Double trouble: exploring risk factors to better predict contralateral breast cancer

Kramer, I.

DOUBLE TROUBLE

Exploring risk factors to better predict contralateral breast cancer

Iris Kramer

Double Trouble

Exploring risk factors to better predict contralateral breast cancer

Iris Kramer

The work presented in this thesis was performed at the Netherlands Cancer Institute – Antoni van Leeuwenhoek, Amsterdam, the Netherlands, in cooperation with the Erasmus Medical Center, Rotterdam, the Netherlands and Leiden University Medical Center, Leiden, the Netherlands.

The research was funded by a grant from Alpe d'HuZes/Dutch Cancer Society (KWF Kankerbestrijding) under grant number A6C/6253.

Financial support for publication of this thesis was kindly provided by the Netherlands Cancer Institute – Antoni van Leeuwenhoek and the Netherlands Comprehensive Cancer Organization (IKNL).

Cover design: Maria Escala Garcia & Iris Kramer
Layout: Ilse Modder | www.ilsemodder.nl
Printing: Gildeprint Enschede | www.gildeprint.nl
ISBN: 978-94-6419-352-7

Double Trouble

Exploring risk factors to better predict contralateral breast cancer

Proefschrift

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof.dr.ir. H. Bijl,
volgens besluit van het college voor promoties
te verdedigen op dinsdag 14 december 2021
klokke 15.00 uur

door

Iris Kramer
geboren te Woerden
in 1994

Promotor:	prof. dr. ir. M.K. Schmidt	
Co-promotor:	dr. M.J. Hooning	*Erasmus MC*
Leden promotiecommissie:	prof. dr. A.M. Stiggelbout	
	prof. dr. J. Wesseling	
	prof. dr. S. Siesling	*Universiteit Twente*
	prof. dr. C.H. van Gils	*UMCU*

Table of contents

CHAPTER 1

General introduction

Introduction

Breast cancer: incidence and mortality

Breast cancer is the most common cancer and leading cause of cancer-related death among women worldwide, with an estimated 2.1 million new cases and 627,000 deaths in 2018[1]. During the last decades, the age-corrected population incidence of invasive breast cancer has been rising in most developed countries, mainly because of changes in reproductive factors and lifestyle[1-4]. Meanwhile, mortality rates have been steadily decreasing in most countries, which may partially be explained by earlier detection by mammographic screening, the increasing use of (neo)adjuvant therapies, and better health care in general[2,3,5]. These trends in breast cancer incidence and mortality were also observed in the Netherlands (Figure 1)[5]. In the Netherlands, the 10-year relative survival of women diagnosed with breast cancer stage I-IV improved from 56% in 1989-1999 to 83% in 2010-2016 (Appendix 1)[5].

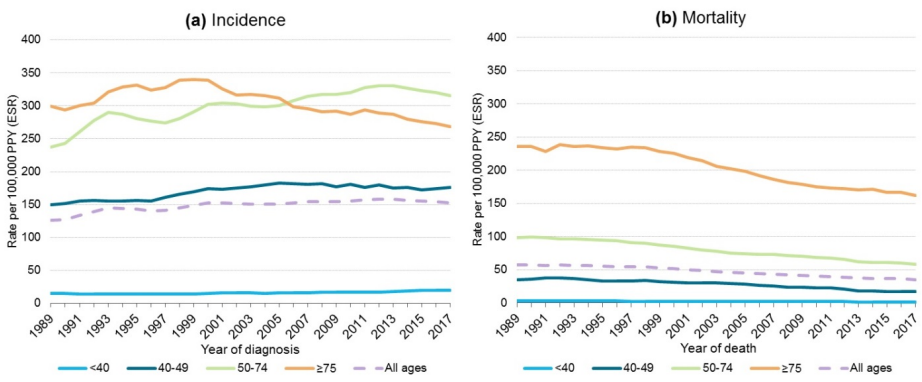


Figure 1. All ages combined and age-specific first primary invasive breast cancer incidence (A) and mortality (B) trends in the Netherlands in the period 1989 to 2017 (Data source: adapted from reference[5], Appendix 1)

Contralateral breast cancer: risk and prevention

Due to the improved breast cancer survival, a larger number of women are at risk of developing a new primary tumor in the opposite breast, which is known as contralateral breast cancer (CBC). CBC is the most common second cancer reported after first breast cancer, comprising between 30 and 50% of all second cancers[6-8]. In the general breast cancer population, around 4 out of 100 women will develop a CBC within 10 years after the first breast cancer diagnosis[9,10]. Patients with CBC have potentially a worse prognosis compared to patients with unilateral breast cancer[11-13].

Even though the incidence of CBC is relatively low in the general breast cancer population, an increasing number of patients with unilateral breast cancer opt for preventive removal of the contralateral breast[10,14], defined as contralateral prophylactic mastectomy. A large population-based cohort study in the United States observed a 3-fold increase in the rate of contralateral prophylactic mastectomy between 2002 (3.9%) and 2012 (12.7%) among breast cancer patients[14]. Patients opt for contralateral prophylactic mastectomy because, for example, they are worried about developing cancer in the other breast or they overestimate their risk[15]. Removal of the other breast significantly reduces the risk of CBC, but the procedure is invasive, irreversible, and can negatively affect women's quality of life[16].

To improve decision making for breast cancer patients, individualized risk estimations of CBC are needed to distinguish between patients who are at high or low risk. To date, accurate risk estimation is lacking, and the prediction of CBC as used in clinical practice is mostly only based on *BRCA1/2* mutation status, family history of breast cancer, and age at first breast cancer diagnosis[17-20]. From various studies we know that additional factors may play a role in the development of CBC, but for some of these factors reliable associations are either lacking or results have been conflicting[20].

In this thesis, we will investigate selected, potentially important breast cancer-related risk and treatment factors to better determine their association with CBC risk. In the next paragraph, we will elaborate on the currently known risk factors and we will address the factors for which there is still less evidence in literature.

Risk factors of (contralateral) breast cancer

Family history and genetic factors

A positive family history is an established risk factor for both primary breast cancer and CBC[20,21]. In a systematic review investigating risk factors associated with CBC results showed that women with a first-degree relative (e.g. mother or sister) with breast cancer had a 1.65- fold increased risk of developing CBC[20].

During the 90s, the breast cancer susceptibility genes *BRCA1* and *BRCA2* were discovered[22,23]. A woman with a *BRCA1/2* germline mutation has 45-72% lifetime risk of developing breast cancer, which is substantially higher than the 12.5% lifetime risk of the general population[24,25]. It is well known that *BRCA1/2* mutation carriers also have higher risks of developing CBC, with approximately two- to fourfold higher risks compared with breast cancer patients without these mutations[20]. A lesser penetrant mutation in the *CHEK2* gene has also been found to be associated with increased risk of both first and CBC[26,27].

Recently, genome-wide association studies have identified multiple common germline variants associated with breast cancer risk[28,29]. Most of these common variants are single nucleotide polymorphisms, which represent a change in a single DNA nucleotide. Although these variants individually have a small impact on breast cancer risk, their effects can be summarized in a polygenic risk score which has been shown to be highly predictive for the development of first primary breast cancer[30,31]. Recently,

Mavaddat et al.[31] developed and validated a polygenic risk score of 313 common variants associated with breast cancer. Whether the polygenic risk score is also predictive for CBC is still unclear and needs to be further investigated.

Primary tumor and treatment factors

A woman's risk of CBC also depends on the tumor and treatment characteristics of the first primary tumor. Breast cancer is a highly heterogeneous disease with a variety of subtypes, with possibly a different etiology.

There are two main most prevalent histological types of breast cancer; ductal carcinoma (most common type) and lobular carcinoma. The majority of the breast cancers are invasive, meaning that the abnormal cells have spread from their place of origin (terminal duct lobular unit) into the surrounding breast tissue, and possibly to the nearby lymph nodes and other parts of the body. When the cells are still contained in the milk duct or lobules, and have not spread into any normal surrounding breast tissue, the breast cancer is called non-invasive. The most common type of non-invasive breast cancer is ductal carcinoma in situ (DCIS). The risk of invasive CBC for women with DCIS has not been widely investigated, but the annual risk is estimated between 0.4% and 0.6%[32-35]. It is still unclear if the risk of CBC is comparable between women diagnosed with invasive breast cancer and women with DCIS.

Breast cancers can also be subdivided by receptor subtypes. Classification of receptor subtypes can be done by evaluating various immunohistochemistry markers such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). There are four main receptor subtypes: Luminal A (ER+ and/or PR+ and HER2−), Luminal B (ER+ and/or PR+ and HER2+), HER2-enriched (ER−/PR−/HER2+), and triple negative (ER−/PR−/HER2−) (Figure 2). Subtyping of breast tumors by ER, PR, and HER2 status has become routine in clinical care and is used to guide treatment decisions. Besides surgery and possibly radiotherapy, treatment for breast cancer is commonly supplemented with adjuvant systemic therapy, i.e., chemotherapy, endocrine therapy, and/or trastuzumab. Endocrine therapy and trastuzumab are widely used to treat breast cancers that are ER-positive or HER2-positive, respectively (Figure 2).

Although adjuvant systemic therapy is intended to lower the risk of breast cancer recurrence, studies have shown that adjuvant chemotherapy and endocrine therapy are also associated with strong reductions in CBC risk[36-40]. Whether specific regimens of chemotherapy (e.g. taxane-containing vs anthracycline-containing chemotherapy) and endocrine therapy (e.g. tamoxifen vs aromatase inhibitors) have different effects on CBC risk, and if trastuzumab use is also associated with a decreased CBC risk, is still unclear.

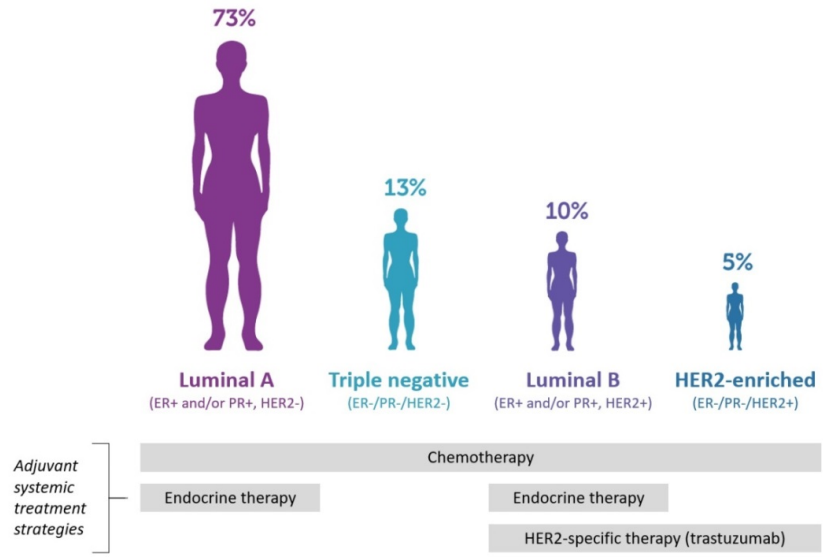


Figure 2. Distribution of breast cancer receptor subtypes and possible strategies for adjuvant systemic therapy
Abbreviations: ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2

Lifestyle, reproductive and other patient-related factors

A younger age at first breast cancer diagnosis has been shown to be a risk factor in a number of population-based studies, both in the sporadic setting as in patients with genetic predisposition[20].

A factor with less evidence for an association with CBC is mammographic density. High mammographic density represents a large amount of fibroglandular tissue (dense tissue), as compared to fat (non-dense tissue)[41]. It is well known that dense breast tissue on a mammogram is a strong risk factor for the development of a first breast cancer[41,42]. For CBC risk, results of the effects of mammographic breast density are limited and contradicting[20].

There is interest in the impact of lifestyle and reproductive factors on CBC risk among clinicians and breast cancer survivors, since these factors are partly modifiable. A systemic review and meta-analysis[43] reported a moderately increased risk of CBC in women being overweight and a slightly elevated risk for alcohol use. In addition, older age at primiparity, being nulliparous, and an older age at menopause were also suggestive of increased risk of CBC[43].

An overview of all known and potential risk factors of CBC can be found in Table 1.

Table 1. Overview of known and potential risk factors associated with contralateral breast cancer risk[20,43]

Family history and genetic factors	Primary tumor and treatment factors		Lifestyle, reproductive and other patient-related factors
Known risk factors			
• Family history for breast cancer	• Tumor size	• **Chemotherapy** (protective effect)	• Young age at 1st breast cancer diagnosis
• *BRCA1/2* mutation	• Lobular histology	• **Endocrine therapy** (protective effect)	• BMI at 1st breast cancer diagnosis
• *CHEK2 c.1100delC* mutation		• Radiotherapy <40 years	
Potential risk factors			
• **Common genetic variants**	• **ER/PR/HER2 expression**	• **Taxane-containing vs anthracycline-containing chemotherapy**	• Breast density
	• **DCIS vs invasive breast cancer**	• **Tamoxifen vs aromatase inhibitors**	• Alcohol use
		• **Trastuzumab**	• Nulliparous
			• Age at primiparity
			• Age at menopause

Abbreviations: ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2; DCIS = ductal carcinoma in situ; BMI = body mass index
In **bold** are those factors that will be further investigated in this thesis

CBC risk prediction model

To make the information on all risk factors useful in clinical practice, the risk factors should not be considered individually in risk prediction, but in combination in a comprehensive risk prediction model[44]. A risk prediction model is a statistical model that uses patients risk factor data to estimate the probability of a healthcare outcome, and often aims to accurately stratify individuals into clinically relevant risk categories (e.g. high or low risk of developing a disease)[44]. Based on the same Dutch Cancer Society funded project, and in another PhD trajectory parallel to the work presented in this thesis, a risk prediction model was developed and validated to predict the risk of CBC based on all known and available risk factors, including patient, primary tumor and treatment characteristics, and *BRCA1/2* mutation status[9].

For a valid risk prediction model to be useful in clinical practice, it needs to be incorporated in a decision support tool. This tool can then support shared decision-making between clinicians and patients for decisions on treatment for the first breast cancer, including potential risk reducing strategies for the contralateral breast or additional follow up for women at high risk, or, to reassure women who are at low risk of CBC.

A key feature of effective shared-decision making is clear risk communication between clinicians and patients. Risk communication is defined as the interactive exchange of information, advice and opinions between experts and people facing threats to their health, with the aim to improve understanding of risk and to promote better decisions about clinical management[45]. One of the challenges to risk communication with patients using decision support tools is to convey quantitative information in a comprehensive form that patients can easily understand. Good quality information and graphics are needed to explain probabilities and options for treatment during clinician-patient consultations. Therefore, it is important to iteratively modify or adjust the tool with potential end users based on their preferences, before its final implementation in clinical practice. A design process that explores the users' needs could potentially increase usage in the future.

Goal of this thesis

The main goal of this thesis was to explore risk factors associated with CBC for which there is insufficient evidence in literature. The results can be useful to improve our understanding of the etiology of CBC and can be used to optimize risk prediction models that predict CBC. Furthermore, as a first step towards implementation of a risk prediction tool, we aimed to explore preferences of breast cancer survivors regarding graphical presentation of probabilities in a CBC risk prediction model.

Outline

We investigated associations between several risk factors and CBC risk using several large population- or hospital-based cohorts (Table 2). In **Chapter 2**, we investigated the association between a polygenic risk score of 313 common germline variants and CBC risk using data from the large breast cancer series of the Breast Cancer Association Consortium. In **Chapter 3**, we performed a population-based cohort study using national data from the Netherlands Cancer Registry (NCR) to investigate the influence of different regimens of adjuvant endocrine therapy, chemotherapy, and trastuzumab on CBC risk. In addition, we investigated if these regimens had different effects on the (hormone) receptor subtype of the CBC. In **Chapter 4**, we estimated the CBC risk in patients with DCIS versus patients with invasive breast cancer in a population-based cohort study with NCR data.

To make a first step towards implementation of a CBC risk prediction model, **Chapter 5** shows the results of an exploratory study, in which we interviewed 19 breast cancer survivors, to investigate preferences for graphical presentation of probabilities in a CBC risk prediction model.

We conclude with a general discussion in **Chapter 6**, where we put our main findings into perspective and discuss clinical implications.

Table 2. Datasets used in this thesis

Chapter	Source	Description	Country	N	Years of primary breast cancer diagnosis	Median follow-up
2	Breast Cancer Association Consortium (BCAC)	Population- and hospital-based studies	USA, US, Europe, Asia, Australia	Cohort: 56,068 Case-case series: 147,437	1947-2017	8.4 years
3	The Netherlands Cancer Registry (NCR)	Population-based study	The Netherlands	83,144	2003-2010	7.7 years
4	The Netherlands Cancer Registry (NCR)	Population-based study	The Netherlands	303,839	1989-2017	11.4 years
5	Interviews with breast cancer survivors	Qualitative study; interview by telephone	The Netherlands	19	1983-2019	N.A.

References

1 Bray, F. *et al.* Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **68**, 394-424, doi:10.3322/caac.21492 (2018).

2 Bray, F., McCarron, P. & Parkin, D. M. The changing global patterns of female breast cancer incidence and mortality. *Breast Cancer Res.* **6**, 229-239, doi:10.1186/bcr932 (2004).

3 Youlden, D. R. *et al.* The descriptive epidemiology of female breast cancer: an international comparison of screening, incidence, survival and mortality. *Cancer Epidemiol.* **36**, 237-248, doi:10.1016/j.canep.2012.02.007 (2012).

4 Heath, A. K. *et al.* Nutrient-wide association study of 92 foods and nutrients and breast cancer risk. *Breast Cancer Res.* **22**, 5, doi:10.1186/s13058-019-1244-7 (2020).

5 van der Meer, D. J. *et al.* Comprehensive trends in incidence, treatment, survival and mortality of first primary invasive breast cancer stratified by age, stage and receptor subtype in the Netherlands between 1989 and 2017. *Int. J. Cancer* **148**, 2289-2303, doi:10.1002/ijc.33417 (2021).

6 Curtis, R. E. *New malignancies among cancer survivors: SEER cancer registries, 1973-2000*. (US Department of Health and Human Services, National Institutes of Health ..., 2006).

7 Soerjomataram, I. *et al.* Risks of second primary breast and urogenital cancer following female breast cancer in the south of The Netherlands, 1972-2001. *Eur. J. Cancer* **41**, 2331-2337, doi:10.1016/j.ejca.2005.01.029 (2005).

8 Evans, H. S. *et al.* Incidence of multiple primary cancers in a cohort of women diagnosed with breast cancer in southeast England. *Br. J. Cancer* **84**, 435-440, doi:10.1054/bjoc.2000.1603 (2001).

9 Giardiello, D. *et al.* Prediction and clinical utility of a contralateral breast cancer risk model. *Breast Cancer Res.* **21**, 144, doi:10.1186/s13058-019-1221-1 (2019).

10 Xiong, Z. *et al.* Patterns of Occurrence and Outcomes of Contralateral Breast Cancer: Analysis of SEER Data. *J Clin Med* **7**, doi:10.3390/jcm7060133 (2018).

11 Schaapveld, M. *et al.* The impact of adjuvant therapy on contralateral breast cancer risk and the prognostic significance of contralateral breast cancer: a population based study in the Netherlands. *Breast Cancer Res. Treat.* **110**, 189-197, doi:10.1007/s10549-007-9709-2 (2008).

12 Vichapat, V. *et al.* Prognosis of metachronous contralateral breast cancer: importance of stage, age and interval time between the two diagnoses. *Breast Cancer Res. Treat.* **130**, 609-618, doi:10.1007/s10549-011-1618-8 (2011).

13 Langballe, R. *et al.* Mortality after contralateral breast cancer in Denmark. *Breast Cancer Res. Treat.* **171**, 489-499, doi:10.1007/s10549-018-4846-3 (2018).

14 Wong, S. M. *et al.* Growing Use of Contralateral Prophylactic Mastectomy Despite no Improvement in Long-term Survival for Invasive Breast Cancer. *Ann. Surg.* **265**, 581-589, doi:10.1097/sla.0000000000001698 (2017).

15 Ager, B., Butow, P., Jansen, J., Phillips, K. A. & Porter, D. Contralateral prophylactic mastectomy (CPM): A systematic review of patient reported factors and psychological predictors influencing choice and satisfaction. *Breast* **28**, 107-120, doi:10.1016/j.breast.2016.04.005 (2016).

16 Carbine, N. E., Lostumbo, L., Wallace, J. & Ko, H. Risk-reducing mastectomy for the prevention of primary breast cancer. *Cochrane Database Syst. Rev.* **4**, Cd002748, doi:10.1002/14651858.CD002748.pub4 (2018).

17 van den Broek, A. J. *et al.* Impact of Age at Primary Breast Cancer on Contralateral Breast Cancer Risk in BRCA1/2 Mutation Carriers. *J. Clin. Oncol.* **34**, 409-418, doi:10.1200/jco.2015.62.3942 (2016).

18 Reiner, A. S. *et al.* Risk of asynchronous contralateral breast cancer in noncarriers of BRCA1 and BRCA2 mutations with a family history of breast cancer: a report from the Women's Environmental Cancer and Radiation Epidemiology Study. *J. Clin. Oncol.* **31**, 433-439, doi:10.1200/jco.2012.43.2013 (2013).

19 Graeser, M. K. *et al.* Contralateral breast cancer risk in BRCA1 and BRCA2 mutation carriers. *J. Clin. Oncol.* **27**, 5887-5892, doi:10.1200/jco.2008.19.9430 (2009).

20 Akdeniz, D. *et al.* Risk factors for metachronous contralateral breast cancer: A systematic review and meta-analysis. *Breast* **44**, 1-14, doi:10.1016/j.breast.2018.11.005 (2019).

21 Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. *Lancet* **358**, 1389-1399, doi:10.1016/s0140-6736(01)06524-2 (2001).

22 Miki, Y. *et al.* A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* **266**, 66-71, doi:10.1126/science.7545954 (1994).

23 Wooster, R. *et al.* Identification of the breast cancer susceptibility gene BRCA2. *Nature* **378**, 789-792, doi:10.1038/378789a0 (1995).

24 Kuchenbaecker, K. B. *et al.* Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. *JAMA* **317**, 2402-2416, doi:10.1001/jama.2017.7112 (2017).

25 Antoniou, A. *et al.* Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. *Am. J. Hum. Genet.* **72**, 1117-1130, doi:10.1086/375033 (2003).

26 Weischer, M. *et al.* CHEK2*1100delC heterozygosity

in women with breast cancer associated with early death, breast cancer-specific death, and increased risk of a second breast cancer. *J. Clin. Oncol.* **30**, 4308-4316, doi:10.1200/jco.2012.42.7336 (2012).
27 Dorling, L. *et al.* Breast Cancer Risk Genes - Association Analysis in More than 113,000 Women. *N. Engl. J. Med.* **384**, 428-439, doi:10.1056/NEJMoa1913948 (2021).
28 Michailidou, K. *et al.* Association analysis identifies 65 new breast cancer risk loci. *Nature* **551**, 92, doi:10.1038/nature24284
https://www.nature.com/articles/nature24284#supplementary-information (2017).
29 Milne, R. L. *et al.* Identification of ten variants associated with risk of estrogen-receptor-negative breast cancer. *Nat. Genet.* **49**, 1767-1778, doi:10.1038/ng.3785 (2017).
30 Mavaddat, N. *et al.* Prediction of breast cancer risk based on profiling with common genetic variants. *J. Natl. Cancer Inst.* **107**, doi:10.1093/jnci/djv036 (2015).
31 Mavaddat, N. *et al.* Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. *Am. J. Hum. Genet.* **104**, 21-34, doi:10.1016/j.ajhg.2018.11.002 (2019).
32 Elshof, L. E. *et al.* Subsequent risk of ipsilateral and contralateral invasive breast cancer after treatment for ductal carcinoma in situ: incidence and the effect of radiotherapy in a population-based cohort of 10,090 women. *Breast Cancer Res. Treat.* **159**, 553-563, doi:10.1007/s10549-016-3973-y (2016).
33 Miller, M. E. *et al.* Contralateral Breast Cancer Risk in Women with Ductal Carcinoma In Situ: Is it High Enough to Justify Bilateral Mastectomy? *Ann. Surg. Oncol.* **24**, 2889-2897, doi:10.1245/s10434-017-5931-2 (2017).
34 Claus, E. B., Stowe, M., Carter, D. & Holford, T. The risk of a contralateral breast cancer among women diagnosed with ductal and lobular breast carcinoma in situ: data from the Connecticut Tumor Registry. *Breast* **12**, 451-456, doi:10.1016/s0960-9776(03)00152-8 (2003).
35 Falk, R. S., Hofvind, S., Skaane, P. & Haldorsen, T. Second events following ductal carcinoma in situ of the breast: a register-based cohort study. *Breast Cancer Res. Treat.* **129**, 929-938, doi:10.1007/s10549-011-1531-1 (2011).
36 Davies, C. *et al.* Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet* **378**, 771-784, doi:10.1016/s0140-6736(11)60993-8 (2011).
37 Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomised trials. *The Lancet* **351**, 1451-1467 (1998).
38 Early Breast Cancer Trialists' Collaborative Group. Polychemotherapy for early breast cancer: an overview of the randomised trials. *Lancet* **352**, 930-942 (1998).
39 Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. *Lancet* **386**, 1341-1352, doi:10.1016/s0140-6736(15)61074-1 (2015).
40 Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* **365**, 1687-1717, doi:10.1016/s0140-6736(05)66544-0 (2005).
41 Lokate, M. *et al.* Mammographic density and breast cancer risk: the role of the fat surrounding the fibroglandular tissue. *Breast Cancer Res.* **13**, R103, doi:10.1186/bcr3044 (2011).
42 McCormack, V. A. & dos Santos Silva, I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. *Cancer Epidemiol. Biomarkers Prev.* **15**, 1159-1169, doi:10.1158/1055-9965.Epi-06-0034 (2006).
43 Akdeniz, D. *et al.* The impact of lifestyle and reproductive factors on the risk of a second new primary cancer in the contralateral breast: a systematic review and meta-analysis. **31**, 403-416 (2020).
44 Janes, H., Pepe, M. S. & Gu, W. Assessing the value of risk predictions by using risk stratification tables. *Ann. Intern. Med.* **149**, 751-760, doi:10.7326/0003-4819-149-10-200811180-00009 (2008).
45 Ahmed, H., Naik, G., Willoughby, H. & Edwards, A. G. Communicating risk. *BMJ* **344**, e3996, doi:10.1136/bmj.e3996 (2012).

CHAPTER 2

Breast cancer polygenic risk score and contralateral breast cancer risk

Am J Hum Genet. 2020 Nov 5;107(5):837-848

Iris Kramer
Maartje J. Hooning
Nasim Mavaddat
Michael Hauptmann
Renske Keeman
Ewout W. Steyerberg
Daniele Giardiello
Antonis C. Antoniou
Paul D.P. Pharoah
Sander Canisius
BCAC collaborators*
Douglas F. Easton
Per Hall
Marjanka K. Schmidt

**For full author list see publication*

Abstract

Previous research has shown that polygenic risk scores (PRS) can be used to stratify women according to their risk of developing primary invasive breast cancer. This study aimed to evaluate the association between a recently validated PRS of 313 germline variants (PRS_{313}) and contralateral breast cancer (CBC) risk. We included 56,068 women of European ancestry diagnosed with first invasive breast cancer from 1990 onwards with follow-up from the Breast Cancer Association Consortium. Metachronous CBC risk (N=1,027) according to the distribution of the PRS_{313} was quantified using Cox regression analyses. We assessed PRS_{313} interaction with age at first diagnosis, family history, morphology, ER-, PR-, and HER2-status, and (neo)adjuvant therapy. In Asian studies, with limited follow-up, CBC risk associated with PRS_{313} was assessed using logistic regression for 340 women with CBC compared with 12,133 women with unilateral breast cancer. Higher PRS_{313} was associated with increased CBC risk: hazard ratio per standard deviation (SD)=1.25 (95%CI=1.18-1.33) for Europeans, and an OR per SD=1.15 (95%CI=1.02-1.29) for Asians. The absolute lifetime risks of CBC, accounting for death as competing risk, were 12.4% for European women at the 10th percentile and 20.5% at the 90th percentile of the PRS_{313}. We found no evidence of confounding by, or interaction with patient characteristics, characteristics of the primary tumor, or treatment. The C-index for the PRS_{313} alone was 0.563 (95%CI=0.547-0.586). In conclusion, the PRS_{313} is an independent factor associated with CBC risk, and may be incorporated in CBC risk prediction models to help improve stratification of patients and optimize surveillance and treatment strategies.

Introduction

Due to the high incidence of breast cancer and improving survival, an increasing number of breast cancer survivors are at risk of developing contralateral breast cancer (CBC). The 10-year cumulative incidence of CBC is ~4%[1,2], however estimates vary widely depending on factors such as germline genetics, family history, and (neo)adjuvant systemic therapy for the first breast cancer[3]. The risk of developing CBC is particularly high in women with rare mutations in certain genes including *BRCA1, BRCA2,* and *CHEK2*, with approximately two- to fourfold higher risks reported compared with women without these mutations[3].

Recently, genome-wide association studies (GWAS) have identified multiple common germline variants that are associated with first primary breast cancer risk[4,5]. These are associated with small differences in risk individually, but their combined effects can be summarized in a polygenic risk score (PRS), which has been shown to stratify women according to their risk of developing breast cancer[6-9]. Using a large GWAS dataset from the Breast Cancer Association Consortium (BCAC), we previously developed and validated a 313-variant PRS (PRS_{313}) among women of European descent. In independent prospective studies, this PRS_{313} predicted the risk of primary invasive breast cancer with an odds ratio (OR) per standard deviation (SD) of 1.61 (95% confidence interval (95%CI)=1.57-1.65)[7]. The PRS_{313} has also been externally validated using the UK Biobank cohort.

The aim of the current study was to evaluate the association between PRS_{313} and CBC risk, using data from BCAC. Other studies have shown associations between risk of CBC and both a 67-variant PRS[10] and individual variants[11], but not yet with PRS_{313}, the most extensively validated PRS. Further, the dataset currently evaluated is larger than those previously tested. We carried out two types of analyses. We conducted a cohort study among studies of European ancestry women with follow-up data available, and performed Cox regression analyses to estimate hazard ratios (HRs) for CBC. Potential confounding and interaction with patient characteristics, characteristics of the primary tumor, or treatment were tested. In addition, to directly compare with the OR reported for PRS_{313} and first breast cancer, we selected case-case series and performed logistic regression analyses comparing the PRS_{313} distribution in women with CBC versus those with unilateral breast cancer. These analyses were conducted separately in European and Asian women (follow-up was too limited to perform a cohort study for the Asian population). Use of PRS_{313} may lead to more accurate CBC risk prediction to support decision making for women who may or may not benefit from additional surveillance and risk-reducing treatment strategies.

Material and Methods

Study subjects

Case-case series

We selected women who were diagnosed with breast cancer and women without any diagnosis of breast cancer from the BCAC including all women of European ancestry, based on genotyping data, selecting only those studies which reported on CBC (62 studies) (Figure S1A, Table S1-S2). BCAC database version freeze 12 was used. All women diagnosed with invasive breast cancer as a first cancer were included in the analysis; the small number of tumors with unknown invasiveness were considered invasive (Table S2). In the case-case series, a CBC was defined as a breast cancer (in situ or invasive) in the contralateral breast irrespective of the time since the first breast cancer. The case-case series comprised 81,000 women with unilateral breast cancer, 3,607 women with CBC, and 62,830 women without any diagnosis of breast cancer (Figure S1A). We also compared women with unilateral breast cancer to women without any diagnosis of breast cancer to reproduce the estimate that was previously reported for first breast cancer risk[7] in our study selection.

We selected for a separate analysis women of Asian ancestry of the BCAC data comprising 12,133 women with unilateral breast cancer, 340 women with CBC, and 13,398 women without any diagnosis of breast cancer from eight studies (Figure S1B, Table S2).

European cohort

In the European cohort we used metachronous CBC as the outcome, defined as a breast cancer in the contralateral breast (in situ or invasive) diagnosed at least three months after the first breast cancer. We used a cut-off of three months to reduce the likelihood that these CBCs represent metastases rather than true second primary tumors. We selected all women diagnosed with breast cancer from the European case-case series and excluded four studies that did not provide follow-up information on vital status (Figure S1A). We did not include Asian women since follow-up was too limited in these studies. We additionally excluded 6,207 women with no follow-up and 2,208 women who developed synchronous CBC, distant metastasis, or who died or last known to be alive within three months after the first breast cancer diagnosis. Since BCAC also included prevalent cases, we excluded 3,796 women who developed CBC or were censored before study entry. The case-case series included women diagnosed between 1947 and 2018. In the European cohort, we excluded 2,235 women who were diagnosed with their first breast cancer before 1990 or who had missing year of first diagnosis. We restricted to women diagnosed from 1990 onwards so that diagnostic procedures and treatment would be more representative of current practice. Moreover, clinico-pathological, treatment and follow-up data were more complete after 1990. In addition, we excluded 16 studies (9,783women) without information about metachronous CBC events (Figure S1A). After these exclusions, the cohort for this analysis comprised data from 42 studies, including 56,068 women with invasive breast cancer among whom 1,027 metachronous CBC occurred (Table S2).

All individuals provided written informed consent, and all studies were approved by the relevant institutional review boards. BCAC data were centrally harmonized and cleaned in communication with the study data managers and principal investigators. Data collection for individual studies is described in Table S1.

Genotyping and PRS

DNA samples from participants were genotyped using the iCOGS array[12,13] or the OncoArray[4,14], with genotypes for variants not on the arrays estimated by imputation[4,13]. The PRS_{313} was calculated as a weighted sum of the minor allele dosages; the variant selection and weights are as given by Mavaddat et al.[7]. We also calculated estimates for a previously published PRS_{77}[6], and estrogen receptor (ER)-specific PRSs (ER-positive PRS_{313} and ER-negative PRS_{313})[7]. The ER-specific PRSs were constructed by defining subtype-specific weights for the 313 variants using a hybrid approach[7]. Variants and corresponding coefficients used to construct the PRS are shown in Table S3. We standardized the PRS in our analyses by dividing it by the SD of the PRS of the controls (PRS_{77} SD=0.45; PRS_{313} SD=0.61; ER-positive PRS_{313} SD=0.65; ER-negative PRS_{313} SD=0.59) exactly as was done in the analyses of the PRS and first breast cancer risk[6,7]. This allows a direct comparison of the magnitude of the CBC relative risk estimation to that of the first breast cancer.

For samples genotyped with both OncoArray and iCOGS array (9,071 samples), OncoArray data were used in preference as the imputation quality was generally higher. The intraclass correlation coefficient (ICC) between the PRS derived from the two platforms was 0.99 (95%CI=0.99-0.99) for the PRS_{77}, and 0.96 (95%CI=0.95-0.96) for PRS_{313} (Figure S2). Given the high correlation between the two platforms, PRS measures from both platforms were used in the analyses without adjustment.

Statistical analysis

European cohort

The primary outcome in the European cohort was the development of metachronous CBC. Cox proportional hazards models were used to estimate HRs for metachronous CBC risk by PRS, stratified by country. Since previous studies have shown that age at first breast cancer diagnosis is an important predictor of CBC[3], the analyses were performed with attained age as the time scale. Time at risk started three months after the first breast cancer diagnosis and ended at the age of CBC diagnosis, distant metastasis (where available), death, or end of follow-up, whichever came first. For patients that had a study entry more than three months after first breast cancer diagnosis, follow-

up started at the age of study entry. We also performed a fixed-effect meta-analysis of country-specific effects using the STATA command *metan*. We performed a fixed-effect meta-analysis over a random-effect meta-analysis since there was no evidence for heterogeneity in effect sizes between countries (I-squared=0%, Figure S3). For some analyses, only invasive CBC was used as the outcome; in these analyses we censored on in situ CBC. Separate analyses were conducted for ER-positive CBC (censored on ER-negative- and ER-unknown CBC) and ER-negative CBC (censored on ER-positive- and ER-unknown CBC).

We evaluated the linearity of the association between PRS_{313} per unit SD and CBC risk using restricted cubic splines with three knots. There was no evidence for violation of the linearity assumption. Therefore, in the main analysis, the PRS_{313} was treated as a continuous covariate, and estimated the HR per unit SD of the $PRS_{313.}$ Violation of the proportional hazard assumption was assessed by inspection of the Schoenfeld residuals[15]. As a second analysis, we used the per SD log HR of the PRS_{313} to calculate the predicted HR at different percentiles of the PRS_{313}, compared to the 50th percentile. Third, the PRS_{313} was categorized into percentile groups (0th to 10th, 10th to 20th, 20th to 40th, 40th to 60th, 60th to 80th, 80th to 90th, 90th to 100th) to illustrate the differences between PRS_{313} subgroups, with the middle quintile (40th to 60th) as the reference.

We also performed multivariable Cox regression analyses to determine whether the log HR of CBC risk by PRS changed when adjusting for year of first breast cancer diagnosis, family history of breast cancer in a first degree relative, and several clinical characteristics of the first breast cancer such as nodal status, tumor size, morphology, ER-, progesterone receptor (PR)- and human epidermal growth factor receptor 2 (HER2)-status, (neo)adjuvant chemotherapy, adjuvant endocrine therapy, and radiotherapy. These analyses were performed in all patients, a complete case set (excluding patients with unknown values for the covariates), and in a set excluding studies oversampling cases with family history. Potential effect modification of the PRS_{313} effect by the same variables was evaluated by fitting interaction terms in different models using complete case sets, including the standardized PRS_{313}, modifier, and interaction.

The discriminative ability of different models; ([model 1] PRS_{313} alone, [model 2] other risk factors (the adjustment variables from the multivariable Cox regression analyses), [model 3] PRS_{313} + other risk factors) was calculated using Harrell's C-index[16]. Since no standard performance measures are currently available to account for left-truncated follow-up time (*i.e.*, to start analyses at age at study entry), we used time since first breast cancer as the time scale to calculate the C-index.

Absolute risks

Absolute risks of developing CBC at PRS_{313} percentiles were calculated using the estimated log HRs per SD from the breast cancer cohort (BCAC) under the log-linear model, assuming the PRS is normally distributed. The PRS_{313}- and age-specific incidences were constrained to the age-specific CBC incidences from women diagnosed with a first invasive breast cancer in the period 2003-2010 from the Netherlands Cancer Registry (NCR)[1]. The procedure for constraining the incidences has been previously described[17]. The age-specific CBC incidences were calculated overall and for age-specific groups, censoring on death and distant metastasis. We used data from the NCR since this registry has complete coverage of all newly diagnosed cancers in the Netherlands. The NCR cohort included all females aged ≥18 years and follow-up for second cancers was complete until February 1, 2016[1]. We then applied the competing risk of dying on the absolute CBC risks. The absolute CBC risk (AR_g) by age t in PRS_{313} category g, taking into account the competing risk of dying was calculated by:

$$AR_g(t) = \sum_{u=0}^{t-1} \mu_g(u) S_g(u) S_m(u)$$

Where $\mu_g\ (t)$ is the CBC incidence associated with PRS_{313} category g, $S_g\ (t)$ the probability of being free of CBC to age t, and $S_m\ (t)$ the probability of surviving to age t.

Case-case series

For the case-case series (European and Asian), logistic regression models were used to estimate the ORs for CBC risk (comparing with unilateral breast cancer) and for unilateral breast cancer risk (comparing with women without any diagnosis of breast cancer) associated with PRS_{313}. All analyses were adjusted for age and country (Table S1). For all unilateral- and contralateral breast cancer patients we used age at first breast cancer diagnosis, and for women without any diagnosis of breast cancer we used age at baseline questionnaire.

For direct comparison with the estimate reported for PRS_{313} and first breast cancer, we also performed logistic regression analyses in the same BCAC study participants included in the validation of the association between PRS_{313} and first breast cancer risk[7]. This validation set comprised a subsample from 24 studies and included 3,781 women with unilateral breast cancer, 94 women with CBC, and 3,753 women without any diagnosis of breast cancer (Table S2). For this analysis, we adjusted for 10 principal components, in line with Mavaddat et al.[7].

For European women who had follow-up time available more than three months after the first breast cancer diagnosis, a sensitivity analysis was performed for metachronous CBC (1,702 CBCs). We also did a separate analysis for invasive CBC (N=3,246), by excluding CBC in situ.

All P-values are two sided; tests with P<.05 are referred to as statistically significant. Analyses were performed using STATA, version 13.1 (StataCorp) and R version 3.3.2.

Results

European (cohort) Cox regression analyses

The European cohort included 56,068 women diagnosed with first invasive breast cancer with 1,027 metachronous CBC events. Median follow-up was 8.4 years. Patient, tumor, and treatment characteristics are summarized in Table S4.

The associations between the different PRSs and CBC risk are shown in Table 1. The HR for CBC per SD of PRS_{313} was 1.25 (95%CI=1.18-1.33). For comparison, the HR per SD for PRS_{77} was 1.21 (95%CI=1.14-1.29). Women within the 0th to 10th and the 90th to 100th percentile of the PRS_{313} had 0.59-fold (95%CI=0.45-0.78) and 1.38-fold (95%CI=1.13-1.69) risks of CBC, respectively, compared with women within the 40th to 60th percentile (Figure 1, Table S5). The predicted HRs of CBC for women at the 10th and 90th percentile of the PRS_{313} were 0.75 and 1.33, respectively, compared to the 50th percentile (Figure 1). Since we observed evidence of departure from the proportional hazards assumption (P=0.02)[15], we also calculated HRs stratified for follow-up duration (<five and ≥five years). The HR by SD of the PRS_{313} was 1.21 (95%CI=1.10-1.32) for CBC diagnosed ≤five years after first breast cancer diagnosis (CBC N=428), and 1.28 (95%CI=1.18-1.38) for CBC diagnosed >five years after first diagnosis (CBC N=599).

Table 1. Association between PRSs and contralateral breast cancer risk in the European cohort (N=56,068)

Polygenic risk score (PRS)		No. of CBC	HR per unit SD[a]	95%CI	P-value
PRS_{77} [b]					
	All CBC	1,027	1.21	1.14-1.29	<.001
	Invasive CBC	923	1.21	1.13-1.29	<.001
PRS_{313} [b]					
	All CBC	1,027	1.25	1.18-1.33	<.001
	Invasive CBC	923	1.24	1.16-1.32	<.001
	ER-positive invasive CBC[d]	275	1.38	1.23-1.55	<.001
	ER-negative invasive CBC[d]	97	0.92	0.75-1.12	.39
ER-positive PRS_{313} [b,c]					
	All CBC	1,027	1.23	1.16-1.31	<.001
	Invasive CBC	923	1.22	1.15-1.30	<.001
	ER-positive invasive CBC[d]	275	1.37	1.22-1.54	<.001
ER-negative PRS_{313} [b,c]					
	All CBC	1,027	1.25	1.17-1.33	<.001
	Invasive CBC	923	1.24	1.16-1.33	<.001
	ER-negative invasive CBC[d]	97	1.06	0.86-1.30	.58

Abbreviations: PRS = polygenic risk score; No. = number; CBC = contralateral breast cancer; HR = hazard ratio; CI = confidence interval; ER = estrogen receptor; SD = standard deviation

[a] All analyses were performed with attained age as time scale

[b] Coefficients to construct the PRSs are shown in Table S3. All PRSs were standardized by the same SD as was used by Mavaddat et al.[7]. The SD was 0.45 for overall breast cancer PRS_{77}, 0.61 for overall breast cancer PRS_{313}, 0.65 for ER-positive PRS_{313}, and 0.59 for ER-negative PRS_{313}

[c] ER-specific PRSs were constructed using a hybrid method, as described by Mavaddat et al.[7]

[d] Patients with ER-unknown CBC (N=551) were censored in these analyses

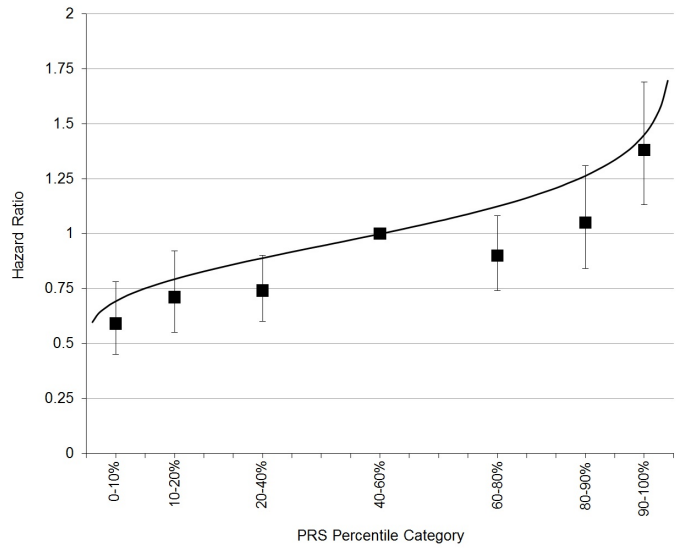


Figure 1. Estimates for contralateral breast cancer risk by percentile categories of the 313-variant PRS (PRS_{313})

The figure shows the hazard ratios per SD and 95% confidence intervals for percentiles of the PRS_{313} relative to the middle quintile (underlying table can be found in Table S5). The solid line denotes the estimates for contralateral breast cancer risk with the PRS_{313} fitted as a continuous covariate. Coefficients to construct the PRS_{313} are shown in Table S3. The PRS_{313} was standardized by SD=0.61, in line with Mavaddat et al.[7]. The analyses were performed with attained age as time scale. Abbreviations: PRS = polygenic risk score; SD = standard deviation

The HR per SD of PRS_{313} for ER-positive invasive CBC was 1.38 (95%CI=1.23-1.55), compared to a HR per SD of the ER-positive PRS_{313} of 1.37 (95%CI=1.22-1.54) (Table 1). For ER-negative invasive CBC, the HR per SD was 0.92 (95%CI=0.75-1.12) for PRS_{313} and 1.06 (95%CI=0.86-1.30) for the ER-negative PRS_{313}.

Sensitivity analysis using the overall PRS_{313} showed a HR per SD of 1.24 (95%CI=1.16-1.32) for invasive CBC risk. When we used time since first breast cancer as the time scale, we found similar results (HR per SD=1.25, 95%CI=1.18-1.33). Meta-analysis of country-specific effects showed a HR per SD of 1.25 (95%CI=1.18-1.33) for CBC risk by PRS_{313} (Figure S3).

The association between the PRS_{313} and CBC risk did not change when adjusting for patient, tumor, and treatment characteristics, nor when excluding studies oversampling cases with a family history (Table S6). When considering potential modifiers of the effect of the PRS_{313} on CBC risk (Table 2), we found that the HR was the lowest in women aged <40 years at first breast cancer diagnosis (HR per SD=1.13; 95%CI=0.98-1.31), and tended to increase with age, although these effects were not statistically significant ($P_{heterogeneity}$=.26; P_{trend}=.05). We found no indication for effect modification by family history ($P_{heterogeneity}$=.63), morphology ($P_{heterogeneity}$=.14), ER-status ($P_{heterogeneity}$=.13), PR-status (P=.26), HER2-status ($P_{heterogeneity}$=.42), chemotherapy ($P_{heterogeneity}$=.60), endocrine therapy ($P_{heterogeneity}$=.79), or radiotherapy ($P_{heterogeneity}$ =.40) (Table 2).

Table 2. Association between the 313-variant PRS (PRS_{313}) and contralateral breast cancer risk for subgroups

Subgroups	No. of patients	No. of CBC	HR per unit SD[a,b]	95%CI	P-value	$P_{hetero-geneity}$[c,d]	P_{trend}[c,e]
All patients	56,068	1,027	1.25	1.18-1.33	<.001	-	-
Age at first breast cancer diagnosis (years)						.26	.05
<40	5,877	171	1.13	0.98-1.31	.09		
40-49	11,928	265	1.25	1.11-1.41	<.001		
50-59	16,882	320	1.22	1.09-1.36	<.001		
60+	21,381	271	1.36	1.21-1.52	<.001		
Family history (first degree relative)						.63	-
no	33,623	618	1.26	1.16-1.36	<.001		
yes	10,369	302	1.22	1.09-1.36	<.001		
Morphology						.14	-
ductal	37,324	621	1.21	1.12-1.31	<.001		
lobular	5,878	118	1.32	1.10-1.59	.002		
mixed (ductal and lobular)	2,174	46	1.52	1.15-2.02	.004		
other	3,344	70	1.20	0.96-1.50	.11		
ER-status						.13	-
negative	9,527	194	1.13	0.98-1.30	.08		
positive	38,090	670	1.28	1.19-1.38	<.001		
PR-status						.26	-
negative	13,098	244	1.16	1.03-1.32	.02		
positive	27,044	554	1.27	1.17-1.38	<.001		
HER2-status						.42	-
negative	23,787	352	1.29	1.17-1.44	<.001		
positive	4,969	60	1.45	1.13-1.85	.004		
(Neo)adjuvant chemotherapy						.60	-
no	18,110	361	1.28	1.16-1.42	<.001		
yes	18,559	363	1.24	1.12-1.37	<.001		
(Neo)adjuvant endocrine therapy						.79	-
no	10,781	242	1.28	1.13-1.44	<.001		
yes	27,322	460	1.30	1.19-1.43	<.001		
Radiotherapy						.40	-
no	11,023	188	1.33	1.15-1.53	<.001		
yes	29,142	617	1.24	1.15-1.34	<.001		

Abbreviations: PRS = polygenic risk score; No. = number; CBC = contralateral breast cancer; HR = hazard ratio; CI = confidence interval; ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2

[a] HR for CBC risk by unit SD of PRS_{313}. All analyses were performed with attained age as time scale

[b] Coefficients to construct the PRS_{313} are shown in Table S3. The PRS_{313} was standardized by standard deviation=0.61, in line with Mavaddat et al.[7]

[c] The interaction between the PRS_{313} and each subgroup was tested in different models including the standardized PRS_{313}, modifier, and interaction. Patients with unknown values were excluded from these analyses. Since attained age was used as time scale in all models, the model with age at first breast cancer only included the PRS_{313} and interaction

[d] P for interaction based on test for heterogeneity across categories

[e] P for interaction based on a trend test with age as continuous variable

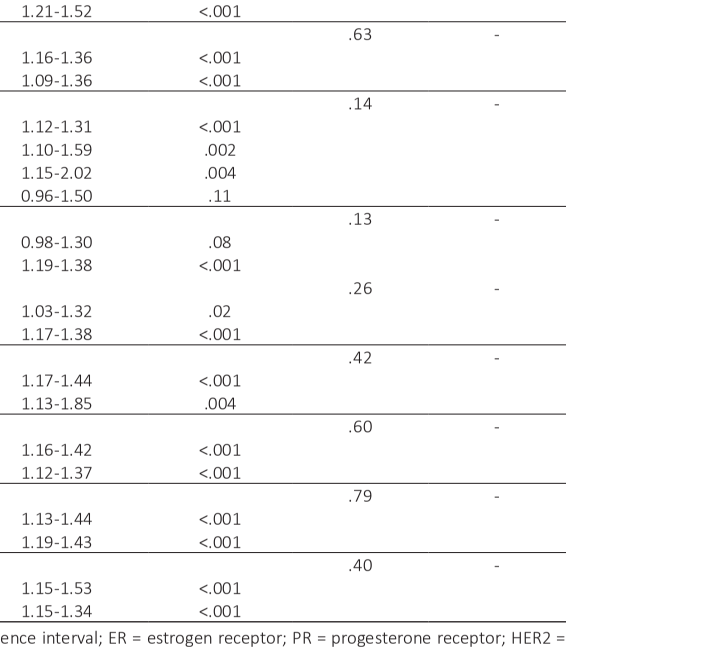


Figure 2. Predicted contralateral breast cancer risk by percentile of the 313-variant PRS (PRS_{313}) with death as competing risk

Coefficients to construct the PRS_{313} are shown in Table S3. The PRS_{313} was standardized by SD=0.61, in line with Mavaddat et al.[7] The CBC incidences were calculated based on incidence data from the Netherlands Cancer Registry[8] and relative risks estimated as described in the Material and Methods. Abbreviations: PRS = polygenic risk score; CBC = contralateral breast cancer

The C-index was 0.563 (95%CI=0.547-0.586) for the model only including PRS_{313}, 0.605 (95%CI=0.591-0.629) for the model only including other risk factors, and 0.623 (95%CI=0.608-0.645) for the complete model (Table 3).

Absolute risks

Based on the HR estimates for PRS_{313}, the predicted CBC risk by age 80 years was 12.4% at the 10th percentile of the PRS_{313}, compared with 20.5% at the 90th percentile of the PRS_{313} (Figure 2), accounting for death as competing risk. When death was not taken into account as competing risk, the corresponding predicted risks by age 80 were 17.0% at the 10% percentile and 27.9% at the 90th percentile of the PRS_{313} (Figure S4). Table 4 shows the five- and 10-year cumulative CBC risks by PRS_{313} for different age groups, accounting for death as competing risk (Table S7 shows results without competing risks).

European and Asian (case-case series) logistic regression analyses

Figure 3 shows the distribution of the PRS_{313} per SD in the European case-case series. Median PRS_{313} was -0.4 (interquartile range [IQR]=1.35) for control women without any diagnosis of breast cancer (N=81,000), 0.2 (IQR=1.36) for women with unilateral breast cancer (N=62,830), and 0.5 (IQR=1.40) for women with CBC (N=3,607). The OR

for unilateral breast cancer per SD of the PRS_{313}, compared to control women, was 1.82 (95%CI=1.80-1.84) (Table S8). The OR for CBC per SD of PRS_{313}, compared to unilateral breast cancer, was 1.30 (95%CI=1.26-1.35) .

In sensitivity analyses, the OR per SD of PRS_{313} was 1.27 (95%CI=1.21-1.33) for metachronous CBC and the OR per SD was 1.29 (95%CI=1.24-1.33) for invasive CBC, compared to unilateral breast cancer. When analyses were restricted to the validation set of Mavaddat et al[7], the OR for unilateral breast cancer per SD of the PRS_{313} was 1.67 (95%CI=1.59-1.76) compared to control women, and the OR for CBC per SD of PRS_{313} was 1.39 (95%CI=1.13-1.70) compared to unilateral breast cancer (Table S8).

For women of Asian descent, the OR for unilateral breast cancer per SD of the PRS_{313} was 1.56 (95%CI=1.52-1.60) compared to control women, and the OR for CBC per SD of PRS_{313} was 1.15 (95%CI=1.02-1.29) compared to women with unilateral breast cancer (Table S8).

Table 3. Discriminatory ability (C-index) of the 313-variant PRS (PRS_{313}) and other risk factors for contralateral breast cancer risk in the European cohort

	C-index (95%CI)[a,b]
Model 1	
PRS_{313}[c] alone	0.563 (0.547-0.586)
Model 2	
Other risk factors[d]	0.605 (0.591-0.629)
Model 3	
PRS_{313}[c] + other risk factors[d]	0.623 (0.608-0.645)

Abbreviations: PRS = polygenic risk score; CI = confidence interval

[a] The Harrell's C-index was obtained by the STATA stcox postestimation command 'estat concordance', using time since first breast cancer on the time scale without taking delayed entry (prevalent cases) into account. We did not consider delayed-entry since no standard performance measures are currently available in the statistical literature to account for left-truncated follow-up time. The median of delayed entry was 0.4 years (standard deviation=2.7) in our study

[b] The 95% CIs were obtained by use of the 'somersd' package in STATA

[c] Coefficients to construct the PRS_{313} are shown in Table S3. The PRS_{313} was standardized by SD=0.61, in line with Mavaddat et al.[7]

[d] Including age at first diagnosis, year of first diagnosis, family history for breast cancer in a first degree relative, and clinical characteristics of the first breast cancer (nodal status, tumor size, differentiation grade, morphology, estrogen receptor status, human epidermal growth factor receptor 2 status, chemotherapy, endocrine therapy, radiotherapy)

Table 4. Five- and ten-year cumulative risks of contralateral breast cancer by the 313-variant PRS (PRS_{313}) for different age groups with death as competing risk

	5-year cumulative CBC risks (%) range by age						10-year cumulative CBC risks (%) range by age					
Age at first breast cancer diagnosis (years)	5th percentile PRS_{313}	10th percentile PRS_{313}	50th percentile PRS_{313}	90th percentile PRS_{313}	95th percentile PRS_{313}		5th percentile PRS_{313}	10th percentile PRS_{313}	50th percentile PRS_{313}	90th percentile PRS_{313}	95th percentile PRS_{313}	
30-34	1.9-3.1	2.1-3.4	2.7-4.5	3.6-5.9	4.0-6.5		3.1-4.1	3.4-4.5	4.5-5.9	5.9-7.7	6.5-8.5	
35-39	0.8-2.1	0.9-2.3	1.2-3.0	1.5-3.9	1.7-4.3		2.1-3.5	2.3-3.8	3.0-5.0	3.9-6.6	4.3-7.2	
40-44	1.5-2.8	1.7-3.1	2.2-4.1	2.9-5.3	3.2-5.9		2.8-4.6	3.1-5.0	4.1-6.6	5.3-8.6	5.9-9.4	
45-49	1.4-2.5	1.5-2.7	2.0-3.6	2.6-4.7	2.9-5.2		2.5-3.9	2.7-4.3	3.6-5.6	4.7-7.4	5.2-8.1	
50-54	1.4-2.8	1.5-3.0	1.9-4.0	2.6-5.2	2.8-5.8		2.8-4.5	3.0-4.9	4.0-6.4	5.2-8.4	5.8-9.3	
55-59	1.6-3.1	1.8-3.4	2.3-4.5	3.1-5.9	3.4-6.5		3.1-4.8	3.4-5.2	4.5-6.9	5.9-9.0	6.5-9.9	
60-64	1.7-3.3	1.9-3.6	2.5-4.7	3.3-6.2	3.6-6.8		3.3-5.0	3.6-5.4	4.7-7.1	6.2-9.3	6.8-10.2	
65-70	1.5-3.2	1.6-3.5	2.1-4.6	2.8-6.1	3.1-6.7		3.2-4.1	3.5-4.5	4.6-5.9	6.1-7.7	6.7-8.5	

Abbreviations: PRS = polygenic risk score; CBC = contralateral breast cancer

Coefficients to construct the PRS_{313} are shown in Table S3. The PRS_{313} was standardized by SD=0.61, in line with Mavaddat et al[7]. The CBC incidences for each age group were calculated based on incidence data from the Netherlands Cancer Registry[1] and relative risks estimated as described in the Material and Methods. Death was taken into account as competing risk

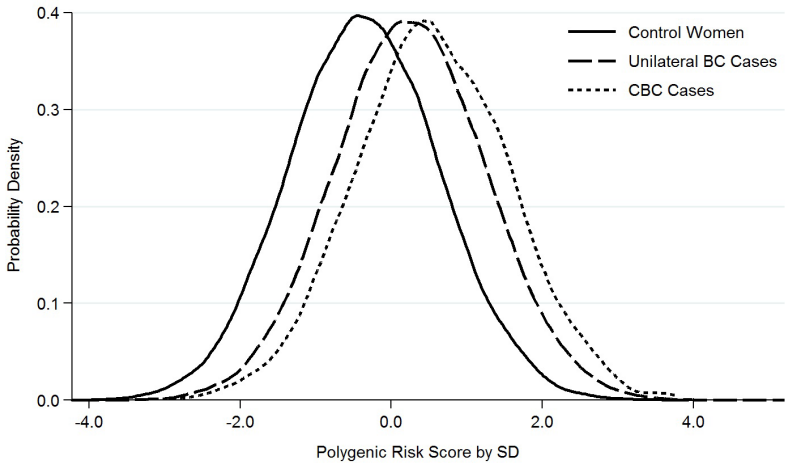


Figure 3. Distribution of the 313-variant PRS (PRS_{313}) in 62,830 control women without any diagnosis of breast cancer, 81,000 women with unilateral breast cancer, and 3,607 women with contralateral breast cancer

Coefficients to construct the PRS_{313} are shown in Table S3. The PRS_{313} was standardized by SD=0.61, in line with Mavaddat et al.[7]. Abbreviations: BC = breast cancer; CBC = contralateral breast cancer; PRS = polygenic risk score; SD = standard deviation

Discussion

Previous studies have shown that a PRS, summarizing the effects of common germline variants, can be used to stratify women with respect to their risk to develop a primary breast cancer[6-9]. In this study, we observed a clear association between the PRS_{313} and CBC risk in women of both European and Asian ancestry. The association was observed in both the case-case series and the European cohort. The HRs per SD of CBC for women at the 10th and 90th percentile of the continuous predicted PRS_{313} were 0.75 and 1.33, respectively, compared to the 50th percentile. This translates to absolute risks at the 10th and the 90th percentile of the PRS_{313} of 12.4% and 20.5%, respectively, by age 80 years. We estimated a C-index for the PRS_{313}, summarizing its discriminatory ability, of 0.563 in the European cohort.

One previous study has investigated the effect of a PRS, including 67 variants, and CBC risk[10]. This study found a risk ratio of 1.75 (95%CI=1.41-2.18) for women in the upper quartile of the PRS compared with women in the lowest quartile. To facilitate comparison, we performed a similar analysis in our case-case series, showing an OR of 1.98 (95%CI=1.79-2.18), adjusted for country and age at first diagnosis, for women in the upper quartile of the PRS_{313}. This indicates the PRS_{313} improves stratification relative to PRSs including fewer variants. Moreover, in our European cohort, the C-index for the PRS alone improved from 0.547 (95%CI=0.536-0.575) for the previously reported PRS_{77}[6] to 0.563 (95%CI=0.547-0.586) for the PRS_{313}.

We found no evidence that the association between the PRS_{313} and CBC risk was confounded by family history, adjuvant therapy, morphology, age, or tumor receptor status of the first breast cancer, nor that there was effect modification by those factors. The absence of notable effect modification is in line with the abovementioned study of a 67-variant PRS and CBC risk; no heterogeneity in association was found by age, family history, morphology, ER-status, and adjuvant treatment[10].

To provide an external validation of our findings, we examined data from UK Biobank, which includes many women diagnosed with breast cancer with data available on the PRS_{313} (Supplemental Note). Unfortunately, UK Biobank has no information available on the laterality of the tumor, and it is, therefore, not possible to distinguish between contralateral and ipsilateral breast cancers. We therefore performed analyses using any second breast cancer as the endpoint. This secondary analysis did confirm the association between the PRS_{313} and second breast cancer risk (HR per SD=1.13, 95%CI=1.01-1.27), but with a lower estimate than in our European cohort. The lower estimate may be explained by the inclusion of the ipsilateral breast cancers, which may be more likely to be recurrences than new primary breast cancers compared to CBCs. Indeed, when we used ipsilateral breast cancer as the outcome in our European cohort, we found no association with the PRS_{313} (HR=1.02, 95%CI=0.90-1.15).

The association between the PRS_{313} and CBC risk (OR per SD=1.30; 95%CI=1.26-1.35) in the BCAC database was weaker (expressed in terms of an OR) than was found for first breast cancer among independent prospective studies (OR per SD=1.61; 95%CI=1.57-1.65). Under a simple polygenic model, the relative risk would be expected to be similar for the second breast cancer. The attenuated estimate for CBC might however be explained by several factors. Some attenuation of the estimate might have been due to dilution in the end-point definition, *i.e.*, if some of the CBCs were metastases. Previous studies investigating the clonal relatedness of first breast cancers and CBCs using tumor sequencing have shown that 6-12% of CBCs represent metastases[18,19]. This hypothesis would be consistent with our finding of a slightly stronger association between the PRS_{313} and late CBCs, diagnosed >five years after the first breast cancer, than for early CBCs, diagnosed ≤five years after the first cancer, since the latter are more likely to be metastases. In addition, 3-5% of the breast cancer patients will have a mutation in the *BRCA1 or BRCA2 gene*[20,21], who have high CBC risks. It has been shown that the relative risk associated with PRS is lower (for the first breast cancer) for women with a *BRCA1* and *BRCA2* mutation than in the general population[22], diluting the overall relative risk for CBC. More generally, it is possible that the CBC association may be attenuated due to the effect of other, unmeasured, genetic or other risk factors. If the risks are high, cases with higher PRS_{313} will have, on average, lower values of other risk factors, due to elimination of the highest risk individuals, again attenuating the CBC association. Finally, given the limited information on family history in our dataset, the estimate could have

been biased due to a family history effect not detected in our data.

There was some suggestion that the relative risk associated with PRS_{313} decreased with younger age, (P_{trend}=.05), and, specifically, was lower for women aged <40 years (HR per SD=1.13; 95%CI=0.98-1.31). Interestingly, Mavaddat et al[7] also found a lower relative risk below age 40 for first breast cancer. This effect may reflect the different characteristics of breast cancers at young ages, both in terms of germline susceptibility and pathology[23,24]. For example, the proportion of ER-negative breast cancers is higher at young ages, and the PRS is less predictive for ER-negative disease[6,7,24].

In the logistic regression analyses in Asian women, the association between the PRS_{313} and CBC risk was slightly weaker than in European women. This finding is consistent with a recent analysis investigating the association between a 287-variant PRS and first breast cancer risk in the Asian population[25], which showed an attenuated OR in Asian women (OR=1.52, 95%CI=1.49-1.56) compared to European women (OR=1.61, 95%CI=1.57-1.66). The lower estimate for Asian women might reflect the fact the PRS_{313} was developed in European populations, and the different LD structure in Asians may attenuate the association since the variants in the PRS are likely to be surrogates for the causal variants. Other explanations for the attenuated estimate may be the slightly younger age at first breast cancer diagnosis and the higher proportion ER-negative CBCs in Asian women compared to European women in our study. Finally, the imputation quality for variants was somewhat lower, on average, for the Asian than for the European dataset, with three variants on OncoArray and four variants on ICOGs with an imputation quality score<0.3 (Table S3). Nevertheless, we included those variants in the PRS for both European and Asian women, to keep the PRS comparable between ethnicities and studies. Future studies including larger numbers of Asian women, and women of other ethnicities, are needed to generate population-specific PRSs and to validate our findings in these groups.

A major strength of this study is the very large sample size in the BCAC dataset, including genotype information for ~150,000 women and a large number of CBC events. A limitation of this study is missing data on the patient, tumor, and treatment characteristics, which reduces the power of the multivariable Cox regression analyses and interaction analyses. In addition, registration of CBC was not complete; the 10-year cumulative CBC incidence was 2.2% in the BCAC dataset, compared to 3.8% using complete data from the Netherlands Cancer Registry[1]. For this reason, we estimated relative risk estimates using the BCAC data and applied these to external registry data to obtain absolute risk estimates. The underreporting of CBC should not bias our HR estimates, given that the event rate is low and reporting of CBC is unlikely to be related to the PRS_{313}. Moreover, we reran the cohort analysis in the subset of countries with a 10-year cumulative CBC incidence ≥3.0% in the BCAC dataset, and the estimates were very similar to the main analyses (HR per SD=1.23, 95%CI=1.14-1.33) (Figure S3).

In conclusion, the PRS_{313} is predictive for the development of CBC. We found no evidence for confounding or effect modification by other previously established CBC risk factors. The PRS_{313} is therefore likely to be an independent risk factor for CBC. Since the predictive ability of the PRS on its own is modest, it should be combined with other breast cancer risk factors to provide more useful CBC risk prediction models. More accurate risk prediction will help identify women at high CBC risk who will benefit from additional surveillance and/or risk reducing mastectomy, and equally important, to identify those women at low risk in order to avoid unnecessary surgeries.

Article information

Funding and acknowledgements

For full list of funding and acknowledgements see publication.

Conflict of interest

Dr. Beckmann conducts research funded by Amgen, Novartis and Pfizer, outside the submitted work. Dr. Fasching conducts research funded by Amgen, Novartis and Pfizer, outside the submitted work. He received honoraria from Roche, Novartis and Pfizer. Dr. Nevanlinna received honorarium from Astra Zeneca outside the submitted work.

Data and code availability

Data used in this manuscript may be requested through the original providers. Data of the Breast Cancer Association Consortium may be requested for non-profit research through an application procedure with the Breast Cancer Association Consortium; more information: http://bcac.ccge.medschl.cam.ac.uk/bcacdata/. Data of the UK Biobank needs to be requested through UK Biobank; more information: https://www.ukbiobank.ac.uk/researchers/

References

1 Kramer, I. *et al.* The influence of adjuvant systemic regimens on contralateral breast cancer risk and receptor subtype. *J. Natl. Cancer Inst.*, doi:10.1093/jnci/djz010 (2019).
2 Xiong, Z. *et al.* Patterns of Occurrence and Outcomes of Contralateral Breast Cancer: Analysis of SEER Data. *J Clin Med* **7**, doi:10.3390/jcm7060133 (2018).
3 Akdeniz, D. *et al.* Risk factors for metachronous contralateral breast cancer: A systematic review and meta-analysis. *Breast* **44**, 1-14, doi:10.1016/j.breast.2018.11.005 (2019).
4 Michailidou, K. *et al.* Association analysis identifies 65 new breast cancer risk loci. *Nature* **551**, 92, doi:10.1038/nature24284 https://www.nature.com/articles/nature24284#supplementary-information (2017).
5 Milne, R. L. *et al.* Identification of ten variants associated with risk of estrogen-receptor-negative breast cancer. *Nat. Genet.* **49**, 1767-1778, doi:10.1038/ng.3785 (2017).
6 Mavaddat, N. *et al.* Prediction of breast cancer risk based on profiling with common genetic variants. *J. Natl. Cancer Inst.* **107**, doi:10.1093/jnci/djv036 (2015).
7 Mavaddat, N. *et al.* Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. *Am. J. Hum. Genet.* **104**, 21-34, doi:10.1016/j.ajhg.2018.11.002 (2019).
8 Brentnall, A. R. *et al.* A case-control evaluation of 143 single nucleotide polymorphisms for breast cancer risk stratification with classical factors and mammographic density. *Int. J. Cancer*, doi:10.1002/ijc.32541 (2019).
9 Shieh, Y. *et al.* Breast cancer risk prediction using a clinical risk model and polygenic risk score. *Breast Cancer Res. Treat.* **159**, 513-525, doi:10.1007/s10549-016-3953-2 (2016).
10 Robson, M. E. *et al.* Association of Common Genetic Variants With Contralateral Breast Cancer Risk in the WECARE Study. *JNCI: Journal of the National Cancer Institute* **109**, djx051-djx051, doi:10.1093/jnci/djx051 (2017).
11 Teraoka, S. N. *et al.* Single nucleotide polymorphisms associated with risk for contralateral breast cancer in the Women's Environment, Cancer, and Radiation Epidemiology (WECARE) Study. *Breast Cancer Res.* **13**, R114, doi:10.1186/bcr3057 (2011).
12 Michailidou, K. *et al.* Genome-wide association analysis of more than 120,000 individuals identifies 15 new susceptibility loci for breast cancer. *Nat. Genet.* **47**, 373-380, doi:10.1038/ng.3242 (2015).
13 Michailidou, K. *et al.* Large-scale genotyping identifies 41 new loci associated with breast cancer risk. *Nat. Genet.* **45**, 353-361, 361e351-352, doi:10.1038/ng.2563 (2013).
14 Amos, C. I. *et al.* The OncoArray Consortium: A Network for Understanding the Genetic Architecture of Common Cancers. *Cancer Epidemiol. Biomarkers Prev.* **26**, 126-135, doi:10.1158/1055-9965.epi-16-0106 (2017).
15 Schoenfeld, D. A. Sample-size formula for the proportional-hazards regression model. *Biometrics* **39**, 499-503 (1983).
16 Harrell, F. E., Jr., Califf, R. M., Pryor, D. B., Lee, K. L. & Rosati, R. A. Evaluating the yield of medical tests. *JAMA* **247**, 2543-2546 (1982).
17 Antoniou, A. C. *et al.* Common breast cancer susceptibility alleles and the risk of breast cancer for BRCA1 and BRCA2 mutation carriers: implications for risk prediction. *Cancer Res.* **70**, 9742-9754, doi:10.1158/0008-5472.Can-10-1907 (2010).
18 Klevebring, D. *et al.* Exome sequencing of contralateral breast cancer identifies metastatic disease. *Breast Cancer Res. Treat.* **151**, 319-324, doi:10.1007/s10549-015-3403-6 (2015).
19 Begg, C. B. *et al.* Contralateral breast cancers: Independent cancers or metastases? *Int. J. Cancer* **142**, 347-356, doi:10.1002/ijc.31051 (2018).
20 Thompson, D. & Easton, D. The genetic epidemiology of breast cancer genes. *J. Mammary Gland Biol. Neoplasia* **9**, 221-236, doi:10.1023/B:JOMG.0000048770.90334.3b (2004).
21 van den Broek, A. J. *et al.* Impact of Age at Primary Breast Cancer on Contralateral Breast Cancer Risk in BRCA1/2 Mutation Carriers. *J. Clin. Oncol.* **34**, 409-418, doi:10.1200/jco.2015.62.3942 (2016).
22 Kuchenbaecker, K. B. *et al.* Evaluation of Polygenic Risk Scores for Breast and Ovarian Cancer Risk Prediction in BRCA1 and BRCA2 Mutation Carriers. *J. Natl. Cancer Inst.* **109**, doi:10.1093/jnci/djw302 (2017).
23 Azim, H. A., Jr. *et al.* Elucidating prognosis and biology of breast cancer arising in young women using gene expression profiling. *Clin. Cancer Res.* **18**, 1341-1351, doi:10.1158/1078-0432.Ccr-11-2599 (2012).
24 Anders, C. K. *et al.* Young age at diagnosis correlates with worse prognosis and defines a subset of breast cancers with shared patterns of gene expression. *J. Clin. Oncol.* **26**, 3324-3330, doi:10.1200/jco.2007.14.2471 (2008).
25 Ho, W. K. *et al.* European polygenic risk score for prediction of breast cancer shows similar performance in Asian women. *Nat Commun* **11**, 3833, doi:10.1038/s41467-020-17680-w (2020).

Supplementary Material

Table S1. Study characteristics of included studies of the Breast Cancer Association Consortium

See online material

Table S2. Studies and samples included in the analyses using the case-case series, cohort, and validation set

	European								Asian		
	Case-case series N studies = 62			Cohort N studies = 42		Validation set N studies = 24			Case-case series N studies = 8		
Studies	Control women[a]	Unilateral BC	CBC	Unilateral BC	CBC	Control women[a]	Unilateral BC	CBC	Control women[a]	Unilateral BC	CBC
ABCFS	738	1,149	127	1,021	93	-	-	-	-	-	-
ABCS	1,567	1,047	54	519	14	-	-	-	-	-	-
ABCS-F	0	861	91	363	17	-	-	-	-	-	-
ABCTB	375	900	17	708	1	74	180	8	-	-	-
BBCC	711	845	58	766	6	49	56	5	-	-	-
BBCS	1,768	1,266	80	466	1	-	-	-	-	-	-
BCEES	-	-	-	-	-	166	133	0	-	-	-
BCFR-NY	27	340	61	-	-	-	-	-	-	-	-
BCFR-PA	0	104	14	69	4	-	-	-	-	-	-
BCFR-UTAH	0	13	87	-	-	-	-	-	-	-	-
BCINIS	-	-	-	-	-	144	262	0	-	-	-
BIGGS	49	713	50	395	2	-	-	-	-	-	-
BREOGAN	725	1,245	19	1,233	15	145	238	4	-	-	-
BSUCH	1,122	900	36	727	3	-	-	-	-	-	-
CBCS	817	530	21	-	-	163	105	4	170	238	10
CCGP	321	598	19	578	8	66	125	7	-	-	-
CGPS	5,250	4,135	60	3,834	17	142	227	3	-	-	-
CNIO-BCS	829	742	5	-	-	-	-	-	-	-	-
CTS	-	-	-	-	-	115	220	0	-	-	-
DIETCOMPLYF	0	704	1	-	-	-	-	-	-	-	-
FHRISK	0	119	2	-	-	-	-	-	-	-	-
GC-HBOC	1,732	2,690	230	1,406	47	-	-	-	-	-	-
GENICA	711	869	26	869	1	56	89	2	-	-	-
GESBC	181	303	3	-	-	-	-	-	-	-	-
GLACIER	0	1,733	230	-	-	-	-	-	-	-	-
HABCS	863	774	84	-	-	173	141	6	-	-	-
HCSC	0	362	13	273	9	-	-	-	-	-	-
HEBCS	1,060	1,632	116	1,578	41	-	-	-	-	-	-
HERPACC	-	-	-	-	-	-	-	-	1,659	756	18
HKBCS	-	-	-	-	-	-	-	-	451	403	12
HMBCS	345	729	28	-	-	-	-	-	-	-	-
HUBCS	116	198	2	-	-	-	-	-	-	-	-
ICICLE	1	138	12	-	-	-	-	-	-	-	-
KARBAC	0	761	46	443	32	-	-	-	-	-	-
KARMA	5,981	2,314	96	2,188	33	597	185	10	-	-	-
KBCP	431	516	9	-	-	-	-	-	-	-	-
KCONFAB/AOCS	898	397	83	305	26	-	-	-	-	-	-

Table S2. Continued

	European								Asian		
	Case-case series N studies = 62			Cohort N studies = 42		Validation set N studies = 24			Case-case series N studies = 8		
Studies	**Control women[a]**	**Unilateral BC**	**CBC**	**Unilateral BC**	**CBC**	**Control women[a]**	**Unilateral BC**	**CBC**	**Control women[a]**	**Unilateral BC**	**CBC**
LMBC	1,821	3,016	208	2,286	92	87	142	14	-	-	-
MABCS	88	80	9	74	2	-	-	-	-	-	-
MARIE	2,066	1,540	115	1,535	53	-	-	-	-	-	-
MBCSG	766	1,015	150	569 8					-	-	-
MCBCS	2,093	1,999	59	1,903	6	35	96	3	-	-	-
MCCS	1,207	1,034	2	-	-	142	86	0	-	-	-
MEC	1,123	1,016	38	988	23	-	-	-	-	-	-
MISS	1,529	582	6	563	3	304	83	0	-	-	-
MMHS	1,635	273	4	-	-	320	48	4	-	-	-
MYBRCA	-	-	-	-	-	-	-	-	4,197	3,652	105
NBCS	212	2,334	31	1,370	4	-	-	-	-	-	-
NBHS	-	-	-	-	-	122	79	0	-	-	-
NC-BCFR	150	614	69	602	5	-	-	-	52	391	33
NCBCS	1,006	1,988	42	-	-	-	-	-	-	-	-
OBCS	414	467	10	445	1	-	-	-	-	-	-
OFBCR	728	1,908	143	1,656	51	-	-	-	-	-	-
ORIGO	0	1,090	89	1,053	69	132	134	15	-	-	-
PBCS	2,082	1,719	40	1,625	9	331	215	2	-	-	-
PKARMA	5,435	4,81	277	4,685	124	1	4	0	-	-	-
POSH	0	1,069	19	1,063	16	-	-	-	-	-	-
PREFACE	0	2,73	90	-	-	-	-	-	-	-	-
PROCAS	1,647	488	9	422	3	-	-	-	-	-	-
RBCS	0	873	152	724	81	-	-	-	-	-	-
SASBAC	1,378	1,118	22	1,086	5	-	-	-	-	-	-
SBCS	848	748	14	691	1	-	-	-	-	-	-
SEARCH	9,056	12,423	118	12,117	59	197	628	0	-	-	-
SEBCS	-	-	-	-	-	-	-	-	2,236	2,080	21
SGBCC	-	-	-	-	-	-	-	-	4,141	1,250	124
SKKDKFZS	29	1,084	71	1,054	41	-	-	-	-	-	-
SMC	-	-	-	-	-	141	244	0	-	-	-
SUCCESSB	0	438	2	-	-	-	-	-	-	-	-
SUCCESSC	0	2,807	29	-	-	-	-	-	-	-	-
SZBCS	489	676	6	409	1	-	-	-	-	-	-
TNBCC	152	1,037	2	-	-	-	-	-	-	-	-
TWBCS	-	-	-	-	-	-	-	-	492	1,250	17
UCIBCS	258	397	1	380	1	51	61	7	-	-	-

Table S2. Continued

	European								Asian		
	Case-case series N studies = 62			Cohort N studies = 42		Validation set N studies = 24			Case-case series N studies = 8		
Studies	**Control women[a]**	**Unilateral BC**	**CBC**	**Unilateral BC**	**CBC**	**Control women[a]**	**Unilateral BC**	**CBC**	**Control women[a]**	**Unilateral BC**	**CBC**
Total	**62,830**	**81,000**	**3,607**	**55,041**	**1,027**	**3,753**	**3,781**	**94**	**13,398**	**12,133**	**340**
Characteristics											
Invasiveness in situ	-	excluded	361	excluded	104	-	3[b]	7	-	excluded	67
invasive	-	79,876	2,200	54,675	670	-	3,777	60	-	11,929	209
unknown	-	1,124	1,046	366	253	-	1	27	-	204	64
ER status negative	-	13,828	446	9,333	105	-	766	8	-	3,457	54
positive	-	52,238	2,048	37,420	289	-	3,001	47	-	7,826	163
unknown	-	14,934	1,113	8,288	633	-	14	39	-	850	123

Abbreviations: BC = breast cancer; CBC = contralateral breast cancer; ER = estrogen receptor

[a] Without any diagnosis of breast cancer

[b] Due to the use of a new freeze of the BCAC data, N=3 breast cancers were now defined as in situ, which had previously been defined as invasive; the original validation dataset contained data of two additional studies[1]

Table S3. Variant information and breast cancer risk coefficients for the 77-variant PRS, 313-variant PRS, and ER-specific PRSs; previously published in Mavaddat et al.[1,2]

See online material

Table S4. Patient, tumor, and treatment characteristics of all women diagnosed with first invasive breast cancer since 1990 (European cohort)

Characteristics		Number of women (%)[a]
Total		56,068 (100)
Median age at first diagnosis in years (range)		56 (18-98)
Year of diagnosis		
	1990-1994	3,029 (5.4)
	1995-1999	10,153 (18.1)
	2000-2004	18,484 (33.0)
	2005-2009	17,575 (31.3)
	2010-2015	6,827 (12.2)
Family history (first degree relative)		
	no	33,623 (76.4)
	yes	10,369 (23.6)
	unknown	12,076
Nodal status		
	negative	29,070 (61.9)
	positive	17,903 (38.1)
	unknown	9,095
Tumor size, cm		
	≤2	28,057 (63.8)
	(2, 5]	14,138 (32.2)
	>5	1,750 (4.0)
	unknown	12,123
Differentiation grade		
	I	8,721 (19.5)
	II	21,621 (48.3)
	III	14,454 (32.3)
	unknown	11,272
Morphology		
	ductal	37,324 (76.6)
	lobular	5,878 (12.1)
	mixed (ductal and lobular)	2,174 (4.5)
	other	3,344 (6.9)
	unknown	7,348
ER-status		
	negative	9,527 (20.0)
	positive	38,090 (80.0)
	unknown	8,451
PR-status		
	negative	13,098 (32.6)
	positive	27,044 (67.4)
	unknown	15,926
HER2-status		
	negative	23,787 (82.7)
	positive	4,969 (17.3)
	unknown	27,312
Surgery		
	yes, breast saving	16,468 (42.3)
	yes, mastectomy	11,315 (29.1)
	yes, type unknown	11,163 (28.7)
	unknown	17,122
(Neo)adjuvant chemotherapy		
	no	18,110 (49.4)
	yes	18,559 (50.6)
	unknown	19,399
(Neo)adjuvant endocrine therapy		
	no	10,781 (28.3)
	yes	27,322 (71.7)
	unknown	17,965
Radiotherapy		
	no	11,023 (27.4)
	yes	29,142 (72.6)
	unknown	15,903

Abbreviations: ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2

[a] Total may not be 100% because of rounding

Table S5. Association between the 313-variant PRS (PRS$_{313}$) and contralateral breast cancer risk in the European cohort

Percentile categories of the PRS$_{313}$	No. of women	No. of CBC	HR per unit SD[a]	95%CI	P-value
0th to 10th	5,607	65	0.59	0.45-0.78	<.001
10th to 20th	5,606	79	0.71	0.55-0.92	.01
20th to 40th	11,214	165	0.74	0.60-0.90	.003
40th to 60th	11,214	224	1.00	Ref.	-
60th to 80th	11,214	208	0.90	0.74-1.08	.25
80th to 90th	5,607	121	1.05	0.84-1.31	.69
90th to 100th	5,606	165	1.38	1.13-1.69	.002

Abbreviations: PRS = polygenic risk score; No = number; CBC = contralateral breast cancer; HR = hazard ratio; CI = confidence interval; SD = standard deviation

[a]The analysis was performed with attained age as time scale. Coefficients to construct the PRS$_{313}$ are shown in Table S3. The PRS$_{313}$ was standardized by SD=0.61, in line with Mavaddat et al.[1]

Table S6. Multivariable Cox regression models of contralateral breast cancer risk by 313-variant PRS (PRS$_{313}$) in all women, all women excluding studies oversampling cases with family history, and those with complete covariate information

		All patients			All women excluding studies oversampling cases with family history			Complete case		
		N=56,068 (CBC=1,027)			N=51,883 (CBC=829)			N=12,065 (CBC=193)		
		HR per unit SD[a]	95%CI	P-value	HR per unit SD[a]	95%CI	P-value	HR per unit SD[a]	95%CI	P-value
Model 1										
PRS$_{313}$[b]		1.25	1.18-1.33	<.001	1.26	1.17-1.34	<.001	1.35	1.17-1.56	<.001
Model 2										
PRS$_{313}$[b]		1.23	1.16-1.31	<.001	1.25	1.17-1.34	<.001	1.33	1.15-1.54	<.001
Family history	yes vs. no	1.43	1.24-1.64	<.001	1.34	1.13-1.59	.001	1.49	1.06-2.09	.02
	unknown vs. no	0.93	0.75-0.16	.54	0.92	0.73-1.16	.47	-	-	-
Model 3										
PRS$_{313}$[b]		1.25	1.18-1.33	<.001	1.26	1.17-1.34	<.001	1.35	1.17-1.56	<.001
Nodal status	positive vs. negative	1.05	0.91-1.20	.50	1.07	0.92-1.25	.37	1.14	0.85-1.53	.37
	unknown vs. no	1.26	1.04-1.53	.02	1.29	1.04-1.60	.02	-	-	-
Model 4										
PRS$_{313}$[b]		1.25	1.18-1.33	<.001	1.26	1.18-1.35	<.001	1.35	1.17-1.56	<.001
Tumor size,	(2-5] vs. ≤2	1.08	0.92-1.25	.34	1.12	0.95-1.32	.20	0.93	0.68-1.27	.66
	>5 vs. ≤2	1.37	0.99-1.89	.06	1.45	1.02-2.07	.04	1.63	0.93-2.85	.09
	unknown vs. ≤2	1.23	1.04-1.47	.02	1.14	0.94-1.39	.18	-	-	-
Model 5										
PRS$_{313}$[b]		1.25	1.17-1.33	<.001	1.25	1.17-1.34	<.001	1.35	1.17-1.57	<.001
Differentiation grade	II vs. I	0.93	0.76-1.13	.45	0.99	0.80-1.24	.94	0.98	0.65-1.48	.93
	III vs. I	0.90	0.73-1.12	.35	0.97	0.76-1.24	.81	1.09	0.70-1.69	.69
	unknown vs. I	1.20	0.96-1.49	.11	1.45	1.13-1.86	.004	-	-	-
Model 6										
PRS$_{313}$[b]		1.25	1.17-1.33	<.001	1.25	1.17-1.34	<.001	1.33	1.16-1.54	<.001
Morphology	lobular vs. ductal	1.26	1.03-1.53	.03	1.34	1.08-1.67	.008	1.48	0.99-2.21	.05
	mixed (ductal and lobular) vs. ductal	1.28	0.94-1.73	.11	1.36	0.98-1.88	.06	1.48	0.87-2.54	.15
	other vs. ductal	1.04	0.81-1.33	.75	0.91	0.66-1.24	.55	1.24	0.69-2.21	.47
	unknown vs. ductal	1.77	1.42-2.19	<.001	1.82	1.44-2.30	<.001	-	-	-
Model 7										
PRS$_{313}$[b]		1.25	1.18-1.33	<.001	1.26	1.18-1.35	<.001	1.35	1.17-1.56	<.001
ER-status	positive vs. negative	0.88	0.75-1.04	.14	0.86	0.72-1.03	.11	0.90	0.62-1.32	.60
	unknown vs. negative	1.16	0.93-0.43	.19	1.11	0.86-1.43	.43	-	-	-
Model 7										
PRS$_{313}$[b]		1.25	1.18-1.33	<.001	1.26	1.18-1.35	<.001	1.35	1.17-1.56	<.001
PR-status	positive vs. negative	0.95	0.81-1.11	.51	0.92	0.78-1.09	.32	0.91	0.66-1.25	.56
	unknown vs. negative	1.15	0.95-1.40	.14	1.10	0.88-1.37	.40	-	-	-

Table S6. Continued

		All patients			All women excluding studies oversampling cases with family history			Complete case		
		N=56,068 (CBC=1,027)			N=51,883 (CBC=829)			N=12,065 (CBC=193)		
		HR per unit SD[a]	95%CI	P-value	HR per unit SD[a]	95%CI	P-value	HR per unit SD[a]	95%CI	P-value
Model 9										
PRS_{313}[b]		1.25	1.18-1.33	<.001	1.26	1.17-1.34	<.001	1.34	1.16-1.55	<.001
HER2-status	positive vs. negative	0.84	0.64-1.11	.22	0.76	0.56-1.05	.10	0.70	0.45-1.10	.12
	unknown vs. negative	1.29	1.11-1.50	.001	1.28	1.08-1.52	.004	-	-	-
Model 10										
PRS_{313}[b]		1.25	1.17-1.32	<.001	1.26	1.17-1.34	<.001	1.35	1.16-1.56	<.001
Chemotherapy	yes vs. no	0.86	0.73-1.01	.06	0.99	0.83-1.19	.92	0.89	0.64-1.25	.51
	unknown vs. no	1.09	0.91-1.31	.34	1.20	0.97-1.47	.09	-	-	-
Model 11										
PRS_{313}[b]		1.25	1.18-1.33	<.001	1.26	1.18-1.35	<.001	1.36	1.17-1.57	<.001
Endocrine therapy	yes vs. no	0.75	0.64-0.88	.001	0.92	0.75-1.12	.41	0.78	0.55-1.11	.17
	unknown vs. no	0.90	0.75-1.09	.28	1.11	0.87-1.41	.39	-	-	-
Model 12										
PRS_{313}[b]		1.25	1.17-1.32	<.001	1.26	1.17-1.34	<.001	1.35	1.17-1.56	<.001
Radiotherapy	yes vs. no	1.00	0.85-1.18	1.00	0.98	0.82-1.18	.85	1.35	0.88-2.08	.17
	unknown vs. no	1.41	1.14-1.74	.001	1.18	0.93-1.50	.17	-	-	-
Model 13										
PRS_{313}[b]		1.25	1.17-1.32	<.001	1.25	1.17-1.34	<.001	1.34	1.16-1.55	<.001
Year of first breast cancer diagnosis		0.95	0.94-0.96	<.001	0.95	0.93-0.96	<.001	0.90	0.86-0.95	<.001
Model 14										
PRS_{313}[b]	full model[c]	1.23	1.16-1.31	<.001	1.25	1.16-1.33	<.001	1.33	1.15-1.53	<.001

Abbreviations: PRS = polygenic risk score; CBC = contralateral breast cancer; HR = hazard ratio; CI = confidence interval; SD = standard deviation; ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2

[a] All analyses were performed with attained age as the time scale

[b] Coefficients to construct the PRS_{313} are shown in Table S3. The PRS_{313} was standardized by SD=0.61, in line with Mavaddat et al.[1]

[c] Adjusted for family history, nodal status, tumor size, differentiation grade, morphology, ER status, HER2 status, chemotherapy, endocrine therapy, radiotherapy, and year of first breast cancer diagnosis

Table S7. Five- and ten-year cumulative risks of contralateral breast cancer by the 313-variant PRS (PRS_{313}) for different age groups

	5-year cumulative CBC risks (%) range by age					10-year cumulative CBC risks (%) range by age				
Age at first breast cancer diagnosis (years)	**5th percentile PRS_{313}**	**10th percentile PRS_{313}**	**50th percentile PRS_{313}**	**90th percentile PRS_{313}**	**95th percentile PRS_{313}**	**5th percentile PRS_{313}**	**10th percentile PRS_{313}**	**50th percentile PRS_{313}**	**90th percentile PRS_{313}**	**95th percentile PRS_{313}**
30-34	1.9-3.3	2.1-3.6	2.8-4.7	3.7-6.2	4.0-6.8	3.3-4.4	3.6-4.8	4.7-6.3	6.2-8.3	6.8-9.1
35-39	0.8-2.2	0.9-2.4	1.2-3.2	1.6-4.2	1.7-4.6	2.2-3.9	2.4-4.2	3.2-5.5	4.2-7.2	4.6-8.0
40-44	1.5-2.9	1.7-3.2	2.2-4.2	2.9-5.5	3.2-6.0	2.9-4.9	3.2-5.3	4.2-7.0	5.5-9.1	6.0-10.0
45-49	1.4-2.5	1.5-2.8	2.0-3.7	2.6-4.8	2.9-5.3	2.5-4.2	2.8-4.5	3.7-6.0	4.8-7.8	5.3-8.6
50-54	1.4-2.9	1.5-3.1	2.0-4.1	2.6-5.5	2.9-6.0	2.9-4.8	3.1-5.3	4.1-6.9	5.5-9.1	6.0-10.0
55-59	1.6-3.3	1.8-3.6	2.4-4.7	3.1-6.2	3.4-6.8	3.3-5.3	3.6-5.7	4.7-7.5	6.2-9.8	6.8-10.8
60-64	1.8-3.5	1.9-3.8	2.6-5.0	3.4-6.5	3.7-7.2	3.5-5.5	3.8-6.0	5.0-7.9	6.5-10.3	7.2-11.3
65-70	1.5-3.5	1.7-3.8	2.2-5.0	2.9-6.6	3.2-7.2	3.5-4.6	3.8-5.0	5.0-6.6	6.6-8.7	7.2-9.5

Abbreviations: PRS = polygenic risk score; CBC = contralateral breast cancer

Coefficients to construct the PRS_{313} are shown in Table S3. The PRS_{313} was standardized by SD=0.61, in line with Mavaddat et al[1]. The CBC incidences for each age group were calculated based on incidence data from the Netherlands Cancer Registry[3] and relative risks estimated as described in the Material and Methods. In contrast to Table 4, death was not taken into account as competing risk

Table S8. Estimates of unilateral- and contralateral breast cancer risk by the 313-variant PRS (PRS_{313}) in the European case-case series and the Asian case-case series

	European						Asian		
	Case-case series[a]			Validation set[b]			Case-case series[a]		
PRS_{313}[c]	**OR per unit SD**	**95%CI**	**P-value**	**OR per unit SD**	**95%CI**	**P-value**	**OR per unit SD**	**95%CI**	**P-value**
Unilateral breast cancer versus control	1.82	1.80-1.84	<.001	1.67	1.59-1.76	<.001	1.56	1.52-1.60	<.001
CBC versus unilateral breast cancer	1.30	1.26-1.35	<.001	1.39	1.13-1.70	.002	1.15	1.02-1.29	.02

Abbreviations: PRS = polygenic risk score; CBC = contralateral breast cancer; OR = odds ratio; SD = standard deviation; CI = confidence interval

[a] Adjusted for country and age. For all women with unilateral- and contralateral breast cancer we used age at first breast cancer diagnosis, and for control women without any diagnosis of breast cancer we used age at baseline questionnaire

[b] The validation set was previously used to develop the PRS_{313}; see details in materials and methods. For analyses in the current paper, this set is nested within the case-case series. These analyses were additionally adjusted for 10 principal components for comparability with the originally published PRS_{313} overall estimates[1]

[c] Coefficients to construct the PRS_{313} are shown in Table S3. The PRS_{313} was standardized by SD=0.61, in line with Mavaddat et al.[1]

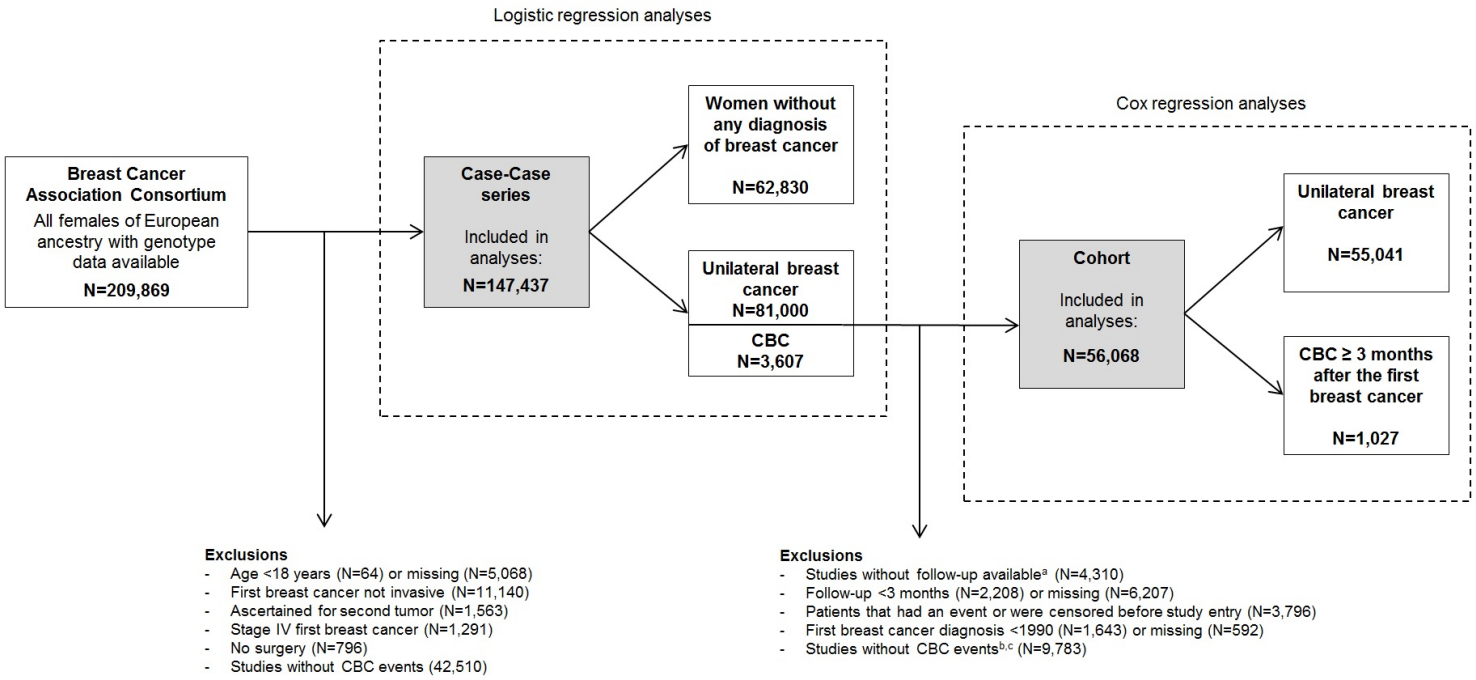


Figure S1A. Overview of the selection of women with breast cancer and control women for the European series
Abbreviations: CBC = contralateral breast cancer. For a complete overview of all studies see Table S1. [a] Excluded studies: CBCS, GLACIER, HMBCS, TNBCC; [b] Excluded studies: BCFR-NY, BCFR-UTAH, CNIO-BCS, DIETCOMPLYF, FHRISK, GESBC, HABCS, HUBCS, ICICLE, KBCP, MCCS, MMHS, NCBCS, PREFACE, SUCCESSB, SUCCESSC; [c] These studies dropped out because for these analyses the definition of CBC is based on the criteria that the CBC was diagnosed at least three months after the first breast cancer diagnosis

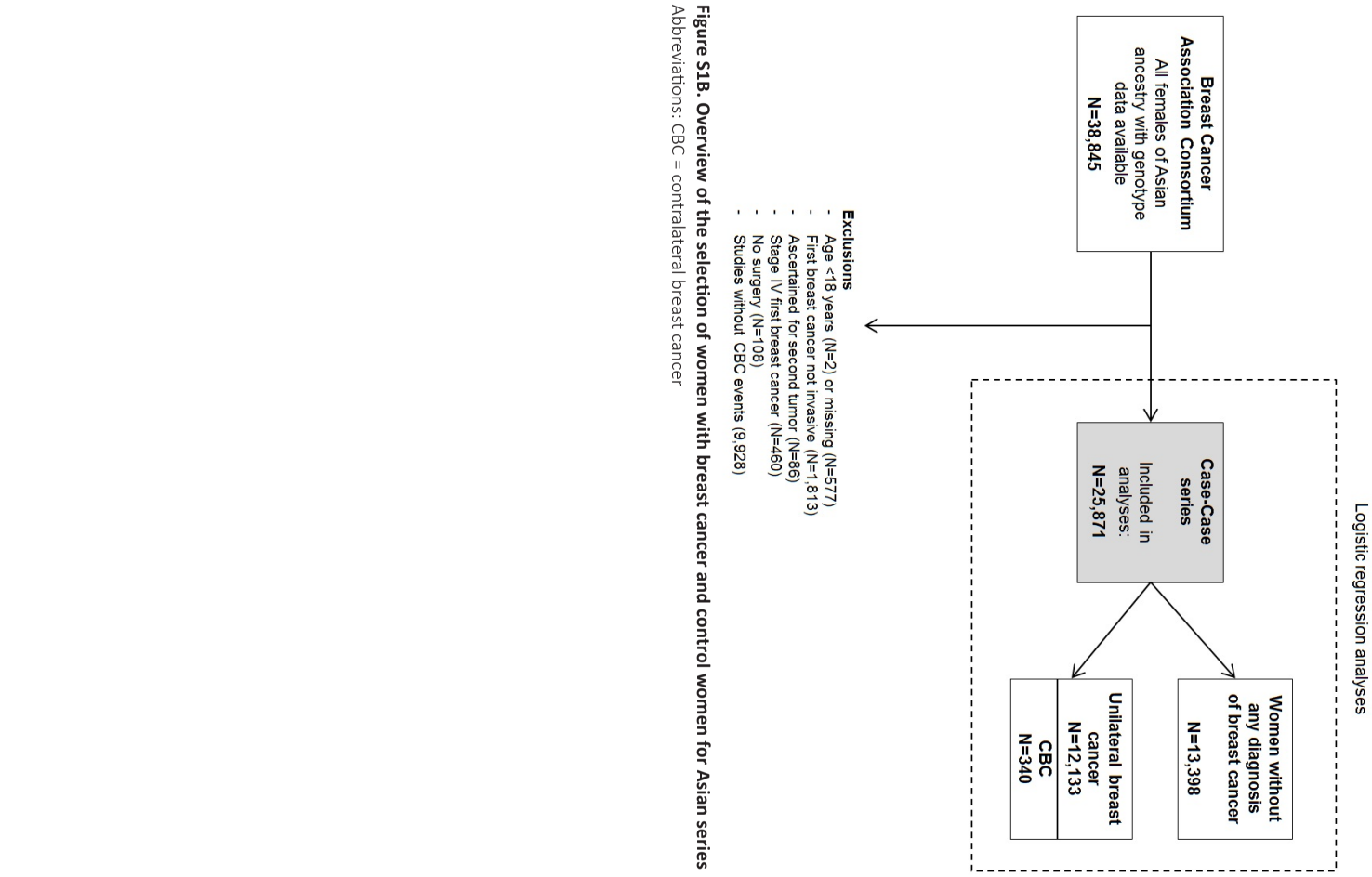


Figure S1B. Overview of the selection of women with breast cancer and control women for Asian series
Abbreviations: CBC = contralateral breast cancer

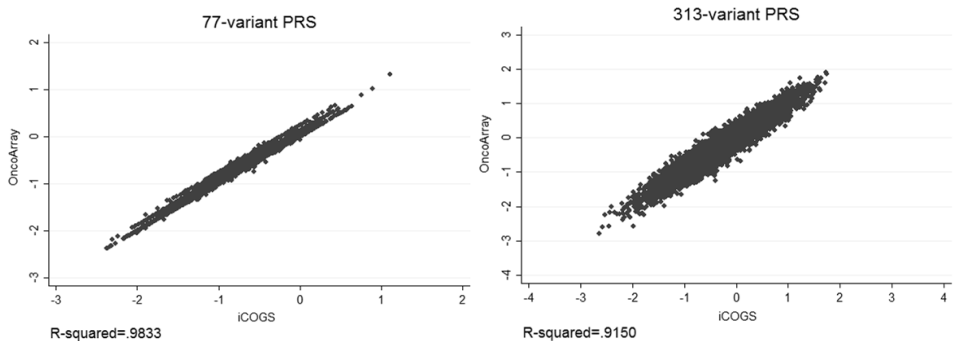


Figure S2. Correlation of total variant scores between the iCOGS array and OncoArray for the 77-variant PRS and the 313-variant PRS[a,b]

Abbreviations: PRS = polygenic risk score; SD = standard deviation. [a] We evaluated consistency between iCOGS and OncoArray using the intraclass correlation coefficient (ICC), showing a ICC of 0.99 (95%CI=0.99-0.99) for the PRS_{77}, and an ICC of 0.96 (95%CI=0.95-0.96) for the PRS_{313}, based on N=9,071 observations; [b] Coefficients to construct the PRSs are shown in Table S3. The PRSs were standardized by the same SD as was used by Mavaddat et al.[1]. The SD was 0.45 for overall breast cancer PRS_{77}, and 0.61 for overall breast cancer PRS_{313}

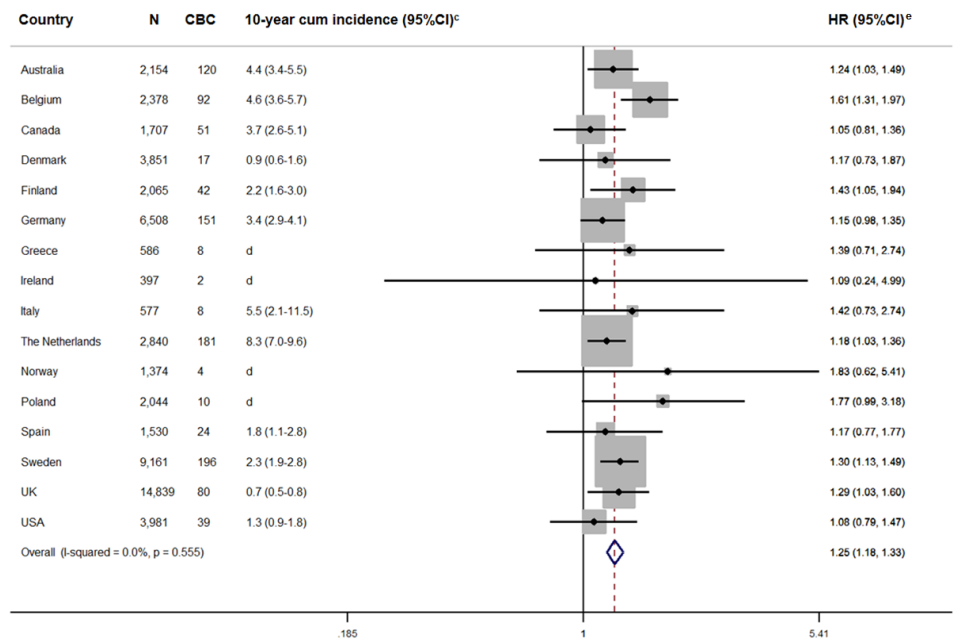


Figure S3. Forest plot of the association between the 313-variant PRS and contralateral breast cancer risk by country[a,b]

Abbreviations: PRS = polygenic risk score; N = number of women; CBC = contralateral breast cancer; cum = cumulative; CI = confidence interval; HR = hazard ratio; SD = standard deviation. Fixed effect meta-analysis was used to calculate I-squared and P-value for heterogeneity. [a] Republic of North Macedonia was left out this plot because of a too small sample size (N=76 women including N=2 CBC events); [b] Coefficients to construct the PRS_{313} are shown in Table S3. The PRS_{313} was standardized by SD=0.61, in line with Mavaddat et al.[1]; [c] The 10-year cumulative incidence of CBC was estimated with time since first breast cancer as time scale, and distant metastases (where available) and death as competing risks; [d] Follow-up too short for calculating 10-year cumulative incidence; [e] HR per SD. The analyses were performed with attained age as the time scale

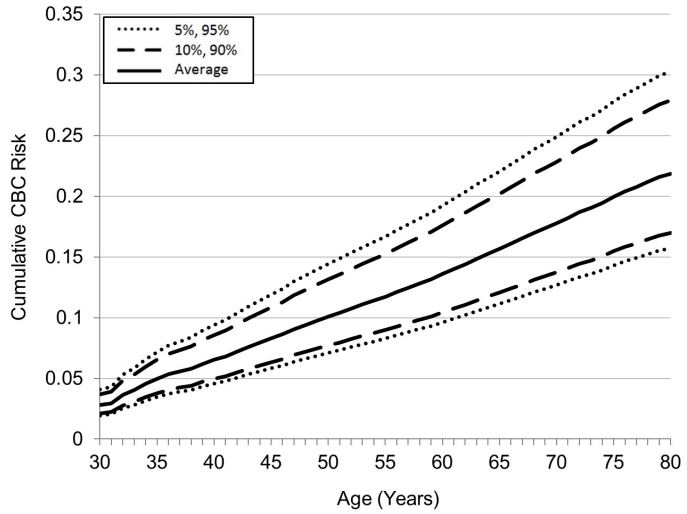


Figure S4. Predicted contralateral breast cancer risk by percentile of the 313-variant PRS (PRS_{313})
Abbreviations: PRS = polygenic risk score, CBC = contralateral breast cancer
Coefficients to construct the PRS_{313} are shown in Table S3. The PRS_{313} was standardized by SD=0.61, in line with Mavaddat et al[1]. The CBC incidences were calculated based on incidence data from the Netherlands Cancer Registry[3] and relative risks estimated as described in the Material and Methods. In contrast to Figure 2, death was not taken into account as competing risk

Supplemental Note

Our initial aim was to externally validate our results using the UK Biobank, which seemed the most suitable cohort given the large number of women diagnosed with breast cancer with information available on the PRS_{313},. However, when we started the analyses, it turned out that the UK Biobank had no information available on the laterality of the second breast tumor. Therefore, we were unable to distinguish between ipsilateral and contralateral breast cancer, and had to define our endpoint in these analyses as 'any second breast cancer'. In addition, in comparison to our analyses in the BCAC, we were unable to exclude patients diagnosed with stage IV invasive first breast cancer from the UK Biobank cohort, and had limited information on metastases developed during follow-up.

The association between the overall breast cancer PRS_{313} and (any) second breast cancer was evaluated among women aged ≥18 years of European ancestry from the UK Biobank cohort who had had a diagnosis of invasive first breast cancer. UK Biobank samples were genotyped using Affymetrix UK BiLEVE Axiom array and Affymetrix UK Biobank Axiom® array and imputed to the combined 1000 Genome Project v3 and UK10K reference panels using SHAPEIT3 and IMPUTE3[4]. The lowest imputation info score for the variants used in these analyses was 0.86. Samples were included for this analysis of the UK BIOBANK study on the basis of female sex (genetic and self-reported) and ethnicity filter (Europeans/White British ancestry subset). Duplicates and individuals with high degree of relatedness (samples which have >10 putative third degree relatives) were removed, and we randomly excluded one of each related pair first-degree relatives. Samples were also excluded on standard quality control criteria. The PRS_{313} was calculated as a weighted sum of the minor allele dosages; the variant selection and weights are as given by Mavaddat et al[1]. The PRS_{313} was standardized by SD=0.61, in line with our BCAC analyses and Mavaddat et al[1].

The final cohort included 10,567 women with invasive breast cancer among whom 302 registry-confirmed second breast cancers developed over 59,260 person-years of follow-up. A Cox proportional hazards model was used to assess the association between PRS_{313} and second breast cancer risk. Time at risk started three months after the age of first breast cancer diagnosis, where this was diagnosed after the baseline questionnaire date, or three months after the baseline questionnaire where first breast cancer was diagnosed before the baseline questionnaire date. Time at risk ended at the age of second breast cancer diagnosis (ipsilateral or contralateral), distant metastasis (where available), death or end of follow-up (at latest December 10, 2016). Potential effect modification of the PRS_{313} by age was evaluated by adding an interaction term (PRS_{313} x age at first breast cancer diagnosis [continuous]) in the model. We performed a separate analysis for invasive second breast cancer (241 breast cancers), where we censored on in situ second breast cancer.

The HR for a second breast cancer (in situ or invasive) per SD of PRS_{313} in the UK Biobank cohort was 1.13 (95%CI=1.01-1.26). We found no indication for interaction with age at first breast cancer diagnosis ($HR_{interaction}$=1.00, 95%CI=0.99-1.01; P=0.87). When analyses were restricted to invasive second breast cancer, the HR per SD was 1.13 (95%CI=1.00-1.29).

Supplemental References

1 Mavaddat, N. *et al.* Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. *Am. J. Hum. Genet.* **104**, 21-34, doi:10.1016/j.ajhg.2018.11.002 (2019).

2 Mavaddat, N. *et al.* Prediction of breast cancer risk based on profiling with common genetic variants. *J. Natl. Cancer Inst.* **107**, doi:10.1093/jnci/djv036 (2015).

3 Kramer, I. *et al.* The influence of adjuvant systemic regimens on contralateral breast cancer risk and receptor subtype. *J. Natl. Cancer Inst.*, doi:10.1093/jnci/djz010 (2019).

4 O'Connell, J. *et al.* Haplotype estimation for biobank-scale data sets. *Nat. Genet.* **48**, 817-820, doi:10.1038/ng.3583 (2016).

CHAPTER 3

The influence of adjuvant systemic regimens on contralateral breast cancer risk and receptor subtype

J Natl Cancer Inst. 2019 Jul 1;111(7):709-718

Iris Kramer
Michael Schaapveld
Hester S.A. Oldenburg
Gabe S. Sonke
Danielle McCool
Flora E. van Leeuwen
Koen K. Van de Vijver
Nicola S. Russell
Sabine C. Linn
Sabine Siesling
C. Willemien Menke- van der Houven van Oordt
Marjanka K. Schmidt

Abstract

Background

An increasing number of breast cancer (BC) survivors are at risk of developing contralateral breast cancer (CBC). We aimed to investigate the influence of various adjuvant systemic regimens on, subtype-specific, risk of CBC.

Methods

This population-based cohort study included female patients diagnosed with first invasive BC between 2003-2010; follow-up was complete until 2016. Clinico-pathological data were obtained from the Netherlands Cancer Registry and additional data on receptor status through linkage with PALGA: the Dutch Pathology Registry. Cumulative incidences (death and distant metastases as competing risk) and hazard ratios (HRs) were estimated for all invasive metachronous CBC and CBC subtypes.

Results

Of 83,144 BC patients, 2,816 developed a CBC; the 10-year cumulative incidence was 3.8% (95% confidence interval [CI]=3.7-4.0%). Overall, adjuvant chemotherapy (HR=0.70, 95%CI=0.62-0.80), endocrine therapy (HR=0.46, 95%CI=0.41-0.52), and trastuzumab with chemotherapy (HR=0.57, 95%CI=0.45-0.73) were strongly associated with a reduced CBC risk. Specifically, taxane-containing chemotherapy (HR=0.48, 95%CI=0.36-0.62) and aromatase inhibitors (HR=0.32, 95% CI=0.23-0.44) were associated with a large CBC risk reduction. More detailed analyses showed that endocrine therapy statistically significantly decreased the risk of estrogen receptor (ER)-positive CBC (HR=0.41, 95%CI=0.36-0.47), but not ER-negative CBC (HR=1.32, 95%CI=0.90-1.93), compared with no endocrine therapy. Patients receiving chemotherapy for ER-negative first BC had a higher risk of ER-negative CBC from 5 years of follow-up (HR=2.84, 95%CI=1.62-4.99), compared with patients not receiving chemotherapy for ER-negative first BC.

Conclusion

Endocrine therapy, chemotherapy, as well as trastuzumab with chemotherapy reduce CBC risk. However, each adjuvant therapy regimen had a different impact on the CBC subtype distribution. Taxane-containing chemotherapy and aromatase inhibitors were associated with the largest CBC risk reduction.

Introduction

Breast cancer (BC) survival has increased considerably, largely as a result of increasing use of (neo)adjuvant therapies[1]. As a consequence, a greater number of women are at risk of developing a second primary tumor in the contralateral breast. Studies have shown that the 10-year risk of contralateral breast cancer (CBC) is 4-7%[2-5].

There is increasing evidence that patients who received adjuvant endocrine therapy or chemotherapy for their first BC have a lower risk of developing CBC. The Early Breast Cancer Trialists' Collaborative Group (EBCTCG) showed that 5-year tamoxifen use was associated with a 38% reduction in CBC risk after 10 years of follow-up[6], and adjuvant chemotherapy with a 20% decrease[7].

CBC patients may have a worse prognosis compared with patients with unilateral BC[2,4,8,9]. An explanation for this worse prognosis, besides having been diagnosed with yet another cancer, may be found in the impact of adjuvant systemic therapy on CBC tumor biology[9], or misclassification of metastatic disease as a CBC[8].

Little is known about the influence of adjuvant systemic therapy on the (hormone) receptor subtype of CBC. Some studies showed a higher proportion of estrogen receptor (ER)-negative CBC among patients who received endocrine therapy for their first BC compared with those who did not[10-14]. The studies that evaluated the effects of adjuvant therapy on subtype-specific CBC risk, however, were based on small numbers. Since adjuvant trastuzumab was introduced for early-stage BC in 2005, the impact on CBC risk has not yet been described.

We therefore aimed to investigate the influence of different regimens of adjuvant endocrine therapy, chemotherapy, and trastuzumab on CBC risk overall and by (hormone) receptor subtype within a large population-based cohort of women diagnosed with invasive BC.

Methods

Study population

The cohort included 83,144 female patients diagnosed with invasive BC, who underwent surgery, in 2003-2010 (Figure 1). Patients were selected from the population-based Netherlands Cancer Registry (NCR), which contains data on all newly diagnosed cancer patients nationwide. Follow-up for all patients started 3 months after first BC diagnosis; therefore, patients who had developed distant metastases or CBC or died within 3 months after diagnosis were excluded.

Patient and tumor characteristics

The NCR provided clinico-pathological data; follow-up on second cancers and vital status

was complete until February 1, 2016, but information of recurrences was complete only for patients diagnosed in 2003-2006 and for 56% in 2007-2008. Pathological information on tumor size, lymph node status, and metastasis collected was coded into tumor stage according to the TNM Classification of Malignant Tumors [15]; if pathological information was missing, clinical stage was used.

Since 2003, the NCR registers receptor status, as determined by immunohistochemistry (IHC). Tumors were defined ER-positive or progesterone receptor (PR)-positive when at least 10% of tumor cells stained positive. In the Netherlands, recommendations for HER2/neu-receptor (HER2) testing and use of adjuvant trastuzumab were implemented from 2005[16]. A tumor was considered HER2-positive if IHC was 3+ (strong and complete membranous expression in >10% of tumor cells) or if IHC was 2+ when additional confirmation with in situ hybridization was available, but considered unknown if in situ hybridization confirmation was missing. To overcome incompleteness in data on receptor status, all CBC patients were linked to the nationwide network and registry of histo- and cytopathology (PALGA)[17] to retrieve where possible information on ER, PR, and HER2 status for both the first BC and CBC.

Data were handled in accordance with privacy regulations for medical research[18]. The review boards of the NCR and PALGA approved the proposal. All data were anonymous to the researchers involved.

Statistical analysis

The primary outcome was the development of metachronous CBC, defined as an invasive BC in the contralateral breast at least 3 months after the first BC diagnosis. Time at risk ended at the date of CBC, distant metastases, death, or end of follow-up, whichever came first. The cumulative incidence of CBC was estimated with distant metastases and death as competing risks.

Multivariable Cox Proportional Hazards (CPH) analysis with time since first BC diagnosis as time-scale was used to examine the effect of adjuvant systemic therapy (chemotherapy, endocrine therapy, trastuzumab, or combined) on CBC risk (hazard ratios [HRs]). Subdistribution HRs were calculated accounting for death and distant metastases as competing risks. We examined the association between specific types of chemotherapy (taxane-containing/anthracycline-containing) and endocrine therapy (tamoxifen/aromatase inhibitors) and CBC risk. All models included all systemic therapies and were adjusted for age and stage (III vs I-II) at first BC diagnosis, factors that were previously shown to be important predictors of CBC in the Dutch population[2], though in our dataset only stage changed the log HR of systemic therapy by greater than 15%. Radiotherapy, ER-status, HER2-status, and year of diagnosis did not change the log HR by more than 15%, and were only included for sensitivity analyses. Using the nlcheck function in STATA, there was no evidence for nonlinearity of age in the multivariable model[19]; therefore, age was continuous in all models, except for Supplementary Tables 1-3 to illustrate the differences between age categories. Because the NCR did not register menopausal status, we used age younger than 50 years and at least 50 years as a proxy for pre- and post-menopausal status[20]. Potential effect modification of menopause was assessed with a specification link test for single-equation models[21,22]. The proportional hazard assumption was assessed using Schoenfeld residuals[23]. We performed sensitivity analyses based on selection of years with complete recurrence information, additional censoring on local/regional recurrence, and a stricter definition of metachronous CBC (≥1 year after first BC).

The effect of adjuvant therapy on subtype-specific CBC was estimated using cumulative incidence curves, additionally accounting for other CBC subtypes as competing risks; for example, to determine risk of ER-positive CBC, the following events were treated as competing risks: ER-negative CBC, ER-unknown CBC, distant metastases, and death. The HER2-specific analysis included only patients diagnosed since 2005. Because there was interaction between treatment and subtype ($P_{interaction}$<.001), we used joint multivariable CPH analyses[24] to determine the association of adjuvant therapies with CBC ER status in separate models for each of the first BC subtypes. We defined subtype as hormone receptor (HR)+/HER2− (ie, ER+ and/or PR+ and HER2−), HR+/HER2+ (ie, ER+ and/or PR+ and HER2+), HR−/HER2+ (ie, ER−/PR−/HER2+), and HR−/HER2− (ie, ER−/PR−/HER2-). Each model included ER-specific CBC (ER-positive/ER-negative/ER-unknown), distant metastases, and death as possible outcome. These subtype-specific models were adjusted for trastuzumab, age, and stage.

All P-values are two sided with the statistical significance level set at less than .05. Tests for heterogeneity between subtypes or follow-up period were performed using the Wald test. Analyses were performed using STATA, version 13.1 (StataCorp).

Results

The cohort included for analyses comprised 83,144 patients diagnosed with invasive first BC with a median follow-up of 7.7 years (range 0.3-13.1) (Figure 1). Median time to develop a CBC (N=2,816) after a first BC was 4.6 years (range 0.3-12.7). Characteristics of the cohort are presented in Table 1. The distributions of adjuvant systemic therapies according to patient and tumor characteristics are presented in Supplementary Table 1.

The 5- and 10-year cumulative incidences of CBC were 1.9% (95% confidence interval [CI]=1.8-2.0%) and 3.8% (95%CI=3.7-4.0%), respectively (Supplementary Table 2). CBC cumulative incidence increased at a rate of 0.4% per year.

In a multivariable CPH model (Table 2), treatment with adjuvant chemotherapy (HR=0.70, 95%CI=0.62-0.80), endocrine therapy (HR=0.46, 95%CI=0.41-0.52), endocrine and chemotherapy (HR=0.35, 95%CI=0.31-0.39), and chemotherapy combined with trastuzumab (HR=0.57, 95%CI=0.45-0.73) were strongly associated with a reduced CBC

risk compared with patients who did not receive systemic therapy. Patients receiving trastuzumab combined with endocrine therapy and chemotherapy were the least prone to develop CBC (HR=0.24, 95%CI=0.17-0.33; P<.05 compared with any other treatment group; Table 2). Adjustment for radiotherapy, year of diagnosis, ER and HER2 status, or taking distant metastases and death as competing risks into account did not substantially alter these results (Supplementary Table 3), neither did additional censoring on local and regional recurrence or a stricter definition of CBC (Supplementary Table 4). Radiotherapy was not associated with CBC risk (HR=0.94, 95%CI=0.86-1.02; Supplementary Table 3). Therapy-specific analysis showed that taxane-containing chemotherapy was strongly associated with a CBC risk reduction (HR=0.48, 95%CI=0.36-0.62; Table 2) compared with patients who did not receive chemotherapy, but not anthracycline-containing chemotherapy (HR=0.91, 95%CI=0.77-1.06). Treatment with aromatase inhibitors (HR=0.32, 95%CI=0.23-0.44) was associated with a stronger CBC risk reduction compared to tamoxifen (HR=0.48, 95%CI=0.44-0.53) ($P_{heterogeneity}$=.01). There was no evidence for effect modification between menopausal status and any adjuvant therapy on CBC risk.

Table 1. Patient, tumor, and treatment characteristics of all patients diagnosed with first BC between 2003-2010 and subsequent CBC

Characteristics	First BC No. (%)*	CBC No. (%)*
Total	83,144 (100.0)	2,816 (100.0)
Age at diagnosis, y		
<35	1,826 (2.2)	22 (0.8)
35-44	9,693 (11.7)	153 (5.4)
45-54	22,154 (26.7)	523 (18.6)
55-64	21,778 (26.2)	801 (28.4)
65-74	17,222 (20.7)	771 (27.4)
75-84	8,242 (9.9)	444 (15.8)
≥85	2,229 (2.7)	102 (3.6)
Median age at diagnosis, y (range)	58.5 (19.4-101.3)	63.9 (24.8-97.0)
Tumor stage		
I	39,676 (47.7)	1,736 (63.3)
II	32,158 (38.7)	703 (25.6)
III	11,310 (13.6)	237 (8.6)
IV	†	68 (2.5)
Unknown	†	72
Histological grade		
Grade 1	17,393 (22.8)	706 (28.9)
Grade 2	34,153 (44.8)	1,091 (44.6)
Grade 3‡	24,632 (32.3)	647 (26.5)
Unknown	6,966	372
Morphology		
Ductal	64,044 (77.0)	2,051 (72.8)
Lobular	9,233 (11.1)	380 (13.5)
Mixed ductal/lobular	3,013 (3.6)	112 (4.0)
Other	6,854 (8.2)	273 (9.7)
ER status		
Positive	64,886 (81.7)	2,200 (81.7)
Negative	14,579 (18.3)	492 (18.3)
Unknown	3,679	124
PR status		
Positive	50,674 (66.1)	1,618 (61.6)
Negative	26,004 (33.9)	1,010 (38.4)
Unknown	6,466	188
HER2 status§		
Positive	11,061 (17.3)	335 (12.6)
Negative	52,956 (82.7)	2,314 (87.4)
Unknown	19,127	167
Subtype‖		
HR+/HER2−	45,441 (71.8)	1,935 (74.9)
HR+/HER2+	6,957 (11.0)	189 (7.3)
HR−/HER2+	3,618 (5.7)	117 (4.5)
HR−/HER2−	7,304 (11.5)	344 (13.3)
Unknown	19,824	231
(Neo)adjuvant therapy for first BC		
No (neo)adjuvant therapy¶	31,290 (37.6)	-
CT	8,889 (10.7)	-
ET	17,359 (20.9)	-
CT + ET	19,923 (24.0)	-
CT + TRA	2,728 (3.3)	-
CT + ET + TRA	2,955 (3.6)	-
(Neo)adjuvant CT		
No CT	48,717 (58.6)	-
Taxane-containing CT#	4,427 (5.3)	-
Anthracycline-containing CT**	6,802 (8.2)	-
Taxane- + anthracycline-containing CT	3,590 (4.3)	-
CT, other or type unknown††	19.608 (23.6)	-
(Neo)adjuvant ET		
No ET	42,861 (51.6)	-
Tamoxifen‡‡	33,862 (40.7)	-
Aromatase inhibitors	2,393 (2.9)	-
Tamoxifen‡‡ + aromatase inhibitors	4,028 (4.8)	-

Abbreviations: BC = breast cancer; CBC = contralateral breast cancer; CT = chemotherapy; ER = estrogen receptor; ET = endocrine therapy; HER2 = human epidermal growth factor receptor2; HR+ = hormone receptor positive; HR− = hormone receptor negative; NCR = Netherlands Cancer Registry; PR = progesterone receptor; TRA = trastuzumab

* Percentages may not total 100 because of rounding

† Excluded

‡ Including 12 first BCs and 1 CBC that were defined as 'undifferentiated' in the NCR

§ HER2 status distribution of first BCs from 2005-2010: positive N=10.388 (17.0%), negative N=50.652 (83.0%), unknown N=2.313

‖ HR+ = ER+ and/or PR+; HR− = ER− and PR−

¶ No chemotherapy, endocrine therapy or trastuzumab (with or without radiotherapy)

The chemotherapeutic combination contains taxanes, but no anthracyclines

** The chemotherapeutic combination contains anthracyclines, but no taxanes

†† All other chemotherapeutic drugs and combinations (e.g. CMF) or type unknown

‡‡ The NCR specifically codes aromatase inhibitors; Tamoxifen is coded as endocrine therapy

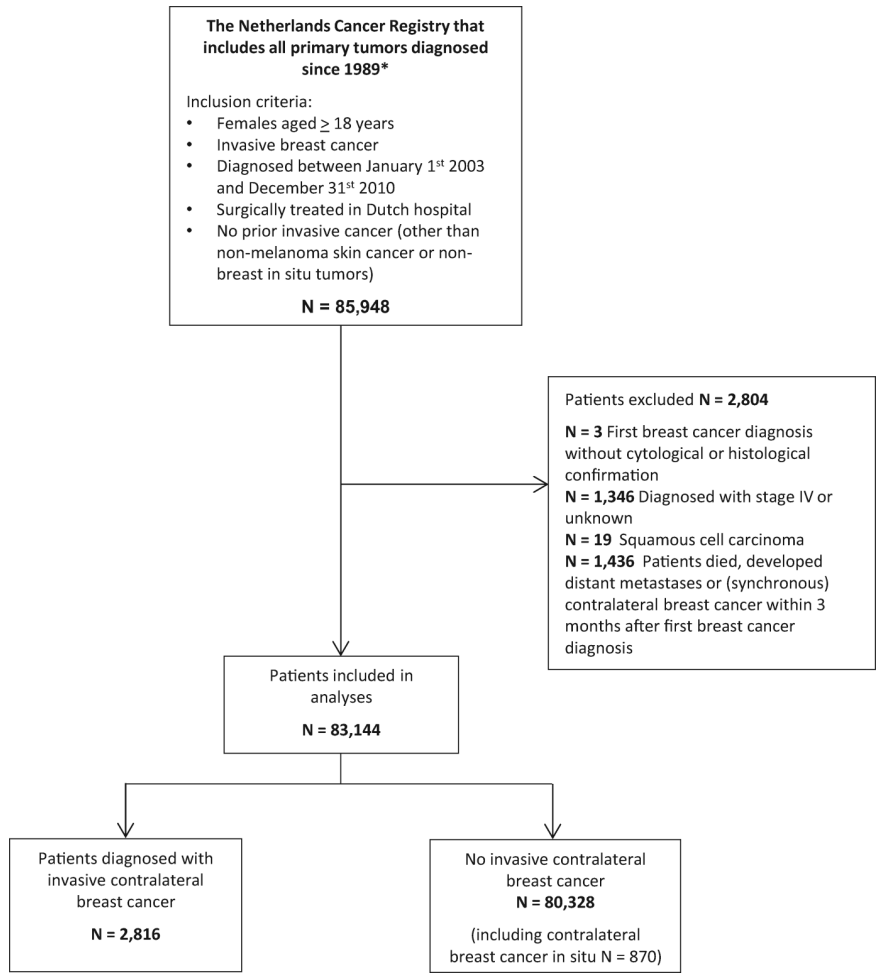


Figure 1. Overview of the selection of breast cancer patients
*After notification by the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA) and the national hospital discharge database, trained NCR personnel collected data directly from patients' files

Because the proportional hazard assumption was violated for chemotherapy, endocrine therapy, and trastuzumab, the multivariable CPH analyses were also performed stratified for follow-up duration up to and including 5 years and longer than 5 years (Table 2)[25], following higher recurrence risk reductions for the period up to and including 5 years shown by the EBCTCG[6,26]. In our study, CBC risk was statistically significantly stronger reduced in the first 5 years of follow-up among patients who had received chemotherapy and endocrine therapy combined ($P_{heterogeneity}$<.001) or chemotherapy and trastuzumab combined ($P_{heterogeneity}$=.04) than in the period longer than 5 years of follow-up. However, overall, systemic therapy remained statistically significantly associated with a reduced CBC risk after 5 years of follow-up.

Patients diagnosed with stage III first BC showed a statistically significantly higher risk of CBC (HR=1.48, 95%CI=1.30-1.69) compared with patients with stage I-II BC, but not if distant metastases and death were considered as competing risks (Supplementary Table 1). CBC risk did not differ by age at first BC, apart from a lower CBC risk for patients aged 45-54 years (HR=0.88, 95%CI=0.80-0.98) and 85 years and older (HR=0.55; 95%CI=0.37-0.81) compared with patients aged 55-64 years.

A greater proportion of CBCs among patients treated with endocrine therapy was ER-negative (23.2%) compared with that among patients without endocrine therapy for ER-positive first BC (6.9%; Supplementary Table 5). The proportion of ER-negativity between the first BC and CBC of all patients did not differ (both 18.3%; Table 1). Among patients with ER-positive first BC (Figure 2A), the difference for ER-positive CBC was 3.4% after 10-years of follow-up between patients who received endocrine therapy (1.8%, 95%CI=1.6-2.0) and those who did not (5.2%, 95%CI=4.9-5.5). Multivariable joint CPH analyses (Table 3) classifying the first BCs in four BC subtypes showed that among patients diagnosed with HR+/HER2− first BC, endocrine therapy statistically significantly decreased the risk of ER-positive CBC (HR=0.41, 95%CI=0.36-0.47), but not ER-negative CBC (HR=1.32, 95%CI=0.90-1.93) ($P_{heterogeneity}$=<.001).

We observed a 10-year cumulative incidence of ER-negative CBC of 1.9% (95%CI=1.6-2.2) for patients who received chemotherapy for ER-negative first BC and 1.2% (95%CI=0.9-1.6) for patients who did not (Figure 2B). Multivariable joint CPH analyses showed that patients diagnosed with HR−/HER2− (triple negative) first BC had a higher risk of triple-negative CBC when they received adjuvant chemotherapy (HR=1.56, 95%CI=1.00-2.42), compared to patients who did not (Supplementary Figure 1). In subsequent analyses within ER-negative tumors (insufficient data for triple negatives), we found no association between chemotherapy received for ER-negative first BC and risk of an ER-negative CBC in the first 5 years of follow-up (HR=1.28, 95%CI=0.84-1.95). However, risk of ER-negative CBC was increased for patients treated with chemotherapy after more than 5 years of follow-up (HR=2.84, 95%CI=1.62-4.99) compared with patients who did not receive chemotherapy for ER-negative first BC. Therapy-specific analyses (Supplementary Table 6) showed a statistically non-significant increased risk of ER-negative CBC for anthracycline-containing chemotherapy (HR=1.32, 95%CI=0.86-2.04), which was the strongest after 5 years of follow-up (HR=1.88, 95%CI=0.91-3.86), but a statistically significant decreased risk for taxane-containing chemotherapy (HR=0.36, 0.17-0.75). The combination of taxane- and anthracycline-containing chemotherapy was associated with a statistically nonsignificant decreased risk of ER-negative CBC (HR=0.59, 95%CI=0.28-1.22; Supplementary Table 6). The proportion of HER2-positive CBC was 33.7% for patients treated with trastuzumab for HER2-positive first BC (Supplementary Table 7) with a 5-year cumulative incidence of 0.4% (95%CI=0.3-0.7; Figure 2C), and this was 12.0% for patients who did not receive trastuzumab, with a 5-year cumulative incidence of 0.2% (95%CI=0.1-0.4; Figure 2C).

Table 2. Multivariable Cox regression analyses of CBC risk related to the (neo)adjuvant systemic therapy for the first BC

(Neo)adjuvant systemic therapy	Total follow-up				≤5 years follow-up					>5 years follow-up					$P_{heterogeneity}$ †
	No. of patients	HR	95% CI	P*	No. of patients	No. of CBC	HR	95% CI	P*	No. of patients	No. of CBC	HR	95% CI	P*	
Model 1: (Neo)adjuvant therapy combined‡															
No (neo)adjuvant therapy§	31,290	1.00	Ref.		4,644	913	1.00	Ref.		26,646	700	1.00	Ref.		
CT	8,889	0.70	0.62-0.80	<.001	2,208	172	0.66	0.56-0.78	<.001	6,681	146	0.79	0.65-0.95	.01	.16
ET	17,359	0.46	0.41-0.52	<.001	3,678	218	0.42	0.36-0.49	<.001	13,681	149	0.50	0.42-0.60	<.001	.13
CT + ET	19,923	0.35	0.31-0.39	<.001	2,287	177	0.26	0.22-0.30	<.001	17,636	227	0.50	0.42-0.59	<.001	<.001
CT + TRA	2,728	0.57	0.45-0.73	<.001	477	43	0.47	0.35-0.64	<.001	2,251	33	0.77	0.54-1.10	.16	.04
CT + ET + TRA	2,955	0.24‖	0.17-0.33	<.001	261	21	0.20	0.13-0.31	<.001	2,694	17	0.31	0.19-0.51	<.001	.19
Model 2: Therapy-specific subgroups¶															
(Neo)adjuvant CT															
No CT	48,717	1.00	Ref.		8,341	1,132	1.00	Ref.		40,376	849	1.00	Ref.		
Taxane-containing CT#	4,427	0.48	0.36-0.62	<.001	620	36	0.42	0.30-0.58	<.001	3,807	24	0.64	0.42-0.97	.04	.13
Anthracycline-containing CT**	6,802	0.91	0.77-1.06	.23	1,127	100	0.85	0.68-1.07	.16	5,675	140	0.98	0.79-1.22	.86	.38
Taxane+anthracycline-containing CT	3,590	0.69	0.52-0.91	.009	475	44	0.71	0.50-0.99	.05	3,115	27	0.66	0.42-1.06	.08	.83
CT, other or type unknown††	19,608	0.70	0.62-0.78	<.001	2,992	232	0.61	0.52-0.71	<.001	16,616	232	0.84	0.71-1.00	.05	.01
(Neo)adjuvant ET															
No ET	42,861	1.00	Ref.		7,319	1,127	1.00	Ref.		35,542	879	1.00	Ref.		
Tamoxifen‡‡	33,862	0.48	0.44-0.53	<.001	5,458	352	0.41	0.37-0.47	<.001	28,404	312	0.57	0.49-0.65	<.001	.001
Aromatase inhibitors	2,393	0.32	0.23-0.44	<.001	407	20	0.31	0.20-0.48	<.001	1,986	18	0.33	0.20-0.53	<.001	.86
Tamoxifen‡‡ + aromatase inhibitors	4,028	0.45	0.36-0.56	<.001	371	45	0.40	0.29-0.55	<.001	3,657	63	0.50	0.38-0.67	<.001	.28

Abbreviations: BC = breast cancer; CBC = contralateral breast cancer; CI = confidence interval; CT = chemotherapy; ET = endocrine therapy; HR = hazard ratio; Ref. = reference group; TRA trastuzumab

* Two-sided Wald test P-value

† Heterogeneity of HRs between ≤5 and >5 years follow-up duration

‡ Adjusted for age and stage at first breast cancer diagnosis

§ No chemotherapy, endocrine therapy or trastuzumab (with or without radiotherapy)

‖ We compared the CT+ET+TRA group versus the other treatment groups by changing the reference (vs. CT, P<.001) (vs. ET, P<.001) (vs. CT+ET, P=.03) (vs. CT+TRA, P<.001)

¶ Adjusted for trastuzumab therapy, age, and stage at first breast cancer diagnosis

The chemotherapeutic combination contains taxanes, but no anthracyclines

** The chemotherapeutic combination contains anthracyclines, but no taxanes

†† All other chemotherapeutic drugs and combinations (e.g. CMF) or type unknown

‡‡ The registry specifically codes aromatase inhibitors; Tamoxifen is coded as hormonal treatment

Table 3. Joint multivariable Cox regression analyses for each of the first tumor subtypes assessing the association of (neo)adjuvant systemic treatment of the first BC with subtype-specific CBC risk*

Subtype first BC	No. of patients	ER-positive CBC				ER-negative CBC				$P_{heterogeneity}$ ‡
		No. of CBC cases	HR	95% CI	P†	No. of CBC cases	HR	95% CI	P†	
Model 1: HR+/HER2−§										
ET										
No ET	18,125	860	1.00	Ref.		54	1.00	Ref.		
ET	27,316	397	0.41	0.36-0.47	<.001	107	1.32	0.90-1.93	.15	<.001
CT										
No CT	28,973	1,048	1.00	Ref.		100	1.00	Ref.		
CT	16,468	209	0.56	0.46-0.67	<.001	61	1.15	0.78-1.70	.48	<.001
Model 2: HR+/HER2+§										
ET										
No ET	2,282	83	1.00	Ref.		8	1.00	Ref.		
ET	4,675	46	0.43	0.28-0.66	<.001	22	1.22	0.50-2.98	.66	.04
CT										
No CT	3,186	96	1.00	Ref.		13	1.00	Ref.		
CT	3,771	33	0.61	0.29-1.27	.19	17	2.33	0.71-6.62	.16	.05
Model 3: HR−/HER2+§										
ET										
No ET	3,538	77	1.00	Ref.		36	1.00	Ref.		
ET	80	0	$7.15 \cdot 10^{-10}$	$1.67 \cdot 10^{-10}$-$3.06 \cdot 10^{9}$	<.001	2	2.19	0.51-9.36	.29	-
CT										
No CT	1,041	19	1.00	Ref.		9	1.00	Ref.		
CT	2,557	58	1.22	0.57-2.64	.61	29	4.01	1.64-9.81	.002	.05
Model 4: HR−/HER2−§										
ET										
No ET	7,126	124	1.00	Ref.		134	1.00	Ref.		
ET	178	4	1.22	0.45-3.32	.69	1	0.30	0.04-2.13	.23	.21
CT										
No CT	2,255	42	1.00	Ref.		32	1.00	Ref.		
CT	5,049	86	0.60	0.38-0.93	.02	103	1.17	0.77-1.78	.47	.02

Abbreviations: BC = breast cancer; CBC = contralateral breast cancer; CI = confidence interval; CT = chemotherapy; ER = estrogen receptor; ET = endocrine therapy; HER2 = human epidermal growth factor receptor 2; HR = hazard ratio; HR+ = hormone receptor positive; HR− = hormone receptor negative; PR = progesterone receptor; Ref. = reference group

* Adjusted for trastuzumab therapy, age and stage at first breast cancer diagnosis

† Two-sided Wald test P-value

‡ Heterogeneity of HRs between ER-positive and ER-negative CBC

§ R+ = ER+ and/or PR+; HR− = ER− and PR−

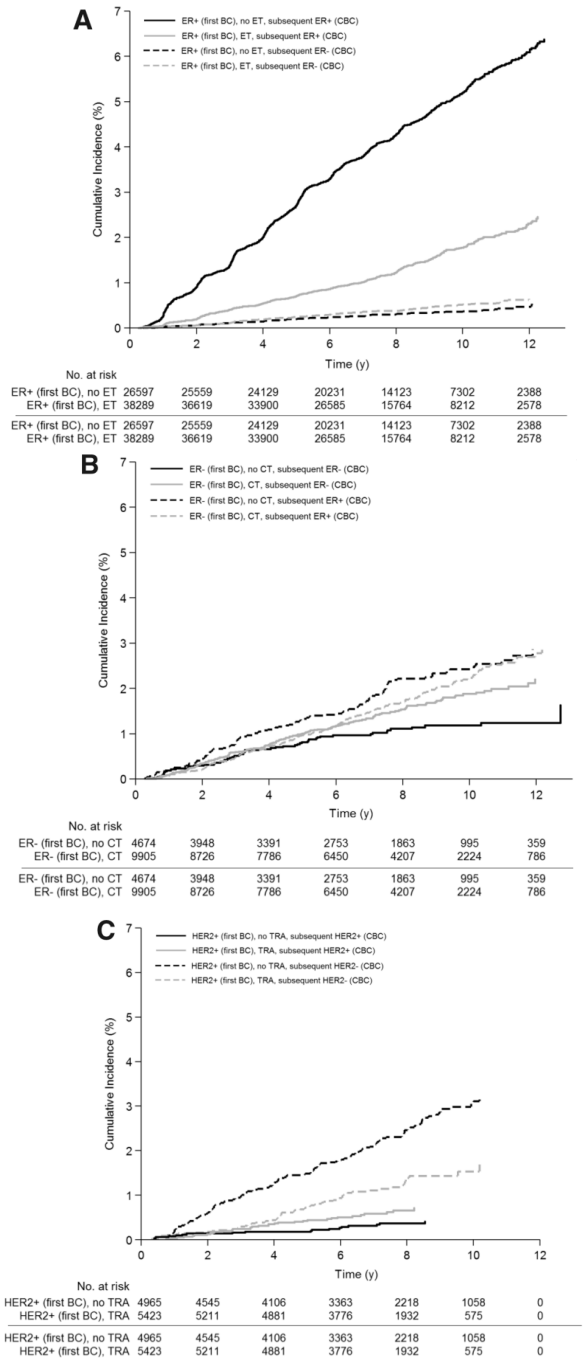


Figure 2. Cumulative incidence of contralateral breast cancer (CBC) by estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2) status

Panel A) Cumulative incidence curves showing the risk of ER-positive or ER-negative CBC after ER-positive first breast cancer (BC), stratified for adjuvant endocrine therapy; **Panel B)** Cumulative incidence curves showing the risk of ER-positive or ER-negative CBC after ER-negative first BC, stratified for adjuvant chemotherapy; **Panel C)** Cumulative incidence curves showing the risk of HER2-positive or HER2-negative CBC after HER2-positive first BC, stratified for adjuvant trastuzumab therapy. For these analyses, patients diagnosed with invasive BC between 2003-2004 were excluded because trastuzumab was not yet widely prescribed in those years. Each panel in the figure consist of two cumulative incidence curves combined (duplicated risk table). In all panels, analyses were performed accounting for death, distant metastases, and CBC-subtype as competing risks (e.g. for the analysis of ER-positive CBC, the following events were treated as competing risks: ER-negative CBC, ER-unknown CBC, distant metastases, and death). Abbreviations: CT = chemotherapy; ET = endocrine therapy

Discussion

In this population-based cohort study comprising 83,144 BC patients, we observed a 10-year cumulative incidence of metachronous CBC of 3.8%. Overall, receipt of adjuvant chemotherapy, endocrine therapy, and/or trastuzumab with chemotherapy was strongly associated with a reduced CBC risk. More detailed analyses showed that endocrine therapy was only associated with a reduced risk of ER-positive CBC and did not protect against the development of ER-negative CBC. Patients receiving chemotherapy for ER-negative first BC had a higher risk of ER-negative CBC after 5 years of follow-up compared with patients who did not receive chemotherapy for ER-negative first BC.

The 10-year cumulative incidence of CBC in our study was relatively low compared with earlier studies[3,4,27], but consistent with other, more contemporary studies[2,5,28,29]. In our study, adjuvant endocrine therapy, chemotherapy, and trastuzumab combined with chemotherapy were associated with overall 54%, 30%, and 43% risk reductions of CBC, respectively. The risk reductions associated with endocrine therapy and chemotherapy in our study are slightly higher than the reductions seen in meta-analyses of the EBCTCG[6,7,26]. Radiotherapy was not associated with an increased CBC risk, which is consistent with other studies with a mean follow-up time less than 15 years[2,29-31].

We observed a strongly reduced CBC risk among patients treated with taxane-containing chemotherapy. The increase in use of taxane-containing chemotherapy coincided with a declining trend in CBC incidence over the years (Supplementary Table 2). Unfortunately, we have no biological explanation for the different effect of taxanes vs anthracyclines. However, our finding is consistent with a randomized adjuvant trial showing an improvement in disease-free survival for docetaxel and cyclophosphamide compared with doxorubicin and cyclophosphamide[32]. The WECARE case-control study[33] found a lesser, statistically nonsignificant CBC risk reduction among patients treated with taxane-containing or with anthracycline-containing chemotherapy of approximately 0.80, but patients were diagnosed in an earlier period (1986-2008) and the study had smaller numbers.

Little is known about the influence of chemotherapy on subtype distribution of CBC. In our study, adjuvant chemotherapy provided for ER-negative first BC was associated with a decreased risk of ER-positive CBC, which might partly be explained by chemotherapy-

induced amenorrhea[34,35]. However, we found an increased risk of ER-negative CBC for patients receiving chemotherapy after 5 years of follow-up. This might possibly be a chance finding. Another possibility is that the ER-negative CBCs that developed after 5 years were chemotherapy-induced tumors. Although effects were not statistically significant because of small numbers, our therapy-specific analyses showed that this increased risk was only seen in the anthracycline-containing chemotherapy group, which is consistent with earlier reports discussing that anthracyclines might increase the risk of development of BC[36,37]. Possibly the risk is only seen for ER-negative CBC because the ER-positive CBCs were prevented due to endocrine therapy irrespectively. The protective effect of taxane-containing chemotherapy seemed attenuated when given in combination with anthracyclines, which might indicate that the increased effect of anthracyclines may be counteracted by taxanes. Thus, anthracycline-containing chemotherapy might induce ER-negative CBC, but further research will be needed to establish the definite role of anthracyclines in second BC development.

We found a larger reduction in CBC risk among patients who received aromatase inhibitors compared to tamoxifen. This finding is consistent with a meta-analysis of randomized trials, which observed that the carryover benefit for CBC was larger for patients randomized to aromatase inhibitors versus tamoxifen[38]. Although endocrine therapy was associated with an overall statistically significantly decreased CBC risk, we and others[33,39,40] showed that it was particularly effective in reducing risk of ER-positive CBC, whereas the risk of ER-negative CBC did not decrease. This is consistent with endocrine therapy selectively inhibiting growth of ER-expressing tumor cells, thus reducing the incidence of ER-positive BCs only[6,41].

It was not possible to investigate the individual effect of trastuzumab on CBC risk, since all patients received trastuzumab combined with chemotherapy. Besides, we were not able to perform multivariable CPH analyses to assess the effect of trastuzumab on HER2-specific CBC because of small numbers of CBCs within this subgroup. Our cumulative incidence curve suggests a slightly higher risk of HER2-positive CBC for patients treated with, compared with patients not treated with trastuzumab. We expected a reduction of HER2-positive CBC after trastuzumab as a consequence of elimination of HER2-overexpressing clones[42].

We observed an increased CBC risk for patients diagnosed with a stage III first BC in the cause-specific CPH model, and there was no association when taking death and distant metastases into account as competing risks. This suggests that part of CBCs were in fact metastases even though these were considered to be by definition a second primary BC. One study, assessing the relationship between first BC and CBC using exome sequencing, has shown that 12% of CBCs represents metastatic spread from the first BC[43]. We attempted to minimize the contribution of metastases to the contralateral breast beforehand by starting follow-up 3 months after first BC diagnosis, only including patients without distant metastasis at initial diagnosis, and censoring for distant metastases during follow-up. Sensitivity analyses showed that additional censoring on local and regional recurrence or a stricter definition of CBC did not alter the results (Supplementary Table 4).

In our study patients younger than 35 years did not have a higher CBC risk compared to older patients, which is in contrast to findings in a previous cohort study using NCR data, including BC patients diagnosed between 1989 and 2002[2]. A potential explanation for these discrepant observations might be the increasing use of adjuvant systemic therapy in the last two decades[2,44]. In our study, 96% of all patients younger than 35 years at first BC diagnosis received adjuvant systemic therapy, whereas this was 59% in the period 1990-2000[45].

This study harbors some limitations. Since this study was observational, patients who received adjuvant systemic therapy differed with respect to some patient and tumor characteristics to patients who did not receive adjuvant therapy (Supplementary Table 1). In the years of diagnosis included, patients with favorable tumor characteristics could avoid systemic therapy following Dutch guidelines[46]. Importantly, in the analyses we adjusted for all these characteristics, but the possibility of some unmeasured residual confounding might still exist. Follow-up on recurrences was not completely recorded by the NCR. This could influence our results, because censoring on distant metastases was not possible for patients outside this period. However, sensitivity analyses showed similar results when including only patients diagnosed between 2003 and 2006 in the analyses (Supplementary Table 4). We lacked data on contralateral prophylactic mastectomy, which could have resulted in an underestimation of the CBC risk. However, our previous cohort study showed that the uptake of contralateral prophylactic mastectomy among BC patients (younger than 50 years) is only approximately 4% in the Netherlands[47]. Therefore, it is unlikely that this missing information affected our main conclusions. Finally, we had no data available on *BRCA1/2* mutation carriership. However, we do not expect that this significantly affected our results, because the proportion of carriers is limited in the general population[47]. We also lacked data on other germline mutations in genes such as *CHEK2* or *PALB2*, or on breast cancer associated single nucleotide polymorphisms. However, because there is no indication that these mutation carriers are treated differently with adjuvant systemic therapy compared to non-carriers[48-50] or that there is interaction with adjuvant systemic therapy[49,51], we do not expect that the absence of these data significantly influenced our results.

The main strengths of this study were the use of a large population-based cohort including all BC patients diagnosed between 2003-2010 in the Netherlands, the comprehensive tumor and therapy information, and active follow-up on CBC occurrence, allowing reliable estimations of CBC risks.

In conclusion, our large population-based study showed a 10-year cumulative CBC incidence of 3.8%. Adjuvant systemic therapy strongly reduced CBC risk in a subtype-dependent manner. According to this study, there is no clear indication to change current

guidelines on adjuvant systemic therapy. Further research disentangling true primary CBCs from metastases may be useful in further personalization of CBC prevention and treatment choices.

Article information

Funding

This work was supported by the Alpe d'HuZes/Dutch Cancer Society (KWF Kankerbestrijding) (2013-6253 ALPE). The sponsor had no role in the design of the study; the collection, analysis, and interpretation of the data; the writing of the manuscript; and the decision to submit the manuscript for publication.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank the registration team of the Netherlands Comprehensive Cancer Organization (IKNL) for the collection of data for the Netherlands Cancer Registry (NCR) as well as IKNL staff for scientific advice. We acknowledge the PALGA foundation for providing us the linkage with the NCR data. We thank all patients whose data we used for this study and the clinicians who treated these patients

References

1 Netherlands Cancer Registry (NCR). Survival and prevalence of cancer (2016). Available from: http://www.cijfersoverkanker.nl/overleving-prevalentie-51.html>

2 Schaapveld, M. *et al.* The impact of adjuvant therapy on contralateral breast cancer risk and the prognostic significance of contralateral breast cancer: a population based study in the Netherlands. *Breast Cancer Res. Treat.* **110**, 189-197, doi:10.1007/s10549-007-9709-2 (2008).

3 Gao, X., Fisher, S. G. & Emami, B. Risk of second primary cancer in the contralateral breast in women treated for early-stage breast cancer: a population-based study. *Int. J. Radiat. Oncol. Biol. Phys.* **56**, 1038-1045 (2003).

4 Healey, E. A. *et al.* Contralateral breast cancer: clinical characteristics and impact on prognosis. *J. Clin. Oncol.* **11**, 1545-1552, doi:10.1200/jco.1993.11.8.1545 (1993).

5 Aalders, K. C. *et al.* Contemporary risks of local and regional recurrence and contralateral breast cancer in patients treated for primary breast cancer. *Eur. J. Cancer* **63**, 118-126, doi:10.1016/j.ejca.2016.05.010 (2016).

6 Davies, C. *et al.* Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet* **378**, 771-784, doi:10.1016/s0140-6736(11)60993-8 (2011).

7 Early Breast Cancer Trialists' Collaborative Group. Polychemotherapy for early breast cancer: an overview of the randomised trials. *Lancet* **352**, 930-942 (1998).

8 Font-Gonzalez, A. *et al.* Inferior survival for young patients with contralateral compared to unilateral breast cancer: a nationwide population-based study in the Netherlands. *Breast Cancer Res. Treat.* **139**, 811-819, doi:10.1007/s10549-013-2588-9 (2013).

9 Hartman, M. *et al.* Incidence and prognosis of synchronous and metachronous bilateral breast cancer. *J. Clin. Oncol.* **25**, 4210-4216, doi:10.1200/jco.2006.10.5056 (2007).

10 Li, C. I., Daling, J. R., Porter, P. L., Tang, M. T. & Malone, K. E. Adjuvant hormonal therapy for breast cancer and risk of hormone receptor-specific subtypes of contralateral breast cancer. *Cancer Res.* **69**, 6865-6870, doi:10.1158/0008-5472.can-09-1355 (2009).

11 Arpino, G., Weiss, H. L., Clark, G. M., Hilsenbeck, S. G. & Osborne, C. K. Hormone receptor status of a contralateral breast cancer is independent of the receptor status of the first primary in patients not receiving adjuvant tamoxifen. *J. Clin. Oncol.* **23**, 4687-4694, doi:10.1200/jco.2005.04.076 (2005).

12 Swain, S. M. *et al.* Estrogen receptor status of primary breast cancer is predictive of estrogen receptor status of contralateral breast cancer. *J. Natl. Cancer Inst.* **96**, 516-523 (2004).

13 Stark, A., Lu, M., Mackowiak, P. & Linden, M. Concordance of the Hormone Receptors and Correlation of HER-2/neu Overexpression of the Metachronous Cancers of Contralateral Breasts. *The breast journal* **11**, 183-187 (2005).

14 Kaas, R. *et al.* The influence of tamoxifen treatment on the oestrogen receptor in metachronous contralateral breast cancer. *Br. J. Cancer* **88**, 707-710, doi:10.1038/sj.bjc.6600746 (2003).

15 Edge, S. B. & Compton, C. C. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann. Surg. Oncol.* **17**, 1471-1474 (2010).

16 de Munck, L. *et al.* Implementation of trastuzumab in conjunction with adjuvant chemotherapy in the treatment of non-metastatic breast cancer in the Netherlands. *Breast Cancer Res. Treat.* **129**, 229-233, doi:10.1007/s10549-011-1451-0 (2011).

17 Casparie, M. *et al.* Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. *Anal. Cell. Pathol.* **29**, 19-24 (2007).

18 Federa. Human Tissue and Medical Research: code of conduct for responsible use 2011. Available from: (https://www.federa.org/sites/default/files/digital_version_first_part_code_of_conduct_in_uk_2011_12092012.pdf).

19 Jann, B. nlcheck: Stata module to check linearity assumption after model estimation, 2008. Available from http://ideas.repec.org/.

20 Hill, K. The demography of menopause. *Maturitas* **23**, 113-127 (1996).

21 Tukey, J. W. One Degree of Freedom for Non-Additivity. *Biometrics* **5**, 232-242, doi:10.2307/3001938 (1949).

22 Pregibon, D. Goodness of Link Tests for Generalized Linear Models. *Journal of the Royal Statistical Society. Series C (Applied Statistics)* **29**, 15-14, doi:10.2307/2346405 (1980).

23 Schoenfeld, D. A. Sample-size formula for the proportional-hazards regression model. *Biometrics* **39**, 499-503 (1983).

24 Xue, X. *et al.* A comparison of the polytomous logistic regression and joint cox proportional hazards models for evaluating multiple disease subtypes in prospective cohort studies. *Cancer Epidemiol. Biomarkers Prev.* **22**, 275-285, doi:10.1158/1055-9965.epi-12-1050 (2013).

25 Kleinbaum, D. G. & Klein, M. *Survival analysis*. Vol. 3 (Springer, 2010).

26 Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomised trials. *The Lancet* **351**, 1451-1467 (1998).

27 Broet, P. *et al.* Contralateral breast cancer: annual incidence and risk parameters. *J. Clin. Oncol.* **13**, 1578-1583, doi:10.1200/jco.1995.13.7.1578 (1995).

28 Bertelsen, L. *et al.* Effect of systemic adjuvant treatment on risk for contralateral breast cancer in the Women's Environment, Cancer and Radiation Epidemiology Study. *J. Natl. Cancer Inst.* **100**, 32-40, doi:10.1093/jnci/djm267 (2008).

29 Hooning, M. J. *et al.* Roles of radiotherapy and chemotherapy in the development of contralateral breast cancer. *J. Clin. Oncol.* **26**, 5561-5568, doi:10.1200/jco.2007.16.0192 (2008).

30 Drooger, J. *et al.* Adjuvant radiotherapy for primary breast cancer in BRCA1 and BRCA2 mutation carriers and risk of contralateral breast cancer with special attention to patients irradiated at younger age. *Breast Cancer Res. Treat.* **154**, 171-180, doi:10.1007/s10549-015-3597-7 (2015).

31 Pierce, L. J. *et al.* Local therapy in BRCA1 and BRCA2 mutation carriers with operable breast cancer: comparison of breast conservation and mastectomy. *Breast Cancer Res. Treat.* **121**, 389-398, doi:10.1007/s10549-010-0894-z (2010).

32 Jones, S. E. *et al.* Phase III trial comparing doxorubicin plus cyclophosphamide with docetaxel plus cyclophosphamide as adjuvant therapy for operable breast cancer. *J. Clin. Oncol.* **24**, 5381-5387, doi:10.1200/jco.2006.06.5391 (2006).

33 Langballe, R. *et al.* Systemic therapy for breast cancer and risk of subsequent contralateral breast cancer in the WECARE Study. *Breast Cancer Res.* **18**, 65, doi:10.1186/s13058-016-0726-0 (2016).

34 Valentini, A. *et al.* Chemotherapy-induced amenorrhea in patients with breast cancer with a BRCA1 or BRCA2 mutation. *J. Clin. Oncol.* **31**, 3914-3919, doi:10.1200/jco.2012.47.7893 (2013).

35 Zavos, A. & Valachis, A. Risk of chemotherapy-induced amenorrhea in patients with breast cancer: a systematic review and meta-analysis. *Acta Oncol.* **55**, 664-670, doi:10.3109/0284186x.2016.1155738 (2016).

36 Henderson, T. O. *et al.* Breast Cancer Risk in Childhood Cancer Survivors Without a History of Chest Radiotherapy: A Report From the Childhood Cancer Survivor Study. *J. Clin. Oncol.* **34**, 910-918, doi:10.1200/jco.2015.62.3314 (2016).

37 Leeuwen, F. E. v. & Ronckers, C. M. Anthracyclines and Alkylating Agents: New Risk Factors for Breast Cancer in Childhood Cancer Survivors? *J. Clin. Oncol.* **34**, 891-894, doi:10.1200/jco.2015.65.0465 (2016).

38 Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. *Lancet* **386**, 1341-1352, doi:10.1016/s0140-6736(15)61074-1 (2015).

39 Bouchardy, C. *et al.* Risk of second breast cancer according to estrogen receptor status and family history. *Breast Cancer Res. Treat.* **127**, 233-241, doi:10.1007/s10549-010-1137-z (2011).

40 Gierach, G. L., Curtis, R. E., Pfeiffer, R. M. & et al. Association of adjuvant tamoxifen and aromatase inhibitor therapy with contralateral breast cancer risk among us women with breast cancer in a general community setting. *JAMA Oncology* **3**, 186-193, doi:10.1001/jamaoncol.2016.3340 (2017).

41 Manna, S. & Holz, M. K. Tamoxifen Action in ER-Negative Breast Cancer. *Sign Transduct Insights* **5**, 1-7, doi:10.4137/sti.s29901 (2016).

42 Guarneri, V. *et al.* Loss of HER2 positivity and prognosis after neoadjuvant therapy in HER2-positive breast cancer patients. *Ann. Oncol.* **24**, 2990-2994, doi:10.1093/annonc/mdt364 (2013).

43 Klevebring, D. *et al.* Exome sequencing of contralateral breast cancer identifies metastatic disease. *Breast Cancer Res. Treat.* **151**, 319-324, doi:10.1007/s10549-015-3403-6 (2015).

44 Kollias, J., Ellis, I. O., Elston, C. W. & Blamey, R. W. Prognostic significance of synchronous and metachronous bilateral breast cancer. *World J. Surg.* **25**, 1117-1124 (2001).

45 Engelhardt, E. G. *et al.* Accuracy of the online prognostication tools PREDICT and Adjuvant! for early-stage breast cancer patients younger than 50 years. *Eur. J. Cancer* **78**, 37-44, doi:10.1016/j.ejca.2017.03.015 (2017).

46 Oncoline, cancer clinical practice guidelines, versie 1.0. Endocriene therapie. Available from: https://www.oncoline.nl/borstkanker.

47 van den Broek, A. J. *et al.* Impact of Age at Primary Breast Cancer on Contralateral Breast Cancer Risk in BRCA1/2 Mutation Carriers. *J. Clin. Oncol.* **34**, 409-418, doi:10.1200/jco.2015.62.3942 (2016).

48 Schmidt, M. K. *et al.* Breast cancer survival and tumor characteristics in premenopausal women carrying the CHEK2*1100delC germline mutation. *J. Clin. Oncol.* **25**, 64-69, doi:10.1200/jco.2006.06.3024 (2007).

49 Kriege, M. *et al.* Survival and contralateral breast cancer in CHEK2 1100delC breast cancer patients: impact of adjuvant chemotherapy. *Br. J. Cancer* **111**, 1004-1013, doi:10.1038/bjc.2014.306 (2014).

50 Cybulski, C. *et al.* Clinical outcomes in women with breast cancer and a PALB2 mutation: a prospective cohort analysis. *Lancet Oncol.* **16**, 638-644, doi:10.1016/s1470-2045(15)70142-7 (2015).

51 Teraoka, S. N. *et al.* Single nucleotide polymorphisms associated with risk for contralateral breast cancer in the Women's Environment, Cancer, and Radiation Epidemiology (WECARE) Study. *Breast Cancer Res.* **13**, R114, doi:10.1186/bcr3057 (2011).

Supplementary Tables

Supplementary Table 1. Distribution of adjuvant chemotherapy, endocrine therapy, and trastuzumab, according to patient and tumor characteristics

Characteristics	Chemotherapy No. (%)		Endocrine therapy No. (%)		Trastuzumab (with chemotherapy)* No. (%)	
	No	Yes	No	Yes	No	Yes
Total	48,717 (58.6)	34,427 (41.4)	42,861 (51.6)	40,283 (48.4)	57,749 (91.2)	5,604 (8.8)
Age at diagnosis, y						
<35	105 (5.8)	1,721 (94.2)	942 (51.6)	884 (48.4)	1,036 (76.8)	313 (23.2)
35-44	2,158 (22.3)	7,535 (77.7)	4,384 (45.2)	5,309 (54.8)	5,972 (82.4)	1,273 (17.6)
45-54	8,387 (37.9)	13,767 (62.1)	10,789 (48.7)	11,365 (51.3)	14,842 (87.7)	2,081 (12.3)
55-64	12,747 (58.5)	9,031 (41.5)	12,066 (55.4)	9,712 (44.6)	15,315 (91.2)	1,473 (8.8)
65-74	14,984 (87.0)	2,238 (13.0)	9,986 (58.0)	7,236 (42.0)	12,894 (96.9)	419 (3.2)
75-84	8,115 (98.5)	127 (1.5)	3,732 (45.3)	4,510 (54.7)	6,044 (99.3)	40 (0.7)
≥85	2,221 (99.6)	8 (0.4)	962 (43.2)	1,267 (56.8)	1,646 (99.7)	5 (0.3)
Tumor stage						
I-II	45,895 (63.9)	25,939 (36.1)	39,533 (55.0)	32,301 (45.0)	50,839 (92.4)	4,163 (7.6)
III	2,822 (25.0)	8,488 (75.0)	3,328 (29.4)	7,982 (70.6)	6,910 (82.7)	1,441 (17.3)
Histological grade						
Grade 1	14,647 (84.2)	2,746 (15.8)	12,261 (70.5)	5,132 (29.5)	13,529 (99.1)	118 (0.9)
Grade 2	22,415 (65.6)	11,738 (34.4)	15,093 (44.2)	19,060 (55.8)	24,734 (94.6)	1,411 (5.4)
Grade 3†	8,554 (34.7)	16,078 (65.3)	12,117 (49.2)	12,515 (50.8)	15,160 (82.6)	3,199 (17.4)
Unknown	3,101 (44.5)	3,865 (55.5)	3,390 (48.7)	3,576 (51.3)	4,326 (83.2)	876 (16.8)
Morphology						
Ductal	36,644 (57.2)	27,400 (42.8)	33,739 (52.7)	30,305 (47.3)	44,168 (90.0)	4,932 (10.0)
Lobular	5,902 (63.9)	3,331 (36.1)	3,468 (37.6)	5,765 (62.4)	6,893 (98.0)	143 (2.0)
Mixed ductal/lobular	1,776 (58.9)	1,237 (41.1)	1,186 (39.4)	1,827 (60.4)	2,127 (95.1)	110 (4.9)
Other	4,395 (64.1)	2,459 (35.9)	4,468 (65.2)	2,386 (34.8)	4,561 (91.6)	419 (8.4)
ER status						
Positive	41,463 (63.9)	23,423 (36.1)	26,597 (41.0)	38,289 (59.0)	48,110 (93.7)	3,221 (6.3)
Negative	4,674 (32.1)	9,905 (67.9)	13,724 (94.1)	855 (5.9)	8,848 (79.2)	2,323 (20.8)
Unknown	2,580 (70.1)	1,099 (29.9)	2,540 (69.0)	1,139 (31.0)	791 (92.9)	60 (7.1)
HER2 status						
Positive	4,637 (41.9)	6,424 (58.1)	6,274 (56.7)	4,787 (43.2)	4,965 (47.8)	5,423 (52.2)
Negative	31,333 (59.2)	21,623 (40.8)	25,420 (48.0)	27,536 (52.0)	50,556 (99.8)	96 (0.2)
Unknown	12,747 (66.6)	6,380 (3z3.4)	11,167 (58.4)	7,960 (41.6)	2,228 (96.3)	85 (3.7)
Subtype‡						
HR+/HER2−	28,973 (63.8)	16,468 (36.2)	18,125 (39.9)	27,316 (60.1)	43,508 (99.8)	66 (0.2)
HR+/HER2+	3,186 (45.8)	3,771 (54.2)	2,282 (32.8)	4,675 (67.2)	3,353 (51.1)	3,214 (48.9)
HR−/HER2+	1,041 (28.8)	2,577 (71.2)	3,538 (97.8)	80 (2.2)	1,191 (35.5)	2,163 (64.5)
HR−/HER2−	2,255 (30.9)	5,049 (69.1)	7,126 (97.6)	178 (2.4)	6,869 (99.6)	28 (0.4)
Unknown	13,262 (66.9)	6,562 (33.1)	11,790 (59.5)	8,034 (40.5)	2,828 (95.5)	133 (4.5)

Abbreviations: ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2; HR+ = hormone receptor positive; HR− = hormone receptor negative

***** Patients diagnosed between 2003-2004 were excluded, since recommendation for HER2 testing and the use of trastuzumab was implemented from 2005 onwards

† Including 12 first breast cancers that were defined as 'undifferentiated' in the Netherlands Cancer Registry

‡ HR+ = ER+ and/or PR+; HR− = ER− and PR−

Supplementary Table 2. Cumulative incidences of CBC for all patients and for patient subgroups

Characteristics	No.	Cumulative Incidence CBC*			
		5-year		10-year	
		%	95% CI	%	95% CI
All patients	83,144	1.9	1.8- 2.0	3.8	3.7- 4.0
Year of first BC diagnosis					
2003	9,853	2.2	1.9- 2.5	4.4	4.0 – 4.8
2004	9,938	2.0	1.7 – 2.3	3.8	3.4 – 4.1
2005	9,945	1.9	1.6 – 2.1	3.8	3.4 – 4.2
2006	10,294	2.0	1.8- 2.3	†	
2007	10,643	1.9	1.6- 2.1	†	
2008	10,706	1.9	1.6- 2.2	†	
2009	10,836	1.6	1.4- 1.9	†	
2010	10,929	1.5	1.3- 1.7	†	
Age, y					
<35	1,826	1.9	1.4- 2.6	3.9	2.9- 5.1
35-44	9,693	1.6	1.4- 1.9	3.6	3.2- 4.0
45-54	22,154	1.6	1.5- 1.8	3.7	3.4- 4.0
55-64	21,778	2.0	1.8- 2.2	4.4	4.0- 4.7
65-74	17,222	2.2	2.0- 2.4	4.1	3.8- 4.5
75-84	8,242	2.0	1.7- 2.3	3.1	2.7- 3.5
≥85	2,229	0.9	0.6- 1.4	1.4	0.9- 2.1
Stage					
I-II	71,834	1.9	1.8- 2.0	4.0	3.8- 4.1
III	11,310	1.6	1.4- 1.8	3.0	2.6- 3.3
Histological grade					
Grade 1	17,393	2.3	2.1- 2.6	4.8	4.4- 5.1
Grade 2	34,153	1.9	1.8- 2.1	4.0	3.7- 4.2
Grade 3‡	24,632	1.5	1.3- 1.6	3.0	2.8- 3.3
Morphology					
Ductal	64,044	1.8	1.7- 1.9	3.7	3.5- 3.9
Lobular	9,233	2.0	1.8- 2.3	4.1	3.7- 4.6
Mixed ductal/lobular	3,013	2.5	2.0- 3.1	5.1	4.2- 6.1
Other	6,854	2.2	1.8- 2.5	3.8	3.4- 4.4
ER status					
Positive	64,886	1.8	1.7- 1.9	3.8	3.6- 4.0
Negative	14,579	2.2	2.0- 2.4	4.2	3.8- 4.6
HER2 status§					
Positive	10,388	1.5	1.3- 1.7	3.1	2.7- 3.6
Negative	50,652	1.9	1.8- 2.0	3.9	3.7- 4.1
Subtype‖					
HR+/HER2–	45,441	2.0	1.8- 2.1	4.1	3.9- 4.4
HR+/HER2+	6,957	1.5	1.2- 1.8	2.9	2.4- 3.4
HR–/HER2+	3,618	2.0	1.6- 2.5	4.3	3.5- 5.2
HR–/HER2–	7,304	2.5	2.2- 2.9	4.8	4.2- 5.5
(Neo)adjuvant therapy¶					
No (neo)adjuvant therapy	31,290	2.9	2.7- 3.1	5.5	5.3- 5.8
CT	8,889	1.9	1.7- 2.2	4.0	3.6- 4.5
ET	17,359	1.3	1.1- 1.4	2.4	2.2- 2.7
CT + ET	19,923	0.9	0.8- 1.0	2.4	2.2- 2.7
CT + TRA	2,728	1.6	1.2- 2.1	3.5	2.7- 4.4
CT + ET + TRA	2,955	0.7	0.5- 1.1	1.8	1.2- 2.7
Radiotherapy					
No radiotherapy	27,265	1.9	1.7- 2.0	3.6	3.3- 3.8
radiotherapy	55,879	1.9	1.7- 2.0	3.9	3.7- 4.1

Abbreviations: BC = breast cancer; CI = confidence interval; CT = chemotherapy; ER = estrogen receptor; ET = endocrine therapy; HER2 = human epidermal growth factor receptor 2; HR = hormone receptor; TRA = trastuzumab

* Accounting for death and distant metastases as competing risk

† Not sufficient follow-up time to report the 10-year cumulative incidence

‡ Including 12 first breast cancers that were defined as 'undifferentiated' in the Netherlands Cancer Registry

§ Patients diagnosed between 2003-2004 were excluded, since recommendation for HER2 testing and the use of trastuzumab was implemented from 2005 onwards

‖ HR+ = ER+ and/or PR+; HR– = ER– and PR–

¶ No chemotherapy, endocrine therapy, and trastuzumab (with or without radiotherapy)

Supplementary Table 3. Multivariable Cox regression analyses (cause-specific hazard ratios and subdistribution hazard ratios), in all patients and those with complete co-variate information, of CBC risk related to adjuvant therapy, stage, age, and receptor status at first BC diagnosis*

Characteristics	Cause-specific hazard ratio (HR)						Subdistribution hazard ratio (SHR)†					
	All patients N = 83,144			Complete case analysis N = 63,251			All patients N = 83,144			Complete case analysis N = 63,251		
	HR	95% CI	P‡	HR	95% CI	P‡	SHR	95% CI	P‡	SHR	95% CI	P‡
(Neo)adjuvant therapy												
No (neo)adjuvant therapy§	1.00	Ref.		1.00	Ref.		1.00	Ref.		1.00	Ref.	
CT	0.65	0.56- 0.77	<.001	0.68	0.56- 0.82	<.001	0.63	0.55- 0.73	<.001	0.66	0.56- 0.78	<.001
ET	0.45	0.40- 0.51	<.001	0.46	0.40- 0.52	<.001	0.45	0.40- 0.50	<.001	0.45	0.39- 0.52	<.001
CT + ET	0.33	0.29- 0.37	<.001	0.27	0.23- 0.31	<.001	0.34	0.30- 0.38	<.001	0.28	0.24- 0.32	<.001
CT + TRA	0.62	0.47- 0.82	.001	0.64	0.48- 0.86	.003	0.68	0.52- 0.89	.005	0.72	0.54- 0.95	.02
CT + ET + TRA	0.27	0.19- 0.39	<.001	0.27	0.19- 0.39	<.001	0.29	0.21- 0.42	<.001	0.29	0.20- 0.42	<.001
Radiotherapy												
No radiotherapy	1.00	Ref.		1.00	Ref.		1.00	Ref.		1.00	Ref.	
Radiotherapy	0.94	0.86- 1.02	.12	0.96	0.87- 1.06	.41	0.98	0.90 – 1.07	.64	1.00	0.90- 1.10	.95
Stage												
I-II	1.00	Ref.		1.00	Ref.		1.00	Ref.		1.00	Ref.	
III	1.48	1.30- 1.69	<.001	1.47	1.27- 1.72	<.001	1.10	0.97- 1.26	.14	1.10	0.95- 1.28	.21
Age												
<35	1.08	0.82- 1.42	.55	1.17	0.86- 1.59	.32	1.10	0.84- 1.44	.48	1.21	0.90- 1.63	.22
35-44	0.98	0.85- 1.12	.72	1.03	0.88- 1.21	.72	0.99	0.87- 1.13	.89	1.04	0.89- 1.22	.63
45-54	0.88	0.80- 0.98	.02	0.85	0.75- 0.96	.007	0.90	0.82 – 1.00	.05	0.86	0.76 – 0.97	.02
55-64	1.00	Ref.		1.00	Ref.		1.00	Ref.		1.00	Ref.	
65-74	0.93	0.84- 1.03	.18	1.00	0.88- 1.13	.98	0.89	0.80 – 0.99	.03	0.96	0.85- 1.09	.54
75-84	0.89	0.76- 1.04	.14	0.93	0.77- 1.11	.40	0.69	0.59 – 0.80	<.001	0.75	0.63 – 0.90	.002
≥85	0.55	0.37- 0.81	.002	0.50	0.32- 0.80	.004	0.31	0.21- 0.45	<.001	0.31	0.20- 0.50	<.001
ER status												
Negative	1.00	Ref.		1.00	Ref.		1.00	Ref.		1.00	Ref.	
Positive	0.96	0.85- 1.09	.55	0.93	0.80- 1.08	.34	1.08	0.96- 1.21	.21	1.04	0.63- 1.19	.52
Unknown	0.58	0.46- 0.73	<.001	-	-	-	0.63	0.50- 0.80	<.001	-	-	-
HER2 status												
Negative	1.00	Ref.		1.00	Ref.		1.00	Ref.		1.00	Ref.	
Positive	0.86	0.74- 1.00	.05	0.82	0.70- 0.96	.02	0.84	0.72 – 0.97	.02	0.79	0.68 – 0.92	.003
Unknown	0.36	0.31- 0.41	<.001	-	-	-	0.38	0.32- 0.45	<.001	-	-	-

Abbreviations: BC = breast cancer; CBC = contralateral breast cancer; CI = confidence interval; CT = chemotherapy; ER = estrogen receptor; ET = endocrine therapy; HER2 = human epidermal growth factor receptor 2; Ref = reference group; TRA = trastuzumab

* Additionally adjusted for year of first breast cancer diagnosis

†Accounting for death and distant metastases as competing risk

‡ Two-sided Wald test P-value

§ No chemotherapy, endocrine therapy or trastuzumab (with or without radiotherapy)

Supplementary Table 4. Sensitivity analyses of CBC risk related to (neo)adjuvant systemic therapy for the first BC (as described in Table 2) based on: selection of years of diagnosis of the first BC, differences of censoring events, and definition of metachronous CBC*

(Neo)adjuvant systemic therapy	Total follow-up				<5 years follow up			≥5 years follow up			$P_{heterogeneity}$‡
	No.	HR	95% CI	P†	HR	95% CI	P†	HR	95% CI	P†	
First BC diagnosed 2003-2010											
No censoring											
No (neo)adjuvant therapy§	31,290	1.00	Ref.		1.00	Ref.		1.00	Ref.		
CT	8,889	0.70	0.62- 0.80	<.001	0.66	0.56- 0.78	<.001	0.79	0.65- 0.95	.02	.17
ET	17,359	0.46	0.41- 0.52	<.001	0.42	0.36- 0.48	<.001	0.51	0.43- 0.61	<.001	.08
CT + ET	19,923	0.35	0.31- 0.40	<.001	0.27	0.23- 0.32	<.001	0.50	0.42- 0.59	<.001	<.001
CT + TRA	2,728	0.56	0.45- 0.73	<.001	0.46	0.35- 0.63	<.001	0.79	0.55- 1.12	.19	.02
CT + ET + TRA	2,955	0.24	0.17- 0.33	<.001	0.20	0.13- 0.31	<.001	0.31	0.19- 0.51	<.001	.19
Censoring: distant metastases											
No (neo)adjuvant therapy§	31,290	1.00	Ref.		1.00	Ref.		1.00	Ref.		
CT	8,889	0.70	0.62- 0.80	<.001	0.66	0.56- 0.78	<.001	0.79	0.65- 0.95	.01	.16
ET	17,359	0.46	0.41- 0.52	<.001	0.42	0.36- 0.49	<.001	0.50	0.42- 0.60	<.001	.13
CT + ET	19,923	0.35	0.31- 0.39	<.001	0.26	0.22- 0.30	<.001	0.50	0.42- 0.59	<.001	<.001
CT + TRA	2,728	0.57	0.45- 0.73	<.001	0.47	0.35- 0.64	<.001	0.77	0.54- 1.10	.16	.04
CT + ET + TRA	2,955	0.24	0.17- 0.33	<.001	0.20	0.13- 0.31	<.001	0.31	0.19- 0.51	<.001	.19
Censoring: distant metastases, local and regional recurrence											
No (neo)adjuvant therapy§	31,290	1.00	Ref.		1.00	Ref.		1.00	Ref.		
CT	8,889	0.70	0.62- 0.80	<.001	0.57	0.48- 0.68	<.001	0.79	0.65- 0.96	.02	.12
ET	17,359	0.47	0.42- 0.53	<.001	0.47	0.40- 0.54	<.001	0.52	0.43- 0.62	<.001	.38
CT + ET	19,923	0.35	0.31- 0.40	<.001	0.22	0.19- 0.27	<.001	0.50	0.42- 0.60	<.001	<.001
CT + TRA	2,728	0.52	0.41- 0.67	<.001	0.37	0.26- 0.51	<.001	0.74	0.52- 1.07	.11	.01
CT + ET + TRA	2,955	0.23	0.17- 0.33	<.001	0.16	0.10- 0.26	<.001	0.32	0.20- 0.52	<.001	.05
Censoring: distant metastases, local and regional recurrence. Only stage I-III CBC included, and follow-up <1 year excluded‖											
No (neo)adjuvant therapy§	30,045	1.00	Ref.		1.00	Ref.		1.00	Ref.		
CT	8,742	0.75	0.65- 0.86	<.001	0.61	0.51- 0.73	<.001	0.81	0.67- 0.98	.03	.03
ET	17,280	0.48	0.43- 0.55	<.001	0.49	0.41- 0.57	<.001	0.52	0.43- 0.62	<.001	.61
CT + ET	19,884	0.36	0.32- 0.41	<.001	0.22	0.18- 0.27	<.001	0.49	0.41- 0.59	<.001	<.001
CT + TRA	2,715	0.55	0.42- 0.71	<.001	0.36	0.25- 0.52	<.001	0.76	0.53- 1.10	.15	.005
CT + ET + TRA	2,948	0.20	0.14- 0.29	<.001	0.13	0.08- 0.23	<.001	0.27	0.16- 0.46	<.001	.07
First BC diagnosed 2003-2006											
No censoring											
No (neo)adjuvant therapy§	16.810	1.00	Ref.		1.00	Ref.		1.00	Ref.		
CT	4.633	0.79	0.68- 0.93	.003	0.77	0.62- 0.96	.02	0.83	0.67- 1.03	.10	.61
ET	7.862	0.46	0.39- 0.53	<.001	0.39	0.31- 0.49	<.001	0.51	0.41- 0.64	<.001	.08
CT + ET	9.074	0.42	0.36- 0.49	<.001	0.29	0.23- 0.37	<.001	0.56	0.46- 0.68	<.001	<.001
CT + TRA	750	0.75	0.53- 1.06	.11	0.71	0.44- 1.15	.16	0.80	0.48- 1.34	.40	.73
CT + ET + TRA	901	0.32	0.20- 0.50	<.001	0.28	0.14- 0.54	<.001	0.37	0.20- 0.68	.001	.52
Censoring: distant metastases											
No (neo)adjuvant therapy§	16.810	1.00	Ref.		1.00	Ref.		1.00	Ref.		
CT	4.633	0.79	0.68- 0.93	.004	0.76	0.61- 0.95	.02	0.83	0.56- 0.78	.09	.61
ET	7.862	0.45	0.39- 0.53	<.001	0.39	0.31- 0.49	<.001	0.50	0.36- 0.49	<.001	.12
CT + ET	9.074	0.41	0.35- 0.48	<.001	0.28	0.22- 0.36	<.001	0.55	0.22- 0.30	<.001	<.001
CT + TRA	750	0.78	0.55- 1.11	.17	0.75	0.47- 1.22	.25	0.81	0.35- 0.64	.43	.83
CT + ET + TRA	901	0.33	0.21- 0.51	<.001	0.28	0.15- 0.55	<.001	0.38	0.13- 0.31	.002	.53
Censoring: distant metastases, local and regional recurrence											
No (neo)adjuvant therapy§	16.810	1.00	Ref.		1.00	Ref.		1.00	Ref.		
CT	4.633	0.79	0.67- 0.92	.004	0.61	0.48- 0.77	<.001	0.85	0.68- 1.05	.14	.45
ET	7.862	0.48	0.41- 0.56	<.001	0.47	0.37- 0.59	<.001	0.54	0.43- 0.67	<.001	.40
CT + ET	9.074	0.43	0.37- 0.50	<.001	0.23	0.18- 0.30	<.001	0.56	0.46- 0.69	<.001	<.001
CT + TRA	750	0.69	0.48- 1.00	.05	0.53	0.32- 0.90	.02	0.76	0.45- 1.27	.29	.35
CT + ET + TRA	901	0.31	0.19- 0.50	<.001	0.19	0.09- 0.40	<.001	0.39	0.21- 0.71	.002	.15
Censoring: distant metastases, local and regional recurrence. Only stage I-III CBC included, and follow-up <1 year excluded‖											
No (neo)adjuvant therapy§	16.647	1.00	Ref.		1.00	Ref.		1.00	Ref.		
CT	4.514	0.83	0.70- 0.98	.03	0.65	0.51- 0.83	.001	0.87	0.70- 1.09	.22	.08
ET	7.818	0.50	0.42- 0.58	<.001	0.49	0.38- 0.62	<.001	0.54	0.44- 0.68	<.001	.50
CT + ET	9.054	0.43	0.37- 0.51	<.001	0.23	0.18- 0.30	<.001	0.56	0.46- 0.69	<.001	<.001
CT + TRA	743	0.66	0.44- 0.98	.04	0.45	0.25- 0.83	.01	0.78	0.46- 1.31	.35	.18
CT + ET + TRA	897	0.22	0.13- 0.40	<.001	0.12	0.05- 0.33	<.001	0.29	0.14- 0.59	.001	.17

Abbreviations: CBC = contralateral breast cancer; CI = confidence interval; CT = chemotherapy; ET = endocrine therapy; HR = hazard ratio; Ref. = reference group; TRA = trastuzumab

* Adjusted for age and stage at first breast cancer diagnosis

† Two-sided Wald test P-value

‡ Heterogeneity of HRs between <5 and ≥5 years follow-up duration

§ No chemotherapy, endocrine therapy and trastuzumab (with or without radiotherapy)

‖ CBC only included tumor stage I-III (excluding those with metastases present <3 months after CBC diagnosis). Follow-up started 1 year after first BC diagnosis (regarding CBC developed <1 year after the first BC as synchronous BC), and ended at the date of: CBC, distant metastasis, local recurrence, regional recurrence, and death (whichever came first)

Supplementary Table 5. ER status of first BC and CBC, synchronous or metachronous, stratified for endocrine therapy

ER status of first BC and endocrine therapy	No. of patients	ER-positive CBC No. of patients (%)	ER-negative CBC No. of patients (%)
Synchronous CBC (<3 months after first breast cancer)			
ER-positive first BC	1,071	994 (92.8)	77 (7.2)
ER-negative first BC	132	80 (60.6)	52 (39.4)
Metachronous CBC (≥3 months after first breast cancer)			
ER-positive first BC			
No endocrine therapy	1,368	1,273 (93.1)	95 (6.9)
Endocrine therapy	737	566 (76.8)	171 (23.2)
ER-negative first BC			
No endocrine therapy	494	278 (56.3)	216 (43.7)
Endocrine therapy	27	19 (70.4)	8 (26.6)

Abbreviations: BC = breast cancer; CBC = contralateral breast cancer; ER = estrogen receptor

Supplementary Table 6. Multivariable Cox regression analysis for ER-negative first BC patients assessing the association between various (neo)adjuvant chemotherapy regimens and ER-negative CBC risk (n = 217)*

(Neo)adjuvant CT	Total follow-up				≤5 years follow-up				>5 years follow-up			
	No. of patients	No. of CBC	HR (95% CI)	P†	No. of patients	No. of CBC	HR 95% CI	P†	No. of patients	No. of CBC	HR 95% CI	P†
No CT	4,674	54	1.00 (Ref)		1,481	38	1.00 (Ref)		3,193	16	1.00 (Ref)	
Taxane-containing CT‡	1,182	9	0.36 (0.17-0.75)	.007	297	5	0.26 (0.09-0.72)	.009	885	4	0.61 (0.19-1.98)	.41
Anthracycline-containing CT§	1,718	56	1.32 (0.86-2.04)	.21	466	31	1.17 (0.66-2.06)	.59	1,252	25	1.88 (0.91-3.86)	.09
Taxane + anthracycline-containing CT	1,149	11	0.59 (0.28-1.22)	.16	248	9	0.67 (0.29-1.55)	.35	901	2	0.48 (0.10-2.41)	.38
CT, other or type unknown ‖	5,856	87	0.68(0.46-1.00)	.05	1,358	54	0.65 (0.40-1.06)	.08	4,498	33	0.83 (0.43-1.59)	.57

Abbreviations: BC = breast cancer; CBC = contralateral breast cancer; CI = confidence interval; CT = chemotherapy; HR = hazard ratio; Ref. = reference group

* Adjusted for year of diagnosis, endocrine therapy, trastuzumab, age and stage at first breast cancer diagnosis

† Two-sided Wald test P-value

‡ The chemotherapeutic combination contains taxanes, but no anthracyclines

§ The chemotherapeutic combination contains anthracyclines, but no taxanes

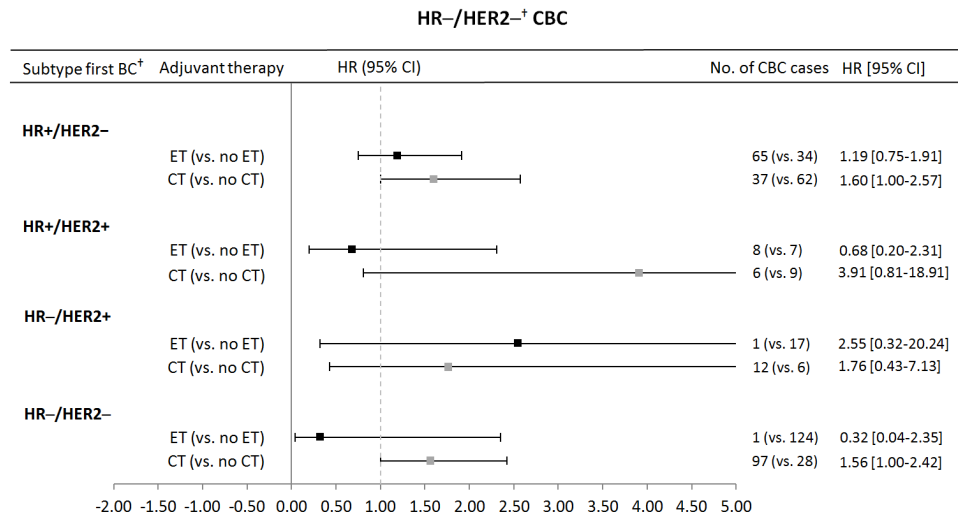
‖ All other chemotherapeutic drugs and combinations (e.g. CMF) or type unknown

Supplementary Table 7. HER2 status of first BC and CBC, synchronous or metachronous, stratified for trastuzumab therapy*

HER2 status of first BC and trastuzumab	No. of patients	HER2-positive CBC	HER-negative CBC
		No. of patients (%)	No. of patients (%)
Synchronous CBC (<3 months after first BC)			
HER2-positive first BC	117	36 (30.8)	81 (69.2)
HER2-negative first BC	875	70 (8.0)	805 (92.0)
Metachronous CBC (≥3 months after first BC)			
HER2-positive first BC			
No trastuzumab	150	18 (12.0)	132 (88.0)
Trastuzumab	101	34 (33.7)	67 (66.3)
HER2-negative first BC			
No trastuzumab	1,490	149 (10.0)	1,341 (90.0)

Abbreviations: BC = breast cancer; CBC = contralateral breast cancer; HER2 = human epidermal growth factor receptor 2

* Patients diagnosed between 2003-2004 were excluded, since recommendation for HER2 testing and the use of trastuzumab was implemented from 2005 onwards



Supplementary Figure 1. Joint multivariable Cox regression analyses for each of the first tumor subtypes assessing the association of (neo)adjuvant systemic therapy of the first BC with triple negative (HR–/HER2–) CBC risk

Adjusted for trastuzumab therapy, age, and stage at first breast cancer diagnosis. Abbreviations: BC = breast cancer; CBC = contralateral breast cancer; CI = confidence interval; CT = chemotherapy; ET = endocrine therapy; HR = hazard ratio; PR = progesterone receptor. † HR+ = ER+ and/or PR+; HR– = ER– and PR–

CHAPTER 4

Contralateral breast cancer risk in patients with ductal carcinoma in situ and invasive breast cancer

NPJ Breast Cancer. 2020 Nov 3;6(1):60

Daniele Giardiello*
Iris Kramer*
Maartje J. Hooning
Michael Hauptmann
Esther H. Lips
Elinor Sawyer
Alastair M. Thompson
Linda de Munck
Sabine Siesling
Jelle Wesseling
Ewout W. Steyerberg
Marjanka K. Schmidt

*authors contributed equally

Abstract

We aimed to assess contralateral breast cancer (CBC) risk in patients with ductal carcinoma in situ (DCIS) compared with invasive breast cancer (BC). Women diagnosed with DCIS (N=28,003) or stage I-III BC (N=275,836) between 1989-2017 were identified from the nationwide Netherlands Cancer Registry. Cumulative incidences were estimated, accounting for competing risks, and hazard ratios (HRs) for metachronous invasive CBC. To evaluate effects of adjuvant systemic therapy and screening, separate analyses were performed for stage I BC without adjuvant systemic therapy and by mode of first BC detection. Multivariable models including clinico-pathological and treatment data were created to assess CBC risk prediction performance in DCIS patients. The 10-year cumulative incidence of invasive CBC was 4.8% for DCIS patients (CBC=1,334). Invasive CBC risk was higher in DCIS patients compared with invasive BC overall (HR=1.10, 95% confidence interval (CI)=1.04-1.17), and lower compared with stage I BC without adjuvant systemic therapy (HR=0.87; 95%CI=0.82–0.92). In patients diagnosed ≥2011, the HR for invasive CBC was 1.38 (95%CI=1.35-1.68) after screen-detected DCIS compared with screen-detected invasive BC, and was 2.14 (95%CI=1.46-3.13) when not screen-detected. The C-index was 0.52 (95% CI=0.50-0.54) for invasive CBC prediction in DCIS patients. In conclusion, CBC risks are low overall. DCIS patients had a slightly higher risk of invasive CBC compared with invasive BC, likely explained by the risk-reducing effect of (neo)adjuvant systemic therapy among BC patients. For support of clinical decision making more information is needed to differentiate CBC risks among DCIS patients.

Introduction

Contralateral breast cancer (CBC) is the most frequent second cancer reported after first invasive breast cancer (BC)[1-3]. The cumulative incidence of invasive CBC for women following invasive BC is ~0.4% per year[4-6]. Several studies have shown a decrease in CBC incidence as a result of (neo)adjuvant systemic therapies[6-8].

Ductal carcinoma in situ (DCIS) is a potential precursor of invasive BC. The incidence of DCIS has increased substantially with widespread introduction of population-based mammography screening including digital mammography and represents 10-25% of all BC patients[9-11]. Since DCIS has an excellent prognosis with a disease-specific survival of more than 98% at 10 years[12-14], a large group of women is at risk of developing CBC.

The risk of invasive CBC for DCIS patients has not been widely investigated, but the annual risk is estimated between 0.4-0.6%[11,13,15,16]. Moreover, it is unclear if the risk of CBC is comparable between patients diagnosed with invasive BC and patients with DCIS. One study in the United States (US), using data from the Surveillance, Epidemiology, and End Results (SEER) database, found a similar relative CBC risk for DCIS patients compared to patients with invasive BC[17]. On the other hand, an indirect assessment between DCIS patients and invasive BC patients has been provided by a CBC risk prediction model developed and validated in the US, showing a higher relative CBC risk for DCIS compared with invasive BC (relative risk: 1.60, 95% confidence interval (CI)=1.42–1.93)[18,19]. The reason for a potential higher CBC risk for DCIS patients is still unclear, but might relate to the risk-reducing effect of adjuvant systemic therapy among invasive BC patients[6,20,21]. In general, relatively few DCIS patients receive adjuvant systemic therapy. In addition, CBC risks may also differ based on the mode of detection of the first BC. Previous research showed that screen-detected invasive breast tumours have a better BC-specific survival than non-screened tumours and hence receive less adjuvant systemic treatment[22].

The aim of this study was to assess the risk of developing invasive CBC in DCIS patients in direct comparison with patients diagnosed with invasive BC using a large population-based cohort of Dutch BC patients, taking age, mode of first BC detection, and (neo)adjuvant systemic therapy into account. In addition, we evaluated the CBC risk prediction performance in patients diagnosed with DCIS.

Methods

Study population

We evaluated 323,285 patients diagnosed with in situ or invasive first BC in 1989-2017, who underwent surgery, from the Netherlands Cancer Registry (NCR) (Supplementary Figure 4). The NCR is an on-going nationwide population-based data registry of all newly diagnosed cancer patients in the Netherlands, with full coverage since 1989[23].

We excluded nine patients with first diagnosis without cytological or histological confirmation, 5,785 with stage IV BC or with incomplete staging information, 66 with squamous cell carcinoma, and 4,145 with in situ BC that was not pure DCIS (i.e. lobular, other subtype, or mixed with ductal). Follow-up for all patients started three months after the first diagnosis; therefore, 9,441 patients who had developed synchronous CBC (invasive or in situ), invasive ipsilateral BC, or died within three months after the first diagnosis were excluded.

Patient and tumour characteristics

Clinico-pathological data were provided by the NCR. After notification by the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA) and the national hospital discharge database, registration clerks of the NCR collect data directly from patients' records. Follow-up information on vital status and second cancers was complete up to January 31, 2018.

Staging was coded according to the TNM Classification of Malignant Tumours using the edition valid at the date of diagnosis, ranging from the 4th to the 8th edition[24]. If pathological stage was missing, clinical stage was used[25].

Receptor status was determined by immunohistochemistry (IHC), and was included in the NCR since 2005. Tumours were defined as estrogen receptor (ER) positive or progesterone receptor (PR) positive when >10% of the tumour cells stained positive (from 2011 the threshold was ≥10%). A tumour was defined human epidermal growth factor receptor 2/neu-receptor (HER2) positive if IHC was 3+ (strong and complete membranous expression in >10% of tumour cells) or if IHC score 2+ when additional confirmation with in situ hybridization was available, but considered unknown if in situ hybridization confirmation was missing.

The NCR did not record information on *BRCA1* and *BRCA2* germline mutation status and family history.

From 2011, the NCR recorded the mode of first BC detection, i.e. if the DCIS or invasive BC was screen-detected or not detected by screening. We did not have detailed information available on the tumours not detected by screening, but these may include interval tumours, non-screen attendant, or screened outside the national program (e.g. due to family history). According to the Dutch guidelines, mammographic follow-up is similar for DCIS and invasive BC[26].

Data used in this study were included in the NCR under an opt-out regime according to Dutch legislation and codes of conduct[25]. The NCR Privacy Review Board approved this study under reference number K18.245. Data were handled in accordance with privacy regulations for medical research[25].

Statistical analyses

The primary outcome was the development of metachronous CBC, defined as an invasive BC in the contralateral breast diagnosed at least three months after the first BC diagnosis (DCIS or invasive BC). Follow-up started three months after the first BC diagnosis, and ended at date of in situ- or invasive CBC, invasive ipsilateral BC, or last date of follow-up (due to death, lost to follow-up, or end of study), whichever occurred first.

Cox proportional hazard models were performed to investigate the association of having DCIS compared with invasive BC as primary diagnosis with the cause-specific hazard of invasive CBC. We also performed analyses with in situ CBC, invasive ipsilateral BC, and death as the outcome. According to the Dutch guideline, DCIS patients do not receive adjuvant systemic therapy. We evaluated the impact of adjuvant systemic therapy by comparing the invasive CBC risk between DCIS patients and patients diagnosed with stage I BC not receiving adjuvant systemic therapy (no chemotherapy, endocrine therapy, nor trastuzumab), i.e., a subgroup of patients that resembles as much as possible the DCIS patient group in terms of treatment conditions. Since hazard ratios (HRs) based on Cox regressions do not have a direct relationship with the cumulative incidence of the event of interest, we also performed competing risks regression to estimate the HRs for the subdistribution hazards of the Fine and Gray model[27,28]. In situ CBC, invasive ipsilateral BC, and death were considered as competing risks. We performed both univariable analyses and analyses adjusted for age- and year of first BC diagnosis. Since 1989, women in the Netherlands aged 50-70 have been invited for biannual screening by mammography, which was extended to women aged 75 since 1998. Based on this, we categorized age at first BC diagnosis into <50 years and ≥50 years. Based on the gradual implementation of the Dutch BC screening, we categorized year at first BC diagnosis into two periods: 1989–1998 (implementation phase) and 1999–2017 (full nationwide coverage; attendance rate is 78.8%[29] and detection rate of invasive BC 6.6 per 1000 in 2017[30]and for DCIS 0.94 per 1000 between 2004-2011[31]). We also performed our analyses stratified by mode of first BC detection. These analyses only included patients diagnosed during or after 2011 and aged 50-75 (eligible for screening).

Cumulative incidence curves of invasive CBC for DCIS patients, all invasive BC patients, and patients with stage I BC not receiving adjuvant systemic therapy were calculated considering in situ CBC, invasive ipsilateral BC, and death as competing risks. These curves were stratified by year of first BC diagnosis (1989-1998 and 1999-2017) and by age (<50 and ≥50 years).

We used joint Cox proportional hazard models[32] to investigate subtype-specific CBC risk (according to stage, grade, ER, PR, and HER2 status) in DCIS patients compared with patients with invasive BC and compared with patients with stage I BC who did not receive adjuvant systemic therapy. Each model included subtype-specific CBC (e.g. ER positive CBC, ER negative CBC, ER unknown CBC), in situ CBC, ipsilateral invasive BC, and death as possible outcomes. Since the NCR actively registered receptor status from 2005, these analyses only included patients diagnosed between 2005-2017.

Multivariable Cox regression was used to quantify the effect of clinico-pathological

and treatment characteristics on CBC risk (all CBC and invasive CBC only) in DCIS patients. In addition, multivariable Fine and Gray regressions were performed to assess the association between every factor and the CBC cumulative incidence. Variables included in the models were age at first DCIS diagnosis, tumour grade, type of surgery (mastectomy or breast conserving surgery), and radiotherapy. The proportional hazard assumption of the models was assessed by examining the Schoenfeld residuals, and restricted cubic splines were used to verify whether linearity of age at first DCIS diagnosis would hold[33]. The discrimination ability of the models to identify patients developing CBC was calculated using the C-index[34]. Missing data were multiply imputed by chained equations (MICE) to avoid loss of information due to case-wise deletion causing bias and reduction in efficiency [35,36]. Multiple imputation accounts for missing data mechanisms assuming that the probability of missingness depends on the observed data namely missing at random (MAR). For every predictor with missing data, every imputation model selects predictors based on correlation structure underlying the data. Details about the imputation model are provided in Supplementary Methods.

Analyses were performed using STATA version 16.0, SAS (SAS Institute Inc., Cary, NC, USA) version 9.4, and R software version 3.5.3.[37].

Results

Patient characteristics

The cohort comprised 28,003 DCIS patients (CBC=1,334) and 275,836 patients with invasive BC (CBC=12,821), including 86,481 patients with stage I BC not receiving adjuvant systemic therapy; i.e. no chemotherapy, endocrine therapy, nor trastuzumab (Table 1). The percentage of patients diagnosed with DCIS, of all BC patients diagnosed in the Netherlands, was 5.7% in the implementation phase of the mammography screening program (1989-1998) and 10.5% in the period of full national coverage (1999-2017). Median follow-up was 11.4 years.

CBC risk for patients diagnosed with DCIS and invasive BC

The 10-year cumulative incidence of invasive CBC was 4.8% (95%CI=4.6–5.2%) for DCIS patients, 4.0% (95%CI=4.0–4.1%) for all invasive BC patients, and 5.6% (95%CI=5.4–5.8%) for patients with stage I BC not receiving adjuvant systemic therapy (Table 1, Figure 1[38]). For comparison, the 10-year cumulative incidence of invasive CBC in patients diagnosed with stage I invasive BC treated with adjuvant systemic therapy was 2.9% (95%CI=2.5-3.3%). Being diagnosed with DCIS was associated with an increased risk of invasive CBC compared with invasive BC overall (HR=1.10, 95%CI=1.04–1.17), and with a lower risk when compared with stage I BC without adjuvant systemic therapy (HR=0.87, 95%CI=0.82–0.92, Table 2). Similar results were observed when using competing risk regression (Table 2).

Table 1. Patient-, tumour- and treatment characteristics of women diagnosed with ductal carcinoma in situ or invasive breast cancer

	DCIS		All invasive BC		Stage I BC without adjuvant systemic therapy[a]	
	N	%	N	%	N	%
Characteristics	**28,003**	**9.2**	**275,836**	**90.8**	**86,481**	**31.4**
Diagnosis, year						
median (range)	2009 (1989- 2017)		2004 (1989- 2017)		2004 (1989- 2017)	
Age, years						
median (range)	59 (21- 95)		59 (18- 102)		61 (18- 99)	
TNM stage						
0	28,003	100.0	-	-	-	-
I	-	-	120,952	43.8	86,481	100.0
II	-	-	124,883	45.3	-	-
III	-	-	30,001	10.9	-	-
Tumour grade						
I (well differentiated)	3,729	16.1	44,690	20.9	27,566	41.9
II (moderately differentiated)	7,864	33.8	95,251	44.6	28,159	42.8
III (poorly/undifferentiated)	11,639	50.1	73,581	34.5	10,036	15.3
missing	4,771	-	62,314	-	20,720	-
ER status						
positive	-	-	133,761	82.7	41,883	90.1
negative	-	-	28,075	17.3	4,598	9.9
missing	28,003	-	114,000	-	40,000	-
HER2 status						
positive	-	-	19,708	14.3	2,324	6.1
negative	-	-	118,409	85.7	35,616	93.9
missing	28,003	-	137,719	-	48,541	-
PR status						
positive	-	-	106,786	67.5	33,862	74.8
negative	-	-	51,437	32.5	11,404	25.2
missing	28,003	-	117,613	-	41,215	-
(Neo)adjuvant chemotherapy						
yes	17	0.1	91,844	33.3	-	-
no	27,986	99.9	183,992	66.7	86,481	100.0
(Neo)adjuvant endocrine therapy						
yes	102	0.4	119,394	43.3	-	-
no	27,901	99.6	156,442	56.7	86,481	100.0
(Neo)adjuvant trastuzumab						
yes	3	0.0	13,994	5.1	-	-
no	28,000	100.0	261,842	94.9	86,481	100.0
Surgery to the breast						
breast conserving surgery	16,396	60.8	142,495	53.4	58,727	70.1
mastectomy	10,571	39.2	124,530	46.6	25,023	29.9
missing	1,036	-	881	-	2,731	-
Radiation to the breast						
yes	13,128	46.9	182,226	66.1	59,354	70.1
no	14,875	53.1	93,610	33.9	27,127	31.4
Follow-up, *years*						
median (IQR)	8.7 (8.5- 8.8)		11.8 (11.7- 11.8)		13.5 (13.4- 13.6)	
Cumulative incidence of invasive CBC, *%*						
5-year (95%CI)	2.4 (2.2- 2.6)		2.0 (2.0- 2.1)		2.9 (2.8- 3.0)	
10-year (95%CI)	4.8 (4.6- 5.2)		4.0 (4.0- 4.1)		5.6 (5.4- 5.8)	
number of invasive CBC	1,334		12,821		5,782	
Cumulative incidence of death, *%*						
5-year (95%CI)	3.8 (3.6- 4.0)		15.0 (14.9- 15.2)		7.8 (7.6- 8.0)	
10-year (95%CI)	9.8 (9.4- 10.2)		29.4 (29.2- 29.6)		19.2 (18.9- 19.5)	
number of death	3,340		91,797		23,899	

Table 1. Continued

	DCIS		All invasive BC		Stage I BC without adjuvant systemic therapy[a]	
	N	%	N	%	N	%
Characteristics	**28,003**	**9.2**	**275,836**	**90.8**	**86,481**	**31.4**
Cumulative incidence of ipsilateral invasive BC *%*						
5-year (95%CI)	1.6 (1.5- 1.8)		0.1 (0.1- 0.1)		0.2 (0.1- 0.2)	
10-year (95%CI)	3.5 (3.3- 3.8)		0.3 (0.2- 0.3)		0.5 (0.4- 0.6)	
number of ipsilateral invasive BC	920		1,471		897	
Cumulative incidence of in situ CBC, *%*						
5-year (95%CI)	1.0 (1.0- 1.1)		0.4 (0.4- 0.5)		0.6 (0.6- 0.7)	
10-year (95%CI)	1.6 (1.5- 1.8)		0.8 (0.7- 0.8)		1.1 (1.0- 1.2)	
number of in situ CBC	427		2,278		1,026	

Abbreviations: DCIS = ductal carcinoma in situ; BC = breast cancer; ER = estrogen-receptor; PR = progesterone-receptor; HER2 = human epidermal growth factor receptor 2; IQR = inter-quartile range; CBC = contralateral breast cancer; CI = confidence interval

[a] The 'stage I BC without adjuvant systemic therapy' group is a subset of the 'all invasive BC' group

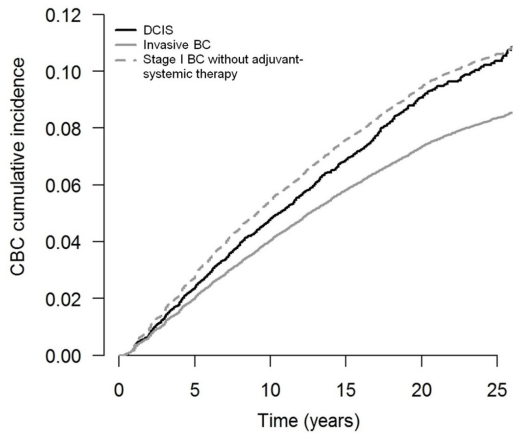


Figure 1. Cumulative incidences of invasive contralateral breast cancer (CBC) in patients diagnosed with ductal carcinoma in situ (DCIS), invasive breast cancer (BC) stage I-III, and stage I BC without (neo)adjuvant systemic therapy

The x-axis represents the time since first BC diagnosis (in years) and the y-axis the cumulative CBC incidence

In sensitivity analyses using different time cut-offs for metachronous CBC, results were similar. The HR for invasive CBC developed at least six months after the first BC was 1.10 (95%CI=1.04-1.17) for DCIS compared with invasive BC, and the HR was 1.09 (95%CI=1.03-1.16) using a 12-month cut-off.

The cumulative incidence of in situ CBC, death, and invasive ipsilateral BC are shown in Supplementary Figures 1-3[38]. The 10-year cumulative incidence of in situ CBC was 1.6% (95%CI=1.5–1.8%) for DCIS patients, 0.8% (95%CI=0.7–0.8%) for invasive BC patients, and 1.1% (95%CI=1.0–1.2%) for patients with stage I BC without adjuvant systemic therapy (Table 1). The risk of death was lower in DCIS patients compared to invasive BC patients (HR=0.47, 95%CI=0.45–0.49, Supplementary Table 1).

Table 2. Relative subsequent contralateral breast cancer risks (invasive and in situ) after diagnosis with ductal carcinoma in situ versus invasive breast cancer using Cox and competing risk regression

Outcome(s)	Type of first BC	Cox regression		Competing risks regression	
		Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
		HR (95% CI)	HR (95% CI)	HR[b] (95% CI)	HR[b] (95% CI)
Invasive CBC	DCIS vs invasive BC	1.08 (1.01-1.14)	1.10 (1.04-1.17)	1.22 (1.15-1.28)	1.20 (1.14-1.27)
	DCIS vs stage I BC without adjuvant systemic therapy	0.87 (0.82-0.92)	0.87 (0.82-0.92)	0.88 (0.83-0.94)	0.87 (0.82-0.93)
In situ CBC	DCIS vs invasive BC	1.92 (1.72-2.13)	1.84 (1.66-2.04)	2.12 (1.92-2.38)	1.98 (1.79-2.20)
	DCIS vs stage I BC without adjuvant systemic therapy	1.49 (1.33-1.67)	1.38 (1.22-1.55)	1.54 (1.37-1.72)	1.40 (1.25-1.58)

Abbreviations: HR = hazard ratio; CI = confidence interval; CBC = contralateral breast cancer; BC = breast cancer; DCIS = ductal carcinoma in situ

[a] Hazard ratios adjusted by age and year at first diagnosis

[b] Hazard ratios for the subdistribution hazards of the Fine and Gray model. Invasive CBC, in situ CBC, invasive ipsilateral BC, and death were taken into account as competing risks

Results by age and screening (period)

Among patients who had their first BC diagnosis during the implementation phase of the national screening program (1989–1998), the risk of invasive CBC was similar in DCIS patients compared with invasive BC patients (HR=0.93, 95%CI= 0.85–1.03, Table 3, Figure 2A-C[38]). In the period of full nationwide coverage of the screening program (1999-2017), the risk of invasive CBC was higher for DCIS patients than for invasive BC patients (HR=1.19, 95%CI=1.10–1.27, Table 3, Figure 2B-D[38]). The risk of invasive CBC was lower in DCIS patients compared with patients with stage I BC not receiving adjuvant systemic therapy in both periods (1989-1998: HR=0.90; 95%CI= 0.81–1.00, and 1999-2017: HR=0.85, 95% CI: 0.79–0.91). The effects were similar stratified by age group (<50 and ≥50 years) (Table 3). The estimated 5- and 10-year cumulative incidences by age and period are shown in Supplementary Table 2.

In a subgroup of patients diagnosed during or after 2011, with information available on the mode of first BC detection, the HR of invasive CBC was 1.53 (95%CI=1.29-1.82) for DCIS patients compared with invasive BC patients, and 0.86 (95%CI=0.71-1.03) compared with patients with stage I BC without adjuvant systemic therapy (Table 4). Among all screen-detected first BCs, the HR of invasive CBC was 1.38 (95%CI=1.35-1.68) for DCIS patients compared with invasive BC patients and 0.81 (95%CI=0.66-1.00) compared with stage I BC without adjuvant systemic therapy (Table 4). When the first BC was not detected by screening, the HR of invasive CBC was 2.14 (95%CI=1.46-3.13) for DCIS patients compared to invasive BC patients and 1.04 (95%CI=0.68-1.59) compared with stage I BC without adjuvant systemic therapy (Table 4). The risk of death in patients with DCIS compared with invasive BC and stage I BC without adjuvant systemic therapy among screen-detected and not screen-detected is shown in Supplementary Table 3.

Table 3. Relative risk of invasive contralateral breast cancer after ductal carcinoma in situ versus invasive breast cancer by period and age at first diagnosis using Cox and competing risks regression

				Cox regression		Competing risks regression	
Period	Type of first BC	N	CBC events	HR	95% CI	HR[a]	95% CI
All							
1989- 1998	DCIS vs invasive BC	81,105	6,488	0.93	0.85- 1.03	1.11	1.01- 1.23
1999- 2017	DCIS vs invasive BC	222,734	7,667	1.19	1.10- 1.27	1.32	1.23- 1.41
1989- 1998	DCIS vs stage I BC without systemic therapy	273,383	2,696	0.90	0.81- 1.00	0.93	0.85- 1.04
1999- 2017	DCIS vs stage I BC without systemic therapy	59,098	3,086	0.85	0.79- 0.91	0.88	0.81- 0.94
Age < 50 years at first diagnosis[b]							
1989- 1998	DCIS vs invasive BC	22,084	2,292	0.94	0.83- 1.09	1.06	0.92- 1.22
1999- 2017	DCIS vs invasive BC	53,570	1,838	1.20	1.06- 1.37	1.26	1.11- 1.45
1989- 1998	DCIS vs stage I BC without systemic therapy	7,192	870	0.90	0.78- 1.04	0.89	0.78- 1.04
1999- 2017	DCIS vs stage I BC without systemic therapy	8,162	472	0.85	0.74- 0.97	0.82	0.71- 0.94
Age ≥ 50 years at first diagnosis[b]							
1989- 1998	DCIS vs invasive BC	59,021	4,196	0.92	0.83- 1.03	1.14	1.03- 1.26
1999- 2017	DCIS vs invasive BC	169,164	5,829	1.18	1.10- 1.26	1.35	1.26- 1.47
1989- 1998	DCIS vs stage I BC without systemic therapy	20,191	1,826	0.89	0.80- 1.00	0.96	0.86- 1.08
1999- 2017	DCIS vs stage I BC without systemic therapy	50,936	2,614	0.85	0.78- 0.92	0.88	0.81- 0.95

Abbreviations: HR = hazard ratio; CI = confidence interval; DCIS = ductal carcinoma in situ; BC = breast cancer

[a] Hazard ratios for the subdistribution hazards of the Fine and Gray model. Invasive CBC, in situ CBC, invasive ipsilateral BC, and death were taken into account as competing risks

[b] Results were based on interaction analyses including the interaction term between age, period, and type of first BC (type of first BC + age + period + age × type of first BC + period × type of first BC)

Subtype-specific CBC risk

DCIS patients had a lower risk of stage IV CBC (HR=0.45, 95%CI=0.22-0.92), and higher risks of grade I invasive CBC (HR=1.55, 95%CI=1.31-1.84) and ER-positive invasive CBC (HR=1.49, 95%CI=1.33-1.66) compared with all invasive BC patients (Supplementary Table 4). Overall, the subtype-specific CBC risk in DCIS patients was comparable to patients with stage I BC not receiving adjuvant systemic therapy (Supplementary Table 4).

Multivariable model

In the multivariable model, no strong predictors of CBC were identified in DCIS patients (Table 5). The C-index of the multivariable model of invasive CBC was 0.52 (standard deviation (SD=0.01) for cause-specific Cox regression; when we considered all CBC (in situ and invasive) the C-index was 0.51 (SD=0.01) (Table 5). When we performed the analyses in a subgroup of patients diagnosed during or after 2011, the C-index was 0.55 (SD=0.01) without information on the mode of first BC detection, and 0.56 (SD=0.01) with information available on the mode of first BC detection (data not shown).

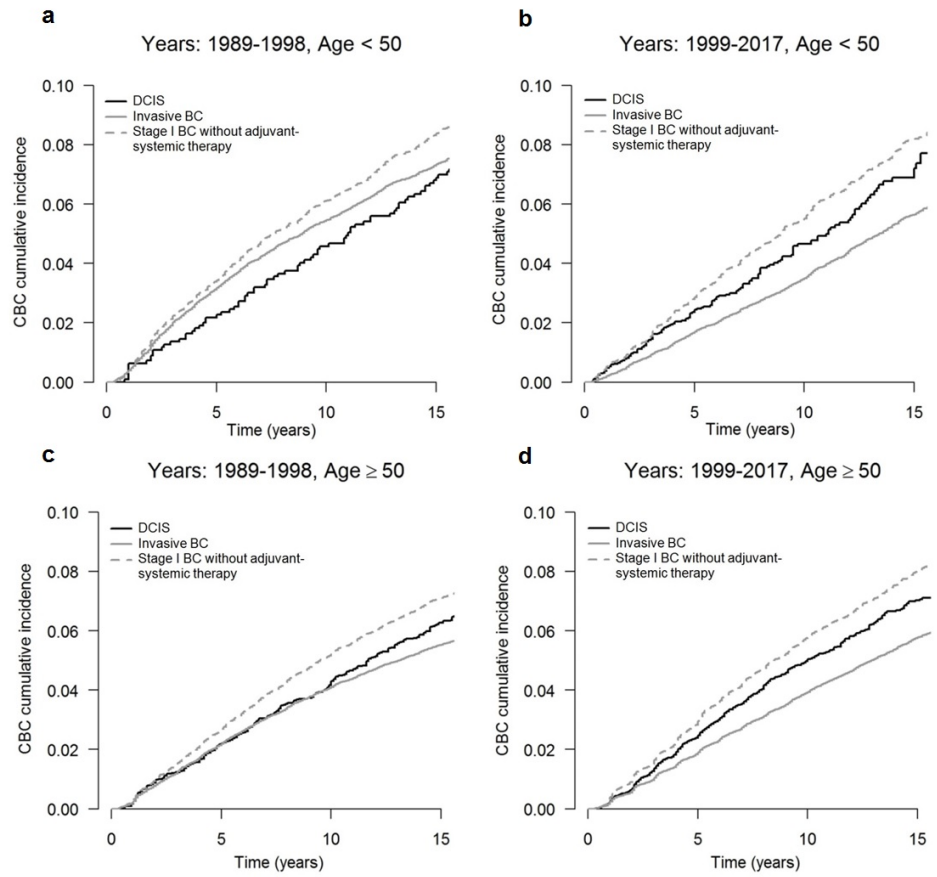


Figure 2. Cumulative incidences of invasive contralateral breast cancer (CBC) in patients diagnosed with ductal carcinoma in situ (DCIS), invasive breast cancer (BC) stage I-III, or stage I BC without (neo)adjuvant systemic therapy

Panel A) patients aged <50 years diagnosed between 1989-1998 (implementation phase Dutch mammography screening program); **Panel B)** patients aged <50 years diagnosed between 1999–2017 (full national coverage of the Dutch mammography screening program); **Panel C)** patients aged ≥50 years diagnosed between 1989–1998; **Panel D)** patients aged ≥50 years diagnosed between 1999–2017. The x-axis represents the time since first BC diagnosis (in years) and the y-axis the cumulative CBC incidence

Table 4. Relative subsequent event risks after diagnosis with ductal carcinoma in situ versus invasive breast cancer by mode of first breast cancer detection for patients diagnosed between 2011-2017[a]

		Overall		By mode of first BC detection[b]		
Outcome	Type of first BC	Cox regression	Competing risks regression		Cox regression	Competing risks regression
		HR (95% CI)[c]	HR[c,d](95% CI)		HR[c] (95% CI)	HR[c,d] (95% CI)
Invasive CBC	DCIS vs invasive BC (n=62,533, events=763)	1.53 (1.29-1.82)	1.55 (1.30-1.85)	screen-detected[e]	1.38 (1.35-1.68)	1.38 (1.13-1.69)
				not screen-detected[e]	2.14 (1.46-3.13)	2.20 (1.50-3.22)
	DCIS vs stage I BC without systemic therapy (n=27,288, events=519)	0.86 (0.71-1.03)	0.86 (0.71-1.03)	screen-detected[e]	0.81 (0.66-1.00)	0.81 (0.65-1.00)
				not screen-detected[e]	1.04 (0.68-1.59)	1.05 (0.68-1.60)
In situ CBC	DCIS vs invasive BC (n=62,533, events=250)	1.99 (1.51-2.63)	2.00 (1.52-2.65)	screen-detected[e]	1.75 (1.26-2.45)	1.75 (1.26-2.45)
				not screen-detected[e]	3.41 (1.98-5.87)	3.46 (2.01-5.97)
	DCIS vs stage I BC without systemic therapy (n=27,288, events=146)	1.51 (1.08-2.10)	1.51 (1.08-2.10)	screen-detected[e]	1.40 (0.96-2.06)	1.41 (0.96-2.06)
				not screen-detected[e]	2.23 (1.14-4.39)	2.25 (1.15-4.41)

Abbreviations: BC = breast cancer; HR = hazard ratio; CI = confidence interval; CBC = contralateral breast cancer; DCIS = ductal carcinoma in situ

[a] The analyses were performed in all patients diagnosed between 2011-2017, since from 2011 we had virtually complete information on the mode of first BC detection

[b] Results were based on interaction analyses including the interaction term between mode of first BC detection and type of first BC (type of first BC + mode of first BC detection + mode of first BC detection × type of first BC)

[c] Adjusted for age at first BC diagnosis

[d] Hazard ratios for the subdistribution hazards of the Fine and Gray model. Invasive CBC, in situ CBC, invasive ipsilateral BC, and death were taken into account as competing risks

[e] Not screen-detected includes interval tumours, non-screen attendant, or screened outside the national program

Table 5. Relative risks of invasive and in situ contralateral breast cancer after diagnosis with ductal carcinoma in situ using multivariable Cox and competing risk regression models

Outcome	Invasive CBC				Invasive and in situ CBC			
	Cox regression		Competing risk regression		Cox regression		Competing risk regression	
	HR	95% CI	HR[a]	95% CI	HR	95% CI	HR[a]	95% CI
Age (years)	1.01[b]	0.93- 1.10	0.78[c]	0.69- 0.89	0.93[b]	0.87- 1.00	0.71[c]	0.63- 0.81
Tumour grade								
Moderately differentiated versus well differentiated	0.93	0.78- 1.12	0.94	0.79- 1.12	0.99	0.85- 1.16	0.99	0.85- 1.16
Poorly differentiated versus well differentiated	0.92	0.76- 1.10	0.93	0.77- 1.11	0.94	0.81- 1.09	0.94	0.81- 1.09
Surgery (Mastectomy versus BCS)	0.96	0.80- 1.16	1.00	0.83- 1.21	1.08	0.92- 1.26	1.13	0.96- 1.32
Radiotherapy to the breast (yes versus no)	1.11	0.94- 1.32	1.12	0.94- 1.33	1.12	0.97- 1.30	1.14	0.98- 1.32
Baseline cumulative incidence at 10 years[d]	0.051		0.044[e]		0.068		0.057[e]	
C-index (SD)	0.520 (0.01)		0.515 (0.01)		0.513 (0.01)		0.526 (0.01)	

Abbreviations: CBC = contralateral breast cancer; HR = hazard ratio; CI = confidence interval; BCS = breast conservative surgery; SD = standard deviation

[a] Hazard ratios for the subdistribution hazards of the Fine and Gray model

[b] parameterized per decade

[c] parameterized as a restricted cubic spline with three knots

[d] The baseline cumulative incidence function is calculated for baseline values of the predictors included in the multivariable models

[e] Baseline cumulative incidence function for the subdistribution hazard of the Fine and Gray model

Discussion

In this large population-based study, the 10-year cumulative incidence of invasive CBC was 4.8% for DCIS patients. The risk of developing invasive CBC was lower for DCIS patients compared with stage I BC patients not receiving adjuvant systemic therapy (HR=0.87), but the risk was slightly higher compared with all invasive BC patients (HR=1.10). A multivariable model, based on the clinical information currently available, was unable to differentiate risks of invasive CBC among DCIS patients.

The slightly higher invasive CBC risk in DCIS patients compared with all invasive BC patients may be explained by the risk-reducing effect of adjuvant systemic therapy among invasive BC patients[6,20,21]. In our previous study using NCR data[6] we showed that adjuvant endocrine therapy, chemotherapy, and trastuzumab combined with chemotherapy were associated with overall 54%, 30%, and 43% risk reductions of CBC, respectively. In our study, a large group (57%) of patients with invasive BC received (neo) adjuvant systemic therapy. According to the Dutch guidelines, DCIS patients are not offered treatment with adjuvant systemic therapy[26]. The potential influence of adjuvant systemic therapy is supported by the CBC risk evaluation in patients diagnosed with stage I BC not receiving adjuvant systemic therapy, showing a higher CBC risk in such patients than in patients diagnosed with DCIS.

To our knowledge, only one previous study in the US investigated the risk of CBC in patients with DCIS in direct comparison with patients diagnosed with invasive BC using SEER data[17]. They found a similar CBC risk (including in situ and invasive) for invasive ductal BC in comparison with DCIS, with a relative risk of 0.98 (95%CI= 0.90–1.06). However, that analysis was based on an earlier, largely pre-screening, period (1973-1996), and lacked information on adjuvant systemic therapy use. Previous studies examining cohorts of DCIS patients have reported a subsequent annual invasive CBC risk of 0.4 to 0.6%[13,15,16], comparable to our finding.

When analyses were restricted to patients with information available on the mode of first BC detection, trends were similar overall. However, the higher CBC risk for DCIS patients compared with invasive BC was more pronounced within the not screen-detected BC group compared with the screen-detected BC group. Tumours not detected by screening could be interval tumours or those arising in women not attending for screening. Certainly, invasive interval tumours tend to be more aggressive than screen-detected BCs and hence receive more often adjuvant systemic treatment[22].

We observed that the invasive CBCs developed within the DCIS group were less aggressive than the invasive CBCs developed after invasive first BC, i.e. more ER-positive, and lower tumour stage and grade. This may be explained by underlying etiological factors and/or be related to the use of adjuvant systemic therapy among invasive BC patients. Studies have shown that adjuvant systemic therapy influences subtype-specific CBC risk, e.g. endocrine therapy strongly reduces the risk of developing ER-positive CBC,

but not ER-negative CBC[6,21]. This is supported by our subgroup analyses in patients with stage I BC not receiving adjuvant systemic therapy, who tended to develop similar CBC subtypes compared with DCIS patients.

The main strength of this study was the use of a large population-based nationwide cohort of DCIS and invasive BC patients, with complete follow-up on CBC over a long period. The NCR did not have follow-up information on distant metastases for all years included and therefore we could not take distant metastasis as a competing event into account. However, in the years where we had information on distant metastases (2003-2006), the median survival was 1.1 years and the 5-year overall survival after distant metastasis was fairly poor (6%). This indicates that death could be used as a proxy for distant metastasis. Since we had complete information on death (as a competing event), we do not expect that the lack of information on distant metastases has led to an underestimation of the CBC risk. We also did not have information available about contralateral prophylactic mastectomy (CPM), which may have resulted in an underestimation of the CBC risk and may not have had equal uptake in all groups. According to Dutch guidelines [26] only women carrying a *BRCA1 or BRCA2* germline mutation are advised to undergo a contralateral preventive mastectomy, since their CBC risk is high with an estimated 10-year risk of ~10-20%[39,40] Unfortunately, information about *BRCA1* and *BRCA2* mutation was lacking. However, we do not expect that this missing information importantly influenced the results since only 1-2% of the DCIS population[41], and 3-5% of the invasive BC population[39,42] will be *BRCA1* or *BRCA2* mutation carriers. Finally, less than 1% of the DCIS patients were not treated according to the Dutch guideline since they received adjuvant chemotherapy, endocrine therapy, and/or trastuzumab. However, since this number is low, we do not expect that this affected our results.

Despite low CBC risks, the use of CPM has increased in recent years, both in patients diagnosed with invasive BC and in patients diagnosed with DCIS, especially in the US[14,43]. Therefore, a need of individualized CBC risk prediction may be as important for patients diagnosed with DCIS as for patients with invasive BC. Currently, CBC risk prediction models have been developed and validated for patients with invasive BC, but these models may not be appropriate for DCIS patients since most of the information available for invasive BC is not routinely collected in DCIS[18,19,44,45]. In our study, we had limited information on biological characteristics of DCIS, e.g. no information on receptor subtypes, and our multivariable model was therefore unable to differentiate CBC risk among DCIS patients. So, based on the clinical information currently available, CBC risk prediction in DCIS patients is insufficiently robust to be clinically actionable. More biological knowledge is needed to improve CBC prediction in DCIS patients.

Based on the results of this study we do not suggest to start treating DCIS patients with adjuvant systemic therapy to prevent CBC since the absolute invasive CBC risk is low. To facilitate patients and physicians in decision making, a comprehensive risk prediction model specifically developed for patients with DCIS would be desirable, including information on genetic, clinical, and lifestyle factors.

Article information

Funding

This work was supported by the Alpe d'HuZes/Dutch Cancer Society (KWF Kankerbestrijding) [grant number A6C/6253] and by Cancer Research UK/KWF Kankerbestrijding [grant numbers C38317, A24043]. The funders had no role in the design of the study, the statistical analyses, interpretation of the data, and writing of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank the registration team of the Netherlands Comprehensive Cancer Organization (IKNL) for the collection of data for the Netherlands Cancer Registry (NCR) as well as IKNL staff for scientific advice. We thank all patients whose data we used for this study and the clinicians who treated these patients.

Data availability statement

The datasets generated and/or analysed during the current study are not publicly available, as the study has used external data from the Netherlands Cancer Registry. The datasets will be made available from the Netherlands Cancer Registry upon reasonable request (data request study number K18.245). To apply for data access, please visit https://www.iknl.nl/en/ncr/apply-for-data. The datasets that support figures 1 and 2, and supplementary figures 1-3, are publicly available in the figshare repository, in the following data record: https://doi.org/10.6084/m9.figshare.12982424[23].

Code availability statement

The codes developed during this study are available upon reasonable request. Analyses were performed using STATA version 16.0, SAS (SAS Institute Inc., Cary, NC, USA) version 9.4, and R software version 3.5.3.

Author contributions

The data used for this study were derived from by the Netherlands Cancer Registry. MKS designed the study; IK prepared and coded the data for analysis; DG performed the statistical analyses; IK, DG, MKS interpreted the results and drafted the first version of the manuscript; all other authors contributed to the interpretation of the results and revisions of the manuscript. DG and IK shared co-first authorship. All authors approved the final manuscript.

References

1 Evans, H. S. *et al.* Incidence of multiple primary cancers in a cohort of women diagnosed with breast cancer in southeast England. *Br J Cancer* **84**, 435-440, doi:10.1054/bjoc.2000.1603 (2001).

2 Soerjomataram, I. *et al.* Primary malignancy after primary female breast cancer in the South of the Netherlands, 1972-2001. *Breast Cancer Res. Treat.* **93**, 91-95, doi:10.1007/s10549-005-4016-2 (2005).

3 Brenner, H., Siegle, S., Stegmaier, C. & Ziegler, H. Second primary neoplasms following breast cancer in Saarland, Germany, 1968-1987. *Eur. J. Cancer* **29a**, 1410-1414, doi:10.1016/0959-8049(93)90013-6 (1993).

4 Portschy, P. R. *et al.* Perceptions of Contralateral Breast Cancer Risk: A Prospective, Longitudinal Study. *Ann Surg Oncol* **22**, 3846-3852, doi:10.1245/s10434-015-4442-2 (2015).

5 Hartman, M. *et al.* Genetic implications of bilateral breast cancer: a population based cohort study. *Lancet Oncol* **6**, 377-382, doi:10.1016/S1470-2045(05)70174-1 (2005).

6 Kramer, I. *et al.* The Influence of Adjuvant Systemic Regimens on Contralateral Breast Cancer Risk and Receptor Subtype. *J Natl Cancer Inst* **111**, 709-718, doi:10.1093/jnci/djz010 (2019).

7 Prater, J., Valeri, F., Korol, D., Rohrmann, S. & Dehler, S. Incidence of metachronous contralateral breast cancer in the Canton of Zurich: a population-based study of the cancer registry. *J Cancer Res Clin Oncol* **142**, 365-371, doi:10.1007/s00432-015-2031-1 (2016).

8 Nichols, H. B., Berrington de Gonzalez, A., Lacey, J. V., Jr., Rosenberg, P. S. & Anderson, W. F. Declining incidence of contralateral breast cancer in the United States from 1975 to 2006. *J Clin Oncol* **29**, 1564-1569, doi:10.1200/JCO.2010.32.7395 (2011).

9 Netherlands Cancer Registry (NCR). *Survival and prevalence of cancer*, <https://www.cijfersoverkanker.nl> (2016).

10 Ernster, V. L. *et al.* Detection of ductal carcinoma in situ in women undergoing screening mammography. *J Natl Cancer Inst* **94**, 1546-1554 (2002).

11 Elshof, L. E. *et al.* Subsequent risk of ipsilateral and contralateral invasive breast cancer after treatment for ductal carcinoma in situ: incidence and the effect of radiotherapy in a population-based cohort of 10,090 women. *Breast Cancer Res. Treat.* **159**, 553-563, doi:10.1007/s10549-016-3973-y (2016).

12 Mariotti, C. Ductal Carcinoma in Situ of the Breast. *Springer International Publishing* (2018).

13 Miller, M. E. *et al.* Contralateral Breast Cancer Risk in Women with Ductal Carcinoma In Situ: Is it High Enough to Justify Bilateral Mastectomy? *Ann. Surg. Oncol.* **24**, 2889-2897, doi:10.1245/s10434-017-5931-2 (2017).

14 Tuttle, T. M. *et al.* Increasing rates of contralateral prophylactic mastectomy among patients with ductal carcinoma in situ. *J Clin Oncol* **27**, 1362-1367, doi:10.1200/JCO.2008.20.1681 (2009).

15 Falk, R. S., Hofvind, S., Skaane, P. & Haldorsen, T. Second events following ductal carcinoma in situ of the breast: a register-based cohort study. *Breast Cancer Res. Treat.* **129**, 929-938, doi:10.1007/s10549-011-1531-1 (2011).

16 Claus, E. B., Stowe, M., Carter, D. & Holford, T. The risk of a contralateral breast cancer among women diagnosed with ductal and lobular breast carcinoma in situ: data from the Connecticut Tumor Registry. *Breast* **12**, 451-456, doi:10.1016/s0960-9776(03)00152-8 (2003).

17 Gao, X., Fisher, S. G. & Emami, B. Risk of second primary cancer in the contralateral breast in women treated for early-stage breast cancer: a population-based study. *Int. J. Radiat. Oncol. Biol. Phys.* **56**, 1038-1045 (2003).

18 Chowdhury, M., Euhus, D., Onega, T., Biswas, S. & Choudhary, P. K. A model for individualized risk prediction of contralateral breast cancer. *Breast Cancer Res Treat* **161**, 153-160, doi:10.1007/s10549-016-4039-x (2017).

19 Chowdhury, M. *et al.* Validation of a personalized risk prediction model for contralateral breast cancer. *Breast Cancer Res Treat* **170**, 415-423, doi:10.1007/s10549-018-4763-5 (2018).

20 Akdeniz, D. *et al.* Risk factors for metachronous contralateral breast cancer: A systematic review and meta-analysis. *Breast* **44**, 1-14, doi:10.1016/j.breast.2018.11.005 (2018).

21 Langballe, R. *et al.* Systemic therapy for breast cancer and risk of subsequent contralateral breast cancer in the WECARE Study. *Breast Cancer Res* **18**, 65, doi:10.1186/s13058-016-0726-0 (2016).

22 Mook, S. *et al.* Independent prognostic value of screen detection in invasive breast cancer. *J Natl Cancer Inst* **103**, 585-597, doi:10.1093/jnci/djr043 (2011).

23 Font-Gonzalez, A. *et al.* Inferior survival for young patients with contralateral compared to unilateral breast cancer: a nationwide population-based study in the Netherlands. *Breast Cancer Res. Treat.* **139**, 811-819, doi:10.1007/s10549-013-2588-9 (2013).

24 Brierley, J. D., Gospodarowicz, M. K. & Wittekind, C. *TNM classification of malignant tumours*. 8th Editor edn, (2017).

25 Foundation Federation of Dutch Medical Scientific Societies. Human Tissue and Medical Research: Code of Conduct for responsible use. (2011).

26 Oncoline. *Borstkanker. Landelijke richtlijn, Versie: 2.0. Available from:* https://www.oncoline.nl/

27 Latouche, A., Allignol, A., Beyersmann, J., Labopin, M. & Fine, J. P. A competing risks analysis should

report results on all cause-specific hazards and cumulative incidence functions. *J Clin Epidemiol* **66**, 648-653, doi:10.1016/j.jclinepi.2012.09.017 (2013).
28 Van Der Pas, S., Nelissen, R. & Fiocco, M. Different competing risks models for different questions may give similar results in arthroplasty registers in the presence of few events. *Acta Orthop* **89**, 145-151, doi:10.1080/17453674.2018.1427314 (2018).
29 RIVM. *Breast Cancer screening program; facts and figures. Available from:* https://www.rivm.nl/en/breast-cancer-screening-programme/background/facts-and-figures
30 IKNL. *National evaluation of breast cancer screening in the Netherlands 2017/2018. Available from:* https://www.iknl.nl/getmedia/8b019b63-0eb1-4afa-a824-31c4d10cc86e/Breast_cancer_screening_in_the_Netherlands_2017-2018_en.pdf
31 Sankatsing, V. D. V. *et al.* Detection and interval cancer rates during the transition from screen-film to digital mammography in population-based screening. *BMC Cancer* **18**, 256, doi:10.1186/s12885-018-4122-2 (2018).
32 Xue, X. *et al.* A comparison of the polytomous logistic regression and joint cox proportional hazards models for evaluating multiple disease subtypes in prospective cohort studies. *Cancer Epidemiol. Biomarkers Prev.* **22**, 275-285, doi:10.1158/1055-9965.epi-12-1050 (2013).
33 Harrell, F. E., Jr. Regression Modeling Strategies with applications to linear models, logistic and ordinal regression, and survival analysis. *Springer Series in Statistics* **2nd edition** (2015).
34 Koziol, J. A. & Jia, Z. The concordance index C and the Mann-Whitney parameter Pr(X>Y) with randomly censored data. *Biom J* **51**, 467-474, doi:10.1002/bimj.200800228 (2009).
35 Van Buuren, S. *Flexible imputation of missing data*. Second edn, (Chapman and Hall/CRC, 2018).
36 Madley-Dowd, P., Hughes, R., Tilling, K. & Heron, J. The proportion of missing data should not be used to guide decisions on multiple imputation. *J Clin Epidemiol* **110**, 63-73, doi:10.1016/j.jclinepi.2019.02.016 (2019).
37 R: A Language and Environment for Statistical Computing (R: Foundation for Statistical Computing, 2020).
38 Giardiello, D. *et al.* Data and metadata supporting the published article: Contralateral breast cancer risk in patients with ductal carcinoma in situ and invasive breast cancer. *figshare*, doi:https://doi.org/10.6084/m9.figshare.12982424 (2020).
39 van den Broek, A. J. *et al.* Impact of Age at Primary Breast Cancer on Contralateral Breast Cancer Risk in BRCA1/2 Mutation Carriers. *J. Clin. Oncol.* **34**, 409-418, doi:10.1200/jco.2015.62.3942 (2016).
40 Kuchenbaecker, K. B. *et al.* Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. *JAMA* **317**, 2402-2416, doi:10.1001/jama.2017.7112 (2017).
41 Claus, E. B., Petruzella, S., Matloff, E. & Carter, D. Prevalence of BRCA1 and BRCA2 mutations in women diagnosed with ductal carcinoma in situ. *JAMA* **293**, 964-969, doi:10.1001/jama.293.8.964 (2005).
42 Thompson, D. & Easton, D. The genetic epidemiology of breast cancer genes. *J. Mammary Gland Biol. Neoplasia* **9**, 221-236, doi:10.1023/B:JOMG.0000048770.90334.3b (2004).
43 Murphy, J. A., Milner, T. D. & O'Donoghue, J. M. Contralateral risk-reducing mastectomy in sporadic breast cancer. *Lancet Oncol* **14**, e262-269, doi:10.1016/S1470-2045(13)70047-0 (2013).
44 Basu, N. N., Ross, G. L., Evans, D. G. & Barr, L. The Manchester guidelines for contralateral risk-reducing mastectomy. *World J Surg Oncol* **13**, 237, doi:10.1186/s12957-015-0638-y (2015).
45 O'Donnell, M. Estimating Contralateral Breast Cancer Risk. *Current Breast Cancer Reports* **10**, 91-97 (2018).

Supplementary Material

Supplementary Table 1. Relative subsequent risks of death and invasive ipsilateral breast cancer after diagnosis with ductal carcinoma in situ versus invasive breast cancer using Cox and competing risk regression

Outcome(s)	Type of first BC	Cox regression		Competing risks regression	
		Unadjusted HR (95% CI)	Adjusted[a] HR (95% CI)	Unadjusted HR[b] (95% CI)	Adjusted[a] HR[b] (95% CI)
Death	DCIS vs invasive BC	0.37 (0.36-0.38)	0.47 (0.45-0.49)	0.36 (0.35-0.37)	0.45 (0.44-0.47)
	DCIS vs stage I BC without adjuvant systemic therapy	0.56 (0.54-0.58)	0.71 (0.69-0.74)	0.53 (0.51-0.55)	0.68 (0.66-0.71)
Invasive IBC	DCIS vs invasive BC	6.67 (6.25-7.14)	6.68 (6.15-7.26)	7.69 (7.14-9.09)	7.79 (7.17-8.47)
	DCIS vs stage I BC without adjuvant systemic therapy	4.17 (3.85-4.54)	4.05 (3.68-4.45)	4.35 (4.00-4.76)	4.28 (3.90-4.71)

Abbreviations: HR = hazard ratio; CI = confidence interval; DCIS = ductal carcinoma in situ; BC = breast cancer; IBC = ipsilateral breast cancer

[a] Hazard ratios adjusted by age and year at first breast cancer diagnosis

[b] Hazard ratios for the subdistribution hazards of the Fine and Gray model. Invasive contralateral breast cancer, in situ contralateral breast cancer, invasive ipsilateral BC, and death were taken into account as competing risks

Supplementary Table 2. Cumulative incidence of invasive contralateral breast cancer at five and ten years in patients with ductal carcinoma in situ or invasive breast cancer by period and age at first diagnosis

	Period[a]	Type of first BC	Five-year cumulative incidence (%) (95% CI)	Ten-year cumulative incidence (%) (95% CI)
All	1989-1998	DCIS	2.2 (1.8-2.7)	4.4 (3.8-5.0)
		Invasive BC	2.5 (2.4-2.6)	4.5 (4.3-4.6)
		Stage I BC without adjuvant systemic therapy	2.9 (2.7-3.1)	5.5 (5.2-5.7)
	1999-2017	DCIS	2.5 (2.2-2.7)	5.0 (4.6-5.4)
		Invasive BC	1.9 (1.8-1.9)	3.8 (3.7-3.9)
		Stage I BC without adjuvant systemic therapy	3.0 (2.8-3.1)	5.8 (5.5-6.0)
Age < 50 years at first diagnosis	1989-1998	DCIS	2.3 (1.5-3.3)	4.6 (3.4-5.9)
		Invasive BC	3.2 (3.0-3.4)	5.5 (5.2-5.8)
		Stage I BC without adjuvant systemic therapy	3.4 (3.0-3.9)	6.1 (5.6-6.7)
	1999-2017	DCIS	2.4 (2.0-3.0)	4.7 (3.9-5.5)
		Invasive BC	1.7 (1.6-1.8)	3.5 (3.3-3.7)
		Stage I BC without adjuvant systemic therapy	2.9 (2.5-3.3)	5.5 (5.0-6.0)
Age ≥ 50 years at first diagnosis	1989-1998	DCIS	2.2 (1.8-2.7)	4.3 (3.7-5.0)
		Invasive BC	2.2 (2.1-2.3)	4.1 (4.0-4.3)
		Stage I BC without adjuvant systemic therapy	2.7 (2.5-2.9)	5.2 (4.9-5.5)
	1999-2017	DCIS	2.5 (2.2-2.7)	5.1 (4.7-5.4)
		Invasive BC	1.9 (1.8-2.0)	3.9 (3.8-4.0)
		Stage I BC without adjuvant systemic therapy	3.0 (2.8-3.1)	5.7 (5.6-6.0)

Abbreviations: CI = confidence interval; DCIS = ductal carcinoma in situ; BC = breast cancer

[a] The two periods were defined according to the gradual implementation of the screening program in the Netherlands: the implementation phase was between 1989 and 1998 and the full screening coverage was reached since 1999

Supplementary Table 3. Relative subsequent event risks after diagnosis with ductal carcinoma in situ versus invasive breast cancer by mode of first BC detection for patients diagnosed between 2011-2017[a]

Outcome	Type of first BC	Overall		By mode of first BC detection[b]		
		Cox regression HR (95% CI)[c]	Competing risks regression HR[c,d] (95% CI)		Cox regression HR[c] (95% CI)	Competing risks regression HR[c,d] (95% CI)
Death	DCIS vs invasive BC (n=62,533, events=2,763)	0.48 (0.42-0.56)	0.48 (0.42-0.55)	screen-detected[e]	0.71 (0.60-0.83)	0.70 (0.60-0.83)
				not screen-detected[e]	0.33 (0.24-0.47)	0.33 (0.23-0.46)
	DCIS vs stage I BC without systemic therapy (n=27,288, events=701)	0.93 (0.79-1.09)	0.93 (0.79-1.09)	screen-detected[e]	1.04 (0.87-1.26)	1.05 (0.87-1.26)
				not screen-detected[e]	0.67 (0.46-0.98)	0.66 (0.45-0.97)
Invasive IBC	DCIS vs invasive BC (n=62,533, events=101)	5.12 (3.46-7.57)	5.17 (3.50-7.65)	screen-detected[e]	3.88 (2.46-6.14)	3.88 (2.46-6.14)
				not screen-detected[e]	10.19 (4.52-22.94)	10.42 (4.63-23.45)
	DCIS vs stage I BC without systemic therapy (n=27,288, events=83)	2.51 (1.62-3.91)	2.52 (1.62-.3.92)	screen-detected[e]	2.34 (1.41-3.88)	2.34 (1.41-3.88)
				not screen-detected[e]	3.46 (1.32-9.10)	3.48 (1.32-9.15)

Abbreviations: BC = breast cancer; HR = hazard ratio; CI = confidence interval; DCIS = ductal carcinoma in situ; IBC = ipsilateral breast cancer

[a] The analyses were performed in all patients diagnosed between 2011-2017, since from 2011 we had virtually complete information on the mode of first BC detection

[b] Results were based on interaction analyses including the interaction term between mode of first BC detection and type of first BC (type of first BC + mode of first BC detection + mode of first BC detection × type of first BC)

[c] Adjusted for age at first BC diagnosis

[d] Hazard ratios for the subdistribution hazards of the Fine and Gray model. Invasive CBC, in situ CBC, invasive ipsilateral BC, and death were taken into account as competing risks

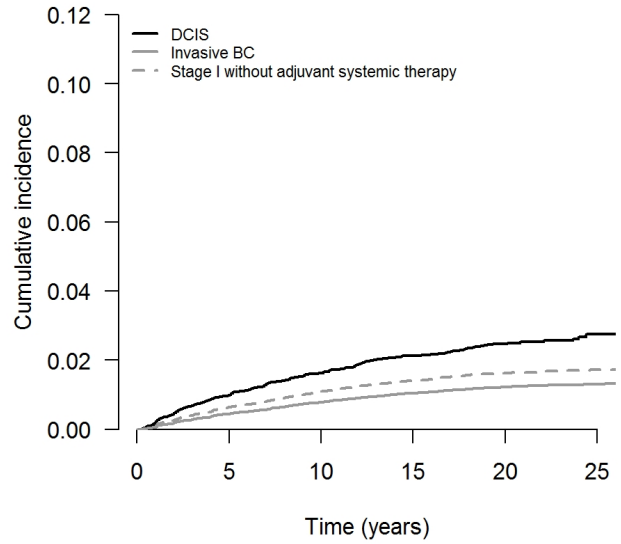
[e] Not screen-detected includes interval tumours, non-screen attendant, or screened outside the national program

Supplementary Table 4. Joint Cox regression analyses assessing subtype-specific invasive contralateral breast cancer risk for patients with ductal carcinoma in situ compared to patients with invasive breast cancer[a]

	DCIS	All invasive BC	Stage I BC without adjuvant systemic therapy	DCIS vs Invasive BC	DCIS vs Stage I BC without adjuvant systemic therapy
CBC subtypes	**N**	**N**	**N**	**HR (95%CI)**	**HR (95%CI)**
TNM stage					
I	330	1,957	1,084	1.35 (1.20- 1.52)	0.74 (0.65- 0.83)
II	146	782	342	1.50 (1.26- 1.79)	1.04 (0.86- 1.26)
III	40	220	78	1.46 (1.04- 2.05)	1.26 (0.86- 1.86)
IV	8	143	29	0.45 (0.22- 0.92)	0.72 (0.33- 1.58)
Tumor grade					
I (well differentiated)	154	797	518	1.55 (1.31- 1.84)	0.72 (0.60- 0.86)
II (moderately differentiated)	245	1,253	652	1.57 (1.37- 1.80)	0.91 (0.79- 1.06)
III (poorly/undifferentiated)	95	675	251	1.13 (0.91- 1.40)	0.93 (0.73- 1.18)
ER status					
positive	386	2,081	1,151	1.49 (1.33- 1.66)	0.81 (0.72- 0.91)
negative	53	471	114	0.90 (0.69- 1.19)	1.12 (0.81- 1.56)
PR status					
positive	314	1,560	943	1.61 (1.43- 1.82)	0.80 (0.71- 0.91)
negative	119	971	311	0.98 (0.81- 1.18)	0.93 (0.75- 1.15)
HER2 status					
positive	51	250	91	1.63 (1.21- 2.20)	1.35 (0.96- 1.91)
negative	375	2,200	1,133	1.36 (1.22- 1.52)	0.80 (0.71- 0.90)

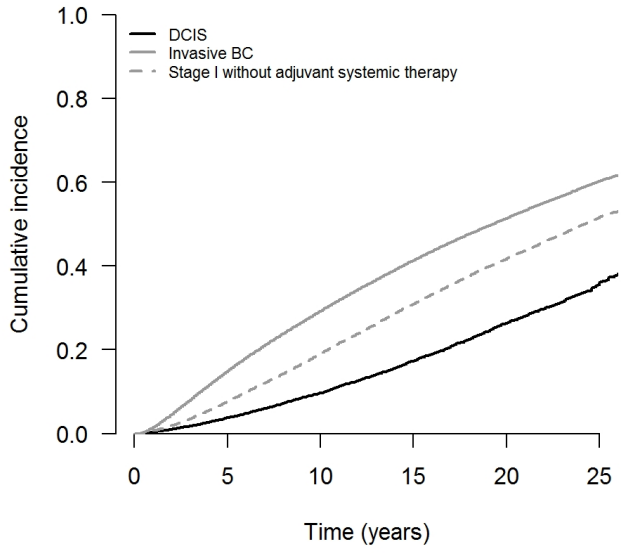
Abbreviations: CBC = contralateral breast cancer; DCIS = ductal carcinoma in situ; BC = breast cancer; HR = hazard ratio; CI = confidence interval; ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2

[a] The analyses were performed only in patients diagnosed between 2005-2017, since from 2005 the Netherlands Cancer Registry actively registered receptor status



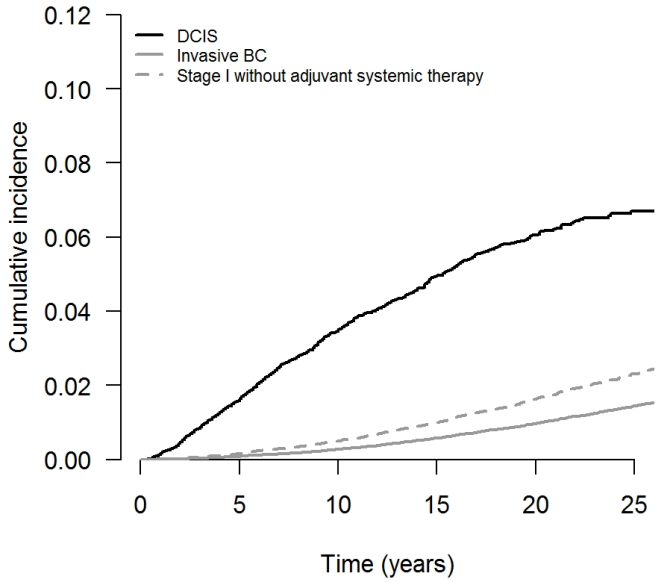
Supplementary Figure 1. Cumulative incidence of in situ contralateral breast cancer in patients diagnosed with ductal carcinoma in situ, invasive breast cancer stage I-III, and stage I breast cancer without (neo) adjuvant systemic therapy

The x-axis represents the time since the first breast cancer diagnosis (in years). The y-axis represents the cumulative incidence of in situ contralateral breast cancer. Abbreviations: DCIS = ductal carcinoma in situ; BC = breast cancer



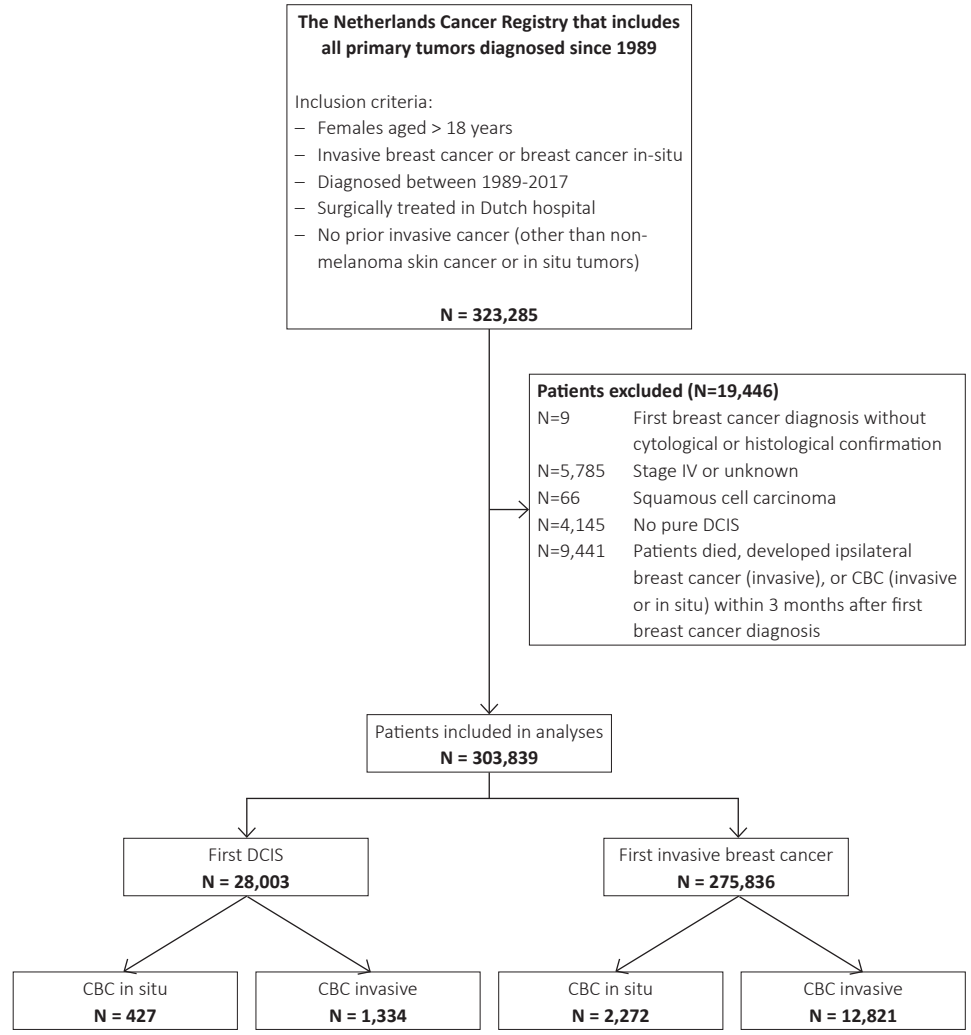
Supplementary Figure 2. Cumulative incidence of death in patients diagnosed with ductal carcinoma in situ, invasive breast cancer stage I-III, and stage I breast cancer without (neo)adjuvant systemic therapy

The x-axis represents the time since the first breast cancer diagnosis (in years). The y-axis represents the cumulative incidence of death. Abbreviations: DCIS = ductal carcinoma in situ; BC = breast cancer



Supplementary Figure 3. Cumulative incidence of invasive ipsilateral breast cancer in patients diagnosed with ductal carcinoma in situ, invasive breast cancer stage I-III, and stage I breast cancer without (neo) adjuvant systemic therapy

The x-axis represents the time since the first breast cancer diagnosis (in years). The y-axis represents the cumulative incidence of invasive ipsilateral breast cancer. Abbreviations: DCIS = ductal carcinoma in situ; BC = breast cancer



Supplementary Figure 4. Study flowchart

Abbreviations: DCIS = ductal carcinoma in situ; CBC = contralateral breast cancer

Supplementary Methods

Multiple imputation of missing values

The predictors for contralateral breast cancer with missing values among patients diagnosed with ductal carcinoma in situ (DCIS) were type of surgery to the breast (3.7%) and tumour grade (17.0%).

We used five imputed datasets based on the multiple imputation chained equations (MICE) using 50 iterations. The visit sequence of the variables was in ascending order of the number of missing values. This technique improves the accuracy and the statistical power assuming missing is at random (MAR)[1]. In the imputation procedure, we also used the year of DCIS diagnosis since this information provides a better correlation structure among covariates used as predictors in the imputation model. Continuous, binary and multiple categorical variables were imputed using predictive mean matching, binary and multinomial logistic regression, respectively. Time-to-event outcome defined as time to contralateral breast cancer, time to death, and time to ipsilateral breast cancer were included in the imputation process through the Nelson-Aalen cumulative hazard estimator[2]. For every variable with missing data, every imputation model selects predictors based on correlation structure underlying the data.

We used the R package mice (version 3.6.0) to impute our data and combine the estimates using Rubin's rules.

Supplementary References

1 Van Buuren, S. *Flexible imputation of missing data*. Second edn, (Chapman and Hall/CRC, 2018).

2 White, I. R. & Royston, P. Imputing missing covariate values for the Cox model. *Stat Med* **28**, 1982-1998, doi:10.1002/sim.3618 (2009).

CHAPTER 5

Preferences for graphical presentation of probabilities in a contralateral breast cancer risk prediction model: an exploratory interview study among breast cancer survivors

Submitted for publication

Iris Kramer
Josephine M.N. Lopes Cardozo
Daniele Giardiello
Hester S.A. Oldenburg
Maartje J. Hooning
Ellen G. Engelhardt*
Marjanka K. Schmidt*

*authors contributed equally

Abstract

Objective

As a first step towards the development of a patient-friendly interface to facilitate clinical implementation of a newly developed contralateral breast cancer (CBC) prediction model to support decision making about contralateral prophylactic mastectomy (CPM), we investigated among breast cancer survivors': (1) preferences for and understanding of graphical presentation of probabilities, (2) which factors are associated with their trust in the risk estimates, and (3) which factors play a role in decision about CPM.

Methods

Semi-structured interviews were conducted with 19 breast cancer survivors. Two researchers independently coded the interview transcripts to identify themes. Discrepancies were resolved using consensus.

Results

Almost all participants (17/19) found a graphical display of added value, but preferences varied regarding which graphical display format was most clear. The majority of participants (13/19) had moderate to good understanding of all display formats and 14/19 highly trusted the probabilities. Participants (11/19) wished to receive information about epistemic uncertainty (e.g., confidence interval), but only four participants had good understanding of the graphical display format containing this information. High probability of developing CBC and fear of future breast cancer were the factors most frequently mentioned as relevant for decision-making about CPM.

Conclusion

No single graphical display format was preferred by all participants. Incorporating multiple display formats into the CBC tool seems to be the best option to meet the needs of a wide range of women considering CPM. Since women wish to receive information about uncertainty associated with the risk estimates, effective ways to graphically communicate this are needed.

Introduction

Active involvement of patients in decisions regarding their health care is widely advocated and shared decision-making is the preferred style in clinical practice nowadays[1,2]. Providing patients and clinicians with personalized probabilities of outcomes can help them when weighing the pros and cons of treatment options. Prediction models including patient-friendly presentation of probabilities can play a key role in clear risk communication, and thereby, support effective *well-informed* and *shared* decision-making. This is particularly important in the context of medical decisions when from a clinical perspective there is no best choice (i.e., preference-sensitive treatment decisions).

A preference-sensitive medical decision where risk communication can play a major role is the context of contralateral prophylactic mastectomy (CPM) for breast cancer patients who are worried about developing breast cancer again in their other (contralateral) breast. Even though the incidence of contralateral breast cancer (CBC) is low in the general breast cancer population (~0.4% per year)[3-5], an increasing number of patients with unilateral breast cancer opt for a CPM, even when they are at low risk[5,6]. One of the most important reasons why patients opt for CPM is the fear of getting breast cancer again[7]. CPM significantly reduces the risk of CBC, but the procedure is drastic, irreversible and can negatively impact women's long-term quality of life[8]. Accurate individualized information about the actual CBC risk is lacking, and e.g., in the Netherlands, CPM is mainly indicated for breast cancer patients carrying a *BRCA1/2* mutation[9], since these women experience high 10-year CBC risks of ~10-20%[10,11].

To support physicians' and patients' decisions about CPM, we recently developed and validated a CBC risk prediction model (PredictCBC) which provides 5- and 10-year individualized probabilities of developing CBC[4]. To make a prediction model useful in clinical practice, the model should be incorporated into a decision support tool, which is not yet available in current practice. Such a tool can help to better identify women at high risk of CBC who may benefit from a CPM, while the estimates can also be used to reassure patients who are at low risk of developing CBC.

It is, however, challenging to effectively communicate probabilistic information. Only a small proportion of people have skills that correspond to minimum statistical literacy in health[12,13]. Literature showed that graphics, e.g., pictographs and bar charts, can improve patients' understanding of probabilistic information[14]. Moreover, patients appear to have a more accurate understanding of risk if probabilistic information is presented as absolute risks (e.g., 10%) rather than verbal labels (e.g., 'a high chance'), and particularly when the information is tailored[14-16]. Whether absolute risks should include a range representing epistemic uncertainty (e.g., 5-15%) is still under debate[17-19]. Conveying the randomness of future outcomes (i.e., aleatory uncertainty) to patients seems to be done more easily by clinicians, and patients generally do not seem to struggle with this as much as they do with epistemic uncertainty[18]. Currently, little is

known about how this can best be communicated[17-19].

Even though many prediction models have been and continue to be developed, very few have been implemented in clinical practice. One of the reasons for this is that they often lack patient-friendly interfaces to facilitate their use during doctor-patient consultations. Moreover, most research on risk communication is performed among healthy participants (e.g., students)[14], and not among patients. For successful implementation of a decision tool, it is important to test the interface within the target end-users. As a first step towards development of a CBC prediction tool that can help clinicians to communicate probabilities to patients, the main aim of this exploratory interview study was to get insights into breast cancer survivors' (i.e., potential end-users) preferences for the graphical presentation of the probabilities, including the epistemic uncertainty, provided by the model. Secondary aims were to evaluate which factors are associated with participants' level of trust in the risk estimates provided, participants' understanding of different graphical display formats, and which factors (in particular probabilities) would play a role in participants' decision on whether to undergo a CPM.

Methods

Design

Study population

Female breast cancer survivors aged ≥18 years were eligible to participate if their invasive breast cancer diagnosis was at least one year prior to the interview (range 2-38 years) and they did not have bilateral breast cancer at primary diagnosis. We chose to exclude women with bilateral breast cancer at primary diagnosis, as these women were no longer at risk of developing CBC during follow-up, and therefore, did not have to consider the prophylactic removal of the contralateral breast. Breast cancer survivors were recruited between March and May 2020 via three different networks; 1) a patient advisory group from the Dutch Breast Cancer Research Group (BOOG), 2) the Dutch Breast Cancer Society, and 3) a breast cancer panel from the Antoni van Leeuwenhoek hospital (AVL; a Dutch cancer hospital). From the applications, we tried to select a heterogeneous sample of participants, i.e., patients with low and high risk of developing CBC (e.g., *BRCA1/2* carriers and Hodgkin lymphoma survivors), a wide age range, and both women who did and those who did not undergo a CPM. We selected a heterogeneous sample to capture the diversity of the population of breast cancer survivors. We did not put any restriction on time since primary breast cancer diagnosis, as women diagnosed long ago have had more time to process and reflect on their breast cancer (treatment) trajectory and can provide input on what is important in the long-term. The Netherlands Cancer Institute-AVL review board approved the study protocol.

Contralateral risk prediction model (PredictCBC)

The PredictCBC risk prediction model quantifies the probability of developing a CBC during follow-up[4]. The model provides individualized estimates of 5- and 10-year CBC probabilities based on patient, primary tumor and treatment (received for the primary tumor) characteristics, and *BRCA1/2* germline mutation status. The PredictCBC model shows an area under the curve (AUC) of 0.63 (95% prediction interval at 5 years, 0.52-0.74; at 10 years, 0.53-0.72)[4].

Graphical display formats

We created five different display formats of the 10-year CBC probability based on formats described in the literature and discussions with experts (Figure 1). All display formats were based on an example patient who had a probability of developing CBC within 10 years after the primary diagnosis of 4% (average risk in the general breast cancer population[3-5]). The probability was visualized using I) text only, II) horizontal bar chart, III) pictograph including graphical representation of randomness, IV) pictograph including epistemic uncertainty by showing the confidence interval around the point estimate, as was described by Raphael et al[17], and V) vertical bar chart including reference lines depicting average risk of the general breast cancer population and *BRCA1/2* mutation carriers. All graphical display formats also included textual explanation of the probabilities, both positively and negatively framed (Figure 1).

Procedures and measures

Interviews

We carried out semi-structured interviews using a video connection (due to the COVID-19 outbreak) after participants electronically provided informed consent. The interviews (Supplementary Information A) were conducted by a research clinician (JMNLC) and took on average 45 minutes (range 34-66 min). The research protocol was developed by two researchers (IK and EGE) based on available literature regarding risk communication principles and input from clinicians. We did not include a patient representative in the development phase as the main aim of this study was to get the perspective of a diverse sample of patients. The interviewer used display format I (Figure 1) to explain the purpose of the model and which factors were included to quantify the probability of developing CBC. The participants were then asked to indicate how much trust they had in the probability provided on a 6-point Likert-type scale, ranging from *no trust at all* to *full trust*, and were asked to elaborate on their answer. Next, participants were shown each of the graphical display formats (display formats II-V, Figure 1) and asked to describe in their own words what the display format depicted (*"Could you explain in your own words what the chances are for this (example) patient to develop breast cancer in the other (tumor-free) breast?"*). Participants were encouraged to verbalize which aspects they liked and which aspects they disliked,

reasons for their preferences, and any changes they would make to improve the display format. Finally, to evaluate which factors, and in particular probabilities, would play a role in participants' decision to undergo a CPM, participants were asked (using an open-ended question) to indicate which factors would play an important role in their decision on whether to undergo a CPM.

Questionnaire

After the interview, all participants completed an electronic questionnaire assessing background information, such as age, educational level, genetic testing, and subjective numeracy (i.e., their ability to use mathematics in everyday life) (Supplementary Information B). We used the Ability subscale from the Subjective Numeracy Scale developed by Fagerlin et al.[20], rated on 6-point Likert-type scales ranging from *not at all good* to *extremely good*. Finally, to better understand whether probabilities play a role in decision-making we asked participants to indicate at what minimum level of risk to develop CBC they would choose to undergo CPM.

Coding and analyses

The interviews were transcribed verbatim. To identify and score the themes that came up during the interviews, an initial codebook was developed by two researchers (IK and EGE) based on three interviews. All interviews were then independently coded by the same two researchers. Items that were coded included factors associated with participants' level of trust, understanding of the graphical display formats, wishes regarding adjustments to display formats, and factors influencing CPM decision. Understanding of the graphical display formats of the participants was scored as 'good', 'moderate', or 'bad' based on the impression of the two researchers (IK and EGE). To score understanding, we looked at whether participants could correctly explain the probabilities visualized in the display formats in their own words *and* if they understood the different aspects of the display format (e.g., for display format V (Figure 1) if they understood the reference lines for *BRCA1/2* carriers and the general breast cancer population). All transcripts were double coded. Discrepancies in coding were resolved through consensus and new codes were added to the initial codebook as encountered. Finally, the two researchers grouped the categories into overarching domains for presentation purposes. All findings and codes were shared and discussed in the project team.

Results

We included breast cancer survivors until we achieved saturation (N=19). Table 1 shows the characteristics of the breast cancer survivors who were interviewed. Mean age was 50 years (range 25-72) at primary breast cancer diagnosis and 59 years (range 34-76) at date of interview. Thirteen of the 19 participants were highly educated, and in general, participants had high confidence in their ability to perform mathematical tasks. Twelve participants underwent breast conserving surgery for their primary breast cancer and three participants had undergone a CPM. The participants who had undergone a CPM were younger than 45 years at primary breast cancer diagnosis, and two of them had been diagnosed with Hodgkin's disease prior to their breast cancer diagnosis, for which they received (mantle field) radiation therapy (i.e., radiation was delivered to a large area including the breasts). The participants without CPM (N=16) indicated that removal of the other breast was not discussed as an option during consultations on their primary breast cancer.

Table 1. Characteristics of participating breast cancer survivors (N=19)

	Number of participants (%)[a]
Personal characteristics	
Mean age in years at interview (range)	59 (34-76)
Education	
Low	2 (11)
Intermediate	4 (21)
High	13 (68)
Breast cancer risk gene testing result at clinical genetic center[b]	
Not tested	5 (26)
Positive	1[c] (5)
Negative	12 (63)
Unknown whether testing has been performed	1 (5)
Non-breast cancer diagnosis prior to primary breast cancer diagnosis[d]	7 (37)
Subjective numeracy[e] (1=not at all good, 6=extremely good), median (range)	
How good are you at working with fractions?	3 (1-6)
How good are you at working with percentages?	5 (1-6)
How good are you at calculating a 15% tip?	5 (1-6)
How good are you at figuring out how much a trouser will cost if it is 25% off?	6 (3-6)
Primary breast cancer and treatment characteristics	
Mean age in years at breast cancer diagnosis (range)	50 (29-72)
TNM stage[f]	
I	4 (21)
II	7 (37)
III	8 (42)
Surgery	
Mastectomy	7 (37)
Breast conserving surgery	12 (63)
Radiotherapy	13 (68)
Chemotherapy	12 (63)
Endocrine therapy	6 (32)
Trastuzumab	3 (16)

[a] May not total 100% because of rounding
[b] The participants were asked to indicate if they were tested for any germline mutation (*BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, etc.)
[c] *BRCA2* carrier
[d] Hodgkin's lymphoma (N=2), basal cell carcinoma (N=2), cervical cancer and anal cancer (N=1), endometrial carcinoma in situ (N=1), oral cancer (N=1)

[e] We used the Ability subscale from the Subjective Numeracy Scale proposed by Fagerlin et al.[20]. In these questions, participants were asked to assess their perceived numerical ability in different contexts. Higher scores denote greater belief in own ability to use mathematics in everyday life

[f] TNM staging source: Brierley JD, Gospodarowicz MK, Wittekind C. TNM Classification of Malignant Tumours. 8th ed. West-Sussex: Wiley-Blackwell; 2017:272

Preferences for model layout and inclusion of information on uncertainty

During the interview, the participants were able to give their opinion on the visualization of probability in the different display formats. Five participants felt that, in all display formats shown, there was too much emphasis on the group of women who do develop a CBC. They mentioned they would have preferred more emphasis on the group of women who do not develop CBC, as a reassuring message, for example by using a more pronounced/vibrant color for that group. One participant said:

> *"Well, especially in this case, it is of course 96% [chance] to remain free of cancer, and that is quite a positive message. But, by making it very light gray, [the positive message] falls away and highlights especially those cases that do develop [breast cancer in the other breast]."*

In display format III and IV, CBC probabilities were visualized using pictographs (Figure 1). Six participants liked the fact that in display format III the female icons were randomly scattered throughout the array. Conversely, five participants preferred a sequential arrangement of the icons (display format IV), mainly because they found the random arrangement messier and more confusing. Some participants indicated that it would help to explicitly mention that the icons are randomly distributed because of chance. The confusion that arose from the random arrangement is illustrated by this quote:

> *"Well, let's see. Yeah, well, I wonder why, uh, those 4 women are … those green women. Why is one on the 3rd row and the other on the 5th row and the other on the 7th row and the other on the last row? I wonder what's the reason or, ...? [interviewer explains why icons are randomly distributed and checks if participant understands this] Well, I would add [to the display format] that… what the meaning is of the place where those women are put. Otherwise, I would think maybe, maybe uh uh, well maybe one is in the 3rd year [of follow-up] and the other in the 5th year [of follow-up] and the other, well… I want to give it a meaning right away and that [the meaning I give it] would not be that it is just randomness. So, it gets it [a wrong interpretation] then... And when you say that they are placed like that to show that it is random, you think, oh yes…"*

Of the 19 participants, 11 thought it was important to show the epistemic uncertainty, as was visualized in display format IV where a confidence interval was shown around the point estimate (Figure 1). However, nine participants mentioned that they did not like the way the confidence interval was currently visualized. They found only coloring in part of the female icons step by step confusing. For example, some participants mentioned *"It is not about getting cancer in your legs, as it looks now".* Six patients mentioned that they would have preferred a fading color to indicate the confidence interval of the icons rather than only coloring in part of the female icons step by step.

In display format V (Figure 1), the CBC probability was visualized using a bar chart including reference lines showing the average CBC risk in the general breast cancer population and in *BRCA1/2* mutation carriers. Fourteen participants mentioned they did not value the reference lines for *BRCA1/2* mutation carriers, since they felt this information was not of added value and/or the dotted lines made the graphical display messier. Ten participants found that the reference line for the general breast cancer population was not of added value either. One participant said:

> *"I think this [display format] contains too much information. It says, 'general breast cancer population', but this lady is not general. She wants to know what her personal risk is. So it should state 'your risk is...'. And if she is not a BRCA1 or BRCA2 mutation carrier… I would not mention it. This [information] is not useful for her."*

Finally, participants were asked to select the display format they most liked. Seventeen of the 19 participants indicated that a graphical display of the probabilities was of added value. Participants had varying preferences and not one graphical display format was clearly preferred. However, 10 participants preferred a bar chart, specifically when oriented vertically.

Trust in risk estimation

Median score on trust in the probability provided by the CBC model was 5 (SD=0.99) based on a 6-point Likert-type scale ranging from *no trust at all* to *full trust* (Figure 2). Eleven participants mentioned that having trust in science in general and/or trusting that the scientific foundation of the predictions is sound were important factors that increase their trust in the model probability (Table 2). The majority of participants mentioned that they would not be able to give a score of six as it is impossible to have 100% certainty; there is always the possibility that you are the unlucky person who does develop a CBC. Five participants had the perception that not all relevant factors were included in the prediction model, which made them score low on trust in the probability (Table 2). Factors they missed in the current model included information on *CHEK2* c.1100del mutation, detailed information about adjuvant treatment (e.g., which type of chemotherapy), number of positive lymph nodes, and the MammaPrint (70-gene signature).

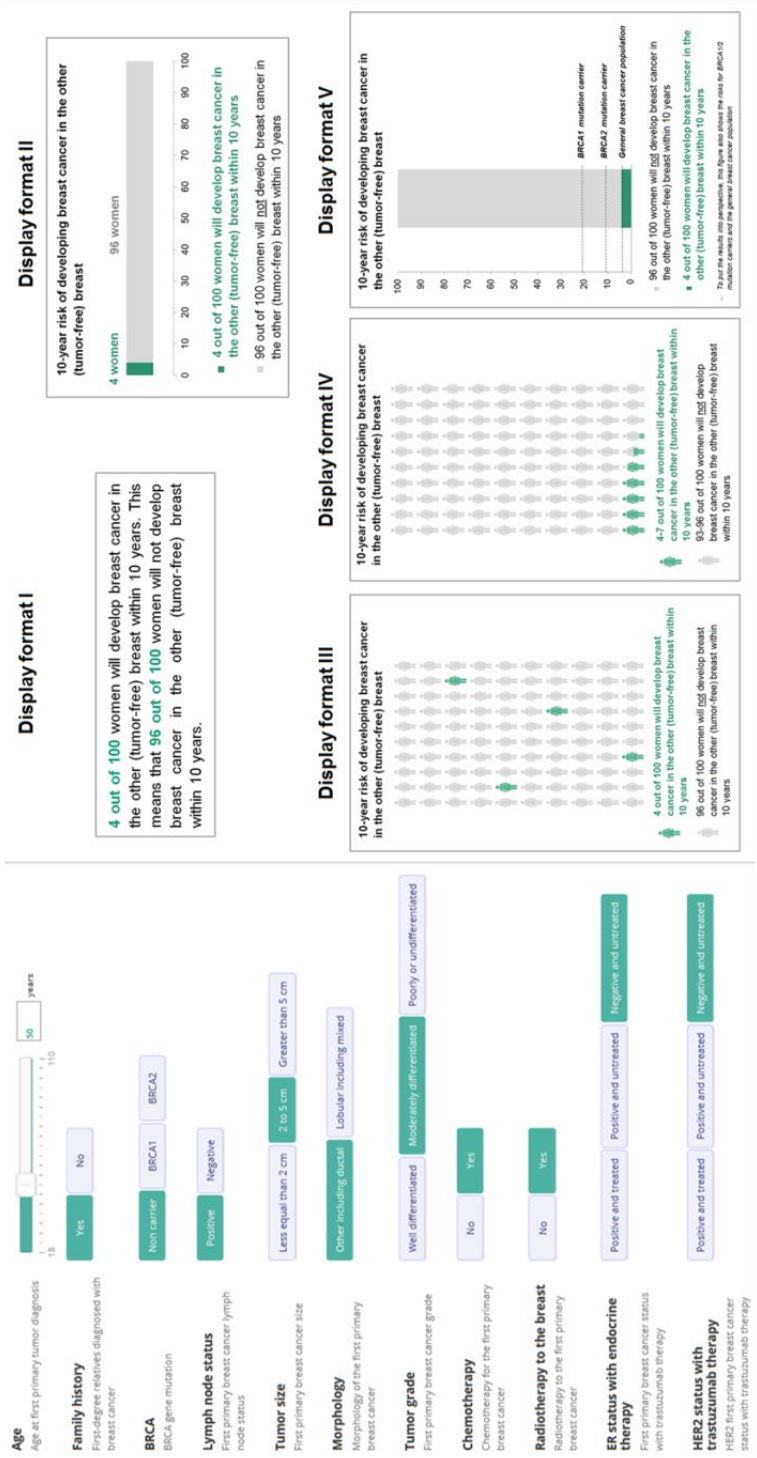


Figure 1. Overview of the display formats of the contralateral risk prediction model (PredictCBC) shown during the interview[a]
Display format 1 – text only
Display format 2 – horizontal bar chart
Display format 3 – pictograph including graphical representation of randomness
Display format 4 – pictograph including epistemic uncertainty by showing the confidence interval around the point estimate as was similar to Raphael et al.[17]
Display format 5 – vertical bar chart including reference lines depicting average risk of the general breast cancer population and *BRCA1* and *BRCA2* mutation carriers
[a] For the purpose of this study, our CBC risk prediction model was implemented in an online tool using the Evidencio platform (https://www.evidencio.com/home)

Table 2. Factors mentioned by the participating breast cancer survivors that influence trust in probabilities provided by the contralateral risk prediction model (PredictCBC)

Mentioned factors[a]	Frequency[b]	Example quotations
Factors that increase trust in the probability shown		
Trust in science and/or scientific foundation of prediction	11	"If I would have known that this [calculation of probability] is based on a very large dataset… Yes, then I would have more trust."
Perception that all relevant factors are included in risk prediction model	3	
Factors that decrease trust in the probability shown		"I cannot fully trust it, but I think that is true for many cancer patients. There will never be complete trust."
You can never be 100% sure/you could always be the unlucky person that experiences the outcome	13	
Perception that not all relevant factors are included in the risk prediction model	5	"What I am actually missing here is [results from] the pathological examination. Because it seems to me that with the pathological examination you should also be able to make a certain prediction. So that makes this incomplete, right?"
Perception that included factors do not discriminate sufficiently between high and low risk	3	

[a] Factors were listed that were mentioned by at least two participants
[b] Rows do not add up to the number of participants (N=19) because some answers contained multiple factors

Understanding of the graphical display formats

We observed that the majority of participants had good understanding of display formats II (horizontal bar chart), III (pictograph including graphical representation of randomness), and V (vertical bar chart including reference lines depicting average risk for other populations) (Figure 3). We found that the participants generally seemed to have difficulty understanding display format IV (pictograph including epistemic uncertainty by showing the confidence interval around the point estimate). Out of the 19 participants, 14 scored moderate on understanding of display format IV and one had poor understanding (Figure 3).

Factors influencing CPM decision

Figure 4 shows factors mentioned by the participants that would influence their decision on whether to undergo a CPM and quotes to illustrate this. Almost all participants (N=18) mentioned that they would choose to have their other (tumor-free) breast removed if the probability of developing CBC was high. In the post-interview survey seven participants (out of 14 participants who answered this question) indicated that the 10-year probability of developing a CBC had to be minimally 10% for them to choose to undergo a CPM. Other factors that were repeatedly mentioned were the reduction of fear of future breast cancer, being a *BRCA1/2* mutation carrier, and the desire to achieve breast symmetry (if the breast of the primary breast cancer is removed).

An important factor for not opting to undergo a CPM was to avoid side-effects of prophylactic surgery (Figure 4). Some participants mentioned that they considered CPM unnecessary as long as they received follow-up check-ups for their primary breast cancer, including mammography of the other breast. Other factors were the negative impact on femininity or body image, or that a breast is a cherished part of the body and/or plays a role in sexuality.

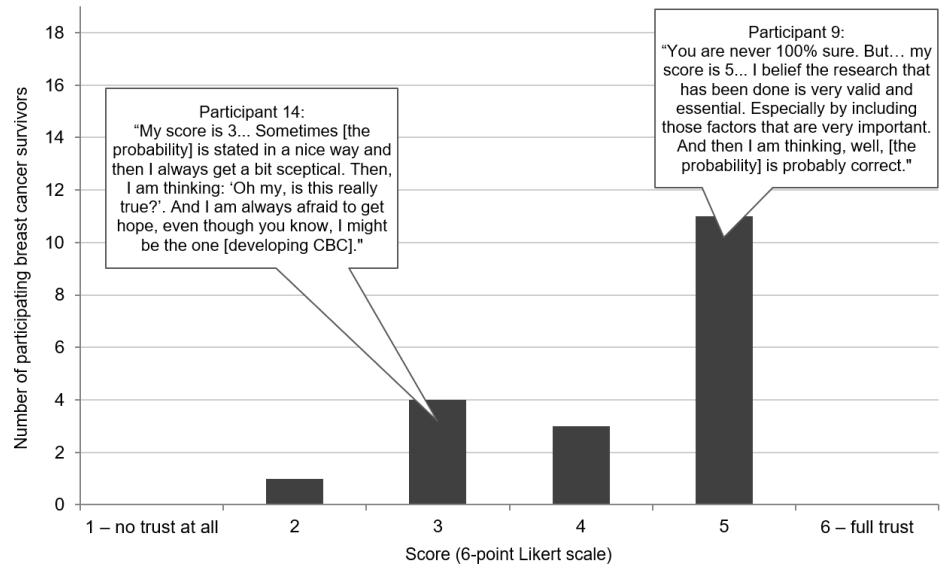
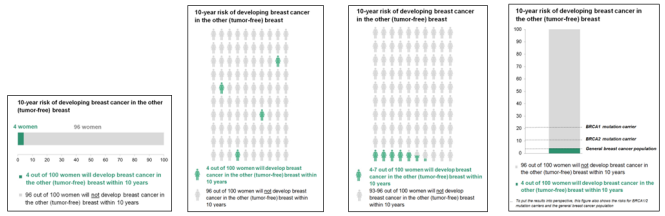


Figure 2. Trust in the probabilities provided by the contralateral risk prediction model (PredictCBC) indicated by the participating breast cancer survivors (N=19) on a 6-point Likert-type scale
Abbreviations: CBC = contralateral breast cancer



	Display format II	Display format III	Display format IV	Display format V
Subjective understanding	N participants (%)	N participants (%)	N participants (%)	N participants (%)
Good	13 (68)	15 (79)	4 (21)	11 (58)
Moderate	0 (0)	3 (16)	14 (74)	5 (26)
Bad	5 (26)	1 (5)	1 (5)	2 (11)
Not clear from answer	1 (5)	0 (0)	0 (0)	1 (5)

Figure 3. Rater's scoring of understanding of the different graphical display formats of the contralateral risk prediction model's (PredictCBC) 10-year probability of developing contralateral breast cancer
To score understanding, we looked at whether participants could correctly explain the probabilities visualized in the display formats in their own words *and* if they understood the different aspects of the display format (e.g., for display format V if they understood the reference lines for *BRCA1/2* carriers and the general breast cancer population)

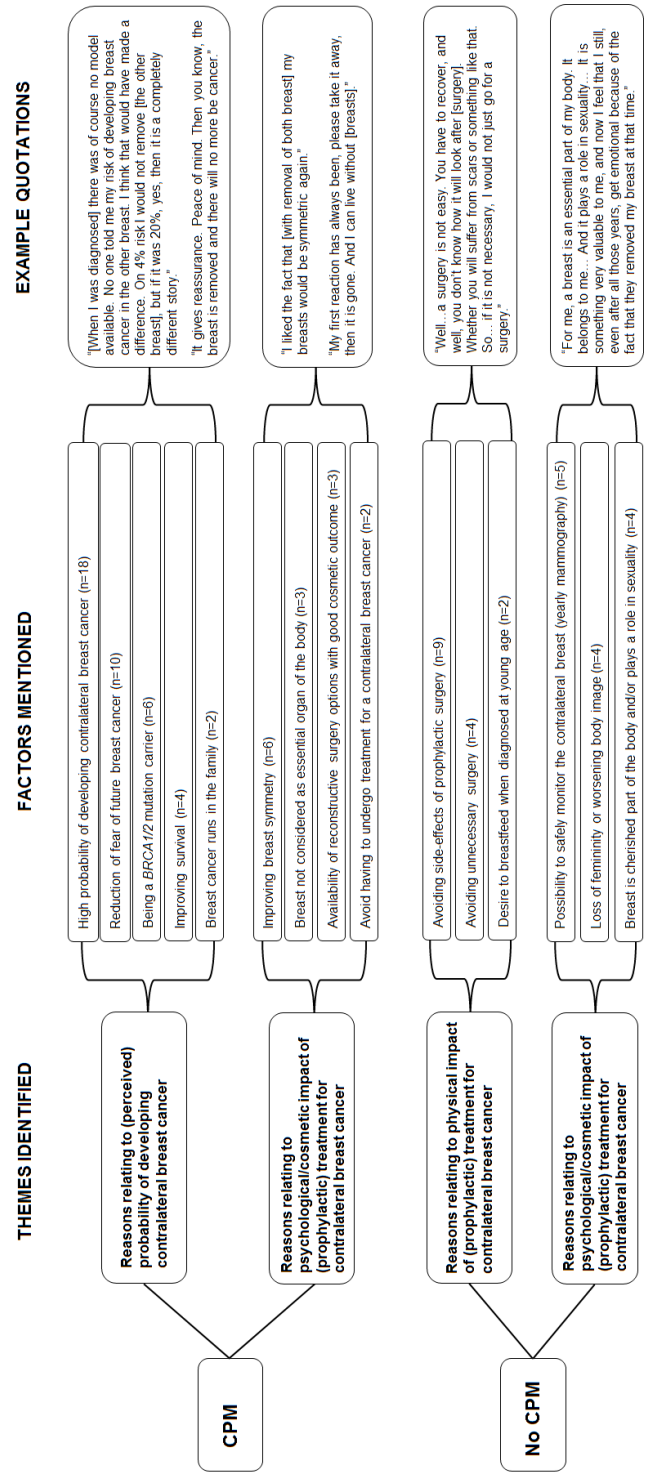


Figure 4. Important factors influencing the participating breast cancer survivors' decision to undergo a contralateral prophylactic mastectomy
Abbreviations: CPM = contralateral prophylactic mastectomy
Factors that were listed in this figure were mentioned by at least two participants. Rows do not add up to the number of participants (N=19) because some answers provided by participants contained multiple factors

Discussion

As a first step towards the development of a CBC prediction tool that can help clinicians to communicate probabilities to patients, the main aim of this exploratory interview study was to get insights into breast cancer survivors' (i.e., potential end-users) preferences for the graphical presentation of the probabilities, including the epistemic uncertainty, provided by the model. Participants in this study preferred graphics to show probabilities in a CBC risk prediction model, but they had varying preferences regarding the type of graphical representation. It is reassuring that participants had high levels of trust in the probabilities shown, which indicates that a CBC risk prediction model can be of added value in helping patients to make decisions. Moreover, probabilities seem to play an important role in decision-making about CPM, as we found that having a high probability of developing a CBC as well as fear of future breast cancer were the factors most frequently mentioned by participants' as relevant for their decision-making. Interestingly, the majority thought it was important to show the epistemic uncertainty associated with risk estimates. However, including the epistemic uncertainty also seems to have its drawback, as only four participants had good understanding of the graphical display format containing this information.

Our findings are in line with previous research showing that textual risk communication is better understood in combination with graphical formats[21-24]. In the literature, no consensus has been reached yet on the optimal graphical format for presenting a single probability. This is reflected in our study where participants had varying preferences, and no display format was a clear favorite. However, a substantial proportion of participants preferred a bar chart, specifically when oriented vertically. The preference for a vertical orientation is in line with some previous studies that showed that vertical graphs were processed slightly faster than horizontal graphs[25-27].

Some studies recommend pictographs as the optimal format to communicate probabilistic information to patients, especially for patients with low numeracy[23,28-30]. They argue that pictographs improve patients understanding of probabilities as they better represent the part-to-whole relationship[23] and they are easier to identify with than bar charts[31,32]. In our study, participants had slightly better understanding of the display format including a pictograph (display format III) compared with a bar chart (display format II). However, the improvement in understanding could potentially be explained by a learning curve, as participants become more familiar with the concept of risk prediction by viewing multiple display formats. Participants had varying preferences between a random and sequential arrangement of the cases in the pictograph. Randomly arranged pictographs have the benefit that they convey the difficult concept of randomness[32], so they are in a way more realistic, but they are generally perceived as more difficult to understand[30]. In our study, participants indicated that when using random arrangement, some additional information on the explanation of the randomness may be a solution to overcome confusion.

In the display format including a vertical bar chart (display format V), we also included reference lines depicting average risk of the general breast cancer population and *BRCA1/2* mutation carriers. The intent was to help patients put their risk in perspective. However, our results suggested that it would be better to leave out these reference lines, as the majority of the participants thought these were not of added value and made the display messier. Moreover, model understanding was slightly worse for display format V compared to display format II (horizontal bar chart) and III (pictograph including graphical representation of randomness). This is in line with the growing evidence that "less is more" in the field of decision-making[33]. For example, a recently published systematic review that evaluated the effect of different ways of communicating treatment risks and benefits to cancer patients, showed that limiting the amount of information in a graphical display improved patients' understanding[34].

In the literature, there is an ongoing debate about whether epistemic uncertainty should be communicated to patients, and if so, how this should be visualized by risk prediction models and in decision aids[17,18]. In current practice, epistemic uncertainty is rarely explicitly communicated[18,19]. In our study, more than half of the participants thought it is important to show epistemic uncertainty, since this information is "more true" and complete. However, the participants seemed to struggle with information about epistemic uncertainty, as understanding of the graphical display format containing the confidence interval was worse. Many participants pointed out they did not like the way the confidence interval was currently visualized (stepwise coloring in part of the female icons) and they recommended a fading color. This is in contrast with another study in the Dutch breast cancer survivor population[17], where the stepwise coloring came out as best format. Future studies should investigate the best way to communicate epistemic uncertainty to patients.

The results of our study indicate that the probability of developing CBC and fear of future breast cancer play an important role in participants' decision on whether to undergo a CPM. This is in line with a systematic review on patient reported and psychological factors influencing the decision on CPM[7]. Our finding highlights that at least some patients have a need for personalized CBC risks. Indeed, we are careful and hesitant in extrapolating our findings to *all* breast cancer patients since our study included a selected group of breast cancer survivors due to the invitation approach and our sampling to achieve a heterogeneous group of participants. The majority of the participants was highly educated, had high confidence in their ability to perform mathematical tasks, and some may have been more actively involved with research than the general breast cancer population. Another limitation is the potential learning curve that participants may have developed by viewing several display formats during the interview. In addition, since this was an exploratory interview study, future large-scale experimental studies are needed to investigate how to effectively design the interface

for a risk prediction tool that meets the diverse needs of end users, and to investigate differences in preferences between subgroups of women. The main strength of this study is that we performed the interviews within the target end-users, breast cancer survivors for whom decision-making about CPM is relevant at different time-points in their survivorship (time since primary breast cancer diagnosis ranged from 2-38 years). As a next step, healthcare professionals' preferences for the CBC model interface should also be investigated as they play a key role in implementation of the model in clinical practice.

In conclusion, our study provided valuable information on preferences for graphical presentation of probability and uncertainty in a CBC prediction model. Graphical components are important to explain probabilities, but there is no single best method for communication of probabilities to patients. Any tool intended for use with patients' needs to allow flexibility in display format (e.g., as done in the frequently used PREDICT prognostication tool [35]). Our study showed that participants valued information on epistemic uncertainty, but future studies are needed to investigate the best way to effectively communicate this type of information. As the probability of developing CBC plays an important role in the participants' decision to undergo a CPM, it is important to carefully design and test the risk prediction model interface prior to implementation. Finding better ways to communicate probabilities will result in better understanding and consequently improve the quality of health decisions and outcomes such as decision regret.

Article Information

Funding

This work was supported by a grant from Alpe d'HuZes/KWF Kankerbestrijding (Dutch Cancer Society) grant number A6C/6253 and by the PRECISION project (Cancer Research UK and KWF Kankerbestrijding ref. C38317/A24043).

Conflict of interest

The authors declare that there is no conflict of interest

Author's contributions

MKS and EGE designed the study; JMNLC conducted the interviews; IK and EGE coded the data; IK drafted the first version of the manuscript; all other authors contributed to the interpretation of the results and revisions of the manuscript. All authors approved the final manuscript.

Acknowledgements

We are very grateful to all the breast cancer survivors who participated in this study. We thank the patient advisory group from the Dutch Breast Cancer Research Group (BOOG), the Dutch Breast Cancer Patient Association, and the breast cancer patient panel from the Antoni van Leeuwenhoek hospital for their help with the recruitment of the breast cancer survivors. We thank Manon Verwijs for her help with the transcription of the interviews.

References

1 Naik, G., Ahmed, H. & Edwards, A. G. Communicating risk to patients and the public. *Br. J. Gen. Pract.* **62**, 213-216, doi:10.3399/bjgp12X636236 (2012).

2 Stiggelbout, A. M., Pieterse, A. H. & De Haes, J. C. Shared decision making: Concepts, evidence, and practice. *Patient Educ. Couns.* **98**, 1172-1179, doi:10.1016/j.pec.2015.06.022 (2015).

3 Giardiello, D. *et al.* Contralateral breast cancer risk in patients with ductal carcinoma in situ and invasive breast cancer. *NPJ Breast Cancer* **6**, 60, doi:10.1038/s41523-020-00202-8 (2020).

4 Giardiello, D. *et al.* Prediction and clinical utility of a contralateral breast cancer risk model. *Breast Cancer Res.* **21**, 144, doi:10.1186/s13058-019-1221-1 (2019).

5 Xiong, Z. *et al.* Patterns of Occurrence and Outcomes of Contralateral Breast Cancer: Analysis of SEER Data. *J Clin Med* **7**, doi:10.3390/jcm7060133 (2018).

6 Wong, S. M. *et al.* Growing Use of Contralateral Prophylactic Mastectomy Despite no Improvement in Long-term Survival for Invasive Breast Cancer. *Ann. Surg.* **265**, 581-589, doi:10.1097/sla.0000000000001698 (2017).

7 Ager, B., Butow, P., Jansen, J., Phillips, K. A. & Porter, D. Contralateral prophylactic mastectomy (CPM): A systematic review of patient reported factors and psychological predictors influencing choice and satisfaction. *Breast* **28**, 107-120, doi:10.1016/j.breast.2016.04.005 (2016).

8 Carbine, N. E., Lostumbo, L., Wallace, J. & Ko, H. Risk-reducing mastectomy for the prevention of primary breast cancer. *Cochrane Database Syst. Rev.* **4**, Cd002748, doi:10.1002/14651858.CD002748.pub4 (2018).

9 Nederland, N. B. O. (2018).

10 van den Broek, A. J. *et al.* Impact of Age at Primary Breast Cancer on Contralateral Breast Cancer Risk in BRCA1/2 Mutation Carriers. *J. Clin. Oncol.* **34**, 409-418, doi:10.1200/jco.2015.62.3942 (2016).

11 Kuchenbaecker, K. B. *et al.* Evaluation of Polygenic Risk Scores for Breast and Ovarian Cancer Risk Prediction in BRCA1 and BRCA2 Mutation Carriers. *J. Natl. Cancer Inst.* **109**, doi:10.1093/jnci/djw302 (2017).

12 Reyna, V. F. & Brainerd, C. J. The importance of mathematics in health and human judgment: Numeracy, risk communication, and medical decision making. *Learning and Individual Differences* **17**, 147-159 (2007).

13 Gigerenzer, G., Gaissmaier, W., Kurz-Milcke, E., Schwartz, L. M. & Woloshin, S. Helping Doctors and Patients Make Sense of Health Statistics. *Psychol. Sci. Public Interest* **8**, 53-96, doi:10.1111/j.1539-6053.2008.00033.x (2007).

14 Zipkin, D. A. *et al.* Evidence-based risk communication: a systematic review. *Ann. Intern. Med.* **161**, 270-280, doi:10.7326/m14-0295 (2014).

15 Trevena, L. J., Davey, H. M., Barratt, A., Butow, P. & Caldwell, P. A systematic review on communicating with patients about evidence. *J. Eval. Clin. Pract.* **12**, 13-23, doi:10.1111/j.1365-2753.2005.00596.x (2006).

16 Büchter, R. B., Fechtelpeter, D., Knelangen, M., Ehrlich, M. & Waltering, A. Words or numbers? Communicating risk of adverse effects in written consumer health information: a systematic review and meta-analysis. *BMC Med. Inform. Decis. Mak.* **14**, 76, doi:10.1186/1472-6947-14-76 (2014).

17 Raphael, D. B. *et al.* Risk communication in a patient decision aid for radiotherapy in breast cancer: How to deal with uncertainty? *Breast* **51**, 105-113, doi:10.1016/j.breast.2020.04.001 (2020).

18 Engelhardt, E. G. *et al.* Disclosing the Uncertainty Associated with Prognostic Estimates in Breast Cancer. *Med. Decis. Making* **37**, 179-192, doi:10.1177/0272989x16670639 (2017).

19 Han, P. K. J. *et al.* Uncertainty in health care: Towards a more systematic program of research. *Patient Educ. Couns.* **102**, 1756-1766, doi:10.1016/j.pec.2019.06.012 (2019).

20 Fagerlin, A. *et al.* Measuring numeracy without a math test: development of the Subjective Numeracy Scale. *Med. Decis. Making* **27**, 672-680, doi:10.1177/0272989x07304449 (2007).

21 Waters, E. A., Weinstein, N. D., Colditz, G. A. & Emmons, K. Formats for improving risk communication in medical tradeoff decisions. *J Health Commun* **11**, 167-182, doi:10.1080/10810730500526695 (2006).

22 Lipkus, I. M. Numeric, verbal, and visual formats of conveying health risks: suggested best practices and future recommendations. *Med. Decis. Making* **27**, 696-713, doi:10.1177/0272989x07307271 (2007).

23 Zikmund-Fisher, B. J. *et al.* Communicating side effect risks in a tamoxifen prophylaxis decision aid: the debiasing influence of pictographs. *Patient Educ. Couns.* **73**, 209-214, doi:10.1016/j.pec.2008.05.010 (2008).

24 Klein, K. A., Watson, L., Ash, J. S. & Eden, K. B. Evaluation of risk communication in a mammography patient decision aid. *Patient Educ. Couns.* **99**, 1240-1248, doi:10.1016/j.pec.2016.02.013 (2016).

25 McCaffery, K. J. *et al.* The influence of graphic display format on the interpretations of quantitative risk information among adults with lower education and literacy: a randomized experimental study. *Med. Decis. Making* **32**, 532-544, doi:10.1177/0272989x11424926 (2012).

26 Shah, P. & Hoeffner, J. Review of graph comprehension research: Implications for instruction. *Educ. Psychol. Rev.* **14**, 47-69 (2002).

27 Tversky, B. in *Spatial schemas and abstract thought.* 79-111.

28 Hawley, S. T. *et al.* The impact of the format of graphical presentation on health-related knowledge and treatment choices. *Patient Educ. Couns.* **73**, 448-455, doi:10.1016/j.pec.2008.07.023 (2008).

29 Franklin, L., Plaisant, C. & Shneiderman, B. An information-centric framework for designing patient-centered medical decision aids and risk communication. *AMIA Annu Symp Proc* **2013**, 456-465 (2013).

30 Ancker, J. S., Weber, E. U. & Kukafka, R. Effect of arrangement of stick figures on estimates of proportion in risk graphics. *Med. Decis. Making* **31**, 143-150, doi:10.1177/0272989x10369006 (2011).

31 Schapira, M. M., Nattinger, A. B. & McHorney, C. A. Frequency or probability? A qualitative study of risk communication formats used in health care. *Med. Decis. Making* **21**, 459-467, doi:10.1177/0272989x0102100604 (2001).

32 Ancker, J. S., Senathirajah, Y., Kukafka, R. & Starren, J. B. Design features of graphs in health risk communication: a systematic review. *J. Am. Med. Inform. Assoc.* **13**, 608-618, doi:10.1197/jamia.M2115 (2006).

33 Zikmund-Fisher, B. J., Fagerlin, A. & Ubel, P. A. A demonstration of ''less can be more'' in risk graphics. *Med. Decis. Making* **30**, 661-671, doi:10.1177/0272989x10364244 (2010).

34 van de Water, L. F. *et al.* Communicating treatment risks and benefits to cancer patients: a systematic review of communication methods. *Qual. Life Res.* **29**, 1747-1766, doi:10.1007/s11136-020-02503-8 (2020).

35 Predict: Breast Cancer. Version 2.2, Release 1.14. Available from: https://breast.predict.nhs.uk/tool.

Supplementary Information A - Interview protocol

Introduction

- **Word of welcome:** Thank you for participating in this study. Let me introduce myself. The interview will take about 45 minutes.
- **Explanation goal of the study:** Our research group focuses on women diagnosed with breast cancer. We would like to have a better estimate for every woman diagnosed with breast cancer what the probability is of developing a second breast cancer in the other (tumor-free) breast. We are currently developing a risk prediction model. In general, the probability of developing a second breast cancer in the other (tumor-free) breast is small. The purpose of this interview is to understand what patients think of our model and how we can improve the risk visualization, in order to make the model as clear as possible and patient-friendly. The results of this study may be published in scientific journals. From the text, you will not be identifiable. With your permission I will record the interview. Everything you say will be treated confidentially. After analyzing the information, we will delete the recording. Do you give consent for this?

Background

- Before we start with the questions related to the model, I would like to know how you are doing?
- I would like to ask a few more questions to get some background information.
    - How old were you when you were diagnosed with breast cancer? What is your current age?
    - At what stage was the primary breast cancer diagnosed?
    - What treatments did you receive when you were diagnosed with breast cancer? Did you receive:
        - Breast conserving surgery, mastectomy, or no surgery? Was this conform the doctor's advice?
        - Did you and your doctor discuss the possibility for preventive removal of the other (tumor-free) breast?
        - Did you receive chemotherapy?
        - Did you receive endocrine therapy?
        - Did you receive HER2-specific therapy?
        - Did you receive radiotherapy?
    - Was your primary breast cancer diagnosis the first time that you were diagnosed with cancer? If not, may I ask what diagnoses you have had previously?

Model introduction

- As I just explained, we developed a model to estimate the probability that a woman who has been diagnosed with breast cancer will develop breast cancer again in the other (tumor-free) breast. I will give some more information [shows display format I]. We developed a mathematical model with data from a large group of patients. In this example we use a fictitious patient with an average CBC risk. The model contains factors that influence the risk of a second breast cancer. For example, age at first breast cancer diagnosis, tumor characteristics of the primary tumor, and treatment. These factors can be entered for each patient, which will result in a certain risk estimate.

The end goal is that this model can be used by doctors to inform patients about the risk of developing breast cancer in the other (tumor-free) breast. For example, additional treatment may be provided, preventive removal of the other breast, or the model can be used to reassure women who are at very low risk of developing breast cancer in the other breast. The latter will be applicable to most women.

In this example (display format I), 4 out of 100 women, who have the same characteristics as this fictitious patient, will develop breast cancer in the other (tumor-free) breast within 10 years. This means that 96 out of 100 women do not develop breast cancer in the other (tumor-free) breast. Currently, the model is still under development and therefore, it is not used by doctors yet. First, we would like to investigate how we can improve the risk visualization, to make the risk information provided by the CBC prediction model as clear and patient-friendly as possible. Therefore, I would like to ask you some questions based on some examples of model visualizations.

Model trust

- [Show display format I and explain the information shown, and then show the 6-point Likert-type scale to rate trust] Could you indicate on this 6-point scale, ranging from *no trust at all* to *full trust*, how much trust you have in the risk estimates you just viewed?
- Could you elaborate on the score you have given? What could be said to improve your trust?

5

Different display formats of model

- As I just explained, we would like to investigate what type of risk visualization is most clear and patient-friendly. Therefore, I will show you different display formats of the model and ask a few questions.
- [show display format II-V, one by one, and repeatedly ask the following questions] Could you explain in your own words what the chances are for this (example) patient to develop breast cancer in the other (tumor-free) breast? Do you miss specific information?
- [show overview of display format I-V] You have just viewed five different display formats of the model. What display format do you prefer? And why?

Factors contralateral prophylactic mastectomy

- Finally, I would like to talk about important factors that influence patients' decision to opt for preventive removal of the other (tumor-free) breast. We ask you this question to get an idea about what information is important when making such a decision.
- [for patients without CPM] Imagine that you have the choice to have the other (tumor-free) breast removed preventively. What would be reasons to remove the other (tumor-free) breast? And what would be reasons for not removing the other (tumor-free) breast?
- [for patients with CPM] You have had your other (tumor-free) breast removed preventively. What were the reasons to remove the other breast preventively?

End interview

- These were all my questions. I would like to thank you for this interview. If you have any questions left, please contact me at any time.

Supplementary Information B – Questionnaire

Question 1. What is your current age: _____

Question 2. What is your highest level of education you have completed:

- ☐ Elementary school, primary school
- ☐ Pre-vocational secondary education
- ☐ Secondary vocational education, senior general secondary education, pre-university education
- ☐ University of applied sciences (i.e., higher professional education) or university
- ☐ Other, namely _____

With the following questions we want to get insight into whether you or someone in your family has undergone genetic testing. Genetic testing can be used to find out whether someone has an increased risk of developing cancer due to a genetic predisposition. Genetic predisposition does not automatically mean that someone will get cancer.

Question 3. Have you or someone in your family undergone genetic testing?

- ☐ No (as far as I know)
- ☐ I do not know
- ☐ I would rather not answer this
- ☐ Yes, please tick what is applicable below:
  - ☐ I have undergone genetic testing
  - ☐ Son(s) and/or daughter(s)
  - ☐ Father and/or mother
  - ☐ Brother(s) and/or sister(s)
  - ☐ Uncle(s) and/or aunt(s)
  - ☐ Cousin(s)
  - ☐ Grandfather(s) and/or grandmother(s)

Question 4. Has a genetic mutation been found in yourself or someone in your family?

- ☐ Yes
- ☐ No
- ☐ I do not know

Question 5. Please indicate in the table below in whom a genetic mutation has been found and which mutation was found.

			Which genetic mutation has been found?											
A genetic mutation has been found by:			CDKN2A mutation	APC mutation	*BRCA 1* mutation	*BRCA 2* mutation	*P53* mutation	*CHEK 2* mutation	*PALB 2* mutation	*Other, namely:*	I do not know
Myself	☐ Yes	☐ No	☐	☐	☐	☐		☐	☐		☐
If a genetic mutation has been found in someone in your family, please indicate below in which family member the mutation has been found and which genetic mutation was found.											
Family member			CDKN2A mutation	APC mutation	*BRCA 1* mutation	*BRCA 2* mutation	*P53* mutation	*CHEK 2* mutation	*PALB 2* mutation	*Other, namely:*	I do not know
Example: sister						☑	☐	☐	☐	☐	☐
			☐	☐	☐	☐	☐	☐	☐	☐	☐
			☐	☐	☐	☐	☐	☐	☐	☐	☐
			☐	☐	☐	☐	☐	☐	☐	☐	☐
			☐	☐	☐	☐	☐	☐	☐	☐	☐
			☐	☐	☐	☐	☐	☐	☐	☐	☐
			☐	☐	☐	☐	☐	☐	☐	☐	☐
			☐	☐	☐	☐	☐	☐	☐	☐	☐
			☐	☐	☐	☐	☐	☐	☐	☐	☐

Question 6. For each of the following questions, please check the box that best reflects how good you are at doing the following things[1]:

a. How good are you at working with fractions?

1	2	3	4	5	6
O	O	O	O	O	O
Not at all good					**Extremely good**

b. How good are you at working with percentages?

1	2	3	4	5	6
O	O	O	O	O	O
Not at all good					**Extremely good**

c. How good are you at calculating a 15% tip?

1	2	3	4	5	6
O	O	O	O	O	O
Not at all good					**Extremely good**

d. How good are you at figuring out how much a trouser will cost if it is 25% off?

1	2	3	4	5	6
O	O	O	O	O	O
Not at all good					**Extremely good**

Question 7. At what level of probability of developing breast cancer in the other (tumor-free) breast would you choose to have the other (tumor-free) breast removed preventively?

- **1-2 out of 100 women** will develop breast cancer in the other (tumor-free) breast within 10 years
- **3-5 out of 100 women** will develop breast cancer in the other (tumor-free) breast within 10 years
- **5-10 out of 100 women** will develop breast cancer in the other (tumor-free) breast within 10 years
- **More than 10 out of 100 women** will develop breast cancer in the other (tumor-free) breast within 10 years

Supplemental References

1 Fagerlin, A. *et al.* Measuring numeracy without a math test: development of the Subjective Numeracy Scale. *Med. Decis. Making* **27**, 672-680, doi:10.1177/0272989x07304449 (2007).

5

CHAPTER 6

General discussion

Discussion

Contralateral breast cancer (CBC) is becoming an important public health issue because of the increased incidence of breast cancer and improved survival[1-4]. To improve decision making on risk reducing strategies and follow-up decisions for breast cancer patients, accurate individualized risk prediction is needed to distinguish between patients who are at high or low risk of developing CBC. The main goal of this thesis was to explore risk factors associated with CBC for which there is insufficient evidence in literature. Furthermore, as a first step towards implementation of a risk prediction model, we performed an exploratory interview study to investigate preferences for graphical presentation of probabilities in a CBC risk prediction model. In this concluding chapter we will discuss the main findings of the results presented in this thesis and interpret them in a broader context. The methodological challenges of our studies will be discussed and we will highlight some strengths and limitations. Finally, recommendations for future research and clinical implications are given.

Main findings in context of other literature

Risk factors of CBC

During the last decades, numerous studies have investigated risk factors associated with CBC[5,6]. Based on the same Dutch Cancer Society funded project, and in another PhD trajectory parallel to the work presented in this thesis, a risk prediction model was developed and validated to predict the risk of CBC. Data was included of 132,756 women diagnosed with non-metastatic invasive breast cancer between 1990 and 2013 from multiple studies in Europe, USA, and Australia. All known and available risk factors were included in the model, including patient (age, family history of breast cancer), primary tumor (nodal status, size grade, morphology, ER and HER2 status), and treatment (chemotherapy, endocrine therapy, trastuzumab, radiotherapy) characteristics, and *BRCA1/2* mutation status[7]. The calibration of the model was reasonable and discrimination moderate (area under the curve of 0.63)[7]. To improve risk prediction of CBC it is important to investigate and incorporate additional risk factors, as well as looking into their etiology. In **chapter 2** we investigated the association between a polygenic risk score of 313 common germline variants (PRS_{313}) and CBC risk using data from the large breast cancer series of the Breast Cancer Association Consortium. Previous studies had shown associations between risk of CBC and both a 67-variant PRS[8] and individual variants[9], but not yet with PRS_{313}, the most extensively validated PRS. We observed a clear association between the PRS_{313} and CBC risk, which was not biased by patient characteristics, characteristics of the primary tumor, or adjuvant treatment. The association was, however, weaker (in terms of an odds ratio) than was found for the PRS_{313} and first primary breast cancer[10]. In **chapter 3** we investigated the influence of various adjuvant systemic regimens on, subtype-specific, risk of CBC in a nationwide population-based cohort study of the Netherlands Cancer Registry (NCR). Previous research had shown that patients who received adjuvant endocrine therapy or chemotherapy for their first breast cancer have a reduced risk of developing CBC[11-15], which was confirmed by our study. Not many studies had looked into different regimens of adjuvant chemotherapy and endocrine therapy in relation to CBC risk. In our study, we showed that taxane-containing chemotherapy (compared to other chemotherapy regimens) and aromatase inhibitors (compared to tamoxifen) were associated with the largest CBC risk reduction. Adjuvant trastuzumab in combination with chemotherapy was also associated with a strong CBC risk reduction. Unfortunately, we do not have a biological explanation for the different effects between different chemotherapy regimens and between tamoxifen and aromatase inhibitors. However, our findings are consistent with some other studies[13,16]. Our subtype-specific analyses showed that each adjuvant therapy regimen had a different impact on the CBC subtype distribution. This finding may be clinically relevant, since each receptor subtype includes a different treatment strategy and prognosis.

CBC risk in women diagnosed with ductal carcinoma in situ

Most research on CBC is focusing on patients with first invasive breast cancer. However, individualized CBC prediction may also be important for women with ductal carcinoma in situ (DCIS). In **chapter 4** we estimated the CBC risk in women with DCIS versus women with invasive breast cancer in a population-based cohort study of the NCR. Interestingly, we showed that the risk of developing invasive CBC was higher for women with DCIS compared with invasive breast cancer. The higher risk is likely explained by the risk-reducing effect of (neo)adjuvant systemic therapy among invasive breast cancer patients. Indeed, when we compared CBC risk for women with DCIS to women with stage I not receiving adjuvant systemic therapy, CBC risk was lower for women with DCIS. Based on the results of this study we do not suggest to start treating women with DCIS with adjuvant systemic therapy to prevent CBC as the absolute CBC risk is low.

In our study, we had limited information on biological characteristics of DCIS, e.g. no information on receptor subtypes, and our multivariable model was therefore unable to differentiate CBC risk among women with DCIS. So, based on the clinical information currently available, CBC risk prediction in women with DCIS is insufficiently robust to be clinically actionable. More biological knowledge is needed to improve CBC prediction in women with DCIS. It is, for example, still unclear if CBC has the same etiology in DCIS as in invasive breast cancer. If so, a separate CBC risk prediction model for women with DCIS would be desired.

How to (graphically) present probabilities to patients?

The results of **chapter 2, 3 and 4** provided valuable information to improve the prediction accuracy of the CBC risk prediction model. To make a prediction model useful

in clinical practice, the model should be incorporated into a decision support tool, which is not yet available in current practice. Prediction tools can inform patients on their probability of developing a certain disease and help them with associated decision making. Literature showed, however, that patients and doctors have difficulties in understanding/communicating probabilistic information, and therefore, it is important to carefully investigate how to effectively communicate the probabilities in the risk prediction tool before its implementation. Graphics (bar chart, pictographs, etc.) can facilitate communication and may aid accurate understanding of probabilities[17], but there is no consensus on which type of graphical presentation is most effective. In addition, it is unclear whether the epistemic uncertainty (i.e. statistical uncertainty e.g. indicated by a confidence interval) should be communicated to patients, and if so, how to do this effectively[18].

In **chapter 5** we performed an exploratory interview study among 19 breast cancer survivors (i.e., potential end-users) to get insights into their preferences for the graphical presentation of probabilities, including the epistemic uncertainty. Almost all participants preferred a graphical component supporting textual explanation of probabilities, but they had varying preferences regarding the graphical display formats. This suggests that there is likely no single best method for the graphical presentation of probabilities and the final format of our tool may contain different display formats. This was, for example, already done for the frequently used prognostication tool PREDICT[19], where the user has different options for visualization of the output (table, bar chart, pictograph, etc.). Interestingly, the majority of the participants indicated they would like to receive information about epistemic uncertainty, but struggled to understand the display format containing this information. One could argue that communicating the epistemic uncertainty to patients is important since it is more realistic, and in a way more ethical to inform them about the reliability of the risk estimation. However, to really support decision making, we first need to know how to communicate/display this information effectively. Otherwise, communication of the epistemic uncertainty may work counterproductive, as showing information patients do not understand may possibly increase anxiety[20].

Strengths, limitations and methodological challenges

The work described in this thesis provides a combination of quantitative and qualitative research to investigate risk factors associated with CBC and to set a first step towards the implementation of our CBC risk prediction model. While interpreting the findings, it is important to keep in mind the strengths and limitations of the studies we performed; several forms of bias to may apply to epidemiological studies.

Access to large patient cohorts with detailed follow-up

The main strength of the quantitative studies presented in **chapter 2, 3, and 4** is that we made use of breast cancer cohorts with very large sample sizes and detailed follow-up data on CBC. In **chapter 2** we used data from the large breast cancer series in the Breast Cancer Association Consortium, including genotype information for ~150.000 women and a large number of CBC events. In **chapter 3 and 4** we were fortunate to have access to datasets from the NCR, collected by the Netherlands Comprehensive Cancer Organization. The NCR is an on-going nationwide population-based data registry of all newly diagnosed cancer patients in the Netherlands, with full coverage since 1989, including comprehensive tumor and therapy information, and active follow-up on CBC occurrence. The large structure of our patient cohorts enabled us to provide reliable estimations of CBC risks based on the risk factor of interest.

Confounding, effect modification, and missing data

Observational studies are typically susceptible to confounding bias and effect modification, since other risk factors are usually not equally distributed between the group with the risk factor of interest and the group without. For example, in **chapter 3** we observed that patients who received adjuvant systemic therapy differed with respect to patient and tumor characteristics (e.g. they were younger and had higher tumor stage), to patients who did not receive adjuvant systemic therapy. In our studies, we tried to reduce confounding bias and effect modification by building multivariable models accounting for other risk factors and used stratified models when interaction was observed. However, some challenges we encountered were the presence of missing values and the fact that some (potential) risk factors were not available in our datasets. Therefore, residual confounding or effect modification may still have been present. Even so, in **chapter 2 and 3** results were similar when including all patients and when only including those patients without missing values (complete case analyses). Therefore, we do not expect that our conclusions would have been substantially different if we would have had more complete data. In the analyses presented in **chapter 4** we used multiple imputation by replacing missing values with imputed values. Multiple imputation is a valid method for handling missing data in multivariable analyses and is highly recommended to use in observational studies[21].

In our studies presented in **chapter 2, 3, and 4**, we lacked data on contralateral prophylactic mastectomy (CPM), which could have resulted in an underestimation of the CBC risk. According to Dutch guidelines [22] only women carrying a *BRCA1 or BRCA2* germline mutation are advised to undergo a CPM, since their CBC risk is high with an estimated 10-year risk of ~10-20%[23,24]. Unfortunately, information about *BRCA1* and *BRCA2* mutation was lacking in our studies. However, we do not expect that this missing information importantly influenced the results since only 1-2% of the DCIS population[25], and 3-5% of the invasive breast cancer population[23,26] will be *BRCA1* or *BRCA2* mutation carriers.

6

Misclassification bias

An important question that came up during our studies is to what extent the CBCs are new primary tumors, or actually metastases from the first breast cancer. For example, this question was raised in **chapter 2**, where we showed that the PRS_{313} was less predictive for CBC than for a first breast cancer. The attenuated effect may partially be explained by the fact that a small subset of the CBCs may have been metastases, since the PRS_{313} has not shown predictive for metastases.

Some (small) studies investigated the clonal relatedness of first breast cancers and CBCs using tumor sequencing and showed that 6-12% of CBCs represent metastases[27-29]. In our studies, we attempted to minimize the misclassification of metastases to the contralateral breast by only including patients without distant metastasis at initial diagnosis, starting follow-up three months after first breast cancer diagnosis (metachronous CBC), and censoring for distant metastases (when possible) during follow-up.

Index event bias

Another type of bias that may occur studying CBC is 'index event bias', a type of bias that arises in studies that select patients based on the occurrence of an index event (in this case breast cancer) and when the risk (in this case of a CBC), is substantial i.e., a violation of the rare disease assumption[30]. Some studies investigating determinants of subsequent events (e.g. recurrence of the disease) showed that factors that have been well-established as determinants of the index event (e.g. patent foramen ovale and the risk of stroke[30,31]) show an attenuated effect for a subsequent event. This can possibly be explained by the fact that conditioning on the index induces dependence between (known and unknown) risk factors, even when these risk factors are independently distributed in the general population[30]. If individuals with the index event score high on the risk factor of interest (e.g. PRS_{313}), they may have lower levels of other risk factors[32]. The other risk factors include most importantly the polygenic risk that is not captured by the PRS (which only explains part of the total polygenic risk), which by definition was not measured and not corrected for. As a consequence, the association between the individual risk factor and subsequent event will be biased toward the null (index event bias)[30,33]. In **chapter 2** we may have encountered some index event bias, since we observed that the association between PRS_{313} and CBC risk was weaker than was found for first primary breast cancer. Index event bias can be reduced by taking into account all other risk factors that contribute to CBC development. However, in our analyses (**chapter 2**) the association between PRS_{313} and CBC risk did not change when taking into account other (non-genetic) risk factors, but we may have encountered some residual bias. Residual bias will always be a concern in etiological research because unmeasured or unknown risk factors are unavoidable (Figure 1).

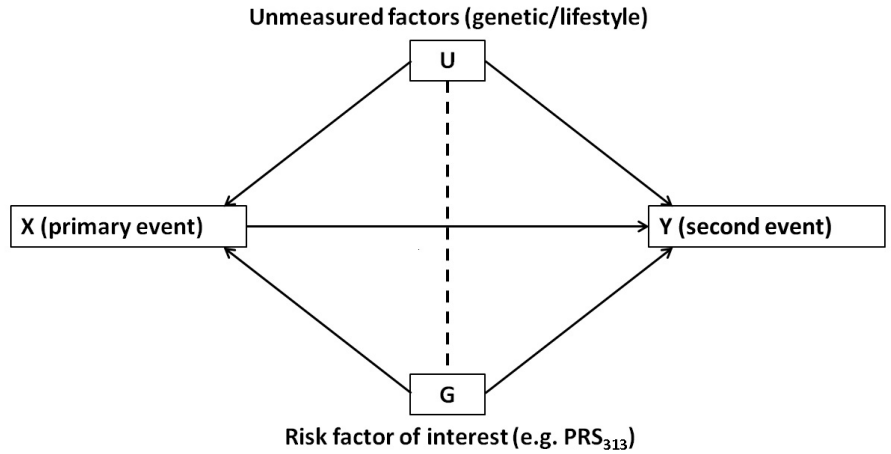


Figure 1. Index event bias. When levels of the risk factor of interest are high (increased G), levels of other (unmeasured) factors may be lower (reduced U), diluting the risk of a second event (Y)

Selection bias

It is highly likely that in our qualitative study presented in **chapter 5** we encountered selection bias due to our invitation approach. Selection bias occurs when some people in a population are systematically more likely to be selected in the study than others. For our interviews, breast cancer survivors were recruited via a patient advisory group, the Dutch Breast Cancer Society, and a breast cancer panel from the Antoni van Leeuwenhoek hospital (AVL). We tried to select a broad range of participants, but the majority of the participants was highly educated and most women were more actively involved with research than women in the general breast cancer population.

Biases and (causal) interpretation of the results

Etiological research provides us knowledge on causes of diseases, but possible biases should be of concern. Biases can obscure true associations, leading to strengthening or weakening of the true associations. Despite that we tried to minimalize bias when possible, we need to be a bit careful in the causal interpretation of our results. On the other hand, causality is not a necessary condition for a risk prediction model to predict an outcome with high validity. The aim of our CBC risk prediction model is not to interpret the (causal) effects of individual variables, but to accurately predict the outcome using a combination of all predictors in the model[34]. Nevertheless, risk prediction models including causal factors will make it more likely that a model is generalizable to other populations that differ from the one in which they were developed.

Clinical implications and suggestions for further research

Suggestions for future research (highlights)

- **Identification of new risk factors**
  Germline mutations (e.g. *ATM*, *PALB2*), breast density, lifestyle factors.
- **Disentangling true primary CBCs from metastases**
  Part of the CBCs represent metastases from the first primary tumor. Disentangling true primary CBCs from metastases is important for prognosis and treatment choices, and to make our future risk prediction analyses more accurate.
- **Exploring factors that predict survival after CBC**
  Studies showed that CBC patients have a worse prognosis compared to patients with unilateral breast cancer. Future studies are needed to investigate which factors are associated with survival after CBC in specific subgroups.
- **Implementation of the CBC risk prediction tool**
  To stimulate successful implementation of our risk prediction tool, it is important to evaluate the usability and patient satisfaction of our tool in the clinical setting. In addition, the tool should be incorporated in clinical guidelines and it needs to be continuously updated by adding new data.

Identification of new risk factors

To get the 'big picture' on CBC development, we will need more data on the effects of other risk factors. For example, more research is needed on the genetic landscape of CBC. From literature we know that carrying a *BRCA1, BRCA2,* or *CHEK2* c.1100delC mutation is associated with strong CBC risks[5]. Moreover, in this thesis, we showed that common genetic variants, summarized in a PRS, are not only predictive for first breast cancer, but also for CBC. Limited information is available for other germline mutations that are shown to be associated with first breast cancer risk, such as *ATM*, *PALB2,* or other variants of the *CHEK2* gene[35]. Future analyses using the data collected in the EU horizon2020 projects B-CAST and BRIDGES will contribute to answering this question.

Another potential risk factor that needs to be further investigated is breast density. The association between high breast density and increased first breast cancer risk has been well-established, with an odds ratio of 4.6 for high versus low density in a large meta-analysis[36], but the association with CBC risk is less clear. If the effect of breast density on CBC risk equals its major effects on first breast cancer risk, breast density could become a highly important target to influence CBC risk. Some case-control studies assessed the association between breast density and CBC risk, but these studies showed inconsistent results[5]. So far, no cohort study has been performed to address this question on a larger scale. Therefore, we collected mammograms (when available) from ~11,000 breast cancer patients diagnosed with first invasive breast cancer between 2005-2017 selected from the AVL tumor registry and ~5,000 patients from the Erasmus Medical Centre. We have access to an algorithm included in a tool (STRATUS) to measure mammographic density (STRATUS)[37]. In future projects, this database can be used to assess the association between breast density and CBC risk.

Little is known about the impact of lifestyle and reproductive factors on CBC risk. Recently, also in the framework of this project, a systemic review and meta-analysis was performed in another PhD trajectory to investigate the impact of multiple lifestyle and reproductive factors and CBC risk[6], but only a few studies were available per studied risk factor. More research on the impact of lifestyle factors and known reproductive risk factors for a first breast cancer on CBC risk is needed to improve individualized CBC risk prediction. Moreover, lifestyle factors may be of particular interest for breast cancer survivors, since these factors are modifiable.

The question remains whether adding results from gene panels, breast density, and lifestyle factors to CBC risk prediction models will significantly improve risk prediction and contribute to clinical practice. For first primary breast cancer, there is evidence that by incorporating these factors much greater levels of breast cancer risk stratification can be achieved both in the general population and in women with a family history of breast cancer. For example, this was investigated for the BOADICEA model, where the PRS, breast density, and lifestyle, hormonal and reproductive risk factors were added to the known risk factors to stratify women on primary breast cancer risk[38,39]. They showed that, apart from family history, the combined effects of PRS, breast density, and lifestyle/hormonal/reproductive factors can identify ~13% of the women in the population who would be classified at moderate or high risk of developing breast cancer, and ~12% at low risk. The results showed that the PRS contributed the most to risk stratification, followed by breast density[39]. In future projects, it would be very interesting to investigate whether these additional risk factors contribute to CBC prediction. It is possible that these factors appear to be less predictive for CBC than for first primary breast cancer (as we have observed for the PRS_{313}).

Disentangling true primary CBCs from metastases

So far, only small studies (max 49 patients) have been performed to investigate clonal relationships between first primary breast cancer and CBCs using tumor sequencing, and a larger cohort to answer this question is desired. During this PhD trajectory, we therefore collected formalin-fixed paraffin-embedded tumor blocks of ~500 patients that were diagnosed with first invasive breast cancer and subsequent invasive CBC between 1990-2016 selected from the tumor registry of the AVL. Disentangling true primary CBCs from metastases is important for prognosis and treatment choices in the first place (i.e. distant metastasis has a different prognosis and needs different treatment strategies than a new primary tumor), but also to make our future risk prediction analyses more accurate.

6

Risk or survival?

In the end, preventing breast cancer mortality is the most important goal. Therefore, an interesting question that came up during our research on CBC was which factors predict survival after a CBC. Several studies showed that CBC patients have a worse prognosis compared to patients with unilateral breast cancer[40-44]. The results presented in this thesis suggested that an explanation for this worse prognosis, besides having been diagnosed with yet another cancer, may be found in the impact of adjuvant systemic therapy on CBC tumor biology, or misclassification of metastatic disease as a CBC. For example, in **chapter 3** we showed that endocrine therapy was particularly effective in reducing risk of ER-positive CBC, whereas the risk of ER-negative CBC did not decrease. A change into a therapy-resistant subtype after adjuvant systemic therapy might negatively affect CBC survival. Future studies are needed to investigate survival after CBC in specific subgroups. Such information could guide counseling strategies following a CBC diagnosis.

Implementation of the CBC risk prediction tool

Once our risk prediction model is upgraded and incorporated in a decision support tool, it can be implemented in clinical practice. A major issue with decision support tools is that many of them do not reach implementation in clinical practice (i.e. they are only developed and evaluated for research purposes)[45]. To stimulate successful implementation of our risk prediction tool, it is important to evaluate the usability and patient satisfaction of our tool in the clinical setting. In addition, future research should elaborate on the wishes of healthcare professionals, for example by using qualitative methods to obtain more insights into barriers and facilitators for adoption. If decision support tools fit the needs of end users, they are more likely to adopt it, which is essential for the successful implementation of these tools in clinical practice. To achieve widespread implementation and long-term relevance, the risk prediction tool should be incorporated in clinical guidelines and it needs to be continuously updated by adding new data[45].

References

1 Bray, F. *et al.* Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **68**, 394-424, doi:10.3322/caac.21492 (2018).

2 Bray, F., McCarron, P. & Parkin, D. M. The changing global patterns of female breast cancer incidence and mortality. *Breast Cancer Res.* **6**, 229-239, doi:10.1186/bcr932 (2004).

3 Youlden, D. R. *et al.* The descriptive epidemiology of female breast cancer: an international comparison of screening, incidence, survival and mortality. *Cancer Epidemiol.* **36**, 237-248, doi:10.1016/j.canep.2012.02.007 (2012).

4 van der Meer, D. J. *et al.* Comprehensive trends in incidence, treatment, survival and mortality of first primary invasive breast cancer stratified by age, stage and receptor subtype in the Netherlands between 1989 and 2017. *Int. J. Cancer* **148**, 2289-2303, doi:10.1002/ijc.33417 (2021).

5 Akdeniz, D. *et al.* Risk factors for metachronous contralateral breast cancer: A systematic review and meta-analysis. *Breast* **44**, 1-14, doi:10.1016/j.breast.2018.11.005 (2019).

6 Akdeniz, D. *et al.* The impact of lifestyle and reproductive factors on the risk of a second new primary cancer in the contralateral breast: a systematic review and meta-analysis. **31**, 403-416 (2020).

7 Giardiello, D. *et al.* Prediction and clinical utility of a contralateral breast cancer risk model. *Breast Cancer Res.* **21**, 144, doi:10.1186/s13058-019-1221-1 (2019).

8 Robson, M. E. *et al.* Association of Common Genetic Variants With Contralateral Breast Cancer Risk in the WECARE Study. *JNCI: Journal of the National Cancer Institute* **109**, djx051-djx051, doi:10.1093/jnci/djx051 (2017).

9 Teraoka, S. N. *et al.* Single nucleotide polymorphisms associated with risk for contralateral breast cancer in the Women's Environment, Cancer, and Radiation Epidemiology (WECARE) Study. *Breast Cancer Res.* **13**, R114, doi:10.1186/bcr3057 (2011).

10 Mavaddat, N. *et al.* Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. *Am. J. Hum. Genet.* **104**, 21-34, doi:10.1016/j.ajhg.2018.11.002 (2019).

11 Davies, C. *et al.* Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet* **378**, 771-784, doi:10.1016/s0140-6736(11)60993-8 (2011).

12 Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomised trials. *The Lancet* **351**, 1451-1467 (1998).

13 Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. *Lancet* **386**, 1341-1352, doi:10.1016/s0140-6736(15)61074-1 (2015).

14 Early Breast Cancer Trialists' Collaborative Group. Polychemotherapy for early breast cancer: an overview of the randomised trials. *Lancet* **352**, 930-942 (1998).

15 Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* **365**, 1687-1717, doi:10.1016/s0140-6736(05)66544-0 (2005).

16 Jones, S. E. *et al.* Phase III trial comparing doxorubicin plus cyclophosphamide with docetaxel plus cyclophosphamide as adjuvant therapy for operable breast cancer. *J. Clin. Oncol.* **24**, 5381-5387, doi:10.1200/jco.2006.06.5391 (2006).

17 Trevena, L. J. *et al.* Presenting quantitative information about decision outcomes: a risk communication primer for patient decision aid developers. *BMC Med. Inform. Decis. Mak.* **13 Suppl 2**, S7, doi:10.1186/1472-6947-13-s2-s7 (2013).

18 Bonner, C. *et al.* Current Best Practice for Presenting Probabilities in Patient Decision Aids: Fundamental Principles. *Med. Decis. Making*, 272989x21996328, doi:10.1177/0272989x21996328 (2021).

19 Predict: Breast Cancer. Version 2.2, Release 1.14. Available from: https://breast.predict.nhs.uk/tool.

20 Kattan, M. W. Doc, what are my chances? A conversation about prognostic uncertainty. *Eur. Urol.* **59**, 224, doi:10.1016/j.eururo.2010.10.041 (2011).

21 Van Buuren, S. *Flexible imputation of missing data*. (CRC press, 2018).

22 Oncoline. *Borstkanker. Landelijke richtlijn, Versie: 2.0. Available from:* https://www.oncoline.nl/

23 van den Broek, A. J. *et al.* Impact of Age at Primary Breast Cancer on Contralateral Breast Cancer Risk in BRCA1/2 Mutation Carriers. *J. Clin. Oncol.* **34**, 409-418, doi:10.1200/jco.2015.62.3942 (2016).

24 Kuchenbaecker, K. B. *et al.* Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. *JAMA* **317**, 2402-2416, doi:10.1001/jama.2017.7112 (2017).

25 Claus, E. B., Petruzella, S., Matloff, E. & Carter, D. Prevalence of BRCA1 and BRCA2 mutations in women diagnosed with ductal carcinoma in situ. *JAMA* **293**, 964-969, doi:10.1001/jama.293.8.964 (2005).

26 Thompson, D. & Easton, D. The genetic epidemiology of breast cancer genes. *J. Mammary Gland Biol. Neoplasia* **9**, 221-236, doi:10.1023/B:JOMG.0000048770.90334.3b

(2004).
27 Klevebring, D. *et al.* Exome sequencing of contralateral breast cancer identifies metastatic disease. *Breast Cancer Res. Treat.* **151**, 319-324, doi:10.1007/s10549-015-3403-6 (2015).
28 Begg, C. B. *et al.* Contralateral breast cancers: Independent cancers or metastases? *Int. J. Cancer* **142**, 347-356, doi:10.1002/ijc.31051 (2018).
29 Alkner, S. *et al.* Contralateral breast cancer can represent a metastatic spread of the first primary tumor: determination of clonal relationship between contralateral breast cancers using next-generation whole genome sequencing. *Breast Cancer Res.* **17**, 102, doi:10.1186/s13058-015-0608-x (2015).
30 Dahabreh, I. J. & Kent, D. M. Index event bias as an explanation for the paradoxes of recurrence risk research. *JAMA* **305**, 822-823, doi:10.1001/jama.2011.163 (2011).
31 Kent, D. M. & Thaler, D. E. Is patent foramen ovale a modifiable risk factor for stroke recurrence? *Stroke* **41**, S26-30, doi:10.1161/strokeaha.110.595140 (2010).
32 Smulders, Y. M. [Index event bias: why causal factors appear not to apply to disease recurrence]. *Ned. Tijdschr. Geneeskd.* **155**, A3458 (2011).
33 Smits, L. J. *et al.* Index event bias-a numerical example. *J. Clin. Epidemiol.* **66**, 192-196, doi:10.1016/j.jclinepi.2012.06.023 (2013).
34 van Diepen, M., Ramspek, C. L., Jager, K. J., Zoccali, C. & Dekker, F. W. Prediction versus aetiology: common pitfalls and how to avoid them. *Nephrol. Dial. Transplant.* **32**, ii1-ii5, doi:10.1093/ndt/gfw459 (2017).
35 Dorling, L. *et al.* Breast Cancer Risk Genes - Association Analysis in More than 113,000 Women. *N. Engl. J. Med.* **384**, 428-439, doi:10.1056/NEJMoa1913948 (2021).
36 McCormack, V. A. & dos Santos Silva, I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. *Cancer Epidemiol. Biomarkers Prev.* **15**, 1159-1169, doi:10.1158/1055-9965.Epi-06-0034 (2006).
37 Eriksson, M., Li, J., Leifland, K., Czene, K. & Hall, P. A comprehensive tool for measuring mammographic density changes over time. *Breast Cancer Res. Treat.* **169**, 371-379, doi:10.1007/s10549-018-4690-5 (2018).
38 Antoniou, A. C., Pharoah, P. P., Smith, P. & Easton, D. F. The BOADICEA model of genetic susceptibility to breast and ovarian cancer. *Br. J. Cancer* **91**, 1580-1590, doi:10.1038/sj.bjc.6602175 (2004).
39 Lee, A. *et al.* BOADICEA: a comprehensive breast cancer risk prediction model incorporating genetic and nongenetic risk factors. *Genet. Med.* **21**, 1708-1718, doi:10.1038/s41436-018-0406-9 (2019).
40 Healey, E. A. *et al.* Contralateral breast cancer: clinical characteristics and impact on prognosis. *J. Clin. Oncol.* **11**, 1545-1552, doi:10.1200/jco.1993.11.8.1545 (1993).
41 Font-Gonzalez, A. *et al.* Inferior survival for young patients with contralateral compared to unilateral breast cancer: a nationwide population-based study in the Netherlands. *Breast Cancer Res. Treat.* **139**, 811-819, doi:10.1007/s10549-013-2588-9 (2013).
42 Schaapveld, M. *et al.* The impact of adjuvant therapy on contralateral breast cancer risk and the prognostic significance of contralateral breast cancer: a population based study in the Netherlands. *Breast Cancer Res. Treat.* **110**, 189-197, doi:10.1007/s10549-007-9709-2 (2008).
43 Hartman, M. *et al.* Incidence and prognosis of synchronous and metachronous bilateral breast cancer. *J. Clin. Oncol.* **25**, 4210-4216, doi:10.1200/jco.2006.10.5056 (2007).
44 Langballe, R. *et al.* Mortality after contralateral breast cancer in Denmark. *Breast Cancer Res. Treat.* **171**, 489-499, doi:10.1007/s10549-018-4846-3 (2018).
45 Joseph-Williams, N. *et al.* What Works in Implementing Patient Decision Aids in Routine Clinical Settings? A Rapid Realist Review and Update from the International Patient Decision Aid Standards Collaboration. *Med. Decis. Making*, 272989x20978208, doi:10.1177/0272989x20978208 (2020).

CHAPTER 7

Summary

Due to the increased incidence of breast cancer and improved survival, more women are at risk of developing contralateral breast cancer (CBC). Even though the incidence of CBC is relatively low in the general breast cancer population (10-year risk of ~4%), an increasing number of patients with unilateral breast cancer opt for preventive removal of the contralateral breast. Understanding which risk factors play a role in the development of CBC could improve stratification of breast cancer patients for high and low CBC risk, and hence improve decision making (**chapter 1**). Therefore, the aim of this thesis was to explore risk factors associated with CBC for which there is insufficient evidence in literature (**chapter 2-4**). Furthermore, as a first step towards implementation of a risk prediction model, we performed an exploratory interview study to investigate preferences for graphical presentation of probabilities in a CBC risk prediction model (**chapter 5**).

In **chapter 2** we investigated the association between a recently developed and validated polygenic risk score of 313 germline variants (PRS_{313}) and CBC risk. To answer this question, we performed both cox regression and logistic regression analyses using data from the large breast cancer series of the Breast Cancer Association Consortium. In a cohort of breast cancer patients of European ancestry, we observed a clear association between the PRS_{313} and CBC risk, which was not biased by patient characteristics, characteristics of the primary tumor, or adjuvant treatment. In the logistic regression analyses, we also observed an association between the PRS_{313} and CBC risk for Asian women, but this association was slightly weaker than for European women. The absolute lifetime risks of CBC, accounting for death as competing risk, were 12.4% for European women at the 10^{th} percentile and 20.5% at the 90^{th} percentile of the PRS_{313}.

In **chapter 3** we performed a population-based cohort study to investigate the influence of different regimens of adjuvant systemic therapy on CBC risk. In our study, adjuvant endocrine therapy, chemotherapy, and trastuzumab combined with chemotherapy were associated with overall 54%, 30%, and 43% risk reductions of CBC, respectively. Taxane-containing chemotherapy and aromatase inhibitors were associated with the largest CBC risk reduction. We also investigated if these regimens had different effects on the (hormone) receptor subtype of the CBC. Our results showed that each adjuvant therapy regimen had a different impact on the CBC subtype distribution. Endocrine therapy decreased the risk of estrogen receptor (ER)-positive CBC, but not ER-negative CBC, compared with no endocrine therapy. Patients receiving chemotherapy for ER-negative first BC had a higher risk of ER-negative CBC from 5 years of follow-up, compared with patients not receiving chemotherapy for ER-negative first BC.

In **chapter 4**, we aimed to assess CBC risk in women diagnosed with ductal carcinoma in situ (DCIS) compared with invasive breast cancer. We performed a nationwide population-based cohort study including all women diagnosed with DCIS or

invasive breast cancer stage I-III between 1989-2017 identified from the Netherlands Cancer Registry. The 10-year cumulative incidence of invasive CBC was 4.8% for women with DCIS (CBC=1,334). Invasive CBC risk was higher in women with DCIS compared with invasive breast cancer overall, likely explained by the risk-reducing effect of (neo) adjuvant systemic therapy among women with invasive breast cancer. Indeed, when we compared CBC risk for women with DCIS to women with stage I not receiving adjuvant systemic therapy, CBC risk was lower for women with DCIS. The higher CBC risk for DCIS patients compared to invasive breast cancer was more pronounced in the subgroup of not screen-detected cancers, which may relate to the fact that invasive interval tumors tend to be more aggressive than screen-detected cancers and hence receive more often adjuvant systemic treatment. In our study, we had limited information on biological characteristics of DCIS, e.g. no information on receptor subtypes, and our multivariable model was therefore unable to differentiate CBC risk among DCIS patients.

To set a first step towards implementation of our CBC risk prediction model, **chapter 5** shows the results of an exploratory study where we interviewed 19 breast cancer survivors to get insights into their preferences for the graphical presentation of probabilities, including the epistemic uncertainty, provided by the model. Additionally, we evaluated which factors are associated with participants' level of trust in the probabilities, participants' understanding of different graphical display formats, and which factors (in particular probabilities) would play a role in participants' decision-making about contralateral prophylactic mastectomy (CPM). In our study, there was no consensus among participants regarding the optimal graphical format for presenting a single probability. The majority indicated they want to receive information about epistemic uncertainty, but struggled to understand the display format containing this information. Probabilities seem to play an important role in decision-making about CPM, as we found that having a high probability of developing a CBC as well as fear of future breast cancer were the factors most frequently mentioned by participants' as relevant for their decision-making.

Concluding, we observed clear associations for a polygenic risk score of common germline variants (PRS_{313}) and for different regimens of adjuvant systemic therapy with (subtype-specific) CBC risk. These factors may be incorporated in CBC risk prediction models together with other known and available risk factors. For support of clinical decision making more biological information is needed to understand CBC development in women with DCIS. Our exploratory interview study provided valuable information for preferences for graphical presentation of probability in a CBC risk prediction model. In future studies, the prediction model should be incorporated in a decision support tool and implemented in clinical practice. This tool can then help to better identify women at high risk of CBC who may benefit from prophylactic surgery, while the estimates can also be used to reassure patients who are at low risk of developing CBC.

Nederlandse samenvatting

Door de toegenomen incidentie van borstkanker en de verbeterde overleving lopen steeds meer vrouwen het risico om ook borstkanker te krijgen in de andere borst. Hoewel de incidentie van zo'n 'contralaterale borstkanker' relatief laag is in de algemene borstkankerpopulatie (10-jaar cumulatieve risico is ongeveer 4%), kiezen steeds meer vrouwen voor preventieve verwijdering van de contralaterale borst. Het risico op contralaterale borstkanker kan echter behoorlijk verschillen van vrouw tot vrouw; tal van factoren spelen namelijk een rol bij het ontstaan van contralaterale borstkanker. Inzicht in dit risico is voor patiënten van groot belang, bijvoorbeeld voor de beslissing om een preventieve amputatie te ondergaan bij hoog risico, of juist om gerust gesteld te kunnen worden wanneer dit risico laag is (**hoofdstuk 1**).

Het doel van de studies in dit proefschrift was om risicofactoren voor contralaterale borstkanker te onderzoeken waarvoor nog onvoldoende bewijs is in de huidige literatuur (**hoofdstuk 2-4**). Om de informatie over alle risicofactoren bruikbaar te maken voor de klinische praktijk, kunnen deze risicofactoren gecombineerd worden in een predictiemodel. In een promotietraject parallel aan dit proefschrift is een predictiemodel voor contralaterale borstkanker ontwikkeld en gevalideerd. Binnen dit proefschrift hebben we ook, als een eerste stap richting implementatie van dit predictiemodel, een exploratieve interviewstudie uitgevoerd om de voorkeuren voor grafische weergave van kansen te bestuderen onder een groep vrouwen die ooit borstkanker hebben gehad (**hoofdstuk 5**).

Uit eerder onderzoek is gebleken dat een zogenoemde 'polygenetische risiscoscore' van veelvoorkomende erfelijke varianten een voorspellende waarde heeft of een vrouw (een eerste) borstkanker zal ontwikkelen. In **hoofdstuk 2** hebben we het verband onderzocht tussen een recent ontwikkelde en gevalideerde polygenetische risicoscore van 313 varianten (PRS_{313}) en het risico op een tweede, contralaterale borstkanker. Om deze vraag te beantwoorden hebben we gebruik gemaakt van gegevens uit de grote borstkanker database van het Breast Cancer Association Consortium. In een cohort van meer dan 56.000 borstkankerpatiënten van Europese afkomst, zagen we een duidelijk verband tussen de PRS_{313} en het risico op contralaterale borstkanker. Dit verband werd niet beïnvloed door andere factoren zoals patiënt-gerelateerde factoren (zoals een familiegeschiedenis van borstkanker), kenmerken van de primaire tumor, of de (neo) adjuvante behandeling. We zagen ook een verband tussen de PRS_{313} en het risico op contralaterale borstkanker voor Aziatische vrouwen, maar dit verband was iets zwakker dan voor Europese vrouwen. Het absolute (levenslange) risico om contralaterale borstkanker te ontwikkelen was 12,4% voor Europese vrouwen die op het 10e percentiel van de PRS_{313} zaten en 20,5% voor vrouwen op het 90e percentiel.

Naast chirurgie en mogelijk radiotherapie, wordt de behandeling van (primaire)

borstkanker vaak aangevuld met (neo)adjuvante systemische therapie zoals chemotherapie, endocriene therapie, en/of doelgerichte therapie (trastuzumab). In **hoofdstuk 3** hebben we gekeken wat de invloed is van deze verschillende adjuvante systemische therapieën op het risico op contralaterale borstkanker. In onze cohortstudie waarbij we data hebben gebruikt van 83.144 vrouwen met invasieve borstkanker verzameld door de Nederlandse Kankerregistratie, gaven adjuvante endocriene therapie, chemotherapie, en trastuzumab in combinatie met chemotherapie, een risicoreductie voor contralaterale borstkanker van respectievelijk 54%, 30% en 43%. Taxaan-bevattende chemotherapie en aromataseremmers waren geassocieerd met de sterkste vermindering van het risico. We hebben ook onderzocht of deze therapieën verschillende effecten hadden op het (hormoon) receptor-subtype van de contralaterale borstkanker. Onze resultaten toonden aan dat elk type adjuvante therapie een andere impact had op deze subtype-distributie. We zagen bijvoorbeeld dat endocriene therapie alleen het risico verminderde op oestrogeenreceptor (ER)-positieve contralaterale borstkanker maar niet op ER-negatieve contralaterale borstkanker.

In **hoofdstuk 4** hebben we het risico op contralaterale borstkanker bij vrouwen met ductaal carcinoma in situ (DCIS) bekeken in vergelijking met vrouwen met invasieve borstkanker. We hebben een landelijke cohortstudie uitgevoerd onder alle vrouwen met de diagnose DCIS of invasieve borstkanker stadium I-III tussen 1989-2017. Het risico op contralaterale borstkanker was iets hoger bij vrouwen met DCIS dan bij vrouwen met invasieve borstkanker. Dit hogere risico kan hoogstwaarschijnlijk verklaard worden door het risico-verlagende effect van (neo)adjuvante systemische therapie bij vrouwen met invasieve borstkanker. Dit zagen wij ook terug in onze analyses; als we het risico van vrouwen met DCIS vergeleken met vrouwen met stadium I borstkanker die niet behandeld waren met (neo)adjuvante systemische therapie, was het risico lager voor vrouwen met DCIS. Het hogere risico op contralaterale borstkanker voor vrouwen met DCIS in vergelijking met invasieve borstkanker was meer uitgesproken wanneer de (eerste) borstkanker niet door screening gedetecteerd was. Dit houdt mogelijk verband met het feit dat invasieve intervaltumoren vaak agressiever zijn dan door screening gedetecteerde tumoren. Deze worden daarom vaker behandeld met (neo)adjuvante systemische therapie.

In onze studie hadden we beperkte informatie over de biologische kenmerken van DCIS en geen informatie over genetische factoren en familiegeschiedenis van borstkanker. Hierdoor was ons multivariabele model niet goed in staat om onderscheid te kunnen maken tussen vrouwen met DCIS die een hoog risico hebben op contralaterale borstkanker en vrouwen die een laag risico hebben.

In **hoofdstuk 5** hebben we ons recent ontwikkelde predictiemodel, weergegeven in een online tool, voorgelegd aan 19 vrouwen die ooit borstkanker hebben gehad. Het doel van deze exploratieve interview studie was om hun voorkeuren te bestuderen voor de grafische weergave van kansen, om zo inzicht te krijgen hoe we de weergave van het model zouden kunnen verbeteren. Aan de hand van vijf verschillende weergaven hebben we gevraagd naar hun voorkeuren. Daarnaast hebben we onderzocht hoeveel vertrouwen ze hadden in de kansen die het predictiemodel genereerde, hoe goed ze de verschillende weergaven begrepen, en welke factoren (in het bijzonder kansen) voor hun een rol zouden spelen bij het wel of niet ondergaan van preventieve amputatie van de contralaterale borst. In onze studie was er geen consensus over de optimale grafische weergave voor het presenteren van kansen op contralaterale borstkanker. De meerderheid van de vrouwen gaf aan informatie te willen ontvangen over de onzekerheid rondom de schattingen, maar over het algemeen hadden ze moeite om de weergave met deze informatie te begrijpen. Kansen lijken een belangrijke rol te spelen bij de besluitvorming over preventieve chirurgie, aangezien een grote kans op het ontwikkelen van een contralaterale borstkanker en de angst voor toekomstige borstkanker het meest werden genoemd als factoren relevant voor hun besluitvorming.

Concluderend, binnen de onderzoeken in dit proefschrift zagen we een duidelijk verband tussen verschillende factoren, o.a. een polygenetische risicoscore en verschillende (neo) adjuvante systemische therapieën, en het krijgen van contralaterale borstkanker. Deze factoren kunnen samen met andere bekende risicofactoren worden opgenomen in predictiemodellen. Voor vrouwen met DCIS is meer (biologische) informatie nodig om de ontwikkeling van contralaterale borstkanker beter te begrijpen binnen deze groep. Onze exploratieve interviewstudie onder vrouwen die ooit borstkanker hebben gehad, gaf inzicht in de voorkeuren voor de grafische weergave van kansen in een predictiemodel voor contralaterale borstkanker. In toekomstige studies zou het predictiemodel moeten worden opgenomen in een beslissingsondersteunende tool zodat deze geïmplementeerd kan worden in de klinische praktijk. Deze tool kan vervolgens helpen om vrouwen met een hoog risico op contralaterale borstkanker beter te identificeren die baat kunnen hebben bij preventieve chirurgie, terwijl de schattingen ook gebruikt kunnen worden om patiënten met een laag risico gerust te stellen.

7

List of publications

van der Meer DJ, **Kramer I**, van Maaren MC, van Diest PJ, C Linn S, Maduro JH, J A Strobbe L, Siesling S*, Schmidt MK*, Voogd AC*. Comprehensive trends in incidence, treatment, survival and mortality of first primary invasive breast cancer stratified by age, stage and receptor subtype in the Netherlands between 1989 and 2017. Int J Cancer. 2021 May 1;148(9):2289-2303.
**Authors contributed equally*

Kramer I, Hooning MJ, Mavaddat N, Hauptmann M, Keeman R, Steyerberg EW, Giardiello D, Antoniou AC, Pharoah PDP, Canisius S, Abu-Ful Z, Andrulis IL, Anton-Culver H, Aronson KJ, Augustinsson A, Becher H, Beckmann MW, Behrens S, Benitez J, Bermisheva M, Bogdanova NV, Bojesen SE, Bolla MK, Bonanni B, Brauch H, Bremer M, Brucker SY, Burwinkel B, Castelao JE, Chan TL, Chang-Claude J, Chanock SJ, Chenevix-Trench G, Choi JY, Clarke CL; NBCS Collaborators, Collée JM, Couch FJ, Cox A, Cross SS, Czene K, Daly MB, Devilee P, Dörk T, Dos-Santos-Silva I, Dunning AM, Dwek M, Eccles DM, Evans DG, Fasching PA, Flyger H, Gago-Dominguez M, García-Closas M, García-Sáenz JA, Giles GG, Goldgar DE, González-Neira A, Haiman CA, Håkansson N, Hamann U, Hartman M, Heemskerk-Gerritsen BAM, Hollestelle A, Hopper JL, Hou MF, Howell A; ABCTB Investigators; kConFab Investigators, Ito H, Jakimovska M, Jakubowska A, Janni W, John EM, Jung A, Kang D, Kets CM, Khusnutdinova E, Ko YD, Kristensen VN, Kurian AW, Kwong A, Lambrechts D, Le Marchand L, Li J, Lindblom A, Lubiński J, Mannermaa A, Manoochehri M, Margolin S, Matsuo K, Mavroudis D, Meindl A, Milne RL, Mulligan AM, Muranen TA, Neuhausen SL, Nevanlinna H, Newman WG, Olshan AF, Olson JE, Olsson H, Park-Simon TW, Peto J, Petridis C, Plaseska-Karanfilska D, Presneau N, Pylkäs K, Radice P, Rennert G, Romero A, Roylance R, Saloustros E, Sawyer EJ, Schmutzler RK, Schwentner L, Scott C, See MH, Shah M, Shen CY, Shu XO, Siesling S, Slager S, Sohn C, Southey MC, Spinelli JJ, Stone J, Tapper WJ, Tengström M, Teo SH, Terry MB, Tollenaar RAEM, Tomlinson I, Troester MA, Vachon CM, van Ongeval C, van Veen EM, Winqvist R, Wolk A, Zheng W, Ziogas A, Easton DF, Hall P, Schmidt MK. Breast Cancer Polygenic Risk Score and Contralateral Breast Cancer Risk. Am J Hum Genet. 2020 Nov 5;107(5):837-848.

Giardiello D*, **Kramer I***, Hooning MJ, Hauptmann M, Lips EH, Sawyer E, Thompson AM, de Munck L, Siesling S, Wesseling J, Steyerberg EW, Schmidt MK. Contralateral breast cancer risk in patients with ductal carcinoma in situ and invasive breast cancer. NPJ Breast Cancer. 2020 Nov 3;6(1):60.
**Authors contributed equally*

Kramer I, Schaapveld M, Oldenburg HSA, Sonke GS, McCool D, van Leeuwen FE, Van de Vijver KK, Russell NS, Linn SC, Siesling S, Menke-van der Houven van Oordt CW, Schmidt MK. The Influence of Adjuvant Systemic Regimens on Contralateral Breast Cancer Risk and Receptor Subtype. J Natl Cancer Inst. 2019 Jul 1;111(7):709-718.

Not in this thesis:

Giardiello D, Hauptmann M, Steyerberg EW, Adank MA, Akdeniz D, Blom JC, Blomqvist C, Bojesen SE, Bolla MK, Brinkhuis M, Chang-Claude J, Czene K, Devilee P, Dunning AM, Easton DF, Eccles DM, Fasching PA, Figueroa J, Flyger H, García-Closas M, Haeberle L, Haiman CA, Hall P, Hamann U, Hopper JL, Jager A, Jakubowska A, Jung A, Keeman R, Koppert LB, **Kramer I**, Lambrechts D, Le Marchand L, Lindblom A, Lubiński J, Manoochehri M, Mariani L, Nevanlinna H, Oldenburg HSA, Pelders S, Pharoah PDP, Shah M, Siesling S, Smit VTHBM, Southey MC, Tapper WJ, Tollenaar RAEM, van den Broek AJ, van Deurzen CHM, van Leeuwen FE, van Ongeval C, Van't Veer LJ, Wang Q, Wendt C, Westenend PJ, Hooning MJ, Schmidt MK. Prediction of contralateral breast cancer: external validation of risk calculators in 20 international cohorts. Breast Cancer Res Treat. 2020 Jun;181(2):423-434.

Giardiello D, Steyerberg EW, Hauptmann M, Adank MA, Akdeniz D, Blomqvist C, Bojesen SE, Bolla MK, Brinkhuis M, Chang-Claude J, Czene K, Devilee P, Dunning AM, Easton DF, Eccles DM, Fasching PA, Figueroa J, Flyger H, García-Closas M, Haeberle L, Haiman CA, Hall P, Hamann U, Hopper JL, Jager A, Jakubowska A, Jung A, Keeman R, **Kramer I**, Lambrechts D, Le Marchand L, Lindblom A, Lubiński J, Manoochehri M, Mariani L, Nevanlinna H, Oldenburg HSA, Pelders S, Pharoah PDP, Shah M, Siesling S, Smit VTHBM, Southey MC, Tapper WJ, Tollenaar RAEM, van den Broek AJ, van Deurzen CHM, van Leeuwen FE, van Ongeval C, Van't Veer LJ, Wang Q, Wendt C, Westenend PJ, Hooning MJ, Schmidt MK. Prediction and clinical utility of a contralateral breast cancer risk model. Breast Cancer Res. 2019 Dec 17;21(1):144.

About the author

Iris Kramer was born on May 23rd, 1994 in Woerden, the Netherlands. In 2012, she graduated from pre-university education (VWO) at the Kalsbeek College in Woerden. Thereafter she enrolled the bachelor program Health and Life Sciences at the VU University in Amsterdam. During her Bachelor of Science degree, she performed an internship at the Netherlands Cancer Institute under supervision of prof.dr.ir. Marjanka Schmidt and dr. Michael Schaapveld on a project investigating the influence of adjuvant systemic therapy on (subtype-specific) contralateral breast cancer risk. After obtaining her bachelor degree in 2015, she moved to Maastricht to follow the Master's program in Health Education and Promotion at the Maastricht University. She performed an internship at the department of Health Promotion on personal characteristics in early childhood and weight status. After obtaining her master's degree in 2016, she joined the group of prof.dr.ir. Marjanka Schmidt as a PhD student, where she continued research on contralateral breast cancer as described in the current thesis. She presented the results on several national and international conferences (e.g. at the San Antonio Breast Cancer Symposium, USA).

Dankwoord

Zo aan het einde van dit bijzondere traject wil ik graag iedereen bedanken die een bijdrage heeft geleverd aan de inhoud van dit proefschrift of die mij de afgelopen 4 à 5 jaar op een andere mogelijke manier heeft gesteund. Uiteraard had mijn proefschrift zonder jullie nooit tot stand kunnen komen! Een aantal mensen wil ik graag in het bijzonder bedanken.

Marjanka, wat heb ik het getroffen met jou als promotor en dagelijks begeleider; ik heb dankzij jou veel meer bereikt dan dat ik ooit had verwacht. Heel veel dank voor je vertrouwen, je altijd kritische blik, maar ook de goede gesprekken die we gevoerd hebben. Maartje, wat was het fijn om jou als co-promotor te hebben, bedankt voor je steun en input. Ik vond het erg leuk om af en toe eens langs te komen in het Erasmus MC.

Ik zou ook graag alle patiënten willen bedanken die hun gegevens beschikbaar hebben gesteld voor de studies in dit proefschrift. Daarnaast wil ik alle analisten, datamanagers, en behandelend artsen bedanken voor de betrokkenheid bij de opzet en/of uitvoering van de studies.

De tijd op het NKI-AVL was natuurlijk lang niet zo leuk geweest zonder de lieve en leuke collega's om mij heen. Maria, Daniele, and Anna, I really enjoyed doing the PhD at the same time. I think we helped each other a lot during the years! Dear other members of the 'Marjanka Group': Delal, Felipe, Yuwei, Mary Ann, Ellen, Josephine, Sander, Susanne, Renée, Miriam, Aaike, Renske, Sten, Sandra, Heleen, and Marcelo, thank you so much for the support, the nice conversations, and after-work activities. I will miss you! Maartje, Sina, en Stefan, wat heb ik genoten van jullie als collega's. De goede klik die wij hadden is bijzonder en ik zal onze vele borrels en natuurlijk de wintersport reis nooit meer vergeten.

Ik wil ook graag heel de C2-afdeling bedanken met in het bijzonder Ellen, Suzanne, Renée, Mathilde, Jelle, en Esther. Bedankt voor de altijd fijne sfeer op de afdeling! Michael, veel dank voor het aannemen van mij als bachelor student in 2015. Ik had het destijds nooit verwacht, maar die stage heeft geresulteerd in dit mooie PhD-traject.

Ik wil graag mijn familie en vrienden bedanken voor jullie interesse, luisterend oor, en de leuke dingen die we samen doen na werktijd. Bart en Isabella, bedankt voor jullie wijze raad en gezelligheid. Als laatste wil ik graag mijn ouders bedanken. Jullie hebben altijd in mij geloofd en me gesteund waar nodig. Dank jullie wel!

APPENDIX 1

Comprehensive trends in incidence, treatment, survival and mortality of first primary invasive breast cancer stratified by age, stage and receptor subtype in the Netherlands between 1989–2017

Int J Cancer. 2021 May 1;148(9):2289-2303

Daniël J. van der Meer
Iris Kramer
Marissa C. van Maaren
Paul J. van Diest
Sabine Linn
John H. Maduro
Luc Strobbe
Sabine Siesling*
Marjanka K. Schmidt*
Adri C. Voogd*

* authors contributed equally

Abstract

This study aimed to provide a comprehensive overview of trends in incidence, survival, mortality and treatment of first primary invasive breast cancer (BC), according to age, stage and receptor subtype in the Netherlands between 1989–2017. Data from all women diagnosed with first primary stage I–IV breast cancer (N=320,249) were obtained from the Netherlands Cancer Registry. BC mortality and general population data were retrieved from Statistics Netherlands. Age-standardized incidence and mortality rates were calculated with annual and average annual percentage change statistics (APC and AAPC). The relative survival (RS) was used as estimator for disease-specific survival. The BC incidence for all BC patients combined increased until 2013 from 126 to 158 per 100,000 person-years, after which a declining trend was observed. Surgery became less extensive, but (neo)adjuvant systemic treatments and their combinations were given more frequently. The RS improved for all age groups and for most stages and receptor subtypes, but remained stable for all subtypes since 2012–2013 and since 2000–2009 for stage IV BC at 15–years of follow-up. Overall, the five- and ten-year RS increased from 76.8% (95% confidence interval [CI]:76.1, 77.4) and 55.9% (95%CI:54.7, 57.1) in 1989–1999 to 91.0% (95%CI:90.5, 91.5) and 82.9% (95%CI:82.2, 83.5), respectively, in 2010–2016. BC mortality improved regardless of age and overall decreased from 57 to 35 per 100,000 person-years between 1989–2017. In conclusion, the BC incidence in the Netherlands has steadily increased since 1989, but the latest trends show promising declines. Survival improved markedly for most patients and the mortality decreased regardless of age.

Introduction

Breast cancer (BC) is the most common cancer and leading cause of cancer related death among women in most countries worldwide[1]. It accounts for almost one in four cancers (24.2%) in women, with an estimated 2.1 million new cases globally in 2018[1]. The incidence of BC has been rising for decades in most developed countries and is expected to continue to rise[1]. Meanwhile, mortality rates have been steadily decreasing in most European, American and other high-income countries, while weak-to-moderate increases in mortality have been observed in some lower-to-middle income countries[2-4]. Worldwide, BC is responsible for 15.0% of all cancer-related deaths in women, with an estimated 627,000 deaths in 2018[1]. However, BC survival has improved significantly in recent decades for all age groups in most countries[4].

The rising trends in BC incidence are attributed to the increased presence of known risk-factors, including early age at menarche, late age at menopause, low parity, nulliparity, not breastfeeding, use of oral contraceptives, hormone replacement therapy and older age at first childbearing[5,6]. Other factors that have been implicated to influence BC incidence include changes in lifestyle factors such as excessive alcohol intake, increasing prevalence of obesity and a decrease in physical activity[5,7,8]. Moreover, screening programmes could influence incidence, but can also influence stage distribution and improvements in BC survival and eventually mortality[4]. Improvement in survival could also be explained by earlier detection outside screening, improvements in treatment, access to appropriate healthcare and increasing disease awareness[4,5].

In the Netherlands, incidence, survival and mortality trends of BC are generally comparable to those observed globally, as shown by various studies[9-13]. However, studies describing and interpreting these endpoints simultaneously are scare and many of the currently available trend studies in the Netherlands or elsewhere in Europe are no longer up-to-date. Additionally, receptor subtype specific trends have remained largely unexplored, while these subtypes have become increasingly important in recent years as targets of new personalised ([neo-]adjuvant) treatment strategies[13-15]. Comprehensive trend analyses are useful for medical doctors to better inform patients about their disease and are of great interest to breast cancer researchers, policy makers, and patient advocates. Therefore, this study aimed to provide an up-to-date and comprehensive overview of first primary invasive breast cancer trends in incidence, treatment, survival and mortality in the Netherlands between 1989–2017. Trend evaluation was performed for all BC patients combined and stratified by age group, stage and receptor subtype.

Materials and Methods

Data sources

Data from all women aged ≥18 years, diagnosed with tumour, node and metastasis (TNM) stage I–IV first primary invasive BC between 1989–2017 were obtained from the nationwide population-based Netherlands Cancer Registry (NCR), hosted by the Netherlands Comprehensive Cancer Organisation (IKNL). The NCR contains records on pathologically confirmed cancers after notification by the National Pathology Archive (PALGA). Yearly linkage with the national discharge register data ensures high completeness. All tumours in the registry are coded according to the International Classification of Diseases for Oncology (ICD-O). Patient-, tumour- and treatment-related characteristics were collected from medical records from all Dutch hospitals by trained tumour registrars from the NCR. Information on vital status and date of death is regularly obtained through linkage with the Dutch Municipal Personal Records database and was updated until 31 January 2018. Data on invasive BC mortality cases and data on the general Dutch female population were obtained from Statistics Netherlands[16,17].

Tumour stage, receptor subtype and treatment

The Union for International Cancer Control (UICC) TNM classification of malignant tumours was used to categorize BC stage. From 1989 to 2017, various editions have been introduced, ranging from the 4th to the 8th edition, and resulted in changes in the definition of tumour stage[18]. Most noticeably, going from the 5th to the 6th edition in 2003, a shift from stage II to stage III BC occurred as tumours with more than three positive lymph nodes were categorized as stage III according to the 6th edition, whereas they were previously categorized as stage II disease. All tumours were classified according to the TNM classification valid at the date of diagnosis. If pathological stage was missing, clinical stage was used.

Oestrogen receptor (ER) and progesterone receptor (PR) status were determined by immunohistochemistry (IHC) and were actively registered by the NKR since 2005. Tumours were defined as ER/PR-positive (ER+/PR+) when >10% of the tumour cells stained positive (from 2011 the threshold was ≥10%). Human epidermal growth factor receptor 2 (HER2) was introduced and registered since 2006. Tumours were defined HER2-positive (HER2+) if IHC was 3+ (at least 10% of cells showed strong intensity membrane staining) or when confirmed positive with in situ hybridization (FISH/CISH). HER2-negativity (HER2-) was declared by IHC when less than 10% of the cells showed membrane staining or when FISH/CISH test outcome was negative. Tumours with IHC 2+ without FISH/CISH confirmation available were considered unknown. For the analyses, we grouped receptor subtypes into: hormone receptor (HR)+/HER2- (e.g., ER+ and/or PR+ and HER2-), HR+/HER2+ (i.e., ER+ and/or PR+ and HER2+), HR-/HER2+ (i.e., ER-/PR-/HER2+) and HR-/HER2- (i.e., ER-/PR-/HER2-).

Treatment data on surgery, radiotherapy, chemotherapy and endocrine therapy were included in the NCR since 1989 on an aggregated level. Type of chemotherapy (e.g. taxane-based and/or anthracycline-based) and endocrine therapy (e.g. tamoxifen and/or aromatase inhibitors) were specified by the NCR since 2003. Targeted therapy was included in the NCR since 2005 and almost exclusively existed of trastuzumab (~99%). Treatment proportions were determined based on specific treatments received by patients at any time during their treatment process, irrespective of duration or whether it was completed. Type of endocrine therapy (tamoxifen and/or aromatase inhibitors) was specified based on the first administered treatment, as information on treatment in the NCR was only available up to one year after diagnosis.

Statistical analyses

Annual crude and age-standardized incidence and mortality rates for the period 1989–2017 were calculated per 100,000 person-years (PY) using the general population size, as obtained from Statistics Netherlands, as person-time denominator[19]. Crude rates were calculated as three-year moving averages with two-year moving averages calculated at both ends of the study period and rates were age-standardized (European Standardized Rates, ESR) to the 2013 European Standard Population 95+ (2013 ESP 95+)[20,21].

Trend changes over time were evaluated with joinpoint regression analyses, with each model representing a series of connected straight lines on a log scale and with each joinpoint denoting a statistically significant change in trends. Annual Percentage Changes (APC) were determined for each trend segment and provide an overview of all trend changes over time. The Average Annual Percentage Change (AAPC) provides a good summary measure of the overall trend and was determined over the whole period[22,23]. Both APCs and AAPCs were calculated from the slope coefficients of the underlying joinpoint models and were determined with the freely available Joinpoint Regression Program version 4.7.0.0 and based on the previously determined age-standardized incidence and mortality rates[24]. Two-sided significance was determined at an α=0.05 level. Analyses were performed using the "Uncorrelated Error Model" and the "Grid Search Method" setting, with the number of points placed between observed x-values set at 3. For model selection, the recommended Bayesian Information Criteria 3 method was used[23]. The minimum allowed number of joinpoints was set at zero. The maximum allowed number of joinpoints to be tested was based on the algorithmic recommendation table included in the Joinpoint help manual 4.7.0.0 (available at https://surveillance.cancer.gov/joinpoint), allowing a maximum of five joinpoints for overall, age- and stage-specific rates and a maximum number of two joinpoints for the subtype-specific rates. The parametric method was used to calculate 95% Confidence Intervals (CI). Further programme parameters were kept at their default settings.

The relative survival (RS) was used as an estimator of disease-specific survival

and is the ratio between the observed BC survival of the patients and the expected survival in the general Dutch population, matched by attained age, sex and calendar year. Expected survival was determined using nationwide lifetables of the general Dutch population adapted from Statistics Netherlands, containing survival probability data of women aged 0–99 years in 1989 to 2018. Outcomes were age-standardized using the traditional method with cumulative weights based on the age-distribution in the 2013 ESP 95+[20]. Used weights were 0.47, 0.14, 0.30 and 0.09 for the <40, 40–49, 50–74, and ≥75 age groups, respectively[25]. The RS was calculated using the Ederer II approach[26]. Brenner's period analysis was used to derive more up-to-date estimates of the RS by exclusively considering the survival time data of patients during a (recent) time period of interest by left-truncating all observations at the start of the time period and right-censoring them at its end. This in contrast with the traditional cohort methodology, which provides outdated long-term survival estimates based on patients that were diagnosed many years ago without consideration of ongoing improvements. A more detailed description of the period analysis methodology is provided elsewhere[27]. End of follow-up was defined as year of death, year of emigration or 2016, whichever came first. We limited survival analyses to 2016 to avoid potential overestimation of long-term survival outcomes following period analyses[27].

All data analyses were performed using the Stata Software Package, version 14.2 and are presented for all BC patients combined and stratified by age group (<40, 40–49, 50–74, and ≥75), stage and receptor subtype when sample size allowed. Patients with missing or unknown values were excluded from the analyses. Likewise, women with unavailable treatment data (e.g. due to not receiving any treatment or incomplete registration) were excluded. To overcome difficulties in trend recognition over time due to the changes in tumour stage classification, stages II and III BC were analysed individually as well as grouped together. Cut-off points for the age groups were based on the age at invitation to the current Dutch national mammographic screening programme (50–74 years), with younger and older women grouped separately.

Results

Study population

In total, 320,249 women were diagnosed with first primary invasive BC in the Netherlands between 1989–2017 and of all women who died (N=2,027,353), 97,187 died from BC (4.8%). The median age at diagnosis was 61 years (range 18–107 years). All population characteristics are presented in Table 1. Data on the yearly number of BC deaths are included in supplementary Table S1.

Incidence

The BC incidence for all BC patients combined significantly increased from 126 to 153 per 100,000 PY (AAPC=0.7% [95%CI:0.6, 0.9]) between 1989–2017 (Figure 1A and Table S2). Age-specific results showed an increase in BC incidence from 15 to 20 (AAPC=1.0% [95%CI:0.5, 1.5]) in women aged <40 years, 150 to 176 (AAPC=0.5% [95%CI:0.2, 0.7]) for 40-49 years, and 237 to 315 per 100,000 PY (AAPC=1.1% [95%CI:0.8, 1.3]) in women aged 50-74 years at time of diagnosis. In women aged ≥75, the incidence decreased from 300 to 269 per 100,000 PY (AAPC=−0.3% [95%CI:−0.5, −0.2]) between 1989–2017.

In some sub-periods, significant declines in BC incidence were observed for all BC patients combined; in the period 1993–1997 the incidence declined from 145 to 141 (APC=−1.3% [95%CI:−2.1, −0.5]) and in the period 2013-2017 from 158 to 153 per 100,000 PY (APC=−0.8% [95%CI:−1.1, −0.5]). In women aged 40–49 years, the BC incidence significantly declined from 2006 onward from 182 to 176 per 100,000 PY (APC=−0.4% [95%CI:−0.6, −0.2]) and in women aged 50–74 years it declined from 330 to 315 per 100,000 PY (APC=−1.1% [95%CI:−1.6, −0.7]) between 2013 and 2017. In women aged ≥75, BC incidence decreased since 1998 from 339 to 269 per 100,000 PY (APC=−1.2% [95%CI:−1.3, −1.1]) in 2017 (Table S2).

Tumour stage

The stage-specific incidence rates of stage I BC for all BC patients combined increased from 36 to 72 per 100,000 PY (AAPC=2.6% [95%CI:2.1, 3.0]) between 1989–2017. In the same period, the combined incidence of stages II and III BC decreased from 80 to 72 per 100,000 PY (AAPC=−0.3% [95%CI:−0.5, −0.1]). The incidence of stage IV BC remained stable around 8 per 100,000 PY (AAPC=−0.2% [95%CI:−0.6, 0.2]) (Figure S1 and Table S3).

Prior to the shift from the 5th to 6th edition of the TNM classification, the incidence of stages II and III combined increased from 80 to 84 per 100,000 PY (AAPC=0.5% [95%CI:0.2, 0.7]) between 1989 and 2003 and declined from 84 to 72 per 100,000 PY (AAPC=−1.1% [95%CI:−1.3, −0.8]) after the shift in 2003–2017. Similar declines after the shift were observed for stages II and III individually (Table S4).

Table 1. Patient, tumour and treatment characteristics of all women diagnosed with first primary invasive breast cancer in the Netherlands between 1989–2017

	1898-1992		1993-1996		1997-2000		2001-2004		2005-2008		2009-2012		2013-2017		Total	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Age at diagnosis																
Median	61.7		61.0		60.1		59.2		59.7		60.5		61.4		60.5	
Range	21.8– 100.3		20.4– 107.3		18.7– 104.2		18.7–100.4		18.9–102.1		19.2–103.1		18.5–103.8		18.5–107.3	
<40	2,364	7.3	2,463	6.6	2,519	6.2	2,829	6.4	2,682	5.7	2,660	5.2	3,378	5.1	18,895	5.9
40–49	6,085	18.8	7,028	18.8	7,639	18.8	8,423	18.9	8,986	19.0	9,160	17.9	10,880	16.3	58,201	18.2
50–74	17,547	54.1	20,492	54.8	22,223	54.7	24,949	56.0	27,385	57.9	30,789	60.3	41,930	62.7	185,315	57.9
≥75	6,440	19.9	7,444	19.9	8,265	20.3	8,333	18.7	8,215	17.4	8,450	16.5	10,691	16.0	57,838	18.1
Tumour stage																
I	9,992	31.4	13,348	36.6	14,972	37.5	16,558	37.6	18,848	40.1	22,816	44.8	31,212	46.7	127,746	40.3
II	16,505	51.8	17,863	49.0	19,430	48.7	20,107	45.7	19,114	40.7	19,046	37.4	24,688	37.0	136,753	43.1
III	3,282	10.3	3,282	9.0	3,401	8.5	5,211	11.8	6,814	14.5	6,673	13.1	7,255	10.9	35,918	11.3
IV	2,083	6.5	1,996	5.5	2,103	5.3	2,145	4.9	2,236	4.8	2,344	4.6	3,633	5.4	16,540	5.2
Unknown	574		938		740		513		256		180		91		3,292	
Tumour size†																
T1	14,190	45.2	18,101	50.2	20,640	52.3	23,797	54.6	26,702	57.2	30,041	59.5	40,559	61.2	174,030	55.4
T2	12,741	40.6	13,491	37.4	14,387	36.5	15,852	36.3	16,264	34.9	16,587	32.9	20,126	30.3	109,448	34.9
T3	1,617	5.1	1,608	4.5	1,616	4.1	1,738	4.0	1,886	4.0	2,125	4.2	3,346	5.0	13,936	4.4
T4	2,866	9.1	2,835	7.9	2,820	7.1	2,232	5.1	1,793	3.8	1,737	3.4	2,291	3.5	16,574	5.3
Unknown	1,022		1,392		1,183		915		623		569		557		6,261	
Lymph node status†																
N0	17,679	56.8	21,621	60.2	23,032	59.0	24,682	57.1	27,959	60.1	31,518	62.3	44,087	66.3	190,578	60.9
N1	12,708	40.8	13,640	38.0	15,427	39.5	15,247	35.3	12,959	27.8	13,849	27.4	17,742	26.7	101,572	32.5
N2	659	2.1	590	1.6	528	1.4	2,125	4.9	3,440	7.4	3,052	6.0	2,545	3.8	12,939	4.1
N3	104	0.3	49	0.1	59	0.2	1,165	2.7	2,199	4.7	2,183	4.3	2,159	3.2	7,918	2.5
Unknown	1,286		1,527		1,600		1,315		711		457		346		7,242	
Histological grade§																
Grade 1	1,022	8.1	1,611	10.3	4,248	16.1	6,988	19.4	9,196	22.7	10,295	24.3	14,031	24.5	47,391	20.6
Grade 2	4,207	33.5	5,824	37.3	11,179	42.3	16,202	45.0	18,032	44.5	19,275	45.6	28,176	49.2	102,895	44.6
Grade 3	7,330	58.4	8,163	52.3	10,997	41.6	12,833	35.6	13,249	32.7	12,723	30.1	15,027	26.3	80,322	34.8
Unknown	19,877		21,829		14,222		8,511		6,791		8,766		9,645		89,641	
ER-status																
Positive									37,675	81.7	41,961	83.9	55,964	84.7	135,600	83.6
Negative									8,433	18.3	8,073	16.1	10,116	15.3	26,622	16.4
Unknown									1,160		1,025		799		2,984	

Table 1. Continued

	1898-1992		1993-1996		1997-2000		2001-2004		2005-2008		2009-2012		2013-2017		Total	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
PR-status																
Positive									29,126	65.9	33,193	67.5	45,736	69.3	108,055	67.8
Negative									15,104	34.1	16,011	32.5	20,269	30.7	51,384	32.2
Unknown									3,038		1,855		874		5,767	
HER2-status																
Positive									6,435	15.3	6,892	14.3	8,766	13.7	22,093	14.3
Negative									35,617	84.7	41,291	85.7	55,143	86.3	132,051	85.7
Unknown									5,216		2,876		2,970		11,062	
Receptor subtype‡																
HR+/HER2-									30,594	73.4	35,865	74.8	48,453	75.9	114,912	74.9
HR+/HER2+									3,929	9.4	4,481	9.3	5,843	9.2	14,253	9.3
HR-/HER2+									2,384	5.7	2,329	4.9	2,908	4.6	7,621	5.0
HR-/HER2-									4,765	11.4	5,287	11.0	6,652	10.4	16,704	10.9
Unknown									5,596		3,097		3,023		11,716	
Surgery + Radiotherapy#																
BCS + RT	8,480	26.1	12,749	34.1	15,315	37.7	19,608	44.0	22,796	48.2	25,494	49.9	36,977	55.3	141,419	44.2
BCS + No RT	486	1.5	781	2.1	1,028	2.5	847	1.9	712	1.5	754	1.5	1,256	1.9	5,864	1.8
Mastectomy + RT	4,433	13.7	5,653	15.1	6,041	14.9	6,063	13.6	5,910	12.5	6,276	12.3	8,579	12.8	42,955	13.4
Mastectomy + No RT	7,609	23.5	13,662	36.5	14,591	35.9	14,105	31.7	13,518	28.6	13,715	26.9	13,215	19.8	90,415	28.2
Surgery (Unspecified) + RT	3,855	11.9	500	1.3	32	0.1	13	0.0	5	0.0	2	0.0	3	0.0	4,410	1.4
Surgery (Unspecified) + No RT	4,416	13.6	610	1.6	82	0.2	11	0.0	3	0.0	4	0.0	8	0.0	5,134	1.6
No surgery + RT	730	2.3	574	1.5	412	1.0	395	0.9	302	0.6	263	0.5	295	0.4	2,971	0.9
No surgery + No RT	2,427	7.5	2,898	7.7	3,145	7.7	3,492	7.8	4,022	8.5	4,551	8.9	6,546	9.8	27,081	8.5
Chemotherapy																
Taxane-containing CT¶	4	0.0	8	0.0	68	0.2	159	0.4	3,589	7.6	5,947	11.6	3,175	4.7	12,950	4.0
Anthracycline-containing CT**	6	0.0	26	0.1	125	0.3	3,673	8.2	3,299	7.0	2,016	3.9	1,195	1.8	10,340	3.2
Taxane- and anthracycline-containing CT††	0	0.0	0	0.0	6	0.0	127	0.3	2,297	4.9	10,521	20.6	22,024	32.9	34,975	10.9
CT (Other/ Unspecified)§§	4,329	13.3	5,483	14.6	9,849	24.2	11,245	25.3	8,804	18.6	4,029	7.9	306	0.5	44,045	13.8
No CT	28,097	86.6	31,910	85.3	30,598	75.3	29,330	65.9	29,279	61.9	28,546	55.9	40,179	60.1	217,939	68.1

Table 1. Continued

	1898-1992		1993-1996		1997-2000		2001-2004		2005-2008		2009-2012		2013-2017		Total	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Endocrine therapy‡‡																
Tamoxifen first	18	0.1	38	0.1	143	0.4	9,649	21.7	21,043	44.5	26,104	51.1	29,774	44.5	86,769	27.1
Aromatase inhibitors first	0	0.0	1	0.0	2	0.0	863	1.9	2,335	4.9	3,999	7.8	8,950	13.4	16,150	5.0
Ovarian ablation##	9	0.0	91	0.2	194	0.5	184	0.4	118	0.2	113	0.2	186	0.3	895	0.3
ET (Other/ Unspecified)¶¶	9,708	29.9	11,436	30.6	14,630	36.0	9,145	20.5	0	0.0	0	0.0	4	0.0	44,923	14.0
No endocrine therapy	22,701	70.0	25,861	69.1	25,677	63.2	24,693	55.4	23,772	50.3	20,843	40.8	27,965	41.8	171,512	53.6
Systemic therapy																
Chemotherapy only	3,829	11.8	4,670	12.5	6,683	16.4	6,490	14.6	4,448	9.4	4,633	9.1	5,598	8.4	36,351	11.4
Endocrine therapy only	9,225	28.4	10,719	28.6	11,604	28.5	11,191	25.1	11,747	24.9	14,436	28.3	20,616	30.8	89,538	28.0
Chemotherapy and Endocrine therapy	510	1.6	847	2.3	3,365	8.3	8,605	19.3	9,843	20.8	12,965	25.4	14,105	21.1	50,240	15.7
Targeted therapy***	0	0.0	0	0.0	0	0.0	115	0.3	3,751	7.9	5,075	9.9	7,220	10.8	16,161	5.0
No systemic therapy	18,872	58.2	21,191	56.6	18,994	46.7	18,133	40.7	17,479	37.0	13,950	27.3	19,340	28.9	127,959	40.0
Total	32,436	100.0	37,427	100.0	40,646	100.0	44,534	100.0	47,268	100.0	51,059	100.0	66,879	100.0	320,249†††	100.0

Abbreviations: HR= hormone receptor, HER2= human epidermal growth factor receptor2, ER= oestrogen receptor, PR= progesterone receptor, CT= chemotherapy, ET= endocrine therapy, RT= radiotherapy, NCR= Netherlands Cancer Registry, BCS= breast-conserving surgery, AI= aromatase inhibitor

*****The various elements included in the table were collected by the NCR at different moments in time. ER and PR-status were routinely collected and included in the NCR since 2005 and HER2-status since 2006. Specification of chemotherapy and endocrine therapy regimens in the NCR has been done since 2003. Target therapy was routinely collected and included in the NCR since 2005 (mainly trastuzumab). Treatment data in the NCR is collected up to one year after initial cancer diagnosis. The data may still include 162 in situ BC cases due to discrepancies in registration within the dataset. Percentages may not total to 100% due to rounding.

† Tumour size and Lymph node status are based on the pathological stage, but clinical stage was used if pathological stage was unavailable.

§ Including 502 first primary BCs that were defined as "undifferentiated" in the NCR.

‡ HR+ = ER+ and/or PR+, HR- = ER- and PR-.

Patients that received both BCS and mastectomy were included in the mastectomy group.

¶ The chemotherapy regimen contains taxanes, but no anthracyclines.

** The chemotherapy regimen contains anthracyclines, but no taxanes.

†† The chemotherapy regimen contains both taxanes and anthracyclines.

§§ All other chemotherapy regimens (e.g. cyclophosphamide/cisplatine containing regimes) and/or chemotherapy not further specified.

‡‡ The NCR codes aromatase inhibitors specifically; tamoxifen is coded as hormonal treatment. Both treatments were included when provided as initial treatment.

Ovarian ablation includes LHRH agonist treatment, radiotherapy and/or surgical removal of the ovaries to reduce oestrogen production in pre-menopausal women.

¶¶ All other hormonal treatments (e.g. fulvestrant) and/or not further specified.

*** Patients received targeted therapy either alone or in combination with CT, ET, or both.

††† Total numbers provided do not correspond with those for the ER, PR, HER2 and the receptor subtype groups due to their inclusion since 2005-2009.

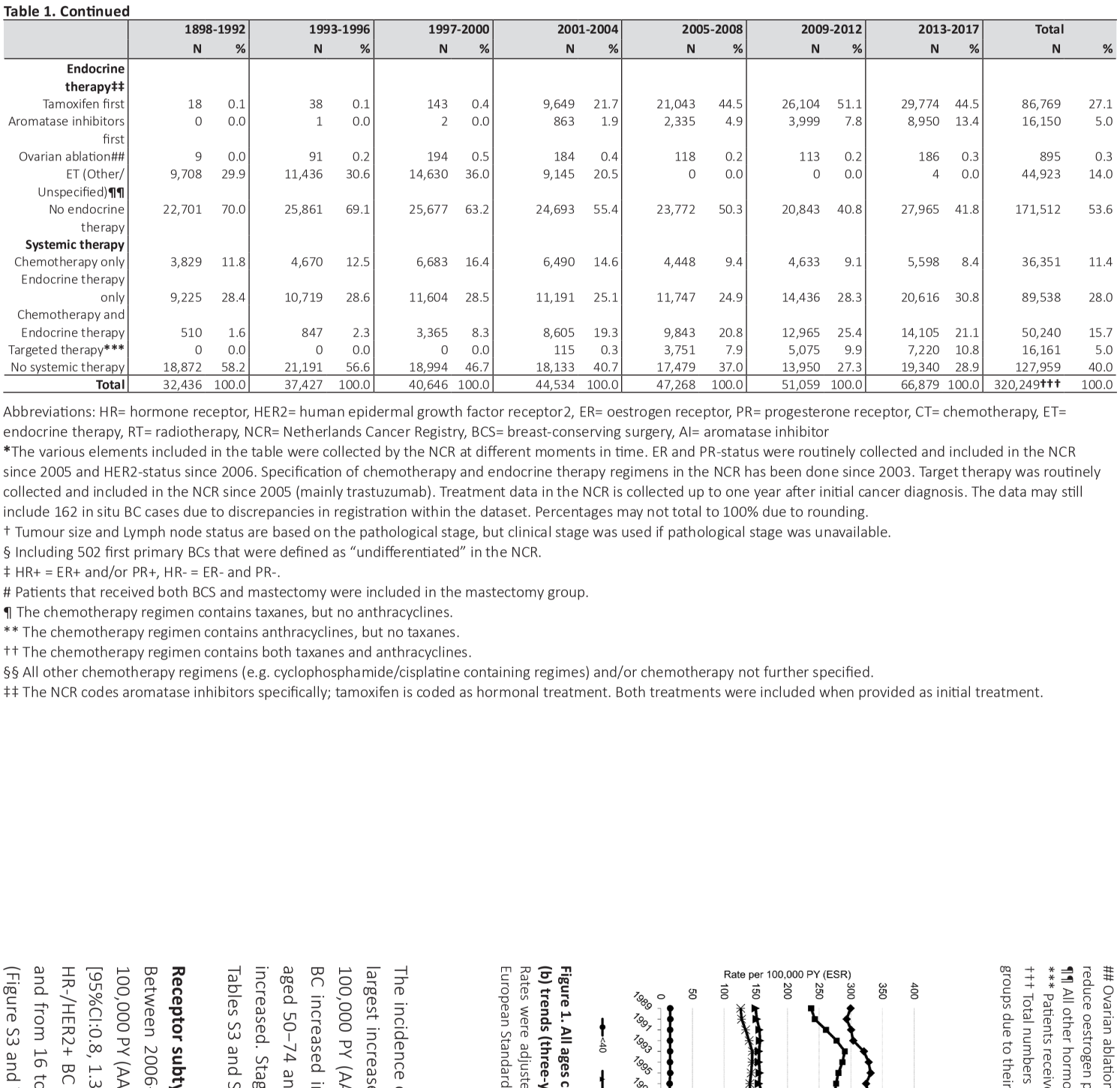


Figure 1. All ages combined and age-specific first primary invasive breast cancer incidence (a) and mortality (b) trends (three-year moving averages) in the Netherlands in the period 1989–2017
Rates were adjusted for age (European Standard Rates, ESR) by direct standardization according to the 2013 European Standard Population 95+ and calculated per 100,000 person-years (PY)

The incidence of stage I BC increased for all age groups between 1989–2017, with the largest increase observed in women aged 50–74 years, increasing from 69 to 176 per 100,000 PY (AAPC=3.5% [95%CI:3.0, 3.9]). The combined incidence of stages II and III BC increased in women aged <40 and 40–49 years, whereas it decreased in women aged 50–74 and ≥75 years. In women aged 40–49 years, the incidence of stage IV BC increased. Stage IV incidence remained stable for the other age groups (Figure S2 and Tables S3 and S4).

Receptor subtype

Between 2006–2017, the incidence of HR+/HER2- BC increased from 104 to 112 per 100,000 PY (AAPC=0.7% [95%CI:0.5, 0.9]) and from 12 to 13 per 100,000 PY (AAPC=1.0% [95%CI:0.8, 1.3]) for HR+/HER2+ BC for all ages combined. Meanwhile, the incidence of HR-/HER2+ BC declined from 8 to 7 per 100,000 PY (AAPC=–0.9% [95%CI:–1.7, –0.2]) and from 16 to 15 per 100,000 PY (AAPC=–0.3% [95%CI:–0.6, –0.0]) for HR-/HER2- BC (Figure S3 and Table S5).

HR+/HER2- BC incidence decreased slightly from 123 to 121 per 100,000 PY (AAPC=−0.3% [95%CI:−0.6, −0.0]) in women aged 40–49 years, whereas it significantly increased among women aged <40 and 50–74 years between 2006–2017. The incidence of HR+/HER2+ BC increased for women aged <40, 40–49 and 50–74 years. No changes in incidence of HR+/HER2- and HR+/HER2+ BC were observed since 1989 among women aged ≥75 years Concurrently, the incidence of HR-/HER2+ BC decreased from 15 to 13 (AAPC=−1.8% [95%CI:−2.3, −1.3]), and HR-/HER2- BC decreased from 29 to 27 per 100,000 PY (AAPC−0.7 [95%CI:−1.1, −0.3]) in women aged 50−74 years. The HR-negative BC incidence remained stable for the remaining age groups (<40, 40–49 and ≥75 years) regardless of HER2-status (Figure 2 and Table S5).

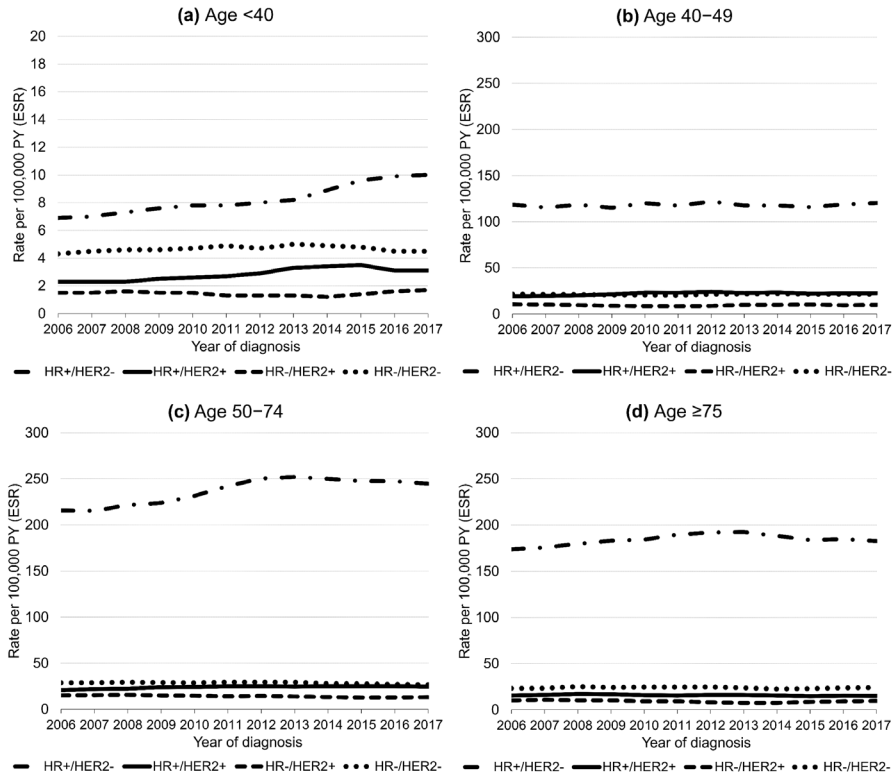


Figure 2. Incidence trends in the Netherlands stratified by receptor subtype between 2006–2017 in women diagnosed with first primary invasive breast cancer

Rates were adjusted for age (European Standard Rates, ESR) by direct standardization according to the 2013 European Standard Population 95+ and calculated per 100,000 person-years (PY). HR+= ER+ and/or PR+, HR-= ER- and PR-. Information on ER/PR and HER2-status was routinely collected by the Dutch cancer registry since 2005 and 2006, respectively. Note the different scaling in (a)

Treatment strategies

Surgery and radiotherapy

The proportion of women with BC that underwent surgery remained stable around 90% since 1989. Breast-conserving surgery (BCS) became the preferred surgical intervention since 2003 with 60.1% of all surgically treated patients undergoing BCS in 2017 (Figure 3). Radiotherapy use increased from 55.4% in 1989 to 70.1% in 2017 and was almost exclusively given in combination with surgical treatment (up to 99.6% in 2013−2017) (Figure 3 and Table 1). The most commonly provided local treatment was BCS followed by radiotherapy, with 55.3% of BC patients receiving this combination in 2013−2017 (Table 1).

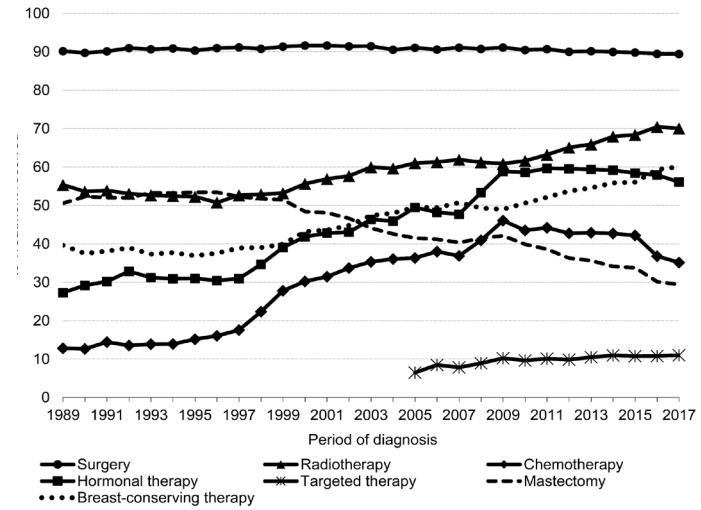


Figure 3. Proportion of treatment received by patients with first primary invasive breast cancer in the Netherlands between 1989–2017

Targeted therapy (mainly trastuzumab) was routinely collected by the NCR since 2005. Cumulative proportion were calculated per treatment strategy and based on treatment received (yes/no). Proportions of mastectomy and breast-conserving surgery were calculated based on the proportion of patients receiving surgery. Patients that received both surgical treatments were included in the mastectomy group

Systemic treatment

The use of any systemic treatment increased from 41.8% in 1989−1992 to 71.1% in 2013−2017. Most women received endocrine therapy only (28.4% in 1989−1992 and 30.8% in 2013−2017). The proportion of women that received both chemotherapy and endocrine therapy increased from 1.6% in 1989−1992 to 25.4% in 2009−2012, but slightly declined to 21.1% in 2013−2017. The use of targeted therapy (mainly trastuzumab) increased from 7.9% in 2005−2008 to 10.8% in 2013−2017 (Figure S4).

Trends in systemic treatment use over time according to age, stage and receptor subtype are included in Figure S5.

Chemotherapy

The overall proportion of women that received chemotherapy increased from 12.8% in 1989 to 46.0% in 2009, and decreased to 35.1% in 2017 (Figure 3). Chemotherapy use likewise decreased since 2009 for most age groups and stages, and for the HR+/HER2- subtype, but remained stable in women aged ≥75 years (2–3%) and in women with stage IV BC (41–43%), as shown in Figure S6. Among all women receiving chemotherapy, the proportion treated with both taxane and anthracycline containing regimens increased from 5.7% in 2003–2005 to 79.3% in 2015–2017 (Figure S7a).

Endocrine therapy

Endocrine therapy use increased from 27.3% in 1989 to 59.6% in 2011, and slightly decreased to 56.1% in 2017 (Figure 3). Most patients received tamoxifen as initial endocrine therapy. Use of tamoxifen for all BC patients combined was stable at 88.2–91.8% between 2003–2005 and 2009–2011, and subsequently decreased to 74.5% in 2015–2017. The use of aromatase inhibitor as initial endocrine therapy increased from 11.2% to 25.0% between 2003–2005 and 2015–2017 (Figure S7b). Endocrine therapy use increased among women of all ages and for most BC stages (stage I–III), as shown in Figure S8.

Relative survival

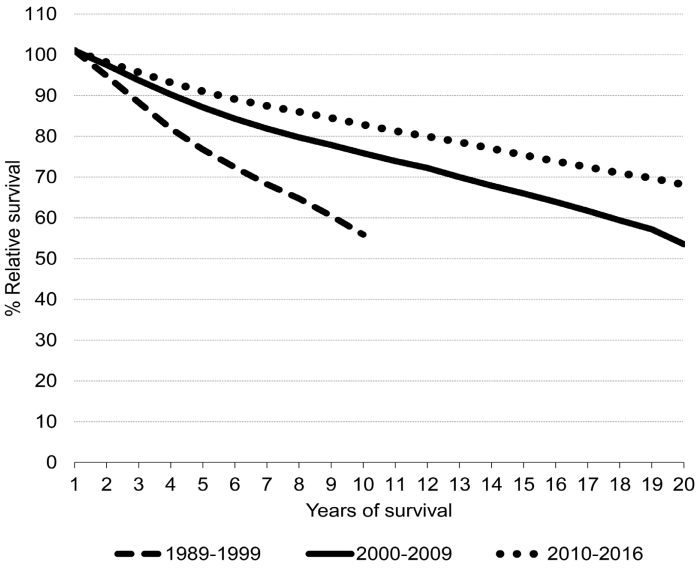
The RS at five and ten years of follow-up for all BC patients combined was 76.8% (95%CI:76.1, 77.4) and 55.9% (95%CI:54.7, 57.1) in 1989–1999, respectively, and increased to 91.0% (95%CI:90.5, 91.5) and 82.9% (95%CI:82.2, 83.5) in 2010–2016. Between 2000–2009 and 2010–2016, the 15-year RS increased from 66.0% (95%CI:65.2, 66.7) to 75.4% (95%CI:74.6, 76.2) and the 20-year RS increased from 53.5% (95%CI:52.2, 54.8) to 68.1% (95%CI:67.1, 69.1) (Figure 4).

The RS improved for all age groups and most stages between 1989–1999 and 2010–2016, but the 15-year RS remained stable for stage IV BC between 2000–2009 (RS=4.6% [95%CI:3.1, 6.4]) and 2010–2016 (RS=7.2% [95%CI:4.6, 10.5]). The survival of all receptor subtypes improved between 2006–2011 and 2012–2013, but no further improvements were observed in the subsequent period 2014–2016 (Figure 5 and Table S6).

The RS improved for all women aged <40, 40–49 and 50–74 years with stages I to III BC between 1989–1999 and 2010–2016 for all years of follow-up. The RS at ten and 15 years of follow-up remained stable for those with stage IV BC since 2000–2009 and likewise did not improve since 2000–2009 in women aged ≥75 years with any stage BC (Figure S9 and Table S7). The five-year RS of all receptor subtypes remained stable since 2012–2013 irrespective of age(Figure S10 and Table S8). Survival outcomes were overall slightly lower in women aged ≥75 years in comparison to other age groups and deteriorated with advancing stage for all age groups (Figures 5 and S9, and Tables S6 and S7).

Mortality

The BC mortality for women of all ages decreased from 57 to 35 per 100,000 PY (AAPC=–1.8% [95%CI:–1.9, –1.7]) between 1989–2017. Similar trends were observed for all age groups, as shown in Figure 1B and Table S2.



Time period	1989–1999	2000–2009	2010–2016
Years of survival	% RS (95%CI)		
5	76.8 (76.1, 77.4)	87.1 (86.7, 87.6)	91.0 (90.5, 91.5)
10	55.9 (54.7, 57.1)	75.9 (75.2, 76.5)	82.9 (82.2, 83.5)
15		66.0 (65.2, 66.7)	75.4 (74.6, 76.2)
20		53.5 (52.2, 54.8)	68.1 (67.1, 69.1)

Figure 4. Age-standardized relative survival (RS) outcomes with corresponding 95% confidence intervals (95% CI) of first primary invasive breast cancer in the Netherlands between 1989–2017

Relative survival was adjusted for age by direct standardization according to the 2013 European Standard Population 95+

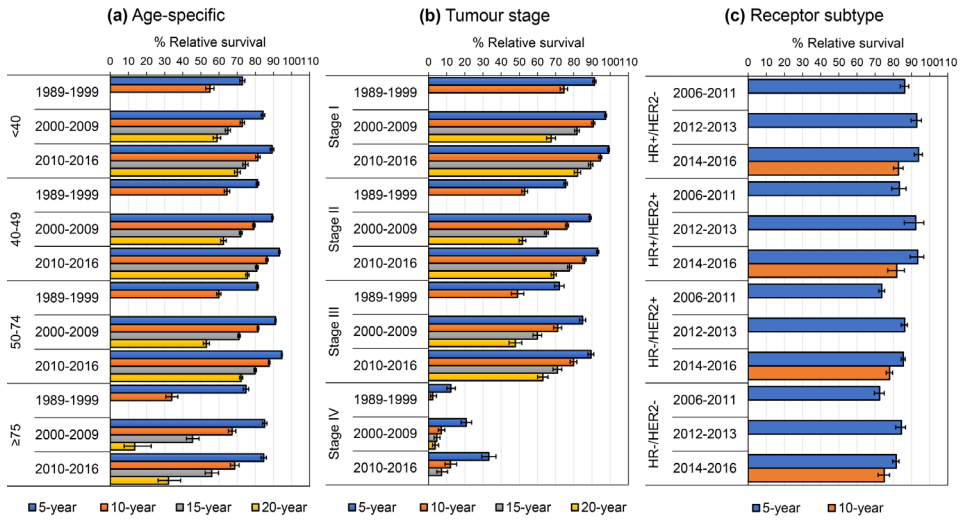


Figure 5. Age-specific (a) and age-standardized stage (b) and receptor subtype-specific (c) relative survival outcomes with corresponding 95% confidence intervals of first primary invasive breast cancer patients in the Netherlands diagnosed between 1989–2016

Relative survival was adjusted for age by direct standardization according to the 2013 European Standard Population 95+. HR+= ER+ and/or PR+, HR-= ER- and PR-. Information on ER/PR and HER2-status was routinely collected by the Dutch cancer registry since 2005 and 2006, respectively. For stage IV BC, the 20-year relative survival in 2010–2016 could not be estimated due low patient numbers

Discussion

This study provides a comprehensive overview of first primary invasive BC incidence, survival, mortality and treatment trends stratified by age, stage and receptor subtype in the Netherlands between 1989–2017, using population-based data on 320,249 women with first primary invasive BC from the NCR. BC incidence in the Netherlands has steadily increased between 1989–2013. However, in recent years the latest time trends (APCs) revealed noticeable declines in BC incidence for the entire patient population, in women aged 40–49 and 50–74 years, and in women with stage I disease. In women aged ≥75 years, BC incidence has been declining since 1998. Systemic treatment increasingly involved a combination of chemotherapy, endocrine therapy and targeted therapy. The relative survival improved markedly over time for all years of follow-up for most patients, but remained stable for all receptor subtypes since 2012–2013 and since 2000–2009 in women with stage IV BC at 15–years of follow-up. BC mortality steadily decreased in women of all age groups since 1989.

Breast cancer incidence

The rising trends in BC incidence are consistent with those found in previous Dutch and global (trend) studies[1,4,5,9-12] and can be attributed in part to changes in the prevalence of known risk and lifestyle factors that have been shown to influence BC incidence[4,5]. In a recent case-control study, the increasingly common use of both oral contraceptives (for more than 10 years) and hormone replacement therapy (for more than three years) has been shown to increase the risk of BC (relative risk=3.2 [95%CI: 1.4, 7.4]) in women aged <55 years[6]. Together with the increased alcohol consumption among younger people this might explain the rising BC incidence in women aged <40 years in this study[7]. The worldwide rise in overweight and obesity in recent decades is also likely to have contributed to the increase in BC incidence in both pre- and post-menopausal women[8]. In the US, decreases in BC incidence in 2002 and 2003 were attributed to the declining use of hormone-replacement therapy in post-menopausal women following unfavourable publicity[28]. However, similar trends were not observed in the Netherlands until 2005 and are likewise not observed now[29].

The observed trends in BC incidence are probably also influenced by the population-based mammography screening programme, which has been operational in the Netherlands since 1989 and for which women aged 50–74 years are invited biennially. Screening is intended to favourably change the stage at diagnosis and leads to a strong temporary increase in BC incidence due to the detection of (mainly) slow growing tumours followed by a decline in more advanced BC stages[4,5]. This corresponds with the observed increase in the incidence of stage I BC and the decline in incidence of stage II/III BC, which was most prominent in women aged 50–74 years. The decline in BC incidence observed since 1998 in women aged ≥75, who are no longer offered screening (compensatory drop), might also reflect screening practices[30].

The decline in BC incidence shown by the latest trends (2013–2017 for all patients combined) might be associated with the transition from screen-film to digital mammography between 2003–2010. In the period when digital mammography was implemented an increase in BC incidence was observed in women aged 50–74 (2004–2013, APC=1.2% [95%CI: 1.0, 1.5]) and in women with stage I BC (2005–2012, APC=3.4% [95%CI: 3.0, 3.8]), whereas no rise in incidence was observed prior to digital mammography implementation. A similar pattern was observed in women aged 50–74 year with HR+/HER2+ BC. In all cases, incidence rates either decreased or remained stable in the subsequent period, which might suggest a temporal increase after implementation of digital mammography[31]. However, in our study, the relation to screening was not directly taken into account in the analyses since mode of detection was not registered in the NCR until 2011. A recent study based on actual screening attendance did show that the incidence of stage III and IV BC was significantly higher in non-screened versus screened women (94 versus 38 per 100,000 PY, respectively; Odds Ratio[OR]=2.86, 95%CI:[2.72, 3.00])[32]. In our data, 56% of all women aged 50–74 years were diagnosed through screening between 2011–2017. Thus, screening has at least partially affected the BC incidence. Alternatively, the observed decline in BC incidence

in women aged 40–49 years might partly relate to the increase in prophylactic bilateral mastectomies, which significantly lowers the BC incidence in unaffected high risk women with *BRCA* mutations (85–100%)[33] and recently showed a significant increase in uptake in women (mean age 41.8 years) who received genetic testing after 2008 (32.7% in the Netherlands)[34].

Treatment strategies

Therapeutic approaches of BC in the Netherlands have changed drastically since 1989. BCS with adjuvant radiotherapy became the preferred treatment over mastectomy after the publication of landmark trials[35,36]. The steep increase in both adjuvant chemotherapy and endocrine therapy use between 2007–2009 can be explained by the broadening of their indications following the 2008 revision of the Dutch evidence-based guidelines and the introduction of the decision tool 'Adjuvant! Online', which was developed to predict the potential benefit of systemic treatment for individual BC patients[37].

The decline in chemotherapy use after 2009 is likely also related to changes in the Dutch evidence-based guidelines for the management of breast cancer (www.oncoline.nl), which now recommends endocrine therapy instead of chemotherapy in post-menopausal women with grade 2 tumours >1.1 cm and ER/PgR >50%. Possibly also related to the decline in chemotherapy use is the increased use of the 70-gene signature (70-GS, "MammaPrint") and other measures used to assess tumour aggressiveness (Ki67 immunohistochemistry, PgR status, etc.), together with a growing focus on shared decision making and a more reluctant attitude of clinicians towards the use of chemotherapy in low risk patients[38,39].

Breast cancer survival and mortality

Advances in treatment and more personalized therapeutic guidelines likely also contributed to the improvements in BC survival and mortality[3-5]. The sharp increase in the proportion of women that received both taxane and anthracycline containing regimens from 2003–2005 to 2015–2017 may provide some explanation for the observed improvements in survival, as use of combination chemotherapy has been shown to improve survival in metastatic BC since the late 1960s[14]. Improvements in survival and mortality may also relate to more personalized therapy (adjuvant endocrine therapy and anti-HER2 therapy) facilitated since the beginning of this century by the use of information on tumour biology (HR and HER2-status), which has improved treatment allocation to patients that will more likely benefit based on their tumour characteristics, even for stage IV disease[40]. The gains in survival and mortality may also in part be attributed to the changed composition of women who receive endocrine therapy, following changes in the Dutch national guidelines. Before 1999, endocrine therapy was given to all post-menopausal women with N+ BC and was provided, irrespective of menopausal status, to all women with N+ and ER+ BC. The similar survival of women with either HER2-positive or HER2-negative BC, irrespective of HR-status, likely relates to the use of trastuzumab, which was recommended in the Netherlands since 2005[15]. When not treated with trastuzumab, the overall survival of HER2-positive BC is poorer compared to HER2-negative BC[41].

Stage at diagnosis has also remained one of the most important determinants for BC survival, with survival becoming increasingly worse with advancing stage. Improvements in stage-specific survival have been described previously[9,11] and may partly be explained by stage migration, due to advances in detecting distant metastases, but also evolutions in TNM classification[42]. In clinical practice, the impact of stage migration has been observed after implementation of FDG-PET in lung cancer care, which resulted in an increase in stage IV classification[43]. Improvements in the detection of distant metastases at time of BC diagnosis likewise resulted in stage migration[44]. It is therefore possible that stage migration contributed to the observed improvements in stage-specific survival observed here. Poorer adherence to treatment guidelines in older patients, together with the fact that these women are no longer included in population screening, may be responsible for the higher stage II–IV rates at diagnosis in women aged ≥75 years and might to some extent explain the lower survival observed in these women compared to the younger age groups[45].

Decreases in BC mortality have been observed previously in most European, North-American and other high-income countries[3-5]. In the south-eastern region of the Netherlands, mortality rates declined annually with 2% between 1995 and 2004[9]. In the current study, a similar annual decline was observed for the entire Netherlands between 1989–2017. The declines in BC mortality and improvements in survival have mainly been related to advances in early diagnosis[3-5]. Worldwide, early detection (mainly due to the more widespread use of mammography screening) has been suggested to be causal in the decline in BC mortality in high-income countries[2,4]. Findings in the Netherlands have led to the same conclusions[12,46,47]. Projections from a simulation study based on six distinct models on BC mortality trends in the US further showed that screening was on average associated with 44% (model range: 35%–60%) and 37% (model range: 26%–51%) of the observed decline in overall BC mortality among women aged 30–79 years in 2000 and 2012, respectively. The remaining decline in mortality in 2012 was on average attributed to chemotherapy; 31% (model range: 22%–37%), endocrine therapy; 27% (model range: 18%–36%) and trastuzumab; 4% (model range: 1%–6%)[48]. However, the data do not support the viewpoint that screening has a substantial effect on breast cancer mortality, as declines in BC mortality in the Netherlands have been present since the late 1980s, prior to the implementation of a nationwide screening programme[49]. Moreover, in this study declines in mortality were slightly higher in women aged <40 and 40–49 years than in older women where organized screening is expected to influence the mortality. Also, declines were already observed in the period shortly after screening implementation, which is not expected due to the usual time lag before screening effects

become apparent[50]. Advances in treatment are therefore more likely to have caused this effect[49].

Strengths and limitations

The major strength of this study was the use of a large population-based dataset from the NCR spanning almost three decades of BC data. Data of all new BC patients were collected by trained registrars, leading to high completeness and ruling out selection bias. This study is among the first to include a detailed description on BC trends according to receptor subtype in Europe, which is another major strength. However, data on receptor subtype was still limited and consequently, we could not detect clear trends based on receptor subtype. Furthermore, we did not have information available on risk and lifestyle factors, and were therefore not able to directly assess trends in incidence according to these factors. We experienced some difficulties in the assessment of trends due to the changing definition of tumour stage. In particular, the change from the 5th to 6th TNM classification resulted in a noticeable shift from stage II to III disease, which complicated trend recognition and comparisons over time. We tried to address this shortcoming by combining both stages for analyses and by assessing pre-shift and post-shift time trends separately with joinpoint regression analyses. Finally, we did not have information available on the BC-specific survival and therefore we used RS as an estimator. Nonetheless, the RS is an appropriate method to use in population-based studies on survival in the absence of cause of death information and does not suffer from misclassification.

Conclusion

This study provides a comprehensive overview of first primary invasive BC trends in the Netherlands since 1989. The incidence of BC for the entire patient population has steadily increased between 1989–2013, but has been declining since. Whether this declining trend continues, should be confirmed by future trend studies covering subsequent time periods. Meanwhile, the relative survival improved for all age groups and for most stages and receptor subtypes, and the the mortality of first primary invasive BC has decreased substantially since 1989. The observed trends in BC incidence, mortality and survival likely result from the combined effect of preventive measures, earlier diagnosis (population screening and better disease awareness), advances in treatment, national implementation of personalized treatment guidelines and changes in the exposure to known risk factors.

Article information

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank the registration team of the Netherlands Comprehensive Cancer Organisation (IKNL) for the collection of data for the Netherlands Cancer Registry as well as staff for scientific advice. In particular, the authors would personally like to thank Linda de Munck and Janneke Verloop for their helpful contribution during the conduction of this study.

Data Availability Statement

The data that support the findings are available from the Netherlands Cancer Registry upon reasonable request (data request study number K18.244, www.iknl.nl).

Ethics statement

The study was reviewed and approved by the Privacy Review Board of the Netherlands Cancer Registry.

References

1 Bray, F. *et al.* Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **68**, 394-424 (2018).

2 Hashim, D. *et al.* The global decrease in cancer mortality: trends and disparities. *Ann. Oncol.* **27**, 926-933 (2016).

3 Bosetti, C. *et al.* The decline in breast cancer mortality in Europe: an update (to 2009). *The Breast* **21**, 77-82 (2012).

4 Youlden, D. R. *et al.* The descriptive epidemiology of female breast cancer: an international comparison of screening, incidence, survival and mortality. *Cancer Epidemiol.* **36**, 237-248 (2012).

5 Bray, F., McCarron, P. & Parkin, D. M. The changing global patterns of female breast cancer incidence and mortality. *Breast Cancer Res.* **6**, 229-239, doi:10.1186/bcr932 (2004).

6 Brinton, L. A. *et al.* Breast cancer risk among women under 55 years of age by joint effects of usage of oral contraceptives and hormone replacement therapy. *Menopause* **25**, 1195-1200 (2018).

7 Heath, A. K. *et al.* Nutrient-wide association study of 92 foods and nutrients and breast cancer risk. *Breast Cancer Res.* **22**, 1-12 (2020).

8 Picon-Ruiz, M., Morata-Tarifa, C., Valle-Goffin, J. J., Friedman, E. R. & Slingerland, J. M. Obesity and adverse breast cancer risk and outcome: Mechanistic insights and strategies for intervention. *CA Cancer J. Clin.* **67**, 378-397 (2017).

9 Louwman, W. J. *et al.* On the rising trends of incidence and prognosis for breast cancer patients diagnosed 1975–2004: a long-term population-based study in southeastern Netherlands. *Cancer Causes Control* **19**, 97-106 (2008).

10 van der Waal, D. *et al.* Breast cancer diagnosis and death in the Netherlands: a changing burden. *The European Journal of Public Health* **25**, 320-324 (2015).

11 Vondeling, G. T. *et al.* Burden of early, advanced and metastatic breast cancer in The Netherlands. *BMC Cancer* **18**, 262, doi:10.1186/s12885-018-4158-3 (2018).

12 Otten, J. D. M. *et al.* Impressive time-related influence of the Dutch screening programme on breast cancer incidence and mortality, 1975-2006. *Int. J. Cancer* **123**, 1929-1934 (2008).

13 Sukel, M. P. P. *et al.* Substantial increase in the use of adjuvant systemic treatment for early stage breast cancer reflects changes in guidelines in the period 1990–2006 in the southeastern Netherlands. *Eur. J. Cancer Clin. Oncol.* **44**, 1846-1854 (2008).

14 Zurrida, S. & Veronesi, U. Milestones in Breast Cancer Treatment. *Breast J* **21**, 3-12, doi:10.1111/tbj.12361 (2015).

15 de Munck, L. *et al.* Implementation of trastuzumab in conjunction with adjuvant chemotherapy in the treatment of non-metastatic breast cancer in the Netherlands. *Breast Cancer Res. Treat.* **129**, 229-233 (2011).

16 Statistics Netherlands (CBS). *Statline: Bevolking; geslacht, leeftijd en burgerlijke staat*. Available from: https://opendata.cbs.nl/statline/#/CBS/nl/dataset/7461bev/table?dl=1B894

17 Statistics Netherlands (CBS). *Statline: Overledenen; belangrijke doodsoorzaken (korte lijst), leeftijd, geslacht*. Available from: https://opendata.cbs.nl/statline/#/CBS/nl/dataset/7052_95/table?dl=1BAAC

18 Brierley, J. D., Gospodarowicz, M. K. & Wittekind, C. *TNM Classification of Malignant Tumours*. 8th ed edn, 272p (Wiley-Blackwell, 2017).

19 Consonni, D., Coviello, E., Buzzoni, C. & Mensi, C. A command to calculate age-standardized rates with efficient interval estimation. *The Stata Journal* **12**, 688-701 (2012).

20 Pace, M., Lanzieri, G., Glickman, M. & Zupanič, T. *Revision of the European Standard Population: report of Eurostat's task force*. (Publications Office of the European Union, 2013).

21 Naing, N. N. Easy way to learn standardization: direct and indirect methods. *The Malaysian journal of medical sciences* **7**, 10-15 (2000).

22 Clegg, L. X., Hankey, B. F., Tiwari, R., Feuer, E. J. & Edwards, B. K. Estimating average annual per cent change in trend analysis. *Stat. Med.* **28**, 3670-3682 (2009).

23 Kim, H. J., Fay, M. P., Feuer, E. J. & Midthune, D. N. Permutation tests for joinpoint regression with applications to cancer rates. *Stat. Med.* **19**, 335-351 (2000).

24 Kuchenbaecker, K. B. *et al.* Evaluation of Polygenic Risk Scores for Breast and Ovarian Cancer Risk Prediction in BRCA1 and BRCA2 Mutation Carriers. *J. Natl. Cancer Inst.* **109**, doi:10.1093/jnci/djw302 (2017).

25 Pokhrel, A. & Hakulinen, T. Age-standardisation of relative survival ratios of cancer patients in a comparison between countries, genders and time periods. *Eur. J. Cancer* **45**, 642-647 (2009).

26 Dickman, P. W. & Coviello, E. Estimating and modeling relative survival. *The Stata Journal* **15**, 186-215 (2015).

27 Brenner, H., Gefeller, O. & Hakulinen, T. Period analysis for 'up-to-date'cancer survival data: theory, empirical evaluation, computational realisation and applications. *Eur. J. Cancer* **40**, 326-335 (2004).

28 Ravdin, P. M. *et al.* The decrease in breast-cancer incidence in 2003 in the United States. *N. Engl. J. Med.* **356**, 1670-1674 (2007).

29 Soerjomataram, I., Coebergh, J. W. W., Louwman, M. W. J., Visser, O. & van Leeuwen, F. E. Does the Decrease in Hormone Replacement Therapy Also Affect Breast Cancer Risk in the Netherlands? *J. Clin. Oncol.* **25**, 5038-5039, doi:10.1200/jco.2007.13.7281 (2007).

30 Jørgensen, K. J. & Gøtzsche, P. C. Overdiagnosis in publicly organised mammography screening programmes: systematic review of incidence trends. *BMJ* **339**, b2587 (2009).

31 Sankatsing, V. D. V. *et al.* Detection and interval cancer rates during the transition from screen-film to digital mammography in population-based screening. *BMC Cancer* **18**, 256, doi:10.1186/s12885-018-4122-2 (2018).

32 de Munck, L. *et al.* Is the incidence of advanced-stage breast cancer affected by whether women attend a steady-state screening program? *Int. J. Cancer* **143**, 842-850 (2018).

33 Alaofi, R. K., Nassif, M. O. & Al-Hajeili, M. R. Prophylactic mastectomy for the prevention of breast cancer: Review of the literature. *Avicenna journal of medicine* **8**, 67-77, doi:10.4103/ajm.AJM_21_18 (2018).

34 Metcalfe, K. *et al.* International trends in the uptake of cancer risk reduction strategies in women with a BRCA1 or BRCA2 mutation. *Br. J. Cancer* **121**, 15-21, doi:10.1038/s41416-019-0446-1 (2019).

35 van Dongen, J. A. *et al.* Long-Term Results of a Randomized Trial Comparing Breast-Conserving Therapy With Mastectomy: European Organization for Research and Treatment of Cancer 10801 Trial. *JNCI: Journal of the National Cancer Institute* **92**, 1143-1150, doi:10.1093/jnci/92.14.1143 (2000).

36 Fisher, B. *et al.* Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer. *N. Engl. J. Med.* **347**, 1233-1241 (2002).

37 Struikmans, H. *et al.* Guideline'Treatment of breast cancer 2008'(revision). *Ned. Tijdschr. Geneeskd.* **152**, 2507-2511 (2008).

38 Kuijer, A. *et al.* Using a gene expression signature when controversy exists regarding the indication for adjuvant systemic treatment reduces the proportion of patients receiving adjuvant chemotherapy: a nationwide study. *Genet. Med.* **18**, 720-726, doi:10.1038/gim.2015.152 (2016).

39 van Steenhoven, J. E. C. *et al.* The Changing Role of Gene-Expression Profiling in the Era of De-escalating Adjuvant Chemotherapy in Early-Stage Breast Cancer. *Ann. Surg. Oncol.* **26**, 3495-3501, doi:10.1245/s10434-019-07511-8 (2019).

40 de Abreu, F. B., Schwartz, G. N., Wells, W. A. & Tsongalis, G. J. Personalized therapy for breast cancer. *Clin. Genet.* **86**, 62-67, doi:10.1111/cge.12381 (2014).

41 Tai, W., Mahato, R. & Cheng, K. The role of HER2 in cancer therapy and targeted drug delivery. *J. Control. Release* **146**, 264-275, doi:https://doi.org/10.1016/j.jconrel.2010.04.009 (2010).

42 Feinstein, A. R., Sosin, D. M. & Wells, C. K. The Will Rogers phenomenon: stage migration and new diagnostic techniques as a source of misleading statistics for survival in cancer. *N. Engl. J. Med.* **312**, 1604-1608, doi:10.1056/nejm198506203122504 (1985).

43 Schuurman, M. S. *et al.* Temporal trends and spatial variation in stage distribution of non-small cell lung cancer in the Netherlands. *Screening* **4**, 9-10 (2014).

44 Polednak, A. P. Increase in distant stage breast cancer incidence rates in US women aged 25–49 years, 2000–2011: the stage migration hypothesis. *J. Cancer Epidemiol.* **2015**, 710106, doi:10.1155/2015/710106 (2015).

45 Bastiaannet, E. *et al.* Breast cancer in elderly compared to younger patients in the Netherlands: stage at diagnosis, treatment and survival in 127,805 unselected patients. *Breast Cancer Res. Treat.* **124**, 801-807, doi:10.1007/s10549-010-0898-8 (2010).

46 Otto, S. J. *et al.* Mammography Screening and Risk of Breast Cancer Death: A Population-Based Case–Control Study. *Cancer Epidemiol. Biomarkers Prev.* **21**, 66-73, doi:10.1158/1055-9965.Epi-11-0476 (2012).

47 van der Waal, D., Ripping, T. M., Verbeek, A. L. M. & Broeders, M. J. M. Breast cancer screening effect across breast density strata: A case–control study. *Int. J. Cancer* **140**, 41-49, doi:10.1002/ijc.30430 (2017).

48 Plevritis, S. K. *et al.* Association of Screening and Treatment With Breast Cancer Mortality by Molecular Subtype in US Women, 2000-2012. *JAMA* **319**, 154-164, doi:10.1001/jama.2017.19130 (2018).

49 Botha, J. L., Bray, F., Sankila, R. & Parkin, D. M. Breast cancer incidence and mortality trends in 16 European countries. *Eur. J. Cancer* **39**, 1718-1729 (2003).

50 Lee, S. J. *et al.* Time lag to benefit after screening for breast and colorectal cancer: meta-analysis of survival data from the United States, Sweden, United Kingdom, and Denmark. *BMJ* **346**, e8441, doi:10.1136/bmj.e8441 (2013).

Supplementary Material

For supplementary material see publication