalities on recurrence

In our studies, type of surgery did not influence the rate of locoregional recurrence (***chapters 2 and 3***). This is in contrast with the results from previous publications evaluating patients treated in earlier years, like the landmark studies from Veronesi and Fisher. These studies reported higher local recurrence rates after breast conserving surgery (BCS) when compared to mastectomy, yet without an effect on overall survival[36–38]. Several factors could have played a role in this discrepancy; better selection of patients for BCS due to increased knowledge on tumor biology as described above, developments in radiotherapy techniques, advancements in systemic therapy, or a combination of these factors.

Radiotherapy improves locoregional control, especially after BCS[39]. We could not evaluate the influence of various radiotherapy techniques and schedules, such as the introduction of a boost to the tumor bed, as this information was not available in the Netherlands Cancer Registry. The administration of a boost dose is likely to have played an important role in the reduction of LR during our study period (2003-2008). National guidelines at the time advised the routine administration of an additional radiotherapy boost to the primary tumor bed, and for the population of young women the Young Boost trial[NCT00212121] was conducted.

The observed decrease in LR and RR in the present study were accompanied by a similar decline in the risk of developing DM. Although this observation will mainly be the effect of advancements in systemic treatment strategies, radiotherapy will have played an important role as well given the described domino effect of preventing breast cancer recurrence with regards to breast cancer specific survival[40].

The improved survival of breast cancer patients is largely attributed to the developments in (neo) adjuvant systemic therapy[41]. The introduction and updates of breast cancer guidelines have streamlined the systemic treatment approach. Furthermore, increased insight in tumor biology

has offered the possibility to tailor treatment either on the basis of risk or the presence of specific biomarkers, for instance trastuzumab treatment in HER2+ disease. Apart from the introduction of biomarker driven therapeutic approaches like endocrine treatment and trastuzumab, increased knowledge on tumor biology as well as the introduction of GEPs have also resulted in better selection of patients for adjuvant systemic therapy. Therefore, although the proportion of patients that received chemotherapy and hormonal treatment has remained stable for the vast majority of the studied period, both treatment modalities have evolved extensively in the last decade with the introduction of aromatase inhibitors, taxane/anthracycline combination chemotherapy and HER2 blockade. A large report by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) has demonstrated that chemotherapy and endocrine treatment both reduce the rate of locoregional recurrence by more than one third[42].

The time period (2003-2008) evaluated in ***chapters 2 and 3*** presents an interesting time frame, since the routine assessment of HER2-status was implemented in the Netherlands mid-2005 and treatment with trastuzumab was reimbursed as of 2006. This allowed us to observe the effect of the introduction of trastuzumab treatment. During this time period, other treatment modalities (eg. breast-conserving surgery and SNB) remained stable and determination of the estrogen (ER) and progesterone (PR) status were already part of daily practice. In our study, the introduction of trastuzumab coincided with the decreasing locoregional recurrence rates (*Chapter 2)*. Treatment with trastuzumab in HER2+ disease has been reported to reduce the risk of LR and RR substantially[43,44]. This is supported by the observation that rates of LR and RR were lower in patients with a known biomarker subtype in comparison with patients with an unknown subtype (*Chapter 3)*. These latter patients mainly received treatment in earlier years when HER2 testing and treatment with trastuzumab was not routinely applied.

Over the last two decades, the administration of neoadjuvant chemotherapy has increased. Originally introduced as a strategy for locally advanced breast cancer with the aim of downstaging the tumor to allow more conservative surgery, it is now increasingly applied in early stage breast cancer patients requiring systemic treatment. The earlier (pre-operative) administration of chemotherapy allows in-vivo' monitoring of the efficacy of the treatment, enhances breast conservation and provides the option of a more conservative approach towards the axilla (eg. using the MARI procedure)[45–49]. Neoadjuvant chemotherapy does not result in an improved disease-free or overall survival when compared to treatment in the adjuvant setting[49]. The achievement of a pathologic complete response (pCR) after neoadjuvant treatment is associated with improved long-term survival, mainly for patients with Her2 positive or triple negative breast cancers[50]. In ***chapter 7*** we demonstrated that different levels of partial response, as defined by the Neoadjuvant Response Index (NRI) proposed by Rodenhuis et al.[51], also provide prognostic information with regards to recurrence-free survival in a population

based cohort. The response to neoadjuvant chemotherapy varied with biomarker subtype, as is known from the higher rates of pCR reported in HER2-enriched and triple negative tumors[50]. Although the association between pCR and survival is widely described, little is known about the distribution and location of distant metastasis after achievement of pCR as compared to after non-pCR. We observed a higher incidence of central nervous system metastases and incidence of skeletal metastases in patients with pCR as compared to non-pCR. These differences in metastatic patterns might have been expected on the basis of breast cancer subtype, considering the sensitivity and known relapse patterns of the different intrinsic subtypes although we attempted to correct for subtype bias.

Future perspectives

Increased insight in tumor biology and subsequent adaptions to the management of breast cancer patients has resulted in improved outcome. This personalized approach will intensify in the coming years given the continuing efforts in dissecting the biology of breast cancer and combining this knowledge with both medical and technical options.

In some patients the extent of treatment may be safely scaled down due to the indolent character of their disease. One example of this involves the efforts in older breast cancer patients with small, luminal A breast tumors in whom surgery may possibly be the only required treatment (eg. TOP 1 study)[52]. De-escalation of treatment can also be achieved through the combination of knowledge on tumor biology, systemic therapy and technical advances such as imaging techniques and non-invasive procedures. The increasing pCR rates following neoadjuvant systemic treatment strategies have opened the door to trials evaluating whether surgical resection might be safely omitted in selected cases (eg. The MICRA trial; NTR6120). A first step towards this de-escalation is the identification of a reliable method to determine the presence of complete remission of the tumor following neoadjuvant treatment, either through multiple core biopsies or perhaps even on the basis of imaging[53].

A personalized approach in breast cancer also entails de-escalation of chemotherapy treatment. Risk assessment using tools combining classical clinicopathological factors as well as the introduction of GEPs have resulted in better defined chemotherapy indications. In the future, targeted agents may render the use of chemotherapy obsolete or merely reserved for selective cases where targeted therapies are not available or have failed.

Defining the background and basis for the development of breast cancer are essential in improving the care of patients. Advances in molecular biology have resulted in growing

knowledge on mechanisms and mediators involved in the development of cancer, as well as on compensatory mechanisms as a basis for therapy resistance.
The identification of cell signaling pathways and the various mediators involved (starting from receptors on the cellular membrane), allowed the development of targeted agents (such as CDK4/6, mTOR and PI3K inhibitors) that can prohibit cell cycle progression and thus tumor growth and proliferation.

The immune system also plays an important role in the development and course of cancer, for example through the activation of T-cells. T-cells are activated in response to the detection of tumor antigens, resulting in infiltration of the tumor and an antitumor immune response. The use of immune modulating agents has been successful in other types of cancer, such as melanoma and lung cancer, and is currently being evaluated in breast cancer.

A key biological process involved in (breast) cancer development is angiogenesis, i.e. establishing blood supply. In the absence of blood supply, tumors are not able to grow and fail the opportunity for hematogenous metastatic spread. Several targeted agents have been developed to interrupt this process, of which bevacizumab is the most investigated. In the addendum to this chapter we have included a review on anti-angiogenic therapy in breast cancer as an example of the complex process of developing, evaluating and implementing a targeted therapy approach.

The rapid developments in new targeted therapy raises the issue of how to properly evaluate their efficacy and safety. Trials evaluating new drugs generally require large numbers of patients and extensive follow-up to demonstrate meaningful improvements in outcome. This is further complicated when increasingly more targeted agents require the presence of specific biomarkers, necessitating a multiple-step study entry and higher number of patients that needs to be screened for participation.

There is a need for alternative strategies to identify signs of efficacy of a new agent, to confirm the new treatment is promising enough for further testing. The I-SPY platform, acronym for Investigation of Serial Studies to Predict Your Therapeutic Response through Imaging and Molecular Analysis, answers this need. Patients are included in the study and have several aspects of their tumor biology determined in order to help randomize them in one of the multiple neoadjuvant treatment arms (adaptive randomization) testing the efficacy of a new therapeutic modality through evaluation of pCR rates, influences on biomarker expression and evaluation of downstaging with the use of imaging[54].

Cancer originates from DNA damage resulting in mutations. Accumulating driver mutations can give raise to abnormal proliferation of a single cell. During progression additional mutations

can occur resulting in cells with differing characteristics. Cells with the greatest abilities for proliferation, survival and invasion will become the dominant clones through clonal selection. Breast cancer treatment is complicated by this heterogeneity inherent to cancer[55], which exists between different patients but can also be observed within the same patient; between different lesions in the breast (inter-tumor heterogeneity), between the primary and its metastasis and even between cells of the same tumor (intra-tumor heterogeneity)[56]. Treatment strategies aimed at the dominant clones in the tumor (for example endocrine treatment in hormone receptor positive disease) may eradicate all sensitive cells while any resistant cells remain and have the potential to grow and become the dominant clone in future macrometastatic disease. Consequently, the biology of the metastatic disease is considered more complex than the biology of the primary tumor[57]. Performing biopsies of metastatic lesions is becoming part of daily practice to obtain more insight in the biology of the relapse and determine the most appropriate treatment of metastatic disease.

The MINDACT Relapses project has been designed in order to obtain more insight in the biology and molecular landscape of metastatic disease. In this translational substudy of the MINDACT trial, we aim to collect metastatic tissue obtained through biopsy within the context of routine clinical practice from patients who were enrolled in the MINDACT trial and who have developed relapsed disease of any kind or a new primary breast cancer. The study will consist of two parts; a retrospective and prospective part. In the retrospective part, we will collect information and left-over tissue from patients already diagnosed with a relapse who underwent a biopsy (following informed consent). In the prospective part, we will collect tissue material as well as a blood sample at time of diagnosis of disease recurrence and another blood sample at the time of progression. The tissue from the metastatic lesion will be compared with archived tissue from the primary tumor of the same patient by means of traditional pathology evaluation, RNA sequencing and DNA whole genome sequencing. The project is expected to start inclusion in the second half of 2018.

Characterizing the breast cancer genome through sequencing likely results in the detection of both clinically relevant mutations for treatment selection as well as the identification of actionable mutations for the development of new therapeutic strategies to further improve personalization of breast cancer treatment with the aim of improved outcome of our patients.

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ADDENDUM:

Anti-angiogenic treatment in breast cancer: Facts, successes, failures and future perspectives

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Cancer Treat Rev. 2017; 53: 98-110

Abstract

Angiogenesis is one of the hallmarks of cancer and a crucial requisite in the development of tumors. Interrupting this process by blocking the vascular endothelial growth factor (VEGF) with the monoclonal antibody bevacizumab has been considered a possible breakthrough in the treatment of various types of cancer, especially for advanced disease. However in breast cancer, studies have shown ambivalent results causing debate about the value of this drug. In this article, we review the evidence for anti-angiogenic treatment options for breast cancer, as well as discuss the possible factors limiting the effectiveness of anti-angiogenic agents and offer a recommendation regarding the future research on these therapies for the treatment of breast cancer.

Introduction

The importance of angiogenesis in the development of tumors and metastases in breast cancer is well established [1]. Interrupting this process has been considered a promising therapeutic option, initially focusing on metastatic disease [2]. The most extensively studied strategy in anti-angiogenic treatment consists of blocking the vascular endothelial growth factor (VEGF) using the monoclonal antibody bevacizumab. Bevacizumab received FDA approval for metastatic breast cancer in 2008 after it was shown to nearly double progression-free survival (PFS) in combination with chemotherapy over chemotherapy alone in the first line setting [3,4]. However, relatively soon after, other trials found only modest gains in PFS and, more importantly, demonstrated no effect on overall survival (OS), resulting in withdrawal of FDA approval [3]. Another strategy to target the VEGF signaling is to inhibit the signal transduction, especially of the VEGF-receptor, with tyrosine kinase inhibitors (TKI), such as sunitinib and sorafenib [5,6].

Here, we review the background and evidence for the use of the anti-angiogenic treatment in breast cancer, and offer a recommendation regarding the future research on these therapies for the treatment of breast cancer.

Role of angiogenesis in breast cancer

Tumor growth and metastasis are depending to a large extend on angiogenesis [1,7]. In the absence of blood supply, tumors are not able to grow or become necrotic, while at the same time connection to the systemic circulation enables hematogenous meta- static spread [2,8]. Angiogenesis is stimulated via substances produced by tumor [1,9]. Several studies have indicated that these angiogenic activators play an important role in the development of tumors [10].

Hypoxia induces tumor cells to produce excessive amounts of VEGF via hypoxia-inducible factor 1 (HIF-1). The released VEGF binds to its receptor (VEGFR) on the surface of endothelial cells [11]. This results in activation of a downstream cascade of proteins transmitting a signal to the nucleus of the endothelial cell, initiating new endothelial cell growth, the first important step towards new vessel formation. These new endothelial cells migrate into the extracellular matrix where they start forming hollow tubes gradually evolving into a mature network of newly formed blood vessels [11,12]. Angiogenesis is further stimulated by the ability of VEGF to recruit endothelial progenitor cells from bone marrow and induce vascular permeability, vasodilation and inhibit endothelium apoptosis [13,14].

The concentration of HIF-1 was observed to increase progressively during the transition from normal breast tissue to invasive ductal carcinoma [15]. Moreover, the level of HIF-1 was shown to be higher in poorly differentiated than in well differentiated lesions, is associated with increased proliferation and plays an important role in the formation of breast cancer metastases [16]. Specifically, in triple-negative breast cancer mouse models inhibition of HIF-1 blocks the development of lung metastases [17]. Studies have also shown, that high-levels of HIF-1 are predictive of poor clinical outcome even for patients with lymph node negative disease and that it constitutes an independent negative prognostic marker for patients with ER-/HER2+ tumors [18,19].

The VEGF family and their relevant receptors represent the most important angiogenic factors and were found to be expressed in the majority of human cancers [13,20,21]. VEGF is an endothelial mitogen, also known as vascular permeability factor since it was originally found to increase permeability of microvessels for plasma proteins [22]. It can exist in four different isoforms (due to alternative splicing of mRNA) with different biological proper- ties; VEGF121, VEGF165, VEGF189 and VEGF206 [23,24].

The level of angiogenesis in breast cancer is considered a prognostic factor for survival [25–27]. Elevated levels of angiogenic factors, such as VEGF, in different cancer types reflect their aggressiveness indicating high-risk and consequently poor prognosis [7,28,29]. Additionally, the number of vessels in tumor sections at pathology was described as an independent predictor of outcome [13,27] and this microvessel density correlates with the expression of VEGF. Increased VEGF expression has also been linked to a poorer response to systemic treatments and radiotherapy [30]. Tumors that exhibit overexpression of HER2 also overexpress VEGF, as VEGF is a downstream target in the HER2 pathway [31]. The unfavorable prognosis of (untreated) HER2-positive patients has been linked to the increased angiogenesis [32]. Previous studies showed higher levels of angiogenic factors and endothelial proliferation in inflammatory breast cancer (IBC) compared to non-IBC [33,34], suggesting these patients could be good candidates for combined chemotherapy and anti-angiogenic treatment.

Apart from its effect on angiogenesis, the protein VEGF possesses various other tumorigenic effects [35]. The elevated expression of VEGF in breast cancer has been associated to the inactivation of tumor-suppressor p53 [36] and VEGF has also been suggested to possibly play a role in the suppression of dendritic cell maturation impeding anti-tumor immune response [37]. Furthermore, the identification of VEGF receptors on tumor cells them- selves revealed the presence of pro-tumorigenic effects of VEGF through the autocrine signaling pathway; proliferation, tumor cell survival by protection from apoptosis, cell adhesion and migration, and invasion [35,38,39].

There have been reports about a possible rebound effect after discontinuation of anti-VEGF treatment, for example in high- grade gliomas, metastatic colorectal and renal cell carcinomas [40–42]. This hypothesis was supported by preclinical data in mice describing complete revascularization 7 days after discontinuation of anti-VEGF treatment consisting of the small molecules AG- 013736 (axitinib) or AG-028262 [43]. The rebound effect could be the result of increased endothelial cell proliferation [42] after discontinuation of sunitinib in renal cell carcinoma. Furthermore, the use of anti-angiogenic agents results in an hypoxic tumor environment which could increase tumor invasiveness and promote metastatic spread, potentially through the stimulation of cancer stem cells through HIF [44,45]. However, a pooled analysis of different phase III trials investigating bevacizumab in various cancer types did not find increased rates of disease progression or mortality in patients who discontinued treatment [46]. Nevertheless, for the different TKIs the true relevance of this effect remains unclear.

Below we summarize the most important studies with anti- angiogenic agents that have been performed in metastatic and early-stage breast cancer. We also attempt to provide an insight on the clinical impact of such treatments, discuss the possible factors limiting the effectiveness of anti-angiogenic agents and suggest strategies to improve the risk- benefit ratio of such treatments.

Bevacizumab

Agents that target the angiogenesis pathway have been studied extensively in the treatment of breast cancer and have been a particularly attractive focus of research in the triple-negative subtype, which is characterized by increased VEGF expression and enhanced angiogenesis [36]. The anti-VEGF monoclonal antibody bevacizumab has so far been the most extensively evaluated agent.

Bevacizumab is a humanized monoclonal antibody that binds and inactivates all isoforms of VEGF-A. After the promising preclinical results of a VEGF-targeting antibody back in 1993, this strategy was believed to be a breakthrough treatment for patients with advanced cancer [47]. Bevacizumab was the first approved anti- angiogenic agent for human cancers, starting with FDA approval for advanced colorectal cancer in 2004 and first approval in Europe in 2005 for the same indication, based on statistically significant improvement in PFS and OS (OS: 20.3months for IFL+Bv vs. 15.6 months for IFL + placebo, HR 0.66, $p < 0.001$) [3,48,49]. Approval for the use of bevacizumab in metastatic HER2-negative breast cancer was granted by the EMA in 2007 and FDA in 2008 after the E2100 trial demonstrated a significantly improved PFS (11.8 vs. 5.9 months, HR 0.60, $p < 0.001$) [3,4]. Remarkably, this clearly prolonged PFS did not translate into

an OS benefit, resulting in controversy over the approval based on PFS as a surrogate efficacy endpoint. As a result of trials contradicting the previously described favorable effects as well as demonstrating increased toxicity, in November 2011 FDA withdrew the approval of bevacizumab for metastatic HER2 negative breast cancer [50,51]. However, based on the increased PFS the drug did remain approved by the EMA in combination with capecitabine or paclitaxel (Fig. 1).

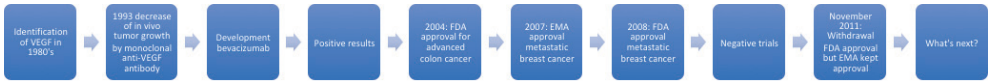

Figure 1. Evolution pathway of treatment with bevacizumab in metastatic breast cancer.

Data from completed clinical trials

Metastatic breast cancer trials

First line treatment studies

Bevacizumab in combination with chemotherapy.

To date five randomized phase III trials have tested the addition of bevacizumab to a backbone of standard chemotherapy as first-line treatment for HER2-negative metastatic breast cancer. All these studies have shown improvements in response rates and PFS, however, none of them succeeded to show an OS benefit (Table 1).

The Eastern Cooperative Oncology Group (ECOG) E2100 trial [4] randomized 673 women with metastatic breast cancer to paclitaxel plus bevacizumab or paclitaxel alone. PFS was significantly increased after addition of bevacizumab (11.8 vs. 5.9 months, HR 0.60, $p < 0.001$) without impact on OS (26.7 vs. 25.2 months, HR 0.88, $p = 0.16$). An independent review of the E2100 trial by Gray et al. described a >50% risk reduction of disease progression or death [52]. As a confirmatory study after FDA approval, the AVADO trial [50] randomly assigned 736 patients with HER2-negative meta- static disease on a 1:1:1 basis to either 7.5 mg/kg bevacizumab plus docetaxel, 15 mg/kg bevacizumab plus docetaxel or docetaxel plus placebo. Primary endpoint of the study was PFS, with OS as a secondary endpoint. After a median follow-up of 25 months, PFS was 9.0 months in the bevacizumab 7.5 mg/kg arm vs. 10.1 months in the bevacizumab 15 mg/kg arm and 8.2 months for the placebo group (HR 0.80; $p = 0.045$ and 0.67; $p < 0.001$ vs. placebo arm, respectively). Again, OS was not affected (30.8 vs. 30.2 vs. 31.9 months, HR 1.03; $p = 0.85$ for bevacizumab 7.5 mg/kg and 1.05; $p = 0.72$ for bevacizumab 15 mg/kg).

Between 2005 and 2007, the RIBBON-1 [51] trial enrolled 1,237 HER2-negative patients with locally recurrent or metastatic breast cancer untreated with chemotherapy. Patients were randomized 2:1 between chemotherapy plus bevacizumab or chemotherapy plus placebo.

Table 1. Summary of randomized phase III trials examining the effect of first-line bevacizumab in metastatic breast cancer.

	Treatments	No. of patients	PFS			OS	
			Median FUP (months)	Median (months)	HR (p) vs. no bevacizumab	Median (months)	HR (p) vs. no bevacizumab
COMBINED WITH CHEMOTHERAPY							
E2100[4,52] **(2001-2004)**	P ± Bv	673 (347 vs. 326)	41.6 vs. 43.5	11.8 vs. 5.9	0.60 (p<0.001)	26.7 vs. 25.2	0.88 (p=0.16)
AVADO[50] **(2006-2007)**	T+Bv 7.5mg/kg vs. T+Bv 15mg/kg vs. T+plus placebo	736 (248 vs. 247 vs. 241)	25	9.0 vs. 10.1 vs. 8.2	0.80 (p=0.045 for Bv 7.5mg/kg)	30.8 vs. 30.2 vs. 31.9	1.05 (p=0.72 for Bv 7.5mg/kg)
					0.67 (p<0.001 for Bv 15mg/kg)		1.03 (p=0.85 for Bv 15mg/kg)
RIBBON-1[51] **(2005-2007)**	CAPE ±Bv vs. taxane or anthracycline based CTx ±Bv	1,237 (409 cape+Bv, 206 cape+placebo,	Cape cohort 15.6	8.6 vs. 5.7 (cape cohort)	0.69 (p<0.001 cape cohort)	29 vs 21.1 (cape cohort)	0.85 (p=0.27 cape cohort)
		415 tax/anthra+ Bv, 207 tax/ anthra+placebo)	Tax/Anthr cohort 19.2	9.2 vs. 8.0 (tax/anthra cohort)	0.64 (p<0.001 tax/anthra cohort)	25.2 vs. 23.8 (tax/anthra cohort)	1.03 (p=0.83 tax/anthra cohort)
MERiDiAN[53] **(2012-2013)**	P ± Bv	481 (239 vs. 242)	15.0 vs. 14.8	11.0 vs. 8.8	0.68 (p=0.0007)	NR	NR
SAKK 24/09[57] **(2010-2012)**	Bv + mCTx vs Bv + P	147 (74 vs. 73)	26.1	8.5 vs. 10.3	(p=0.83)	18.7 vs. 25.6	(p=0.24)
AVEREL[58] **(2006-2010)**	T+H ± Bv	424 (216 vs. 208)	26.0 vs. 25.8	16.5 vs. 13.7	0.82 (p=0.0775)	>38 for both	1.01 (p=0.9543)
COMBINED WITH ENDOCRINE TREATMENT							
LEA[61] **(2007-2011)**	ET (L or F) ± Bv	374 (190 vs. 184)	23.7	19.3 vs. 14.4	0.83 (p=0.126)	52.1 vs. 51.8	0.87 (p=0.518)
CALGB 40503[62] **(2008-2011)**	L ± Bv	343 (173 vs. 170)	39	20.2 vs. 15.6	0.75 (p=0.016)	47.2 vs. 43.9	0.87 (p=0.188)

Abbreviations: P= paclitaxel, Bv=bevacizumab, T=docetaxel, H=trastuzumab, , CAPE=capecitabine, CTx=chemotherapy, mCTx= metronomic chemotherapy, L=letrozole, F=fulvestrant, NR=not reported/data immature

Type of chemotherapy was chosen by the investigators before randomization and could be capecitabine (cape cohort) or a taxane- or anthracycline-based (tax/anthra cohort) regimen, administered every 3 weeks. PFS was significantly prolonged in patients receiving bevacizumab, compared to placebo (8.6 vs. 5.7 months, HR 0.69; $p < 0.001$ in cape cohort and 9.2 vs. 8.0 months, HR 0.64; $p < 0.001$ in tax/anthra cohort). However, OS and 1-year survival did not significantly differ between the treatment arms, consistent with the other two studies.

In the MERiDiAN study, metastatic HER2-negative breast cancer patients were stratified according to baseline plasma VEGF concentration (VEGF-high vs. VEGF-low) and randomized to paclitaxel with or without bevacizumab [53]. After a median follow-up of 15 months, an improved PFS was observed in the bevacizumab group with an absolute difference of 2.2months (11.0 vs. 8.8 months, HR 0.6, $p = 0.0007$). Data on OS were immature with only 41% of patients that had died at primary analysis [53].

The addition of bevacizumab results in increased toxicity when compared to chemotherapy alone [54]. Metronomic chemotherapy is the frequent, even daily, administration of low-dose chemotherapy without prolonged treatment intervals with the benefit of decreased toxicity [55]. Furthermore, this strategy has been associated with an increased anti-angiogenic chemotherapy effect by prohibiting endothelial repair during the recovery period and a decrease in plasma VEGF thereby enabling apoptosis of endothelial cells induced by the chemotherapy [55]. This strategy of was tested in the adjuvant setting by the IBCSG 22-00 study in which metronomic chemotherapy was given as maintenance after standard chemotherapy in triple negative breast cancer without a clear benefit [56]. However, the combination of bevacizumab and metronomic chemotherapy could optimize the anti-angiogenic effects, also when compared with the combination of bevacizumab with standard chemotherapy, with the added benefit reduced toxicity [57]. This hypothesis was tested in the SAKK 24/09 [58] trial, randomizing 147 HER2-negative patients with advanced breast cancer to bevacizumab with paclitaxel or bevacizumab with metronomic capecitabine and cyclophosphamide. The primary endpoint of grade 3–5 adverse events did not differ between the two arms (24% vs. 25%, $p = 0.96$). There was also no significant difference in efficacy between the two regimens, although there appeared to be a numerical benefit for the standard chemotherapy combination (PFS 8.5 vs. 10.3 months, $p = 0.83$ and OS 18.7 vs. 25.6 months, $p = 0.24$, Table 1).

As previous studies had proven a synergistic mechanism between HER2 and VEGF both in vitro and in vivo, the AVAREL study was undertaken to investigate the effect of adding bevacizumab to trastuzumab and chemotherapy in HER2-positive locally recurrent or metastatic breast cancer [59]. After a median follow-up of 26 months there was no statistically significant difference in both PFS (16.5 vs. 13.7 months, HR 0.82, $p = 0.0775$) and OS (>38 months for both, HR 1.01, $p = 0.9543$, Table 1) [59].

The TURANDOT trial [60,61], running from 2008–2010, aimed to prove non-inferiority of the combination bevacizumab/- capecitabine when compared to the bevacizumab/paclitaxel

combination. The final analyses of the primary endpoint, evaluating 564 randomized HER2-negative metastatic breast cancer patients, suggested non-inferiority of bevacizumab/capecitabine compared with bevacizumab/paclitaxel in terms of OS (26.1 vs. 30.2 months, HR 1.02, p = 0.0070) although this was not supported by the unstratified analyses.

Bevacizumab in combination with endocrine treatment.

Bevacizumab has also been evaluated as first-line treatment in combination with endocrine therapy (ET) in the LEA [62] and CALGB 40503 trial [63]. Several studies have reported on the interaction between angiogenesis and endocrine regulation mediated by VEGF [30,64,65]. Higher levels of VEGF were associated with a decreased response to ET [65,66], suggesting that the combination of anti- VEGF treatment with ET could increase efficacy. The LEA trial [62], investigating ET (either letrozole or fulvestrant) with or with- out bevacizumab in post-menopausal advanced BC patients, did not show significant differences in PFS (19.3 vs. 14.4 months, HR 0.83, p = 0.126) and OS (52.1 vs. 51.8 months, HR 0.87, p = 0.518) between the two regimens. In the phase III CALGB 40503 [63], the combination of letrozole and bevacizumab in hormone receptor-positive MBC patients resulted in a 5-month improvement of PFS compared to letrozole monotherapy (20.2 vs. 15.6 months, HR 0.75, p = 0.016). Yet, this study also did not show a significant OS difference (47.2 vs. 43.9months, HR 0.87, p = 0.188, Table 1).

Second line treatment studies

The AVF2119G trial [67] assessed the safety and efficacy of adding bevacizumab to capecitabine in second and third line setting. In this study, 462 pretreated metastatic breast cancer patients were randomized to receive capecitabine alone or in combination with bevacizumab at 15 mg/kg every 3 weeks. The study showed an improved response rate (19.8% vs. 9.1%, p = 0.001), but no benefit in PFS (4.86 vs. 4.17, HR 0.98, p = 0.857) or OS (15.1 vs. 14.5, NR, Table 2).

The RIBBON-2 [68] study compared the efficacy and safety of bevacizumab in combination with standard chemotherapy versus chemotherapy alone in HER2-negative previously treated metastatic breast cancer patients. This second-line study enrolled 684 patients with 225 assigned to the chemotherapy plus placebo arm and 459 to the chemotherapy plus bevacizumab arm. Chemotherapy backbone was a taxane (docetaxel or nab-paclitaxel or paclitaxel), gemcitabine, capecitabine or vinorelbine. The median follow-up period was 15 months. There was a statistically significant, but moderate, increase of PFS (7.2 vs. 5.1 months, HR 0.78; p = 0.0072) and non-significant 10% improvement in overall response rate (ORR) (39.5% vs. 29.6%, p = 0.0193), without OS benefit (18.0 vs. 16.4, HR 0.90, p = 0.3741, Table 2) [68].

It was hypothesized that the limited benefit of bevacizumab in previously treated metastatic breast cancer patients could perhaps be explained by the already established angiogenesis in this highly refractory patient population, suggesting that future research should aim at the use of bevacizumab in less advanced disease [67].

Table 2. Summary of randomized phase III trials examining the effect of second-line bevacizumab in metastatic breast cancer

	Treatments	No. of patients	PFS			OS	
			Median FUP (months)	Median (months)	HR (p) vs. no bevacizumab	Median (months)	HR (p) vs. no bevacizumab
AVF2119G[66] **(2000-2002)**	CAPE ± Bv	462 (232 vs. 230)	NR	4.86 vs. 4.17	0.98 (p=0.857)	15.1 vs. 14.5	NR
RIBBON-2[67] **(2006-2008)**	CTx (CAPE or Tax or Gem or Vino) + Bv vs. CTx + placebo	684 (459 vs. 225)	15	7.2 vs. 5.1	0.78 (p=0.0072)	18.0 vs. 16.4	0.90 (p=0.3741)
MAINTENANCE TREATMENT							
IMELDA*[69] **(2009-2011)**	T+Bv à Progression free patients: Bv+CAPE vs. Bv alone	185 (91 vs. 94)	31.6 vs. 30.4 (maintenance period)	11.9 vs. 4.3	0.38 (p<0.0001)	29.0 vs 23.7	0.43 (p=0.003)
TANIA[70]	CTx ± Bv	494 (247 vs. 247)	16.1 vs. 15.9	6.3 vs. 4.2	0.75 (p=0.0068)	NR	NR
AROBASE[¥][76]	Tax+Bv vs. E+Bv	117 (59 vs. 58)	21.4 vs. 20.6	8.1 vs. 7.6	(p=0.998)	NR	NR

Abbreviations: CAPE=capecitabine, Bv=bevacizumab, CTx=chemotherapy, Tax=taxane, Gem=gemcitabine, Vino=vinorelbine, T=docetaxel, E=exemestane, NR=not reported/data immature

*Trial terminated early due to the withdrawal of FDA approval of combined docetaxel/bevacizumab treatment in 2011

[¥] Trial terminated early due to futility at interim analysis

Maintenance therapy

The optimal duration of bevacizumab treatment has not yet been established. A meta-analysis by Gennari et al. [69] demonstrated an improved outcome after prolonged first-line chemotherapy in metastatic breast cancer patients. This consideration combined with the key role of VEGF in angiogenesis and hypotheses on a rebound effect after discontinuation of bevacizumab, could suggest maintenance therapy with bevacizumab in the metastatic setting would result in improved outcomes.

In the IMELDA trial [70], HER2-negative metastatic breast cancer patients without progression on initial treatment with bevacizumab and docetaxel were randomized to subsequent maintenance bevacizumab in combination with capecitabine or bevacizumab alone. Unfortunately, the accrual of the trial was pre- maturely terminated due to the withdrawal of FDA approval of combined docetaxel/bevacizumab treatment in 2011. Of the 284 enrolled patients, 185 were randomized between the two maintenance strategies. After a median follow-up of 2.5 years, PFS was significantly longer in the combination group compared to the bevacizumab monotherapy group (11.9 vs. 4.3 months, HR 0.38, $p < 0.001$). OS was also significantly improved in the bevacizumab/capecitabine group (29.0 vs. 23.7months, HR 0.43, $p = 0.003$) [70]. As this study was designed to establish the benefit of longer duration of maintenance chemotherapy and did not contain a capecitabine alone arm, no strong conclusions can be drawn as to the use of maintenance bevacizumab.

The simultaneously published TANIA trial [71] included 494 HER2-negative patients with locally recurrent or metastatic dis- ease who had relapsed following earlier treatment with bevacizumab plus chemotherapy. Patients were randomized to receive next line chemotherapy alone (n=247) or with bevacizumab ($n = 247$). After a median follow-up of 16 months, PFS was significantly improved in the bevacizumab group (6.3 vs. 4.2 months, HR 0.75, $p = 0.0068$, Table 2). These results were similar to those reported for the RIBBON-2 study. So far there was no OS difference between the two groups (40% vs. 41% at a median of 16 months) although longer follow-up is required for definite conclusions. Although the difference in PFS is statistically significant, the absolute gain of 2 months is of limited clinical significance and must be balanced against the increased toxicity and cost of bevacizumab.

Although in early treatment setting of metastatic disease, OS benefit is difficult to prove, because of the possible influence of additional treatment after progression, duration of follow-up and number of patients needed to have enough power to establish presence of treatment effect [72], the question remains whether the currently demonstrated absolute gain in PFS of approximately 2–3 months [51,59,73] should be considered sufficient to adapt treatment strategy in view of the added toxicity and high cost of this treatment. Various renowned guidelines offer diverse

recommendations on the place of bevacizumab in metastatic breast cancer. The NCCN guidelines support the use of bevacizumab combined with paclitaxel in recurrent or metastatic disease [74], while the ASCO and ESO-ESMO ABC guidelines state considering bevacizumab only as an option in selected cases [75,76].

As anti-VEGF treatment could increase the efficacy of endocrine treatment, the AROBASE trial was designed to investigate continuation of bevacizumab in combination with the initial taxane chemotherapy or combined with endocrine therapy in patients with ER-positive, HER2-negative, unresectable locally advanced or metastatic breast cancer with a response or disease stabilization after first-line bevacizumab and chemotherapy [77]. Enrollment was stopped after interim analysis due to futility, but included patients were allowed to continue treatment and follow-up according to protocol. After a median follow-up of 21 months there was no difference in PFS (8.1 vs. 7.6, $p = 0.998$) nor was there a difference in OS at 35 months, while the toxicity profile was in favor of the ET/Bv arm.

Early-stage breast cancer trials

The results of the trials in metastatic disease are sometimes interpreted as indicative that the anti-angiogenic blockage is not effective in macro-metastases since these lesions have already established blood-flow, whereas angiogenesis is potentially most essential in helping microscopic tumor lesions grow beyond 2– 3 mm [1]. Furthermore, VEGF has been described to mediate far more early events essential for metastatic spread of tumor cell lines, such as vascular permeability to macromolecules, lymphangiogenesis, increased expression of the chemokine receptor CXCR4 mostly associated with lymphatic and pulmonary metastases through binding of SDF-1 and inhibiting the anti-tumor immune response [14,78]. These facts led to the hypothesis that bevacizumab treatment could be of higher value as an addition to chemotherapy in patients with early stage breast cancer eligible for (neo)adjuvant treatment. However, studies in early stage disease have again offered disappointing results (Table 3).

Neoadjuvant trials

The NSABP B-40 [79] trial was a six-arm study that evaluated whether the addition of either capecitabine or gemcitabine ± pre- and postoperative bevacizumab to the standard neoadjuvant chemotherapy scheme of docetaxel followed by doxorubicin/cyclophosphamide would improve the outcome in 1186 patients with operable HER2-negative breast cancer. Bevacizumab was administered at 15 mg/kg IV every 3 weeks for 12 (neo)adjuvant doses. The primary endpoint was rate of pCR which was significantly higher after the addition of neoadjuvant bevacizumab (34.5% vs. 28.2%, $p = 0.02$) with the greatest effect in the hormone receptor positive population (15.1% vs. 23.2%, $p = 0.007$) [80]. After a median follow-up of 56.4 months, in spite of the predefined secondary endpoint DFS not being significantly affected (HR 0.80; $p = 0.65$) [79], OS was increased in patients treated with bevacizumab (HR 0.65; $p = 0.004$).

Table 3. Summary of randomized phase III trials examining the effect of bevacizumab in neo-adjuvant and adjuvant setting in early breast cancer

	Treatments	Patient population	No. of patients	Median FUP (months)	pCR rates (p) vs no bevacizumab	DFS HR (p) vs. no bevacizumab	OS HR (p) vs. no bevacizumab
NEOADJUVANT							
NSABP-B40[78] **(2007-2010)**	T à AC	HER2-	199	56.4	34.5% vs. 28.02% (p=0.02)	0.80 (p=0.06) for all chemotherapy regimens	0.65 (p=0.004) for all chemotherapy regimens
	T+Bv à AC+Bv à Postop Bv		195				
	T+CAPE à AC		204				
	T+CAPE+Bv à AC+Bv à Postop Bv		196				
	T+GC à AC		191				
	T+GC+Bv à AC+Bv à Postop Bv		201				
GeparQuinto[81] **(2007-2010)**	EC-T ± bevacizumab	HER2-	1948 (974 vs. 974)	45.6	18.4% vs. 14.9% (p=0.042)	1.03 (p=0.7837)	0.97 (p=0.8422)
ARTemis[83]	T-FEC ± Bv	HER2-	800 (399 vs. 401)		22% vs. 17% (p=0.03)		
ADJUVANT							
BEATRICE[90] **(2007-2010)**	Chemotherapy ± Bv	TNBC	2591 (1301 vs. 1290)	32.0 vs. 31.5,.		0.87 (p=0.18)	0.84 (p=0.23)
NSABP-B44 BETH[91] **(2008-2010)**	T+C+H ± Bv à H±Bv	HER2+	3231 (1614 vs. 1617)	38		1.02 (p=0.8791)	0.87 (p=0.4387)
	T+H ±Bv à FEC à H±Bv		278 (138 vs. 140)				
E5103[92] **(2007-?)**	Arm 1: AC à P+placebo						
	Arm 2: AC+Bv à P+Bv	HER2-	4994	47.5		Arm 3 vs. 1: 0.87 (p=0.17)	Arm 3 vs. 1: HR 0.89 (p=0.41)
	Arm 3: AC+Bv à P+Bv à Bv						

Abbreviations: T=docetaxel, AC=doxorubicin and cyclophosphamide, Bv=bevacizumab, CAPE=capecitabine, GC=gemcitabine, EC-T=epirubicin/cyclophosphamide followed by docetaxel, C=carboplatin, H=trastuzumab, FEC= 5-fluorouracil/epirubicin/cyclophosphamide, P= paclitaxel, TNBC= triple negative breast cancer

The GeparQuinto [81,82] trial randomized 1948 HER2-negative breast cancer patients to neoadjuvant epirubicin/cyclophosphamide followed by docetaxel (EC-T) with or without bevacizumab 15 mg/kg every 3 weeks. Patients without a clinical response after 4 cycles of therapy were again randomized to receive paclitaxel with or without everolimus. The pCR rate increased slightly with the use of bevacizumab (18.4% vs. 14.9%, $p = 0.042$) [81,82] with the greatest effect in the triple-negative breast cancer subtype (39.3% vs. 27.9%, $p = 0.0003$) [83]. After a median follow-up of 3.8 years there was no difference in DFS (3- yr DFS 80.0 vs. 81.5%, HR 1.03, $p = 0.7837$) or OS (3-yr OS 90.7% vs. 88.7%, HR 0.97, $p = 0.8422$), neither in the total nor the triple- negative population [82]. Remarkably, DFS and OS were lower in patients that achieved a pCR after treatment with bevacizumab compared to pCR patients treated without bevacizumab, although this difference was not statistically significant (HR 2.02; $p = 0.067$ for DFS and HR 2.00; $p = 0.193$ for OS).

In 2015 the first results of the ARTemis trial [84], investigating the addition of bevacizumab to neoadjuvant docetaxel followed by fluorouracil, epirubicin, and cyclophosphamide for women with HER2-negative early breast cancer, were published. The addition of bevacizumab increased the rate of pCR (22% vs. 17%, $p = 0.03$). Longer follow-up will have to determine whether this difference will translate towards favorable survival outcome [84]. Meanwhile, in the phase 2 CALGB 40603 trial the addition of bevacizumab to chemotherapy did not result in a statistically significant though numerical improvement in pCR rates in breast and axilla (pCR breast/axilla 52% vs. 44%, OR 1.36, $p = 0.057$) [85].

The randomized phase 2 randomized AVATAXHER [86] trial evaluating the addition of bevacizumab to neoadjuvant chemotherapy and trastuzumab in HER2-positive early stage breast cancer patients who were predicted as non-responders based on the relative change in FDG uptake by the tumor as measured by 18F-FDG PET scanning, demonstrated a positive influence of bevacizumab on the achievement of a pathologic response (43.8% vs. 24.0% without bevacizumab). Results on survival were immature and will be reported at a later time.

The single-arm phase II BEVERLY-1 [87] and BEVERLY-2 [88] trials evaluated the addition of bevacizumab to neoadjuvant chemotherapy in patients with inflammatory breast cancer, following the hypothesis that these tumors are good candidates for bevacizumab because of being highly angiogenic. In the BEVERLY-1, bevacizumab was added to neoadjuvant chemotherapy and trastuzumab in HER2+ patients, while BEVERLY-2 evaluated the combination of neoadjuvant chemotherapy and bevacizumab in HER2-negative patients. In BEVERLY-1 only 19% of patients achieved a pCR and with a DFS of 57% and OS of 75% at 3 years, the trial consequently does not suggest a clinical benefit of bevacizumab in this setting. The results of the BEVERLY-2 trial were more promising with a pCR-rate of 63% and a 3-year DFS rate of 68% and OS of 90% [89,90].

Adjuvant trials

In the adjuvant setting, the BEATRICE [91] trial evaluated the addition of bevacizumab to standard chemotherapy (anthracycline, taxane or combination-therapy) in triple negative operable breast cancer (T1b-3N P 1). A total of 2190 patients were included, with a median follow-up of 32 months. The primary endpoint invasive disease-free survival did not differ between the two arms (3-yr IDFS 83.7% vs. 82.7%, HR 0.87, p = 0.18) as was the case for OS (HR 0.84, p = 0.23, Table 3) [91].

These results were supported by the preliminary results of the multinational NSABP-B44 BETH trial [92]. In this study, patients with HER2-positive, node-positive or high-risk node-negative disease, were divided in two cohorts. The first randomized patients between the nonanthracycline regimen TCH (docetaxel, carbo- platin, and trastuzumab [Herceptin]) and TCH plus bevacizumab, while in the second cohort patients were randomly assigned to anthracycline-based therapy with T-FEC-H (docetaxel, fluorouracil, epirubicin, cyclophosphamide, plus trastuzumab) with or without bevacizumab. There was no statistically significant difference in IDFS after a median follow-up of 38 months in patients treated with or without bevacizumab with a rate of 92% in both groups of cohort 1 and for cohort 2–89% in the anthracycline-containing arm without bevacizumab and 91% with bevacizumab (Table 3).

In the E5103 trial evaluating the addition of bevacizumab to adjuvant chemotherapy in lymph node-positive or high-risk lymph node-negative, HER2-negative breast cancer patients, bevacizumab also did not improve IDFS (HR 0.87, p = 0.17) nor OS (HR 0.89, p = 0.41, Table 3) while toxicity was increased in the bevacizumab groups. Additionally, more patients than hypothesized were taken off bevacizumab treatment due to all causes (+/-24% in arm 2 and +/-55% in arm 3) [93].

The positive results of the neoadjuvant NSABP B-40 trial were hypothesized to be the result of the postoperative continuation of bevacizumab treatment. However, the negative results of adjuvant trials make this explanation highly unlikely [94].

Toxicity

Treatment with bevacizumab can result in a variety of side effects, the most important being cardiovascular and hemorrhagic events [95]. A meta-analysis including a total of 3784 patients, showed that the addition of bevacizumab to chemotherapy is associated with an increased risk of grade P 3 proteinuria, hypertension, left ventricular dysfunction and hemorrhagic events. No significant relationship was found for fatal events, febrile neutropenia, GI perforation, arterial or venous thromboembolic events [54]. The suggested cardiotoxicity of bevacizumab was evaluated by a meta-analysis by Choueri et al. [96], which included five trials, comprising a total of 3784

patients with metastatic breast cancer. A significant increase in the risk of congestive heart failure (CHF) was observed in bevacizumab-treated patients (RR 4.74, $p = 0.001$). Importantly, all of the trials excluded patients with uncontrolled hypertension, clinically significant congestive heart failure, cerebrovascular disease or peripheral vascular disease, and unstable angina or recent history of MI. The trials included patients with prior anthracycline exposure and one trial included patients on concomitant anthracycline. Of the 2366 patients who received bevacizumab, 36 had high-grade congestive heart failure, for an overall incidence rate of 1.6% (95% CI 1.0%-2.6%), whereas among the 1418 control or placebo patients the incidence rate of CHF was 0.4% (95% CI 0.2–1.0%) [96]. The exact pathogenesis of bevacizumab-related cardiotoxicity has not yet been established [97].

Anti-angiogenic tyrosine kinase inhibitors

Another category of anti-angiogenic drugs are the tyrosine kinase inhibitors (TKI), such as sunitinib, sorafenib and axitinib, that target growth factor receptors—the most important of which are the VEGF receptor (mainly VEGFR2), platelet-derived growth factor (PDGF) receptor, and KIT receptor [98–100]. TKIs can pass through the cellular membrane where they can interfere with downstream signaling pathways [6].

Sunitinib

Sunitinib is a type I, ATP-competitive, TKI that inhibits VEGFR and PDGFR when they are activated by binding to the ATP-site on these receptors through presentation of several hydrogen bonds that mimic those of ATP, thereby preventing signal transduction [6].

FDA and EMA approved sunitinib for the treatment of gastrointestinal stromal tumors (GIST), renal cell carcinoma and well- differentiated pancreatic neuroendocrine tumors (pNET) [101,102]. Previous studies have reported on limited activity both as monotherapy and when combined with capecitabine in advanced breast cancer. So far, randomized phase III studies investigating the use of sunitinib have not been successful in establishing a beneficial effect in advanced breast cancer (Table 4). A phase III trial comparing sunitinib with capecitabine as single-agent treatment in HER2-negative advanced breast cancer was prematurely stopped due to futility following the first interim analysis after 482 patients were randomized. PFS was significantly shorter in the sunitinib group (2.8 vs. 4.2 months, $p = 0.002$) combined with a clearly poorer safety profile [103]. A subsequent study com- paring the combination of sunitinib plus capecitabine with capecitabine alone in pretreated metastatic breast cancer patients [104], after a median duration of 14.3 months, demonstrated no difference in either PFS (5.5 vs. 5.9 months) or OS (16.4 vs. 16.5 months). Another phase III trial evaluating docetaxel ± sunitinib as

Table 4. Summary of randomized phase III trials examining the effect of sunitinib in breast cancer

					PFS		OS	
	Treatments	**Setting**	**No. of patients**	**Median FUP (months)**	**Median (months)**	**HR (p) vs. no sunitinib**	**Median (months)**	**HR (p) vs. no sunitinib**
Barrios et al[102]**. (2006-2009)***	Sunitinib vs. capecitabine	Advanced BC	482 (238 vs. 244 resp.)		2.8 vs. 4.2	1.47 (p=0.002)	15.3 vs. 24.6	1.17 (p=0.350)
Crown et al[103]**. (2007-2009)**	Sunitinib plus capecetabine vs. capecitabine alone	Pretreated metastatic BC	442 (221 vs. 221)	14.3	5.5 vs. 5.9	1.22 (p=0.941)	16.4 vs. 16.5	0.99 (p=0.484)
Bergh et al[104]**. (2007-2009)**	Sunitinib plus docetaxel vs. docetaxel alone	First-line advanced BC	593 (296 vs. 297)	18.0	8.6 vs. 8.3	0.92 (p=0.265)	24.8 vs. 25.5	1.21 (p=0.904)
Robert et al[105] **(2006-2009)***	Sunitinib plus paclitaxel vs. bevacizumab plus paclitaxel	First-line advanced BC	485 (242 vs. 243)	8.1	7.4 vs. 9.2	vs. bevacizumab 1.63 (p=0.999)		vs. bevacizumab 1.82 (p=0.996)

*Trial terminated early due to futility

first-line treatment in HER2-negative advanced BC also did not show improvement of PFS nor OS [105]. Additionally, in both studies the sunitinib group displayed higher frequencies of grade 3–4 adverse events and dose reductions [104,105]. In a randomized phase III trial comparing the combination of sunitinib plus paclitaxel with bevacizumab plus paclitaxel in 485 patients with HER2-negative advanced breast cancer, PFS and OS as well as the safety profile were in favor of the bevacizumab-containing regime [106], suggesting bevacizumab would be more suited in this setting.

Although a phase II study by Burstein et al. [107] described promising results in pretreated metastatic breast cancer patients with triple negative disease other studies did not confirm this finding [104,108]. More recent studies are evaluating the addition of sunitinib in less advanced disease (eg. NCT00887575), but thus far have also not been able to demonstrate a beneficial effect [109].

Sorafenib

The multikinase inhibitor sorafenib is a type 2 TKI and has a different mechanism of action, which targets the kinases receptors VEGFR, PDGFR, Raf, and KIT when they are inactive by binding to a hydrophobic site that is directly adjacent to the ATP binding site thereby indirectly competing with ATP and preventing phosphorylation of these kinase targets [6].

Sorafenib is indicated for advanced renal cell carcinoma, inoperable hepatocellular carcinoma and recurrent or metastatic thyroid carcinomas [110,111]. Four phase II trials were conducted in MBC of which two have shown promising results of adding sorafenib to chemotherapy, especially in combination with capecitabine [112– 115]. The phase IIb SOLTI-0701 study [115] randomly assigned 229 Her2-negative patients with advanced disease to capecitabine with or without sorafenib and demonstrated a modest improvement of PFS with the addition of sorafenib (6.4 vs. 4.1 months, $p = 0.001$) although no difference in OS was observed. This trial formed the basis for the multinational phase III RESILIENCE (NCT01234337) trial [116] investigating the addition of sorafenib to capecitabine either as first or second line treatment in patients with HER2-negative advanced or metastatic breast cancer. This study, provided evidence against a possible role of sorafenib in the metastatic setting as there was no difference is PFS or OS between the two regimens while there was a substantially higher rate of toxicity in the sorafenib arm [117].

Pazopanib

Pazopanib also competes with ATP by forming hydrogen bonds thereby inhibiting the activation of its target kinase receptors, such as VEGFR and PDGFR [118].

This TKI has been investigated in combination with lapatinib in two phase II trials in patients with advanced and inflammatory HER2-positive breast cancer and demonstrated no beneficial effect on PFS and increased toxicity compared to lapatinib alone [119,120].

Toxicity

The most common adverse effects of these TKI are diarrhea, fatigue, hand- foot syndrome, hypertension, stomatitis, hypothyroidism and myelotoxicity. Cardiac adverse effects are less frequent although these agents have induced cardiotoxicity to a certain degree. In imatinib-resistant, metastatic GIST patients treated with sunitinib, 11% suffered a cardiovascular event, with congestive heart failure occurring in 8% of patients [121].

Anti-VEGFR antibodies

Ramucirumab is a human monoclonal antibody (IgG1) that binds to VEGFR2 thereby blocking the binding between the receptor and its ligand VEGF. Currently, ramucirumab has EMA and FDA approval for the use in advanced gastric cancer, metastatic colorectal cancer and advanced non-small cell lung cancer [122,123]. The multicenter phase III ROSE/TRIO-12 randomly assigned patients with HER2-negative, unresectable locally recurrent or metastatic breast cancer to receive ramucirumab plus docetaxel or placebo plus docetaxel in first-line setting. The addition of ramucirumab did not result in improvements in PFS or OS, while the rate of grade P 3 toxicities, such as fatigue, hypertension, stomatitis, hand-foot syndrome and febrile neutropenia, was higher in the ramucirumab arm [124].

Predictive markers

The inability of bevacizumab to generate significant clinical benefit may be at least partially explained by the fact that there is no specific marker that can predict in which patients the drug could be efficacious [125,126]. All the above reported trials have been conducted in unselected breast cancer populations. Future research efforts have been initiated to identify predictive biomarkers for the efficacy of anti-angiogenic agents.

Pretreatment plasma VEGF concentrations have been reported to play a prognostic role [59,73,127]. Patients with high concentrations at baseline displayed a poorer prognosis and had a larger treatment effect of bevacizumab compared to those with low VEGF [59,73]. The AVAGAST [128] trial in metastatic gastric cancer reported a potential prognostic and predictive value of plasma VEGF concentrations with the median of 111 ng/L (equal to 111 pg/mL) as cut-

off value. However, exploratory biomarker analysis performed in the BEATRICE trial found no prognostic or predictive value of circulating VEGF when using the median value (i.e. 77.0 pg/mL) as the cutoff value, although there was a non-significant trend towards increased IDFS when applying a cutoff similar to the median applied in the AVADO and AVAREL trials (i.e. 133.6pg/mL, AVADO: 125.0 pg/mL, AVAREL 129.1 pg/mL) [73,91]. The MERiDiAN trial was designed to prospectively evaluate the predictive value of VEGF for the efficacy of bevacizumab (PFS) [53]. Patients were stratified according to high or low VEGF levels at baseline using 5.05 pg/mL as cut-off, based on results of the AVADO trial (newer version IMPACT assay) [73]. The study did not support a predictive value of baseline plasma VEGF for benefit of treatment with bevacizumab (VEGF/treatment interaction test: $p = 0.4619$) [53].

The AVAGAST trial also demonstrated prognostic and predictive values of tumor neuropilin-1 (NRP-1) [128]. The transmembrane glycoprotein neuropilin-1 is a multifunctional receptor that also binds VEGF [128,129]. Patients with low baseline levels of NRP-1 had a shorter OS and in this group a larger treatment effect was observed with regards to both PFS and OS [128]. The exact staining and grading method for NRP-1 is however not yet established and requires further assessment and validation [127,128]. Moreover, its role in metastatic breast cancer is unknown.

Baseline plasma concentrations of VEGFR2, the biologically dominant VEGF receptor, might also have a potentially predictive value for the efficacy of bevacizumab [73,91]. The currently recruiting phase IIB Triple B study [NCT01898117] aims to evaluate the predictive value of the level of plasma VEGFR on PFS in triple- negative breast cancer patients randomized to receive first-line carboplatin-cyclophosphamide or paclitaxel with or without bevacizumab.

Carbonic anhydrase IX (CAIX) is a protein that becomes overexpressed in reaction to hypoxia in solid tumors [130]. A biomarker sub-analysis from the GeparQuinto trial has shown predictive ability of CAIX [131]. Patients with low serum CAIX levels at baseline significantly less often achieved a pCR after neoadjuvant chemotherapy, which improved significantly after the addition of bevacizumab (12.2% for NCT vs. 21.3% for NCT-B, $p = 0.006$). Additionally, a non-significant improvement of DFS was seen (5-year DFS: 69.5% vs. 80.4%). This effect was not demonstrated in patients with high serum CAIX levels. These initial results must now be validated in independent series.

Conclusions and future directions

Although the scientific rationale for anti-angiogenics appears to be well supported, so far studies have not demonstrated clinically significant benefits of adding these therapeutic agents. As reported, angiogenesis is crucial for allowing tumors to grow beyond 2– 3 mm [1], suggesting anti-angiogenic agents could be able to block or even reverse growth beyond this size. Although these agents possess various anti-tumorigenic capacities outside their role in angiogenesis, the effect of anti-angiogenic monotherapy appears to be limited [70,103]. This would indicate that any residual microscopic lesions will not be eradicated by these agents alone and could eventually develop into macrometastatic disease. This could explain why previous studies demonstrated improved PFS and pCR rates but without influence on OS, and supports the need for com- bination treatment with chemotherapy or endocrine treatment. However, these agents fail to show significant clinical benefit even when used in combination regimens.

Previous studies evaluated the use of anti-angiogenics in unselected patient populations, not taking into account the heterogeneity of breast cancer and in the absence of a predictive biomarker. Future research should continue to try and identify biomarkers since, in principle, a higher therapeutic benefit is likely to occur in selected patients. Due to the multiple mediators that play a role, identifying a single biomarker has proven to be a difficult process. Genomic and proteomic techniques could be valuable in order to identify multiple markers that combined could potentially be more reliable than a single biomarker, given the complexity of the angiogenic pathway.

The current modest efficacy of anti-angiogenics should be weighed against the background of high costs which are associated with most targeted agents, in particular monoclonal antibodies. Previous studies have demonstrated that the use of bevacizumab in combination with taxane as 1st line therapy for advanced breast cancer is not cost-effective [132,133]. Considering this is the only drug with reported statistically significant PFS benefit, other anti- angiogenic agents will likely prove to be even less attractive in terms of health economics [134]. Moreover, all anti-angiogenic drugs are accompanied with increased toxicity, although generally acceptable and manageable. The anticipated identification of a validated predictive biomarker could also improve the cost- effectiveness and limit unnecessary toxicity.

In conclusion, previous studies with anti-angiogenic agents have so far not displayed a clinically significant benefit in both the metastatic as well as the early setting, neither as monotherapy nor in combination with chemotherapy or endocrine treatment, nor as maintenance therapy. Although small increases in pCR and PFS have been shown with bevacizumab, this did not translate into improved long-term outcomes such as DFS and OS. The increased toxicity reported when adding

anti-angiogenic agents to other anticancer agents, as well as the higher costs are therefore not out- weighed by an improved prognosis and may even decrease patients' quality of life. Hence, bevacizumab should be considered only on a case-by-case basis, mainly as first or second line treatment for metastatic breast cancer and only in combination with chemotherapy as in these settings some PFS benefit has been demonstrated [75]. The results in the neoadjuvant setting are contradictory [79,81,82]. Longer follow-up will be needed to demonstrate whether the increased pCR rates in some studies will translate in improvements in long-term outcome. Finally, we would currently not recommend the use of bevacizumab adjuvant setting, at least until a specific marker is discovered to predict which patients may derive a sufficient benefit.

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CHAPTER 10

Summary

Summary

Breast cancer is the most common type of cancer in women worldwide. In the Netherlands, over 14.000 women a year are diagnosed with invasive breast cancer. Fortunately, the prognosis of breast cancer patients is improving thanks to increased insight in tumor biology and continuing improvements in systemic therapy. In this thesis we evaluated the rate of breast cancer recurrence as well as associated prognostic factors. This knowledge can help us to personalize treatment with the aim of avoiding over and under treatment, and thereby improving outcome and reducing unnecessary toxicity.

In order to assess for which patients de-escalation of treatment is possible or on the other hand determine where there is a need for more intensive treatment, it is necessary to know what the current breast cancer relapse rate is. Therefore, in ***chapter 2*** of this thesis we evaluated the overall rates of local (LR) and regional (RR) breast cancer recurrence as well as the occurrence of contralateral breast cancer (CLC) in a relatively recent population of Dutch non- metastatic breast cancer patients treated for primary breast cancer with curative intent between 2003 and 2008. We observed a decrease in the recurrence rates over the study period that ended up very low. There were several clinicopathologic factors associated with the rate of recurrence, such as age, tumour size, lymph node involvement, tumour histologic type, grade, hormone receptor, radiotherapy and systemic treatment.

Patient age at breast cancer diagnosis is an often described factor related to breast cancer. Previous studies have reported young age as an independent prognostic factor for poor outcome. The proportion of patients with tumors displaying unfavorable disease characteristics is generally higher in younger women, with higher frequencies of poor differentiation grade, metastatic lymph node involvement, hormone receptor negative disease, HER2 overexpression, a triple negative molecular subtype and germline mutations. Increasing knowledge on tumor biology, developments in systemic treatment strategies and improved outcome in the overall population raise the question whether young age is still associated with poor prognosis in the modern era. In ***chapters 3 and 4*** we analyzed the rate of recurrence in young women diagnosed with breast cancer. In ***chapter 3*** we demonstrated a relatively low rate of local and regional recurrence in the historically considered high-risk population of women aged <35 at time of diagnosis treated for primary non-metastatic breast cancer treated between 2003 and 2008. The rates of local and regional recurrence varied by biomarker subtype with higher rates in the hormone receptor negative biomarker subtypes. These data suggest that not young age in itself, but the biology of the disease is indicative of the risk of recurrence.

Young women are at a higher risk of being over treated on the basis of age considerations. In our study, over 90% over patients were treated with chemotherapy. It is unclear whether more aggressive therapy is really benefiting the outcome of all young women. Over the last few years, several gene expression profiles have been developed to better predict clinical outcome compared to standard assessment based on clinicopathological characteristics. One of these gene expression profiles is the 70-gene signature MammaPrint® of which the clinical utility has been assessed in the randomized Microarray In Node-negative and 1-3 positive lymph-node Disease may Avoid ChemoTherapy (MINDACT) trial.

In ***chapter 4***, we demonstrated that young women, here defined as <45 years of age, more often were at a high genomic risk according to the 70-gene signature as compared to their older counterparts aged 45-55 and >55. Nevertheless, the 5-year survival without distant metastasis (DMFS) was comparable between the different age categories (<45, 45-55, >55), overall as well as according to MINDACT risk assessment group (clinical low/genomic low, clinical low/genomic high, clinical high/genomic low and clinical high/genomic high).

Axillary lymph node status is an established prognostic factor for long-term outcome. However, the prognostic value of internal mammary (IM) lymph nodes remains unclear. In approximately 20% of sentinel lymph node biopsy procedures there is drainage observed to the IM chain. We evaluated the clinical impact of tumor positive internal mammary (IM) lymph nodes on overall survival in **chapter 5** and did not observe an effect on overall survival when correcting for the presence of axillary metastasis. A tumor-positive IM node approximates the risk associated with one involved axillary lymph node.

Another clinicopathologic factor that has often been debated is the presence of multifocal disease. Current guidelines recommend basing adjuvant systemic treatment decisions on characteristics of the largest lesion thus disregarding possible biological implications of having multifocal disease. Gene expression profiles provide additional prognostic information that can help in the selection of patients for chemotherapy. Therefore, we evaluated the value of using the 70-gene profile in patients with multifocal breast cancer categorized as clinically low-risk based on their largest lesion, and therefore would not have an indication for adjuvant chemotherapy in daily practice. Tumors of patients with multifocal disease were more likely to have a high-risk 70-gene signature profile as compared to unifocal tumors. However, this did not translate in an interaction with prognosis in terms of distant metastasis-free survival, suggesting that performing the 70-gene signature profile in this setting does not result in clinical benefit (***chapter 6***).

Neoadjuvant systemic treatment is a well-established strategy for locally advanced disease with the aim of downstaging the tumor to enable more conservative surgery. However, in early

stage breast cancer patients with small tumors neoadjuvant systemic treatment is increasingly being administered as it allows for 'in vivo' monitoring of the efficacy of administered systemic treatment. The achievement of a pathologic complete response (pCR) has been associated with improved survival, mainly in hormone receptor negative breast cancer. In ***chapter 7*** we demonstrated that different levels of partial response, as defined by the Neoadjuvant Response Index (NRI) proposed by Rodenhuis et al., also provide prognostic information with regards to recurrence- free survival in a population-based cohort. Although the association between pCR and survival is widely described, there is limited data on the association between achievement of a pCR and the pattern of metastatic spread. In ***chapter 8*** we observed a higher incidence of central nervous system metastases and lower incidence of skeletal metastases in patients with pCR as compared to non-pCR.

The improved prognosis of breast cancer patients can be attributed to the developments of different treatment strategies and increase in tailoring treatment to the clinicopathologic characteristics of the individual patient. Traditional clinicopathological factors have been shown to have their limitations in their use to select optimal treatment. Advances in molecular biology have resulted in growing knowledge on mechanisms and mediators involved in the development of cancer, which has resulted in the development of new treatment strategies such as immunotherapy, anti-angiogenic agents and checkpoint inhibitors. In the ***addendum to chapter 9*** we provide an overview of the background and evidence for the use of the anti-angiogenic treatment in breast cancer.

In conclusion, breast cancer management has changed dramatically over the last decades from a 'one size fits all' approach towards tailoring treatment to the individual patient. This personalized approach will intensify in the coming years given the continuing efforts in dissecting the biology of breast cancer and combining this knowledge with both medical and technical options.

CHAPTER 10

Summary

Samenvatting

Borstkanker is de meest voorkomende soort kanker bij vrouwen wereldwijd. In Nederland worden meer dan 14.000 vrouwen per jaar gediagnosticeerd met invasieve borstkanker. Gelukkig verbetert de prognose van borstkankerpatiënten, dankzij toename van inzicht in de biologie van borstkanker en voortdurende verbeteringen in systemische therapie. Dit proefschrift richt zich op het optreden van borstkanker recidief na primaire behandeling van borstkanker met curatieve opzet, en de factoren die daarop van invloed zijn. Deze kennis kan ons helpen bij het personaliseren van borstkanker behandeling om zo over- en onderbehandeling te verminderen en daarmee hopelijk de overlevingskansen van patiënten te verbeteren en onnodige toxiciteit te beperken.

Om te identificeren bij welke patiënten er ruimte is voor de-escalatie van behandeling of juist behoefte is aan meer intensieve therapie, is het noodzakelijk om eerst te weten wat het huidige borstkanker recidief risico is. Daarom hebben we in ***hoofdstuk 2*** van dit proefschrift gekeken naar het optreden van lokaal (LR) en regionaal (RR) recidief en contralaterale borstkanker (CLC) in een relatief recente populatie van Nederlandse niet-gemetastaseerde borstkanker patiënten behandeld voor primaire borstkanker met curatieve opzet tussen 2003 en 2008. We zagen een daling van het recidief risico over de studieperiode die zeer laag eindigde. Verschillende factoren waren gerelateerd met het risico op recidief, zoals leeftijd, tumorgrootte, lymfklierbetrokkenheid, histologisch tumor type, graad, hormoonreceptor status, radiotherapie en systemische behandeling.

Leeftijd ten tijde van diagnose is een vaak beschreven factor in relatie tot borstkanker. Eerdere studies hebben gerapporteerd dat jonge leeftijd een onafhankelijke prognostische factor is voor een slechtere overleving. Het aandeel patiënten met tumoren die ongunstige kenmerken vertonen is over het algemeen hoger bij jongere vrouwen, met hogere frequenties van een slechte differentiatiegraad, metastatische lymfeklierbetrokkenheid, hormoonreceptor-negatieve ziekte, HER2-overexpressie, een triple negatief moleculair subtype en erfelijke genmutaties. De toegenomen kennis over tumorbiologie, ontwikkelingen in systemische behandelstrategieën en verbeterde prognose in de algehele borstkanker populatie doen de vraag rijzen of een jonge leeftijd nog steeds geassocieerd is met een slechte prognose in het moderne tijdperk. Daarom hebben we in ***hoofdstukken 3 en 4*** het recidief risico van jonge borstkanker patiënten geanalyseerd. In ***hoofdstuk 3*** demonstreren we een relatief lage lokaal en regionaal recidief risico in de over het algemeen als hoog-risico ingeschatte populatie van vrouwen die jonger dan 35 jaar waren ten tijde van diagnose en behandeld zijn voor primaire niet-gemetastaseerde borstkanker tussen 2003 en 2008. De lokaal en regionaal recidief risico's varieerden per biomarker subtype, met

hogere risico's in de hormoonreceptor negatieve biomarker subtypen. Deze data suggereren dat niet de jonge leeftijd, maar tumor biologie leidend is voor de prognose.

Jonge vrouwen hebben een hoger risico om overbehandeld te worden op basis van leeftijdsoverwegingen. In onze studie werden meer dan 90% van de patiënten behandeld met chemotherapie. Het is onduidelijk of meer agressieve therapie echt ten goede komt aan de prognose van alle jonge vrouwen. Verschillende genexpressieprofielen zijn ontwikkeld om de kans op terugkeer van ziekte beter te voorspellen in vergelijking met risico-inschatting op basis van traditionele clinicopathologische kenmerken. Een van deze genexpressieprofielen is het 70-genen profiel MammaPrint® waarvan de klinische waarde is onderzocht in de gerandomiseerde Microarray In Node-negative and 1-3 positive lymph-node Disease may Avoid ChemoTherapy (MINDACT) studie.

In ***hoofdstuk 4*** hebben we aangetoond dat jonge vrouwen, hier gedefinieerd als <45 jaar oud, vaker een hoog-risico genexpressie profiel hadden vergeleken met de oudere patiënt categorieën van 45-55 jaar en> 55 jaar. Desondanks was de 5-jaars afstandsmetastase-vrije overleving (DMFS) vergelijkbaar tussen de verschillende leeftijdscategorieën (<45, 45-55,> 55), zowel voor de totale groepen als voor de MINDACT-risicobeoordelingsgroep (klinisch laag/genomisch laag, klinisch laag/genomisch hoog, klinisch hoog/genomisch laag en klinisch hoog/genomisch hoog).

De axillaire lymfklier status is een gevestigde prognostische factor voor de uitkomst op lange termijn. De prognostische waarde van parasternale lymfeklieren blijft echter onduidelijk. Bij ongeveer 20% van de schildwachtklier procedures wordt drainage naar de in de parasternale keten geobserveerd. We evalueerden de klinische impact van tumor-positieve interne borstklier (IM) lymfeklieren op de totale overleving in ***hoofdstuk 5*** van dit proefschrift. We zagen geen effect van parasternale lymfkliermetastasen op de algehele overleving na correctie voor de aanwezigheid van axillaire metastasen. Een tumor-positieve parasternale lymfklier benadert het risico dat gepaard gaat met het hebben van één betrokken axillaire lymfeklier.

Een andere veel besproken clinicopathologische factor is multifocaliteit; de aanwezigheid van meerdere invasieve tumor laesies in hetzelfde kwadrant van de borst. De huidige richtlijnen bevelen aan om de besluitvorming omtrent adjuvante systemische therapie te baseren op kenmerken van de grootste laesie, waarbij dus geen rekening wordt gehouden met mogelijke biologische implicaties van multifocale ziekte. Genexpressie profielen bieden aanvullende prognostische informatie die kan helpen bij de selectie van patiënten voor chemotherapie. Daarom evalueerden we de waarde van het gebruik van het 70-genen profiel bij patiënten met multifocaal borstkanker gecategoriseerd als klinisch laag-risico op basis van hun grootste laesie,

en die dus in de dagelijkse praktijk geen indicatie zouden hebben voor adjuvante chemotherapie. Tumoren van patiënten met multifocale ziekte hadden vaker een hoog-risico 70-genen expressie profiel in vergelijking met unifocale tumoren. Dit vertaalde zich echter niet in een interactie met prognose uitgedrukt als afstandsmetastase-vrij overleving, wat suggereert dat het uitvoeren van het 70-genen profiel in deze setting niet van toegevoegde waarde is (***hoofdstuk 6***).

Neoadjuvante systemische therapie is een gevestigde strategie voor lokaal gevorderde ziekte met als doel de tumor te reduceren om zo meer conservatieve chirurgie mogelijk te maken. Deze strategie wordt ook steeds vaker toegepast bij patiënten met vroeg-stadium borstkanker en kleine tumoren, omdat het 'in vivo' beoordeling van de werkzaamheid van toegediende systemische behandeling mogelijk maakt. Het bereiken van een pathologisch complete respons (pCR) is geassocieerd met een betere overleving, voornamelijk bij hormoonreceptor-negatieve borstkanker. In ***hoofdstuk 7*** hebben we aangetoond dat verschillende niveaus van gedeeltelijke respons zoals gedefinieerd door de Neoadjuvante Response Index (NRI) voorgesteld door Rodenhuis et al., ook prognostische informatie bieden met betrekking tot recidief-vrije overleving in een populatie-gebaseerd cohort. Hoewel de associatie tussen pCR en overleving breed wordt beschreven, is er maar weinig bekend over het verband tussen pCR en het patroon van afstandsmetastasering. In ***hoofdstuk 8*** observeerden we een hogere incidentie van metastasen in het centrale zenuwstelsel en lagere incidentie van skeletmetastasen bij patiënten na pCR in vergelijking met niet-pCR.

De verbeterde prognose van borstkankerpatiënten kan worden toegeschreven aan ontwikkelingen van verschillende behandelingsstrategieën en de toename in afstemming van de behandeling op de clinicopathologische kenmerken van de individuele patiënt. Traditionele clinicopathologische factoren bleken hun beperkingen te hebben in hun gebruik om een optimale behandeling te selecteren. Vooruitgang in de moleculaire biologie heeft geresulteerd in een groeiende kennis over mechanismen en mediatoren die betrokken zijn bij de ontwikkeling van kanker, wat heeft geresulteerd in de ontwikkeling van de hiervoor beschreven genexpressie profielen alsook nieuwe behandelingsstrategieën zoals immunotherapie, anti-angiogene therapie en 'checkpoint' remmers. In het **addendum bij hoofdstuk 9** geven we een overzicht van de achtergrond en het bewijs voor het gebruik van anti-angiogene behandeling bij borstkanker.

In conclusie, de behandeling van borstkanker is de afgelopen decennia dramatisch veranderd van een 'one size fits all'-benadering naar het aanpassen van de behandeling aan de individuele patiënt. Deze gepersonaliseerde aanpak zal de komende jaren intensiveren dankzij de voortdurende inspanningen om de biologie van borstkanker verder te analyseren en deze kennis te combineren met zowel medische als technische ontwikkelingen.

CHAPTER 11

Appendices

Review committee of this thesis

List of publications

Acknowledgements

Curriculum Vitae

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CHAPTER 11

Appendices

Review committee of this thesis

List of publications

Acknowledgements

Curriculum Vitae

List of publications

2018

K.C. Aalders, N. Touati, K. Tryfonidis, M. Annonay, S. Litiere, J. Bergh, A. Bodmer, D.A. Cameron, H.R. Bonnefoi, on behalf of the EORTC 10994/BIG 1-00 Study Investigators. *Do patients whose tumor achieved a pathological response relapse at specific sites? A substudy of the EORTC 10994/BIG-1-00 trial.* Breast Cancer Res Treat. 2018; 169(3):497-505

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*Acknowledgement

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*Acknowledgement

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CHAPTER 11

Appendices

Acknowledgements

This thesis would not have been possible without the support and encouragement from a lot of people: colleagues, family and friends. It has been a great experience and I am very thankful for everyone who has helped me in this journey.

First of all, to my co-promotor and promotors, in Dutch:

Dr. van Dalen, beste Thijs. Via mijn semi-arts stage in 2012 naar ANIOS naar arts-onderzoeker, is mijn pad binnen de chirurgie begonnen in het Diakonessenhuis. Vanuit niets hebben we een heel mooi onderzoekstraject opgezet waarmee mijn enthousiasme voor wetenschappelijk onderzoek is begonnen en heeft geleid tot waar ik nu ben. Ik weet dat ik met mijn eigenwijsheid en neiging alles te willen weten niet altijd de makkelijkste voor je was, maar ik hoop dat je weet hoe dankbaar ik ben voor al je support en onze samenwerking de afgelopen jaren!

Prof. Rutgers, beste Emiel. Drie jaar geleden mocht ik langs komen in een gezellig kennismakingsgesprek om te praten over een eventueel fellowship bij de EORTC Breast Cancer Group en aan het eind van het gesprek was het besluit al genomen. Heel erg bedankt voor de enorm bijzondere kans en ervaring om naar Brussel te gaan. Het heeft zo veel deuren geopend en bevestigd dat ik echt door wil gaan met onderzoek. Bedankt voor je vertrouwen, betrokkenheid en de leuke tijd! Ik vind het heel bijzonder dat je mijn promotor bent.

Prof. Borel Rinkes, beste Inne. Kort, maar krachtig. Vooral in de laatste maanden van mijn promotie samen gewerkt voor het afronden van mijn proefschrift. Bedankt voor de doortastende feedback, hulp en het fijne laagdrempelige contact.

There are so many more people that I would like to thank for their support, trust, inspiration and just being there. A special thanks to:
Dr. F. Cardoso, dr. L. van't Veer, prof. M. Piccart, Dr. M. van der Heiden, prof. L.J. Boersma, Dr. G.S. Sonke, prof. P.J. van Diest, prof. Siesling, prof. D. Cameron, prof. H. Bonnefoi, dr. E. Senkus, Emily Postma, Konstantinos Tryfonidis, Coralie Poncet, Leen Slaets, Saskia Litiere, Nathan Touati, Els van Genbrugge, dr. L.J. Strobbe, dr. E.V.E. Madsen, dr. P.D. Gobardhan, dr. F.W. van der Ent, dr. S.G. Elias, dr. R.A. Valdés Olmes, prof. G. Viale, dr. A.M. Glas, dr. M. Delorenzi, prof. J. Bergh, Dr. A. Bodmer, dr. B. Pistilli, drs. M. Annonay, dr. M.H. Hamaker, Olga Klok, Romy Liesdek, Annelotte van Bommel, Marieke Straver, Anne Kuijer, Pouline van Oort, Marissa van Maaren, Janneke Verloop, Linda de Munck, Jan-Maarten van der Zwan, former colleagues from the EORTC Headquarters, all EORTC Breast Cancer Group members, my fellow Young BCG steering committee members, SABCS jonkies, oud-collega's en stafleden

van het Diakonessenhuis, oud-collega's en stafleden van Ziekenhuis Geldese Vallei, collega's en stafleden van Elisabeth-Tweesteden ziekenhuis, the review committee of this thesis, de Moedt meisjes, Lotte, Marjolein, Linde, Myrthe, mijn paranimfen Merel en Lieveke, mijn gehele familie, Thomas, Mae, Marieke, Arun, Joy, mam, pap & Douwe.

And now it is time for 'mafkees' and 'sukkeltje' to go on an adventure: Canada here we come!

CHAPTER 11

Appendices

Curriculum Vitae

Kim Carmen Aalders was born on April 26th 1988 in Ermelo, the Netherlands. In 2006, she graduated from Christelijk College Nassau Veluwe in Harderwijk. She continued with medical school at the University of Utrecht, where she obtained her medical degree in 2012. Her senior internship was done at the Department of Surgery in Diakonessenhuis Utrecht, where she returned as a surgical resident after graduation. During her residency she developed a strong affinity with surgical oncology. This resulted in the start of a PhD track within the scope of breast cancer recurrence under the supervision of Dr. van Dalen, Prof. Rutgers and Prof. Borel Rinkes. IN 2015 she received the opportunity to go to Brussels for a medical research fellowship for the Breast Cancer Group of the European Organisation for Research and Treatment of Cancer (EORTC). During this period, she worked on various aspects of clinical trials including the MINDACT, LORD, P53, BRAVO, 75111, Male BC and Treat CTC trials. Under the supervision of Dr. Cardoso, she became study co-coordinator of a new translational substudy of the MINDACT, the MINDACT Relapses project, that aims to further characterize breast cancer progression and therapy resistance by performing whole genome sequencing on the primary tumor, metastasis and blood samples. She is also involved in the study team of a new trial in development, evaluating de-escalation of treatment in older breast cancer patients with small Luminal-A breast cancers (EUROPA trial). To develop the protocol of this study, she went to the ECCO-AACR-EORTC-ESMO Workshop: Methods in Clinical Cancer Research in 2016. As of mid-2017 she has returned to the Netherlands and has first worked as a surgical resident at Gelderse Vallei Hospital in Ede before transferring to the Elisabeth-TweeSteden Hospital in Tilburg. In the meantime, she completed her thesis. In the future, she intends to keep combining clinics with scientific research.