Proefschrift Delal Akdeniz

Identifying Risk Factors Associated with Contralateral Breast Cancer

A step towards personalized risk prediction

Identifying Risk Factors Associated with Contralateral Breast Cancer
A step towards personalized risk prediction

Identificatie van risicofactoren geassocieerd met contralaterale borstkanker
Een stap in de richting van gepersonaliseerde risicopredictie

Proefschrift

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Ji bo dîya min û bawê min

In memoriam, Mehmet Nuri "Nurettin" Akdeniz
August 15th,1950 – July 12th, 2013

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CHAPTER 1

INTRODUCTION

Breast cancer prevalence and risk factors

Breast cancer (BC) is the most common type of cancer among women globally; in 2020 around 2.3 million women were diagnosed with BC.[1] Women in the Netherlands have a lifetime risk of 10-14% of developing BC, meaning that 1 out of 7 women will eventually develop BC.[2] Around 5-10% of all BC patients have a hereditary predisposition. Women harboring germline mutations in BReast CAncer (BRCA) 1 or 2 tumor suppressor genes are examples of women having a high risk for BC, with life time risks of up to 80%.[3] Other germline mutations have also been associated with a high BC risk such as *PALB2* mutations for example, or associated with a moderately increased BC risk, such as *CHEK2* c.1100delC and pathogenic mutations in *ATM*.[4-6] Combinations of BC Single nucleotide polymorphisms, so called polygenic risk scores, can also explain elevated BC risks, especially in familial BC cases.[7-9]

Also in the absence of pathogenic germline mutations or polygenic risk scores, there are numerous other factors that can lead to an increased BC risk. A family history of breast cancer as such already leads also to an increased BC risk for reasons yet to be revealed.[3,10,11] High mammographic density, i.e. the ratio of fibro glandular breast tissue to fat tissue, is also strongly associated with increased BC risk[12-14], and is determined by heredity, menopausal status, body mass index and age.[15-18] In western countries the incidence of BC is up to 3.5 times higher than in developing countries.[1,19,20] Especially at population level a change in lifestyle or reproductive factors influencing body hormone status (i.e. estrogen, testosterone and insulin-like growth factor-1)[21,22] have been associated with increasing BC incidence in western countries: young age at menarche, especially in combination with older age at menopause; no or low number of full-term pregnancies; older age at primiparity; no or short period of breast feeding; oral contraceptive use; and overweight.[23-26] Further, also alcohol use, smoking, night shift work, tall stature, and exposure to ionizing radiation of the chest at a young age have been associated with increased BC risk.[27-31]

Treatment for early breast cancer and outcome

Treatment for early (non-metastasized) BC consists of loco-regional treatment of the breast (i.e. surgery and radiotherapy) and can be preceded (neo-adjuvant) or followed by (adjuvant) systemic therapy (chemotherapy, endocrine therapy, targeted therapy).

The exact treatment plan is based on several factors such as patient preference, recurrence risk, tumor characteristics, age, clinical performance, and co-morbidity. For example, whether patients will undergo a lumpectomy or a mastectomy depends on size and location(s) of the tumor and the patients' preference. BC survival outcomes are comparable between patients with breast conserving therapy (i.e. lumpectomy followed by radiotherapy) and mastectomy.[32]

Breast irradiation is, in principal, always indicated if a patient receives a lumpectomy. In patients undergoing a mastectomy, treatment with radiotherapy depends on complete excision of the tumor, tumor size and location, number of lymph nodes involved, tumor grade, the presence of angiolymphatic invasion, Estrogen hormone (ER)/Progesterone hormone (PR)/Her2 neu (Her2) receptor status and age at BC diagnosis. In general, treatment with radiotherapy decreases local recurrence risk by 50%.[33]

Systemic therapy, i.e. chemotherapy, endocrine therapy and/or targeted therapy are indicated in case of increased risk of developing recurrent disease. The risk of recurrent disease increases with higher tumor stage, high grade tumor and/or a young age at BC diagnosis. Factors such as patients' preference, age, clinical condition, and co-morbidity determine if, and if so, which type of systemic treatment can be given. The type of systemic treatment is also dependent on tumor characteristics such as the ER, PR and Her2 receptor status. BC survival increased with the addition of systemic treatment.[34,35]

In general, survival of BC mainly depends on tumor stage, morphology, receptor status and age at BC diagnosis. Over the years, BC survival has substantially improved as a result of population-based screening, and more refined treatment and follow-up methods. This led to earlier diagnosis and improved BC survival.[36] In the United states, BC death rates have dropped by 34% since 1990.[37] In the Netherlands, 10-year BC survival increased from around 60% in 1980-1990 to around 80% in patients diagnosed between 2006-2010.[2]

Contralateral breast cancer

With improved detection and treatment methods for BC, patients have been surviving for longer periods. This has also led to an increasing number of BC survivors at risk of developing a second new BC in the opposite breast during

follow-up, i.e. metachronous contralateral breast cancer (CBC). The annual risk to develop CBC is around 0.5% in the general population and is up to 3% in *BRCA1* and *BRCA2* mutation carriers.[38] In patients without a *BRCA1/2* mutation, but with a positive family history for bilateral BC, the risk is still in the same range, especially if women are diagnosed with their primary breast cancer (PBC) at a young age.[39]

CBC can occur in the same period as the primary breast tumor (synchronous CBC) or it may occur after a certain time period following the primary diagnosis (metachronous CBC). There is however no fixed definition concerning the time lapse to distinguish synchronous from metachronous CBC. In literature, mainly a time frame of 3 to 12 months following PBC diagnosis has been used to define a metachronous CBC. However, outliers of 24 months or even 60 months have also been used.

To reduce the risk of CBC development, preventive removal of the (contralateral) breast tissue is currently offered to women at high risk (i.e. *BRCA1/2* mutation, familial bilateral BC). In these patients, a risk-reducing mastectomy has indeed been associated with improved overall survival.[40] In the general BC population, risk-reducing surgery is not indicated, since the risk of developing CBC is relatively low and no survival benefit has been reported.[41]

Nonetheless, in clinical practice an increasing number of low-risk women have been opting for preventive removal of their contralateral breast tissue. One of the main motivations for women to opt for preventive removal is the fear of having to experience the procedure of a cancer diagnosis and severe treatments all over again.[42,43] From personal interviews it was however noticed that women without a germline mutation in general were overestimating their risk of developing a CBC. These overestimations came in the range of CBC risks for *BRCA1/2* mutation carriers. Clearly, there is an urgent need for accurate risk prediction models for CBC.

CBC Risk prediction model

With a personalized CBC risk prediction model, the problem of overestimations can be addressed potentially leading to less fear and to less overtreatment. In addition, in high risk women there might also be specific situations in which the best treatment option is currently not clear. For these women as well, it would be useful to have personalized CBC risk estimates available to optimize follow-up decisions.

To provide personalized CBC risk estimates an accurate (i.e. taking all relevant factors into account) CBC risk prediction model is needed. Currently, there is no model available with the ability to accurately predict CBC risk. For example, the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) model, is currently a frequently used model, however it does not take PBC treatment into account.[44] In the first phase of building such a risk prediction model, all risk factors for CBC need to be identified and potential interaction between risk factors should be determined. In the second phase, the model has to be validated both internally as well as externally. In the last phase, the model is tested in clinical practice, i.e. the implementation phase: the practical usability and patient satisfaction will be evaluated, and preferably the clinical utility, i.e. to what extent women's health outcome will be improved.

In this thesis we mainly focused on the first phase of a CBC risk prediction model and investigated risk factors of which published literature shows conflicting results in its association with CBC risk.

Risk factors for contralateral breast cancer

Current established factors for CBC risk are germline mutations such as *BRCA1/2* and *CHEK2* c.1100delC, young age at PBC diagnosis, or positive (bilateral) BC family history.[38,39,45] Several other patient, breast tumor and treatment characteristics have been associated with CBC risk. However in the majority of these factors, their reported impact are contradicting.

In principle, risk factors that have been associated with PBC might also be of influence on developing CBC. Primary breast tumor and treatment characteristics could have an additional influence on this risk.

Radiotherapy for PBC has been reported as a CBC risk increasing factor, especially in *BRCA1/2* mutation carriers.[46] Since *BRCA1* and *BRCA2* mutation carriers are already at increased risk of developing CBC, investigating the effects of several factors within these carriers specifically is essential.

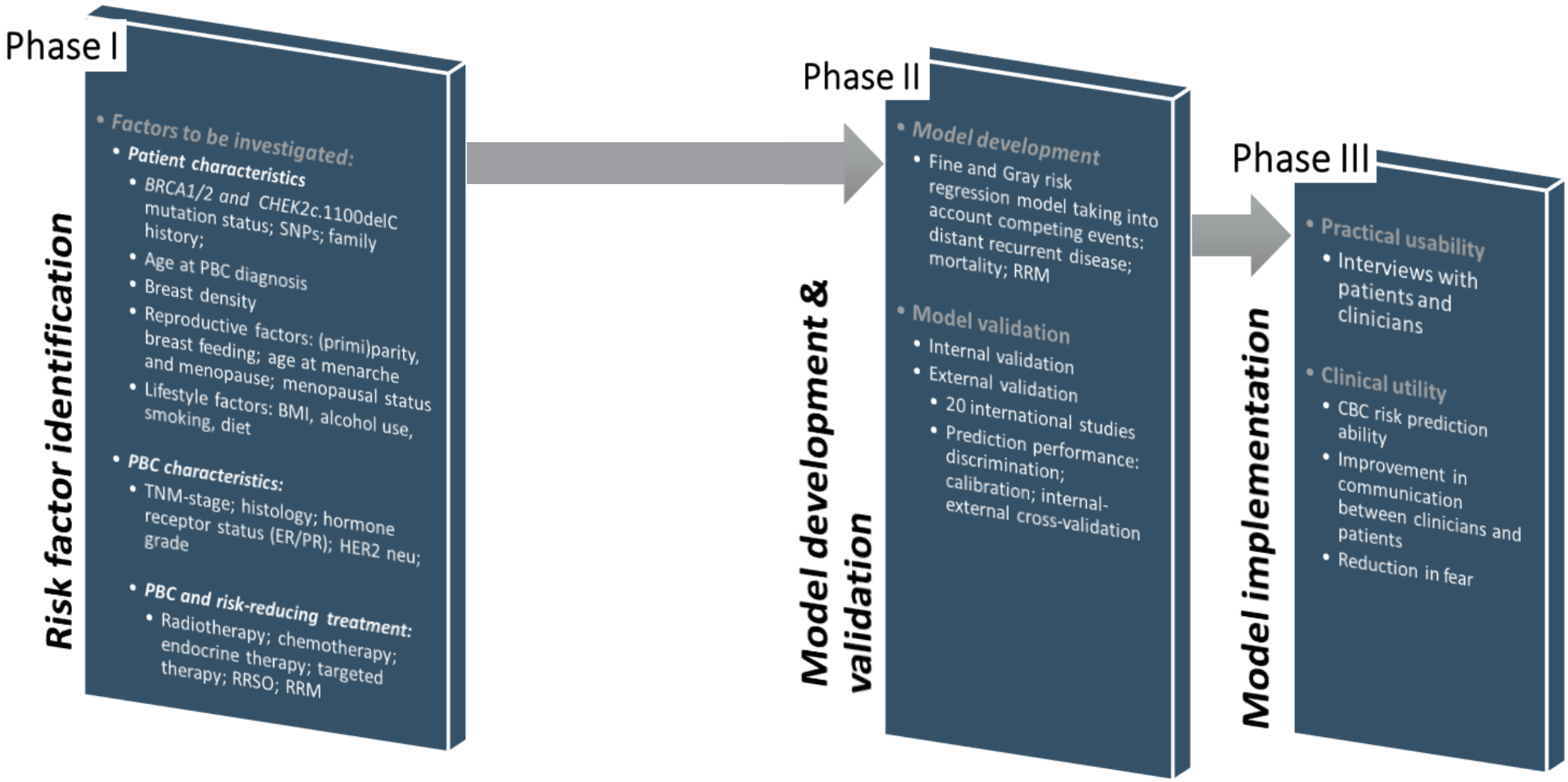

Figure 1. CBC risk prediction model building, validation and implementation

TNM-stage: tumor size, nodal status, distant metastasis; SNPs: single nucleotide polymorphisms; ER: estrogen receptor status; PR: progesterone receptor status; HER2: human epidermal growth factor receptor 2; BMI: body mass index; RRSO: risk-reducing salpingo oophorectomy; RRM: risk-reducing mastectomy.

Different systemic treatment modalities such as chemotherapy and endocrine therapy have been established as CBC risk lowering factors.[47,48] Current evidence for the effect of chemotherapy and endocrine therapy on CBC risk in *BRCA1/2* mutation carriers is mainly based on older studies in which selection and ascertainment bias limit the reliability.[49-53] In addition, due to power issues, *BRCA1* and *BRCA2* mutation carriers have been analyzed as one group, while *BRCA1*-associated breast tumors have more unfavorable characteristics than *BRCA2* tumors, i.e. young age at PBC diagnosis, triple negative phenotype (i.e. ER, PR and HER2 negative receptor status).[38,50,54] Ideally, *BRCA1* and *BRCA2* mutation carriers with BC should be analyzed as separate groups.

PBC characteristics such as TNM-stage, receptor status, grade and lobular histology have also been reported as risk factors.[55-57] Lobular histology is the second most common type of BC and accounts for 10-15% of all BCs. Some lobular mixed types have been associated with poor prognosis.[58] In literature, lobular BCs have been described as being associated with higher risk of CBC.[59-61] More recent studies show however a smaller CBC risk following a lobular PBC[55,57,62], which might be a consequence of adjuvant systemic therapy options.[63] In addition, it has not been investigated yet whether CBC characteristics are different following a lobular as compared to a ductal PBC. This could be a potential reason to screen more actively for CBCs, especially if secondary tumors are more aggressive following a lobular than ductal PBC.

Several lifestyle and reproductive factors have also been investigated with respect to CBC risk. For a majority of these factors results have been either contradicting (i.e. a protective, risk increasing or no association have been observed; examples hereof are BMI, alcohol use, menarche, age at primiparity, having 1 or more versus 0 full-term pregnancies or 2 or more versus 1 full-term pregnancy, menopausal status and age at menopause)[61,64-73] or results have been inconclusive (i.e. a non-significant risk-increasing association was observed; for example in smokers versus non-smokers[61,64,69,70]; or non-significant risk-decreasing results were observed; examples hereof are breastfeeding, having 1-3 versus 0 full-term pregnancies, gravidity and oral contraceptive use [61,66,68,69,74]). Having 4 or more full-term pregnancies is the only factor so far that has been strongly associated with reduced CBC risk.[69,75]

Whether mammographic density or a decrease in mammographic density after systemic treatment for PBC is associated with CBC risk, is not clear: results are conflicting and are based on small study populations.[15,64,76-78]

Aims and outline of this thesis

In this thesis, we aim to identify the main risk factors that are associated with CBC in different genetic risk groups and to provide more accurate estimates for suspected and known factors. The effect estimates can be incorporated into a personalized CBC risk prediction model and can help improve individualized decision-making in women with BC.

As mentioned, there have been several factors inconsistently associated with CBC risk.

In **chapter 2** we searched through the literature to identify reported genetic, patient, tumor and treatment related risk factors for CBC and performed meta-analyses to quantify these associations. We aimed to provide CBC risk estimates for *BRCA1*, *BRCA2* and *CHEK2* c.1100delC mutation carriers, familial women (i.e. hereditary cause suspected but no mutation found) and unselected women (i.e. women from the general population without any preselection) with BC.

In **chapter 3** we again performed a systematic review and meta-analysis, this time focusing on several lifestyle and reproductive factors and their association with CBC risk in unselected patients.

We used the Hereditary Breast and Ovarian cancer research Netherlands (HEBON) cohort to investigate the effects of different types of chemotherapy on CBC risk in *BRCA1* and *BRCA2* mutation carriers. The results hereon are presented in **chapter 4**.

In **chapter 5 & chapter 6** we investigated the effects of radiation therapy on CBC risk in young *BRCA1* and *BRCA2* mutation carriers. In chapter 5 we investigated this question in mutation carriers treated for their BC at the Family Cancer Clinic at the Erasmus MC in Rotterdam. In chapter 6, we used a larger, international cohort: The International *BRCA1/2* Carrier Cohort Study (IBCCS).

In **chapter 7** we aimed to investigate the risk of CBC in patients with lobular PBC using a large nationwide dataset and investigated the effects of systemic therapy. In addition, we inspected whether CBC characteristics are different following a lobular or ductal PBC.

Finally, in **chapter 8** the results of this thesis are discussed and summarized and suggestions for future directions are discussed.

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CHAPTER 2

RISK FACTORS FOR METACHRONOUS CONTRALATERAL BREAST CANCER: A SYSTEMATIC REVIEW AND META-ANALYSIS

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ABSTRACT

Background: The risk of developing metachronous contralateral breast cancer (CBC) is a recurrent topic at the outpatient clinic. We aimed to provide CBC risk estimates of published patient, pathological, and primary breast cancer (PBC) treatment-related factors.

Methods: PubMed was searched for publications on factors associated with CBC risk. Meta-analyses were performed with grouping of studies by mutation status (i.e., *BRCA1*, *BRCA2*, *CHEK2* c.1100delC*)*, familial cohorts, and general population-based cohorts.

Results: Sixty-eight papers satisfied our inclusion criteria. Strong associations with CBC were found for carrying a *BRCA1* (RR=3.7; 95%CI:2.8-4.9), *BRCA2* (RR=2.8; 95%CI:1.8-4.3) or *CHEK2* c.1100delC (RR=2.7; 95%CI:2.0-3.7) mutation. In population-based cohorts, PBC family history (RR=1.8; 95%CI:1.2-2.6), body mass index (BMI) ≥30kg/m^2 (RR=1.5; 95%CI:1.3-1.9), lobular PBC (RR=1.4; 95%CI:1.1-1.8), estrogen receptor-negative PBC (RR=1.5; 95%CI:1.0-2.3) and treatment with radiotherapy <40 years (RR=1.4; 95%CI:1.1-1.7) was associated with increased CBC risk. Older age at PBC diagnosis (RR per decade=0.93; 95%CI:0.88-0.98), and treatment with chemotherapy (RR=0.7; 95%CI:0.6-0.8) or endocrine therapy (RR=0.6; 95%CI:0.5-0.7) were associated with decreased CBC risk.

Conclusions: Mutation status, family history, and PBC treatment are key factors for CBC risk. Age at PBC diagnosis, BMI, lobular histology and hormone receptor status have weaker associations and should be considered in combination with key factors to accurately predict CBC risk.

INTRODUCTION

Due to an increasing incidence of primary breast cancer (PBC) and improved breast cancer (BC) surveillance and treatment methods, an increasing number of women who have survived BC are at risk of developing a contralateral breast cancer (CBC).[1] The annual CBC risk is around 0.5% in the general BC population and up to 3% in *BRCA1/2* mutation carriers.[2,3]

A risk-reducing contralateral mastectomy minimizes the risk of developing a subsequent CBC and may improve survival in patients considered to be at high risk, i.e. hereditary BC patients.[4-6] On the other hand, the percentage of patients opting for a risk-reducing contralateral mastectomy has rapidly increased over the last decades, suggesting that more relatively low-risk BC patients are also treated.[7-9] Fear and overestimation of risk may play a role in the decision-making of these low-risk patients.[10,11]

For both high-risk and low-risk PBC patients, accurate CBC risk prediction is crucial and can be achieved by taking into account the effect of patient, pathological, and treatment-related characteristics. However, CBC risk prediction as used in clinical practice is currently only based on *BRCA1/2* mutation status, family history of BC and age at PBC.[2,12,13] The association of other factors with CBC risk is either lacking or conflicting. Combinations of these factors may improve decision-making regarding surveillance, primary and risk-reducing therapies, and may enable patient-tailored counselling in both high-risk and low-risk patients.

Therefore, we aimed to quantify the association of various patient, pathological, and treatment-related characteristics with metachronous CBC risk.

METHODS

For this systematic review we published an online protocol at Prospero including details on study design (registration number: CRD42015014381, link: http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42015014381) and we followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines.

Search strategy

In collaboration with a research librarian (EdC, see acknowledgements) a search strategy was developed. One reviewer searched PubMed for publications on search terms for metachronous CBC in combination with various predefined patient characteristics (carriership of *BRCA1, BRCA2* and *CHEK2* c.1100delC mutations, family history of (bilateral) BC, mammographic density, factors at PBC diagnosis: age, BMI, menopausal status), PBC characteristics (TN(M)-stage, tumor grade, Estrogen (ER), Progesterone (PR) and HER2 neu receptor status, histological subtype), and PBC treatment-related characteristics (radiotherapy, chemotherapy, endocrine therapy, targeted therapy, risk-reducing salpingo-oophorectomy (RRSO)). We also searched for publications on second BC risk, in the knowledge that a majority (95%) of the second breast cancers are contralateral events rather than ipsilateral breast tumors.[14] Details of the full strategy applied are provided in Supplementary Table A.1.

Abstracts were screened using the following inclusion criteria: experimental and observational studies published in English, between January 1990 and July 4, 2016, investigating CBC risk in women who have no prior history of other invasive malignancies. We included papers only from 1990 onwards to have a long-term follow-up while also being able to investigate the effects of adjuvant treatment options (which were considered mainly from the late eighties onwards). Further, we excluded papers if the reported number of second BC events was less than twenty (arbitrary cut-off), and also if no relative risk (RR) estimates (hazard ratio or odds ratio or relative risk) for CBC risk were provided.

Relevant full-text publications were considered for inclusion and critically appraised, on methodology, and comparability of groups, subgroups and their reference groups. If papers reported on specific subgroups that were non-combinable with other subgroups, these papers were excluded for the meta-analysis. In addition, potential overlap in (part of) patients due to selection from the same registries/ hospitals in the same period was solved by selecting the most relevant cohort (i.e. the factor of interest for the meta-analysis was specifically published on) and/or selecting the most recent cohort with the longest follow-up.

From the included papers, study design characteristics and all the available univariable and multivariable risk estimates were extracted and entered in a Microsoft Access database by four reviewers (DA, MKS, AJvdB, MJH) using a specifically designed data entry form.

Statistical analyses

We investigated the effects of carrying vs. not carrying a *BRCA1*, *BRCA2* or *CHEK2* c.1100delC mutation on the risk of developing CBC. We also investigated the effects of the aforementioned patient, pathological, and treatment characteristics separately in five different groups: 1. *BRCA1* mutation carriers; 2. *BRCA2* mutation carriers; 3. *CHEK2* c.1100delC mutation carriers; 4. Familial BC patients, i.e. patients who tested negative for a *BRCA1/2* or *CHEK2* c.1100delC mutation; 5. Population-based cohorts, i.e. patients from hospitals or official registries representing the general population, that have not been selected on gene mutation carriership or a positive family history for BC.

Papers with only combined results for *BRCA1* and *BRCA2* mutation carriers were excluded from the analyses, as these two groups represent different entities with different characteristics and should be analyzed separately (*BRCA1* mutation carriers are younger at PBC diagnosis and present more often with a triple negative BC phenotype (ER, PR and HER2 receptor negative) than *BRCA2* mutation carriers).[2,15,16]

For the analyses on carriership of a genetic *BRCA1*, *BRCA2* or *CHEK2* c.1100delC mutation, we included studies where the reference group consisted of familial patients (i.e. patients from non-*BRCA1/2*, and/or *CHEK2*-negative BC families) and excluded papers that used a sporadic population as a reference group. After all, studies that compare mutation carriers recruited from Clinical Genetic departments with BC patients from the general population easily lead to overestimations.[17] Since this is no issue in population-based studies with genetic test results generally available, these studies were included as well.

Since various ranges for age were used in the different papers, we estimated the overall effect of age using the method described by Greenland et al.[18], typically defined in the context of dose-response studies. The requirements needed for this method are the risk estimates from every age category, the corresponding confidence levels or the standard errors, and the number of cases and controls or person-time in case of incidence rate data. If these were not given, the continuous age effect was estimated by linearly regressing the category-specific log relative

risks on an age value representative for each age category. Representative values were the median age at PBC diagnosis calculated from female BC patients in the Netherlands Cancer Registry[19], with a 10-year CBC risk of 4% which is comparable with published results from studies from various western countries.[20-22]

All types of relative risk estimates were log transformed and subsequently pooled for every factor of interest. The available univariable and multivariable estimates were analyzed separately (and reported as crude and adjusted analyses, respectively). If only subgroup estimates were available in a paper, we combined these estimates to generate an overall estimate. A random effects model was used to perform the meta-analyses.[23] We tested for heterogeneity using I^2 statistics and the *p*-value for heterogeneity using the Cochran's Q-statistic was reported.

To conduct the meta-analyses, we used Metan from the Stata Statistical Software package (version 14.0). To assess the effects of age at PBC diagnosis, the dosresmeta package from R software (version 3.2.2) was used.

Quality Assessment

We used the QUality In Prognostic Studies (QUIPS) tool for assessing the quality and bias in the included papers.[24] As suggested by the developers of this tool, we modified the domains to be applicable to the specific study questions in our systematic review (Supplementary Table A.2). We excluded one domain, which assessed outcome measurement, since this was performed similarly in all studies and in a following domain we already scored whether a definition for outcome was given.

Using the modified tool, two reviewers (DA, MJH) scored 11 items in five domains. Every item was assigned 0 points if bias was unlikely, 0.5 points if bias was possibly present and 1 point if bias was likely present. When in doubt, the reviewers discussed with the other authors to reach consensus.

The distribution of points for potential bias following the QUIPS tool was inspected using a boxplot (not shown); the overall mean score was 1.8 points (range 0-5.5). Results were comparable for case-control (2.0), cohort studies (1.8) and randomized controlled trials (1.8). Papers that were classified as high-quality papers (i.e. on a

scale of 0-11 a total bias score of <2 was assigned; Supplementary Table A.3), were analyzed separately using a random-effects model.

RESULTS

In total, 100 papers out of 1789 identified records fulfilled the inclusion criteria (Flow diagram, see Figure 1)[2,3,12,13,15,16,20-22,25-115]; study characteristics are depicted in Table 1. Eligibility was validated for 10% of the titles and abstracts by a second reviewer.

Subsequently, potential overlap in patients included in different papers was evaluated and we selected either the most relevant (i.e. on topic) or most recent paper (n=11 excluded). In addition, we evaluated whether risk estimates in their given form were usable and/or combinable (n=21 excluded).

Eventually, 68 papers were used for the meta-analyses and these included between 247 and 205 316 PBC patients and 21 and 6 924 second BCs per study. Twenty studies used data from patients diagnosed in Northern America (USA/Canada) solely, versus 24 European studies. The risk estimates mainly concerned population-based cohorts; for the specific genetic groups of interest and the familial BC group the number of estimates was limited (Supplementary Table A.4).

In the summary estimates reported below, the adjusted estimates are reported (Figures 2-5). Crude estimates are only provided in the main paper if the number of multivariable estimates was insufficient to perform a meta-analysis. An overview of the results from the crude analyses can be found in Supplementary Figure (S Fig.) B.1. Study-specific estimates per factor and per group of interest are provided in S Figs. B.2-B.40.

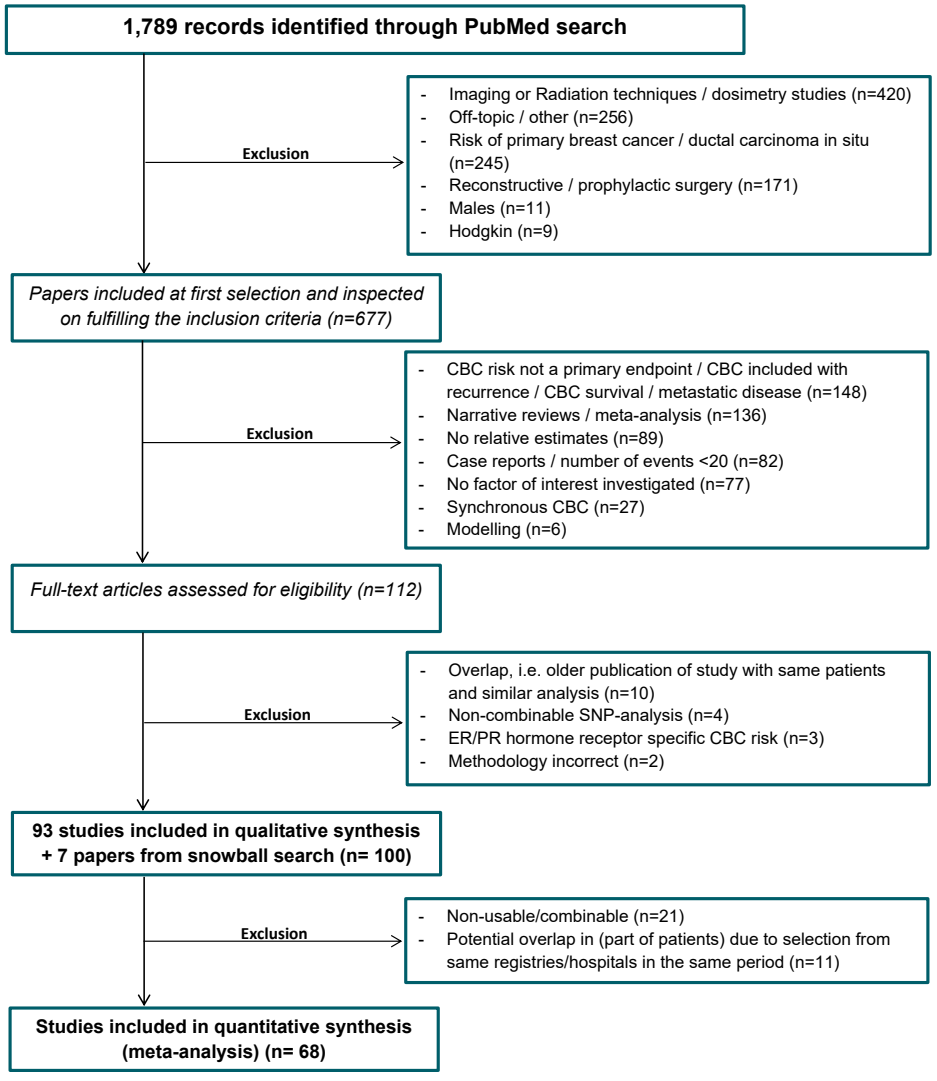

Figure 1. PRISMA flow chart of papers on risk factors for contralateral breast cancer

CBC: Contralateral breast cancer; SNP: Single Nucleotide Polymorphism.

Population-based cohorts: patient characteristics (Figure 2; S Figs. B.2-B.12)

For the analyses concerning patient characteristics we reviewed 30 papers. Having a positive family history of BC was associated with an increased risk of CBC, but heterogeneity was substantial (RR=1.72; 95% CI: 1.15-2.57; I^2 93.1%; S Fig. B.2). The studies performed by Hemminki et al.[79] was the main outlier. They used a non-conventional method to determine CBC risk, by doubling the risk, leading to overestimation. Heterogeneity as well as the relative risk estimate decreased when ignoring this study (RR=1.43; 95% CI: 1.22-1.68; I^2 41.6%).

CBC risk appeared to be higher in first than in second degree relatives (RR=1.54; 95% CI: 1.25-1.90 and RR=1.17; 95% CI: 0.90-1.52, respectively; S Figs. B.3 and B.4). Heterogeneity was also present in the meta-analysis concerning first degree relatives (I^2 60.1% vs. 0% in second degree relatives). Excluding the results from Buist et al.[62], which was the main outlier in this analysis, resulted in a decrease in heterogeneity and small increase in CBC risk (RR=1.61; 95% CI: 1.41-1.85; I^2 15.3%).

Age at PBC diagnosis was associated with a 7% decrease in CBC risk per decade (RR=0.93; 95% CI: 0.88-0.98, I^2 86.9%; S Fig. B.5). Although heterogeneity between studies was substantial, the estimates from the individual papers did not seem to vary widely.

For mammographic breast density (S Figs. B.6 and B.7) and menopausal status (S Fig. B.8) no association with CBC risk was observed.

Being overweight or obese (BMI ≥25 kg/m^2) or being obese (BMI ≥30 kg/m^2) compared to having normal weight (BMI <25 kg/m^2), was associated with an increased risk of developing CBC (RR=1.26; 95% CI: 1.10-1.44; I^2 44.4% and RR=1.54; 95% CI: 1.26-1.87; I^2 0%, respectively; S Figs. B.9 and B.11).

Population-based cohorts:pPathological characteristics (Figure 2; S Figs. B.13-B.19)

For the analyses concerning pathological characteristics we analyzed 15 papers. Having a PBC with a larger size was associated with increased CBC risk (tumor size >2cm vs. ≤2cm; RR=1.17; 95% CI: 1.03-1.34; I^2 21.6%; S Fig. B.13). For nodal status and tumor grade no association with CBC was observed (S Fig. B.14 and B.15, respectively). Both negative ER and PR hormone receptor status (vs. positive) were associated with an increased risk of CBC as well (RR=1.53; 95% CI: 1.04-2.26; S Fig. B.16; and RR=1.23; 95% CI: 1.02-1.48; S Fig. B.17, respectively), although for ER status there was evidence of substantial heterogeneity (I^2 67.3% vs. 0% for PR status). Excluding the outlying estimate reported by Filleron et al.[50] (possibly large effect size due to a small study population available for this factor), resulted in a

decrease in heterogeneity and a non-significant association between ER status and CBC risk (RR=1.32; 95% CI: 0.99-1.76; I^2 38.5%). For Her2 status no association with CBC risk was observed (S Fig. B.18)

Lobular morphology vs. ductal/non-lobular morphology was also associated with an increased risk of developing CBC, which in the forest plot was observed mainly in the older publications (RR=1.43; 95% CI: 1.13-1.82; I^2 42.5%; S Fig. B.19).

Population-based cohorts: treatment-related characteristics (Figure 2; S Figs. B.20-B.27)

Nine papers were included on treatment with adjuvant chemotherapy and 15 studies on adjuvant endocrine therapy; both factors were associated with a lower CBC risk (RR=0.70; 95% CI: 0.62-0.79; I^2 21.3%; S Fig. B.20 and RR=0.61; 95% CI: 0.53-0.72; S Fig. B.21, respectively). Results for patients aged below and above 50 years at PBC diagnosis were similar (data not shown). Heterogeneity was high in the meta-analysis concerning endocrine therapy (I^2 73.6%), but decreased substantially (I^2 19.4%) when we selected papers including only patients with ER-positive tumors (RR=0.57; 95% CI: 0.49-0.66; S Fig. B.22).

Treatment with radiotherapy (vs. no radiotherapy) was analyzed in 8 papers and associated with a modestly increased CBC risk when diagnosed at least five years after PBC (RR=1.10; 95% CI: 1.05-1.15; I^2 0%; S fig. B.24). In patients aged below 40 years at PBC diagnosis this risk appeared to be higher, both for CBCs occurring any time after PBC and for CBCs occurring at least 5 years after PBC diagnosis (RR=1.37; 95% CI: 1.13-1.66; I^2 0% and RR=1.34; 95% CI: 1.07-1.67; I^2 0%, respectively; S Figs. B.26 and B.27). The association appeared to attenuate when the age cut-off was raised to 45 years at PBC diagnosis (RR=1.22; 95% CI: 1.09-1.36, I^2 0.0% and RR=1.20; 95% CI: 1.06-1.35, I^2 0.0%, respectively, data not shown).

Mutation carriers vs. patients from mutation-negative BC families (Figure 3, S Figs. B.28-B.30)

The effect of mutation status on CBC risk was analyzed in 5 papers.[2,3,32,45,70] Carriership of a *BRCA1*, *BRCA2* or *CHEK2* c.1100delC mutation vs. non-carriership was associated with an increased risk of CBC (RR=3.68; 95% CI: 2.76-4.89; I^2 12.4%; RR=2.75; 95% CI: 1.77-4.27; I^2 20.8%; RR=2.68, 95% CI: 1.96-3.65; I^2 0%; S Figs. B.28-B.30; respectively).

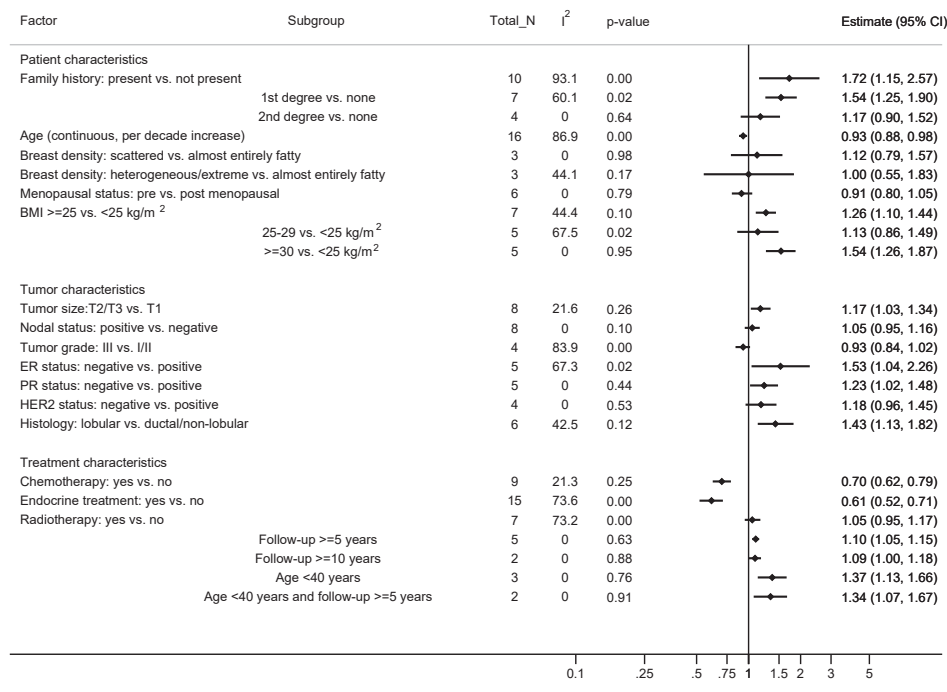

Figure 2. Forest plot of the adjusted meta-analyses per patient, pathological and treatment-related characteristic on the risk of developing contralateral breast cancer in population-based cohorts

BMI: body mass index per kg/m^2; ER: Estrogen hormone receptor; PR: Progesterone hormone receptor; T1: tumor size ≤2cm; T2: tumor size 2.1-5.0cm; T3: tumor size >5.0cm; Total_N: number of papers used for the analysis.
Age concerns the age at primary breast cancer diagnosis; family history concerns the family history of breast cancer; estimate is a relative risk estimate combining hazard ratios, odds ratios and relative risks; I^2 test for heterogeneity; *p*-value for heterogeneity: $p<0.05$ considered significant; patient and pathological factors are assessed at primary breast cancer diagnosis; treatment-related characteristics concern primary breast cancer treatment.

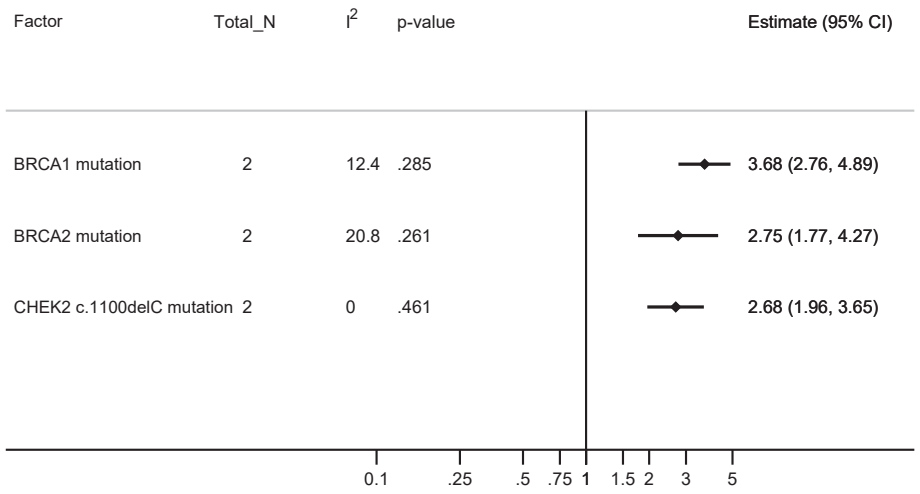

Figure 3. Forest plot of the adjusted meta-analyses comparing carrying a *BRCA1*, *BRCA2* or *CHEK2* c.1100delC mutation with patients who did not have the genetic mutation on the risk of developing contralateral breast cancer

Total_N: number of papers used for the analysis.
Estimate is a relative risk estimate combining hazard ratios, odds ratios and relative risks; I^2: test for heterogeneity; *p*-value for heterogeneity: $p<0.05$ considered significant.

BRCA1 and *BRCA2* mutation carriers (Figure 4; S Figs. B.31-B.40)

Seven papers reported on risk factors in both *BRCA1* and *BRCA2* mutation carriers[3,15,33,40,53,59,80], and one in *BRCA1* mutation carriers only.[2] Although the number of papers was limited for *BRCA1* and *BRCA2* mutation carriers, effects of the meta-analyses pointed in the same direction as in the population based cohorts for family history of BC, age at PBC diagnosis and endocrine therapy. RRSO was associated with a decreased CBC risk in *BRCA*1 mutation carriers (crude RR=0.56; 95% CI: 0.32-0.99; I^2 46.8%; S Fig. B.36).

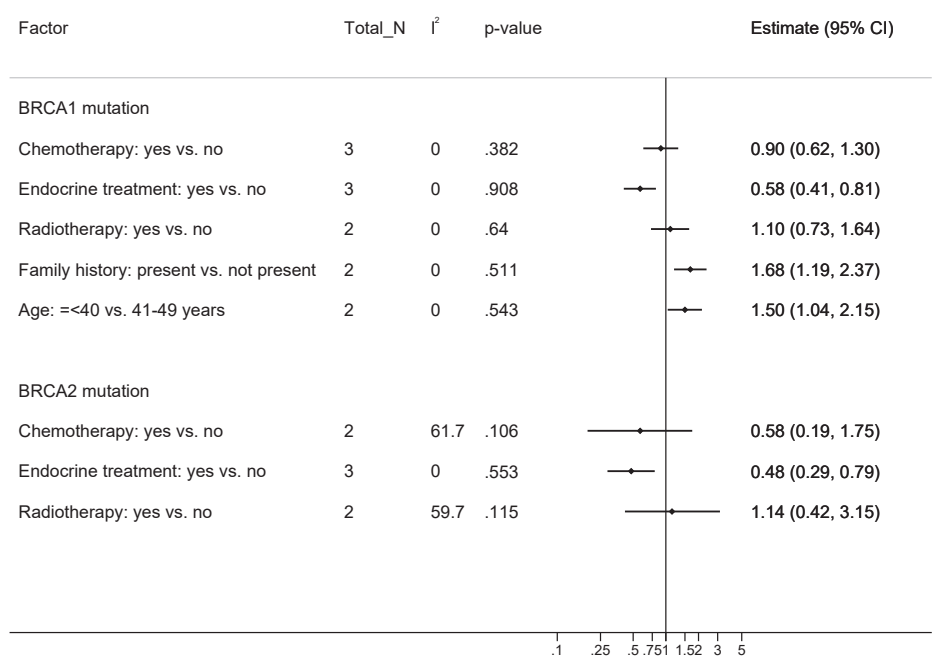

Figure 4. Forest plot of the overall adjusted meta-analyses per patient, pathological or treatment-related characteristic on the risk of developing contralateral breast cancer in *BRCA1* and *BRCA2* mutation carriers

Total_N: number of papers used for the analysis.
Age concerns the age at primary breast cancer diagnosis; family history concerns the family history for breast cancer; estimate is a relative risk estimate combining hazard ratios, odds ratios and relative risks; I^2: test for heterogeneity; *p*-value for heterogeneity: $p<0.05$ considered significant; treatment-related characteristics concerns primary breast cancer treatment.

Quality assessment (Figure 5 and Supplementary Table A.3)

We classified 46 out of 68 papers as being high quality which were subsequently used for the sensitivity analysis (Figure 5).

Following the sensitivity analysis, heterogeneity became 0% in the meta-analysis concerning ER status and BMI (25-29.9 vs <25 kg/m^2) and decreased for age at PBC diagnosis (I^2 58.4%). Further, a significant association between BMI and CBC risk was observed (BMI 25-29.9 vs <25 kg/m^2: RR=1.39; 95% CI: 1.14-1.69), but we no longer observed an association between T2 vs. T1/T0 PBC and CBC risk.

Concerning *BRCA1* and *BRCA2* mutation carriers, an insufficient number of papers remained to perform meta-analyses, especially due to evidence for selection bias.

Funnel plots were generated for the factors with multiple papers available (i.e. family history, age at PBC diagnosis, TNM-stage, treatment); we observed no evidence for publication bias (Supplementary Figures B.42-B.48).

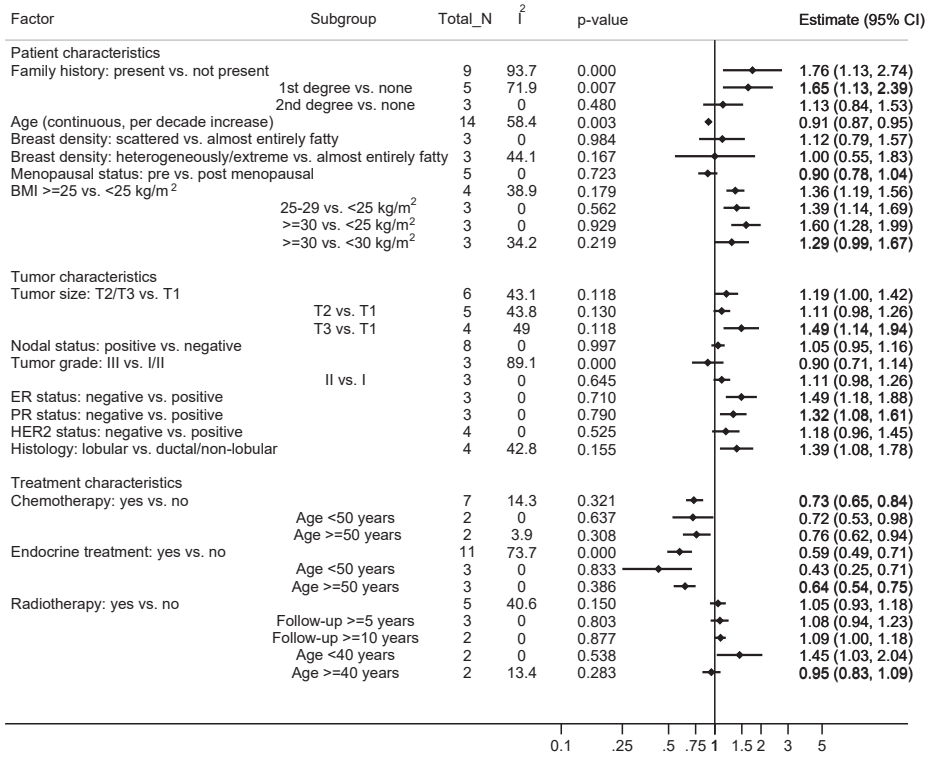

Figure 5. Forest plot of the overall adjusted meta-analyses per patient, pathological or treatment-related characteristic on the risk of developing contralateral breast cancer in population-based cohorts using only high-quality papers following the QUIPS bias scoring tool

BMI: body mass index per kg/m^2; ER: Estrogen hormone receptor; PR: Progesterone hormone receptor; Total_N: number of papers used for the analysis.
Age concerns the age (years) at primary breast cancer diagnosis; family history concerns the family history of breast cancer; estimate is a relative risk estimate combining hazard ratios, odds ratios and relative risks; I^2: test for heterogeneity; *p*-value for heterogeneity: $p<0.05$ considered significant; patient and pathological characteristics are assessed at primary breast cancer diagnosis; treatment-related characteristics concerns primary breast cancer treatment.

DISCUSSION

In this systematic review with meta-analyses, we aimed to quantify the association of several patient, pathological, and treatment-related characteristics and their influence on CBC risk. For the general BC population, confirming current clinical practice, we observed that carrying a *BRCA1*, *BRCA2* or *CHEK2* c.1100delC mutation comprises the strongest predictors for CBC risk. Family history of BC was also associated with increased CBC risk. In addition, a moderately increased risk was observed following lobular PBC, ER/PR negative PBC, radiotherapy for PBC (at young age) or having a high BMI at PBC diagnosis. Administration of adjuvant chemotherapy or endocrine therapy was associated with decreased CBC risk, as well as older age at PBC diagnosis, although to a lesser extent.

For *BRCA1*, *BRCA2* and *CHEK2* c.1100delC mutation carriers, all estimates on risk factors went in the same direction. However, the number of papers was insufficient to draw strong conclusions.

Most importantly, we confirmed the protective effect of adjuvant chemotherapy and endocrine therapy on CBC risk in population-based studies, as reported in large consortia such as the Early Breast Cancer Trialists' Collaborative Group.[104,116] In addition, the protective effect of adjuvant endocrine therapy was also found in *BRCA1/2* mutation carriers, specifically.

Radiotherapy for primary BC was associated with an increased risk of CBC, especially in patients irradiated at younger age (< 40 years). This negative effect of radiotherapy is likely a consequence of scattered radiation dose in the contralateral breast.[68] In addition, in younger patients the cells are at higher risk of damage after radiotherapy due to a higher breast cell proliferation and increased DNA synthesis.[117] The late adverse effects of radiotherapy occur at least 10-12 years after PBC diagnosis, as has been shown by Land et al., who studied atomic bomb survivors, and by Ronckers et al., who investigated the effects of x-rays for spine deformities.[118,119] Interestingly, we observed an increased risk of CBC already 5 years following radiotherapy for PBC.

We observed an increased CBC risk in patients with large tumors, and ER/PR negative PBC. Although we cannot deny these associations, both features are

also associated with worse prognosis of BC, raising the question whether some CBCs were distant PBC metastases. Only recently it became possible to genetically distinguish a true CBC from recurrent disease. In the latter case, we might misclassify a malignant tumor in the contralateral breast as a new entity, while in fact we are dealing with recurrent disease (misclassification of outcome).[120-123] This can lead to overestimation of CBC risk for these features. Furthermore, some studies did not rule out the ascertainment of CBCs in the presence of distant metastasis[47,56,112] or did not mention this. Misclassification of outcome may then occur more often, especially when considering tumor features with high recurrence rate. We can thus not rule out that part of the CBCs were in fact recurrences.

We observed an increased association with CBC risk for lobular PBC, which is in line with some older studies.[112,124] In the papers published before 2000 lobular PBC appeared to be associated with a higher risk of CBC. The effect of lobular histology on CBC risk was less observed in the papers published after 2000, an era in which adjuvant systemic therapy was more widely given (S Fig. B.19). The latter phenomenon has also been reported for CBC in general.[20] In our opinion, this reflects the risk reducing effect of adjuvant systemic therapy, and is in line with our earlier mentioned results on the impact of systemic therapy for PBC on CBC risk.

Results from the QUIPS underscored the importance of interpreting the results of studies in *BRCA1/2* mutation carriers with caution because of several potential forms of bias. In particular, survival bias was observed, which was mainly due to the retrospective design with inclusion of only mutation carriers who were still alive at the time of genetic testing.[125] Additionally, selection bias played a role specifically in the papers published on factors associated with the DNA test result, such as RRSO. These studies showed a protective effect from RRSO in the meta-analyses, but were potentially biased and led to an overestimation of the protective effect.

Our study had some limitations. First, we used reported results rather than individual patient data for the meta-analyses. Nonetheless, for most factors we observed acceptable levels of heterogeneity, which make our results reliable. Second, we cannot completely exclude the possibility of some publication bias, although the funnel plots did not provide evidence for the factors where we had enough papers to inspect this. Last, we only included papers with relative risk estimates, excluding 89 papers which reported cumulative incidences or standardized incidence rates only. However, those papers presented univariable estimates (factors were sometimes only stratified for a potential effect modifier), while we preferred multivariable estimates since these results are potentially less biased.

Implications for future research

Results from our meta-analyses have provided information on multiple CBC risk factors that should be incorporated in a CBC risk prediction model, but have also identified several topics needing further attention. First, although we observed considerable bias according to the QUIPS tool in the studies on *BRCA1*, *BRCA2* or *CHEK2* c.1100delC mutation carriers, the effect of carrying either one of these mutations has the largest impact on CBC risk and remains, therefore, the most important factor in estimating CBC risk. We will need more data on the effects of other risk factors within these groups to provide more personalized CBC risk estimates. This also accounts for familial BC cohorts. Second, concerning treatment, the effects of various adjuvant chemotherapy regimens, various targeted therapies and the long-term effects of radiotherapy (in young patients) should be investigated more extensively.

Third, the effects of breast density on CBC risk should be investigated in large and prospective studies to determine the effects of breast density at PBC diagnosis and changes in density over time, also in relation to adjuvant systemic treatment. Fourth, we propose to investigate SNPs and polygenic risk scores within one large international dataset. This will enable researchers to explore interaction between different SNPs (and between SNPs and other factors) and to further personalize CBC risk estimates.

In general, large cohorts (i.e. multicenter/international studies) with individual patient data and sufficiently long follow-up of at least 10-15 years are needed to accurately predict the risk of CBC.

Clinical implications

CBC risk is a growing concern in patients diagnosed with PBC, not only resulting in a psychological burden, but also determining survival in certain cases.[126,127] Risk-reducing mastectomy may be offered to those at high risk of developing CBC. On the other hand, overtreatment and exposing patients to side-effects of such radical

surgery should be avoided as long as survival benefit has not been demonstrated. Especially in low-risk patients, where the number of patients opting for contralateral risk-reducing mastectomy is increasing, but no survival benefit has been observed, more thorough discussion on the individual CBC risk estimation considering various risk factors is important. This also includes discussing potential alternative risk-reducing options.[128]

For example, extended endocrine treatment (beyond 5 years of initial/standard therapy) has been recently associated with a reduced risk of CBC as well.[25] In specific subgroups where the benefit from contralateral mastectomy is undecided, (extended) endocrine treatment as an alternative to reduce the risk of CBC may be advised. Nonetheless, the side-effects of (extended) endocrine treatment should also be considered.

For young PBC patients it is important to take into consideration the long-term side-effects of radiotherapy. Although local recurrence rates are decreased by more than 50% after radiotherapy in young PBC patients[129], CBC risk after radiotherapy is quite substantial in this group, and options to further reduce the scattered radiation dose towards the contralateral breast, as is done with more recent techniques, should thus be focused on.

Having a high BMI is one of the few modifiable risk factors that we have identified. Physicians should inform overweight patients about weight loss interventions programs that already have gained some success in BC patients.[130,131]

Conclusion

Based on this review with meta-analyses, key prognostic factors for CBC risk are mutation status, family history of BC, and treatment for primary BC. Age at primary BC diagnosis, BMI, lobular histology and hormone receptor status of the primary BC have a weaker association and should be considered in combination with key factors to accurately predict CBC risk.

Table 1. Study characteristics of papers publishing on risk factors for contralateral breast cancer included in the systematic review

First author, Year	Country / Continent[a]	Study Design[a]	Population	Factors[a]	Mean follow-up total group (years)[a,b]	Mean age total group (years)[a]	*N* Patients included	*N* CBCs[c]
van den Broek, 2016 (2)	Netherlands	Cohort	(non-) BRCA1/2	FamHis, Age, Ctx, DNA,	Md. 12.5	N/A	6 294	578
Goss, 2016 (25)[e]	USA, Europe, Canada	RCT	Unselected	Etx	Md. 6.3	Md. 65.1	1 918	43
Aalders, 2016 (26)	Netherlands	Cohort	Unselected	Age, TNM, Grade, ER,PR,HER2, His	5.0	59.0	52 626	1 534
Sisti, 2015 (27)	USA, Denmark, Canada	(Nested) Case-Control	Unselected	Meno	Md. Cases 6.3 Controls: 5.5 (matched)	46.0 (matched)	3 733	1 521
Menes, 2015 (16)[e]	USA, Australia, Canada	Cohort	BRCA1/2	Ctx, Etx, Rtx, RRSO	8.9	41.0	800	86
Kiderlen, 2015 (28)	Netherlands	Cohort	Unselected	Age	Md. 7.2	Md. 74.9	2 926	75
Drooger, 2015 (29)[e]	Netherlands	Cohort	BRCA1/2	Age, Ctx, Etx, Rtx, RRSO, DNA	Md. 8.6	N/A	691	161
Basu, 2015 (30)[e]	United Kingdom	Cohort	BRCA1/2	Age, Meno, RRSO, DNA	Md. 7.8	NA	1 011	202
Rasmussen, 2014 (20)	Denmark	Cohort	Unselected	Age	Md. 5.6	N/A	85 863	3 120
Mellemkjaer, 2014 (31)	Denmark	Cohort	Unselected	Etx	N/A	N/A	37 533	124
Kriege, 2014 (32)	Netherlands	Cohort	(non-) CHEK2	DNA	Md. CHEK2: 6.8 Non-carriers: 7.2	N/A	3 502	197
Gronwald, 2014 (33)	USA, Europe, Canada	(Nested) Case-Control	BRCA1/2	Etx	7.2	50.9 (matched)	1 504	411

Table 1. Continued.

First author, Year	Country / Continent[a]	Study Design[a]	Population	Factors[a]	Mean follow-up total group (years)[a,b]	Mean age total group (years)[a]	*N* Patients included	*N* CBCs[c]
Calip, 2014 (34)	USA	Cohort	Unselected	BMI	Md. 6.3	Md. 63.0	4 216	145 (ipsilateral: n.a.)
van de Water, 2013 (35)	USA, Japan, Europe	RCT	Unselected	Age	Md. 5.1	Md. 64.0	9 766	83
Valuckas, 2013 (36)	Lithunia	Cohort	Unselected	Age, Meno, BMI, TNM, Ctx, Etx, Rtx,	Md. HRtx 10.1 CRT 10.4	Md. 53.4	832	48 (ipsilateral: n.a.)
Sandberg, 2013 (37)	Sweden	(Nested) Case-Control	Unselected	BD	Cases: 8.25 Controls: 8.25 (matched)	(matched)	422	211
Reiner, 2013 (12)	USA, Denmark	(Nested) Case-Control	Non-BRCA1/2	FamHis	(matched)	(matched)	1 713	594
Phillips, 2013 (15)	USA, Australia, New Zealand, Europe, Canada	Cohort	BRCA1/2	Etx	Md. 6.6	N/A	2 464	520
Pacelli, 2013 (38)[e]	Italy	Cohort	Unselected	ER/PR/HER2	Md. 4.9	Md. 53.0	468	24
Metzger-Filho, 2013 (39)[e]	Australia, New Zealand, Europe, India, South-America, Africa	RCT	Unselected	ER/PR/HER2	Md. 12.5	53.9	1 951	75
Mavaddat, 2013 (40)	United Kingdom	Cohort	BRCA1/2	RRSO	Md. 2.6	Md. 39.5	988	61

Table 1. Continued.

First author, Year	Country / Continent[a]	Study Design[a]	Population	Factors[a]	Mean follow-up total group (years)[a,b]	Mean age total group (years)[a]	*N* Patients included	*N* CBCs[c]
Maskarinec, 2013 (41)[f]	USA	Cohort	Unselected	BD	12.9	63.3	607	71
Dellapasqua, 2013 (42)	Italy	Cohort	Unselected	Age, TNM, ER/PR/HER2	Md. 6.3	Md. 52.0	6 971	129
Courdi, 2013 (43)	France	Cohort	Unselected	Rtx	Md. 12.8	N/A	1 630	116
Bernstein, 2013 (44)	USA, Denmark	(Nested) Case-Control	BRCA1/2	Rtx, DNA	(matched)	(matched)	1 802	603
Weischer, 2012 (45)	USA, Australia, Europe, Canada	Cohort	CHEK2	DNA	Md. 6.6	N/A	25 094	647 (ipsilateral: n.a.)
Vichapat, 2012 (46)	Sweden	Cohort	Unselected	Age, TNM, His, Etx	Md. 6.7	N/A	37 393	894
Saltzman, 2012 (47)[f]	USA	(Nested) Case-Control	Unselected	ER, PR, HER2	(matched)	(matched)	1 988	482
Neta, 2012 (48)[f]	USA	Cohort	Unselected	Rtx	10.0	N/A	205 316	6 924
Mavaddat, 2012 (49)	USA, Australia, Europe	Cohort	BRCA1/2	ER	N/A	N/A	6 893	1 022
Filleron, 2012 (50)	France	RCT	Unselected	Age, TNM, Grade	Md. 4.4	Md. 49.0	2 820	58
Brooks, 2012 (51)	USA, Denmark	(Nested) Case-Control	Unselected	BMI	Md. 4.2	Md. 45.0 (matched)	1 510	511
Zhang, 2011 (22)	Italy	Cohort	Unselected	Rtx	8.0	54.7	5 248	261
Vichapat, 2011 (52)	United Kingdom	Cohort	Unselected	FamHis, Age, Meno, TNM, Grade, ER, PR, HER2, His, Ctx, Etx, Rtx	N/A	N/A	4 366	315

Table 1. Continued.

First author, Year	Country / Continent[a]	Study Design[a]	Population	Factors[a]	Mean follow-up total group (years)[a,b]	Mean age total group (years)[a]	*N* Patients included	*N* CBCs[c]
Metcalfe, 2011 (53)	USA, Canada	Cohort	BRCA1/2	FamHis, Age, TNM, Grade, ER, Ctx, Etx, Rtx, RRSO, DNA	11.1	Md. 42.0	846	149
Majed, 2011 (54)	France	Cohort	Unselected	FamHis, Age, Meno, BMI, TNM, Grade, ER, PR, His, Ctx, Etx, Rtx	Md. 10.0	54.0	15 166	1 370
Hackshaw, 2011 (55)[e]	Europe, Asia	RCT	Unselected	Etx	Md. 10.1	Md. 62.0	3 449	118
Bouchardy, 2011 (56)[f]	Switzerland	Cohort	Unselected	FamHis, Age, ER, Etx	Md. 5.2	59.8	4 152	63
Rubino, 2010 (57)[e]	France	Cohort	Unselected	Age, TNM	Md. 10.6	56.0	6 629	673
Rondeau, 2010 (58)	France	Cohort	Unselected	TNM, Grade, Ctx	Md. 12.7	Md. 57.0	919	69
Reding, 2010 (59)	USA, Denmark	(Nested) Case-Control	(non-) BRCA1/2	Ctx, Etx	(matched)	(matched)	1 579	181
Poynter, 2010 (60)[e]	USA, Denmark	(Nested) Case-Control	(non-) BRCA1/2	Meno	(matched)	(matched)	2 103	181
Malone, 2010 (3)	USA, Denmark	(Nested) Case-Control	BRCA1/2	DNA	(matched)	Md. 46.0 (matched)	2 103	705
Cuzick, 2010 (61)[e]	Unknown	RCT	Unselected	Etx	Md. 10.0	Md. 72.0	6 241	178
Buist, 2010 (62)	USA	Cohort	Unselected	FamHis, Age, BD, TNM, Ctx, Etx,	N/A	N/A	17 286	344 (ipsilateral: 54)
Berrington de Gonzalez, 2010 (63)	USA	Cohort	Unselected	Rtx	13.0	N/A	182 057	6 491

Table 1. Continued.

First author, Year	Country / Continent[a]	Study Design[a]	Population	Factors[a]	Mean follow-up total group (years)[a,b]	Mean age total group (years)[a]	*N* Patients included	*N* CBCs[c]
Li, 2009 (64) **A**	USA	(Nested) Case-Control	Unselected	Etx	(matched)	(matched)	1 094	367
Li, 2009 (65)[d] **B**	USA	(Nested) Case-Control	Unselected	BMI	(matched)	(matched)	1 091	365
Graeser, 2009 (13)[e]	Germany	Cohort	BRCA1	DNA	N/A	N/A	2 020	381
Bertelsen, 2009 (66)	Denmark	Cohort	Unselected	Age	8.4	N/A	8 737	466
Alkner, 2009 (67)	Sweden	RCT	Unselected	Age, Etx	Md. 14.0	N/A	564	52
Stovall, 2008 (68)[d]	USA, Denmark	(Nested) Case-Control	Unselected	Rtx	Cases: 5.0 Controls: 5.0 (matched)	51.0 (matched)	1 806	606
Schaapveld, 2008 (69)	Netherlands	Cohort	Unselected	Age, TNM, Ctx, Etx, Rtx	Md. 5.8	N/A	45 229	1 477
Mellemkjaer, 2008 (70)	USA, Denmark	(Nested) Case-Control	(non-) CHEK2	Ctx, Rtx, DNA	5.0	(matched)	2 103	708
Hooning, 2008 (71)	Netherlands	Cohort	Unselected	FamHis, Age, Ctx, Rtx	Md. 13.8	N/A	7 221	503
Bertelsen, 2008 (72)	USA, Denmark	(Nested) Case-Control	Unselected	Ctx, Etx	(matched)	Md. 46.0 (matched)	1 792	634
van der Leest, 2007 (73)[e]	Netherlands	Cohort	Unselected	Ctx, Etx	Md. 8.5	Md. 37.5	758	59
Trentham-Dietz, 2007 (74)[f]	USA	Cohort	Unselected	FamHis, Meno, BMI	7.1	59.4	10 953	488 (ipsilateral: n.a.)

Table 1. Continued.

First author, Year	Country / Continent[a]	Study Design[a]	Population	Factors[a]	Mean follow-up total group (years)[a,b]	Mean age total group (years)[a]	*N* Patients included	*N* CBCs[c]
Schmidt, 2007 (75)[d,f]	Netherlands	Cohort	CHEK2	DNA	Md. 10.1	43.0	1 479	124 (ipsilateral: 13)
Rutqvist, 2007 (76)	Sweden	RCT	Unselected	Etx	Md. 18.0	N/A	2 738	170
Largent, 2007 (77)[e]	USA, Denmark	(Nested) Case-Control	Unselected	Meno (at CBC diagnosis)	Cases: 5.0 Controls: 5.0 (matched)	45.0 (matched)	2 107	708
Kirova, 2007 (78)	France	Cohort	Unselected	Rtx	Md. 10.5	Md. 55.0	16 705	1 343
Hemminki, 2007 (79)	Sweden	Cohort	Unselected	FamHis	N/A	N/A	102 176	5 495
Broeks, 2007 (80)	Netherlands	Case-Only	BRCA1/2, CHEK2	Rtx	N/A	N/A	247	247
Brekelmans, 2007 (81)[e,f]	Netherlands	Cohort	(non-) BRCA1/2	DNA	Md. BRCA1/2: 4.3 NonBRCA: 4.8 Sporadic: 5.1	N/A	498	53
Tilanus-Linthorst, 2006 (82)[e]	Netherlands	Cohort	Non-BRCA1/2	FamHis	Md. 6.1	(matched)	654	51
Pierce, 2006 (83)[e]	USA, Israel	Cohort	BRCA1/2, Unselected	Age, TNM, Ctx, Etx, DNA	Md. BRCA1/2: 7.9 Unselected: 6.7	(matched)	605	48
Levi, 2006 (84)	Switzerland	Cohort	Unselected	Rtx	7.8	N/A	6 119	222
Gronwald, 2006 (85)[d]	USA, Israel, Europe, Canada	(Nested) Case-Control	BRCA1/2	Etx	Etx: 5.7 No Etx: 7.4	N/A	1 007	356

Table 1. Continued.

First author, Year	Country / Continent[a]	Study Design[a]	Population	Factors[a]	Mean follow-up total group (years)[a,b]	Mean age total group (years)[a]	*N* Patients included	*N* CBCs[c]
Dignam, 2006 (86)	USA	Cohort	Unselected	BMI	N/A	N/A	4 077	242
Brekelmans, 2006 (87)[e,f]	Netherlands	Cohort	BRCA1	DNA	Md. 5.1	Md. 39.0 (matched)	669	75
Nordenskjold, 2005 (88)[e]	Sweden	RCT	Unselected	Etx	Md. 10.6	N/A	4 610	138
Roychoudhuri, 2004 (89)	United Kingdom	Cohort	Unselected	Rtx	N/A	N/A	64 782	308
McCaskill-Stevens, 2004 (90)[d]	USA, Australia, South-America, Ireland	RCT	Unselected	Etx	N/A	Md. 50!	10 619	494
Coombes, 2004 (91)[e]	USA, Australia, Europe	RCT	Unselected	Etx	Md. 2.6	64.3	4 742	29
Li, 2003 (92)[f]	USA	Cohort	Unselected	FamHis, Age, BMI, TNM, ER, PR, His	9.0	37.7	1 285	77
Gao, 2003 (21)[d]	USA	Cohort	Unselected	Age, Rtx, His	Rtx: 5.7 No Rtx: 6.8	61.0	134 501	5 679
Dignam, 2003 (93)	USA, Unknown	RCT	Unselected	BMI	Md. 13.8	N/A	3 385	193
Fisher, 2002 (94)	USA, Canada	RCT	Unselected	Etx	Md. 7.2	N/A	1 000	27
Li, 2001 (95)[d]	USA	Cohort	Unselected	Etx	Etx: 3.9 No Etx: 4.2	N/A	8 981	189
Fisher, 2001 (96)	USA, Unknown	RCT	Unselected	Etx	Md. 6.8	56.0	1 172	37
Vaittinen, 2000 (97)	Sweden	Cohort	Unselected	FamHis, Age	6.2	N/A	72 092	2 529
Narod, 2000 (98)[d]	USA, Europe, Canada	(Nested) Case-Control	BRCA1/2	Ctx, Etx, Rtx, RRSO	9.7	40.2 (matched)	593	209

Table 1. Continued.

First author, Year	Country / Continent[a]	Study Design[a]	Population	Factors[a]	Mean follow-up total group (years)[a,b]	Mean age total group (years)[a]	*N* Patients included	*N* CBCs[c]
Matsuyama, 2000 (99)	Japan	Cohort	Unselected	Etx	Md. Etx: 7.6 No Etx: 8.1	51.0	6 148	30
Robson, 1999 (100)[e]	USA	Cohort	BRCA1/2	DNA	Md. 10.3	N/A	305	42
Newcomb, 1999 (101)	USA	Cohort	Unselected	Etx	6.4	N/A	54 821	1 730
Kollias, 1999 (102)	United Kingdom	Cohort	Unselected	FamHis, Age, His	Md. 9.0	Md. 54.0	3 211	83
Broet, 1999 (103)	France	Cohort	Unselected	Ctx	7.9	56.0	6 185	334
Early Breast Cancer Trialists' Collaborative, 1998 (104)[d]	USA, New Zealand, Europe, South-America, Africa, Asia	RCT	Unselected	Etx	2.7	N/A	32 422	839
Swedish Breast Cancer Cooperative Group, 1996 (105)[e]	Sweden	RCT	Unselected	Etx	Md. 5.5	N/A	3 545	51
Cook, 1996 (106)	USA	(Nested) Case-Control	Unselected	FamHis, Meno, BMI, ER, PR, His, Ctx, Rtx,	(matched)	N/A	640	216
Cook, 1995 (107)[d]	USA	(Nested) Case-Control	Unselected	Etx	Md. 3.3 (matched)	(matched)	673	234
Broet, 1995 (108)	Europe	Cohort	Unselected	His, Ctx	Md. 6.7	55.5	4 748	282
Healey, 1993 (109)	USA	Cohort	Unselected	Age, TNM, Ctx, Etx	Md. 7.9	53.0	1 624	77
Storm, 1992 (110)[f]	Denmark	(Nested) Case-Control	Unselected	FamHis, Meno, BMI, Rtx	(matched)	51.0 (matched)	56 540	529

Table 1. Continued.

First author, Year	Country / Continent[a]	Study Design[a]	Population	Factors[a]	Mean follow-up total group (years)[a,b]	Mean age total group (years)[a]	*N* Patients included	*N* CBCs[c]
Boice, 1992 (111)[f]	USA	(Nested) Case-Control	Unselected	Rtx	(matched)	51.7 (matched)	1 844	655
Bernstein, 1992 (112)[f] **A**	USA	Cohort	Unselected	Age, Meno, BMI, His, Ctx, Rtx,	4.3	44.3	4 550	136
Bernstein, 1992 (113) **B**	USA	Cohort	Unselected	FamHis	N/A	N/A	4 660	136
Baum, 1992 (114)	United Kingdom	RCT	Unselected	Etx	Md. 7.8	55.1	1 912	21
Andersson, 1991 (115)[d]	Denmark	RCT	Unselected	TNM, Etx	Md. 7.9	N/A	3 538	143

[a] Age: age at PBC diagnosis, BD: breast density, BMI: body mass index, Ctx: chemotherapy, DNA: *BRCA1/BRCA2/CHEK2* c.1100delC DNA mutation, ER: Estrogen hormone receptor status, Etx: endocrine therapy, FamHis: family history, Grade: tumor grade, HER2: HER2 receptor status, His: histology, Md: median, Meno: menopausal status, N/A: not available, PR: Progesterone hormone receptor status, RCT: Randomized controlled trial RRSO: risk-reducing salpingo-oophorectomy, Rtx: radiotherapy, TNM: TNM-stage, USA: United States of America.

[b] Md.: median if no mean was given; (matched): patients were matched on follow-up; if no mean/median follow-up for the total group was given, the mean/median follow-up for the exposed versus the reference group was given.

[c] If the number of CBC events was not provided, this was calculated from the figure in the paper; (ipsilateral): ipsilateral second breast cancers were included in the analyses.

[d] Studies not used for the analyses due to overlap in patients.

[e] Studies not used for the analyses due to reporting on subgroups that could not be combined with another estimate for the meta-analyses.

[f] Studies that included patients with metastatic disease at primary breast cancer diagnosis as well.

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SUPPLEMENTARY MATERIAL A

Supplementary Table A.1. Search terms that were used to identify papers publishing on risk factors for contralateral breast cancer in PubMed

Search	Query
#1[a]	contralateral breast cancer* [tiab] OR contralateral breast tumor* [tiab] OR contralateral breast tumour* [tiab] OR contralateral breast neoplasm* [tiab] OR CBC [tiab] OR (breast neoplasms [mesh] AND contralateral [tiab]) OR second primary breast cancer [tiab] OR second breast cancer [tiab]
#2	(((salpingectomy [mesh] OR salpingectom* [tiab]) AND (ovariectomy [mesh] OR ovariectom* [tiab] OR oophorectom* [tiab])) AND risk reducing* [tiab]) OR (risk-reducing salpingo-oophorectomy [tiab] OR RRSO [tiab] OR risk-reducing mastectom* [tiab] OR RRM [tiab]) OR ((mastectomy [tiab] OR mastectom* [mesh]) AND risk reducing [tiab])
#3	"Age of Onset"[Mesh] OR "Age Factors"[Mesh]
#4	"Health Behavior"[Mesh] OR "Food Habits"[Mesh] OR "Exercise"[Mesh] OR "Body Mass Index"[Mesh] OR "Obesity"[Mesh] OR Obes* [tiab] OR "Life Style"[Mesh]
#5	"Menopause"[Mesh] OR "Postmenopause"[Mesh] OR "Premenopause"[Mesh] OR "Menopaus* [tiab]
#6	Family[Mesh] OR Family Characteristics[Mesh] OR non-brca[tiab] OR "Checkpoint Kinase 2"[Mesh] OR (("Genes, BRCA1"[Mesh]) OR "Genes, BRCA2"[Mesh]) OR BRCA* [tiab] OR CHEK2* [tiab] OR Sporadic [tiab] OR "Neoplastic Syndromes, Hereditary"[Mesh] OR "Hereditary Breast and Ovarian Cancer Syndrome"[Mesh]
#7	((chemo [tiab] OR hormon* [tiab] OR targeted [tiab] OR radiotherap* [tiab] OR radio-therap* [tiab] OR chemo-therap* [tiab] OR molecular targeted therapy [mesh] OR antineoplastic agents [mesh] OR trastuzumab [tiab] OR combined modality therapy [mesh]) AND (adjuvant* [tiab])) OR chemotherapy, adjuvant [mesh] OR Radiotherapy, adjuvant [mesh] OR chemoradiotherapy, adjuvant [mesh] OR systemic adjuvant [tiab] OR neoadjuvant therap* [tiab] OR neo-adjuvant therap* [tiab] OR (radiotherapy [mesh] OR radiotherap* [tiab] OR radio-therap* [tiab])
#8	((((("Selective Estrogen Receptor Modulators"[Mesh]) OR "Receptors, Progesterone"[Mesh]) OR "Triple Negative Breast Neoplasms"[Mesh]) OR "Histology"[Mesh]) AND "anatomy and histology" [Subheading]
#9	Mammograph* [tiab] OR dens*[tiab] OR mammographic density [tiab]
#10	SNP[tiab] OR "Polymorphism, Genetic"[Mesh]
#11	Ethnic group [Mesh] OR Ethnic* [tiab]

Applied search: #1 AND (#2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11); restriction criteria: publication date from 1990/01/01.
[a]No MeSH term was available for contralateral breast cancer. Therefore, we searched for contralateral breast cancer in title and abstract and on MeSH terms for second primary breast cancer.

Supplementary Table A.2. Modified Quality in Prognostic Studies (QUIPS) tool to assess bias in papers on risk factors for contralateral breast cancer included in the systematic review

Domains	Ratings	
1. Study participation		
A. Equal source	Unlikely	Patients are recruited from the same registry/hospital. In case of case-control studies / RCT: cases and controls should be matched on source or correction/stratification for the participating institutes should have been applied.
	Likely	Patients are recruited from different hospitals/countries and this is not corrected for. Only a correction for country (instead of hospital/region) has been applied.
B. Incident or prevalent cases	Unlikely	A pure prospective analysis has been conducted.
	Likely	Prevalent cases have been included without any adjustment/correction for this in the analysis.
	Possibly	An attempt has been made to correct for prevalent cases (i.e. exclusion of index cases, applying left truncation).
2. Study Attrition		
A. Equal source	Unlikely	Equal source has been used to assess follow-up. Assume equal source if it is not mentioned.
	Likely	Different sources for have been used for the groups of interest to be compared.
B. Type of source	Unlikely	Either medical records or official registries have been used.
	Likely	Only self-reported data has been used.
C. Equal Follow-up	Unlikely	Follow-up is equal for the comparison groups. In case-control studies, patients are matched on follow-up (i.e. incidence density matching). In case of prognostic factor studies, it is not necessary to compare follow-up for every factor included in the model.
	Likely	In cohort studies, Kaplan-Meier curves show different follow-up and no specific period (by using a cut-off) has been selected to compare the groups. Different follow-up observed from the table. In case-control studies, cases and controls are not matched on follow-up.
	Possibly	Unknown whether groups are matched on follow-up and no mean/median (years/months) is given in a paper.
3. Prognostic factor measurement		

Supplementary Table A.2. Continued.

Domains	Ratings	
A. Equal source	Unlikely	The same source has been used to determine the factor of interest. Assume equal source if this is not mentioned.
	Likely	Different sources for the comparison groups have been reported.
B. Source type	Unlikely	Standardized methods have been used to measure the factors of interest (also measuring at the same point in time). Factors such as treatment and menarche and menopause are allowed to be self-reported, because we do not expect that these will be biased.
	Likely	Self-reported data for BMI has been used or it is not mentioned through which sources factors are assessed.
4. Outcome measurement		Domain was not used.
5. Study confounding	Unlikely	Correction/stratification/restrictions have been applied for all possible confounding factors (see below for list of potential confounders). If SIRs are used, the factors that were standardized (such as age) can also be considered as factors that have been corrected for. The same accounts for matching factors in case-control studies. If it has been tested whether a potential confounder altered the risk (e.g. by at least 10%), then all these potential confounders can be interpreted as being corrected for, even if the factor has not been included in the final model.
	Likely	No correction for potential confounders has been applied.
	Possibly	If one particular factor is investigated (e.g. radiotherapy), and in a subsequent multivariable model other factors of interest are included as well, for which the risk of CBC (e.g. treatment) is reported, but potential confounders for these factors have not been corrected for (e.g. ER status in endocrine treatment).
6. Statistical analysis and reporting		
A. Model description	Unlikely	Clear description of the analysis has been given (matching/correction for which factors should be mentioned as well). In addition: in RCTs/cohort studies, time-to-event analysis is described; in case-control studies, the use of a logistic regression model is described
	Likely	No clear description of the statistical analysis is given.

Supplementary Table A.2. Continued.

Domains	Ratings	
B. Censoring	Unlikely	Censoring in case of death, (distant) recurrent disease and second cancers (and contralateral prophylactic mastectomy in studies concerning mutation carriers and familial patients). Competing risk analyses, treating the outcomes mentioned above as a competing risk.
	Likely	Nothing has been mentioned on censoring.
	Possibly	Only (distant) recurrent disease has not been censored for. Start of second course of treatment as a censoring moment.
C. Event description	Unlikely	An adequate definition of CBC has been provided, i.e. mentioning the period of time required between PBC – CBC. In papers where only CBC events are included that appear after a certain number of years of follow-up, the number of years will be interpreted as a definition of time between PBC and CBC.
	Likely	No definition for CBC (including time between PBC-CBC) has been provided.

Handling of confounders that were considered in the QUIPS evaluation. The factors under investigation (bold) and corresponding possible confounders that were required to be corrected for in the analyses.

Age (all optional)
- Adjustment for family history (depends on study design)
- Mutation
- Treatment (i.e. chemotherapy and endocrine treatment).

BMI
- Treatment (optional), i.e. chemotherapy and endocrine
- Menopausal status

Menopausal status
- Treatment (chemotherapy)

Family history
- Mutation status (specific to study design, i.e. if it concerns mutation carriers)

Mutation status
- Age
- Family history (optional)

Breast density
- Menopausal status (study-dependent)
- BMI
- Systemic treatment (optional)

Chemotherapy
- Age
- Endocrine treatment

Endocrine treatment
- ER status of PBC
- Chemotherapy
- Age/menopausal status

Radiotherapy
- Age

RRSO
- Age
- Mutation status

TNM stage
- No factors required

Receptor status (different for ER/PR and HER2)

- Endocrine treatment
- Age
- Mutation status (optional)

Tumor grade

- Age
- Mutation status (optional)
- Chemotherapy/year of diagnosis

Tumor histology

- Treatment

BMI: body mass index; CBC: contralateral breast cancer; ER: Estrogen receptor; HER2: human epidermal growth factor 2 receptor; PBC: primary breast cancer; PR: progesterone receptor; RCT: randomized controlled trial; SIR: standardized incidence ratio.

Supplementary Table A.3. Scores assigned per domain using the modified QUIPS tool to papers on risk factors for contralateral breast cancer included in the systematic review

First author & year	Domain 1: selection of participants		Domain 2: Study attrition			Domain 3: Prognostic factor measurement		Domain 5: Confounding	Domain 6: Model				Total[d]
	1A. Equal Source	1B. Incident vs. Prevalent	2A. Equal Source	2B. Type of Source	2C. Equal length of follow-up	3A. Equal Source	3B. Type of Source	5. Confounding	6A. Model description	6B. Censoring	6C. Event description	6D. PH assumption	
van den Broek, 2016 (2)	0	0	0	0	0	0	0	0	0	0	0	Yes	**0**
Goss, 2016 (25) [b]	0	0	0	0	0	0	0	0	0	0	1	No	**1**
Aalders, 2016 (26)	0	0	0	0	0	0	0	0	0	0	1	No	**1**
Sisti, 2015 (27)	0	1	0	0	0	0	0	0	0	0,5	0	No	**1,5**
Menes, 2015 (16) [b]	0	0,5	0	0	0	0	0	1	0	0,5	0	No	**2**
Kiderlen, 2015 (28)	0	0	0	0	0	0	0	0	0	0	1	No	**1**
Drooger, 2015 (29) [b]	0	0,5	0	0	0	0	0	0,5	0	0	0	No	**1**
Basu, 2015 (30) [b]	0	0,5	0	0	0	0	0	1	0	0,5	1	No	**3**
Rasmussen, 2014 (20)	0	0	0	0	0	0	0	0	0	0	0	No	**0**
Mellemkjaer, 2014 (31)	0	0,5	0	0	0	0	0	0	0	0	0	No	**0,5**
Kriege, 2014 (32)	0	0	0	0	0	0	0	0	0	0	0	Yes	**0**
Gronwald, 2014 (33)	1	1	0	0	0	1	0	1	0	0,5	1	No	**5,5**
Calip, 2014 (34)	0	0	0	0	0	0	1	0	0	0	0	No	**1**
van de Water, 2013 (35)	0	0	0	0	0	0	0	0	0	0	1	No	**1**
Valuckas, 2013 (36)	0	0	0	0	0	0	0	1	0	0,5	0	No	**1,5**
Sandberg, 2013 (37)	0	0	0	0	0	0,5	0	0,5	0	0	0	No	**1**
Reiner, 2013 (12)	0	1	0	0	0	0	0	0	0	0,5	0	No	**1,5**
Phillips, 2013 (15)	1	0,5	0	1	0	0	0	1	0	0,5	0	No	**4**
Pacelli, 2013 (38) [b]	0	0	0	0	0	0	0	1	0	1	1	No	**3**
Metzger-Filho, 2013 (39) [b]	0	0	0	0	0	0	0	0	0	0	1	No	**1**
Mavaddat, 2013 (40)	1	0	0	0	0	0	0	0	0	1	0	Yes	**2**
Maskarinec, 2013 (41) [c]	0	0	0	0	0	0	0	1	0	0,5	0	No	**1,5**
Dellapasqua, 2013 (42)	0	0	0	0	0	0	0	0,5	0	0	1	No	**1,5**
Courdi, 2013 (43)	0	0	0	0	0	0	0	1	1	1	1	No	**4**
Bernstein, 2013 (44)	0	1	0	0	0	0	0	0	0	0,5	0	No	**1,5**
Weischer, 2012 (45)	0	0,5	0	1	0	0	0	0	0	0,5	1	Yes	**3**
Vichapat, 2012 (46)	0	0	0	0	0	0	0	0	0	0,5	0	No	**0,5**
Saltzman, 2012 (47) [c]	0	0	0	0	0	0	0	0	0	1	0	No	**1**
Neta, 2012 (48) [c]	0	0	0	0	1	0	0	1	0	1	0	No	**3**
Mavaddat, 2012 (49)	1	0	1	0	0	0	0	1	0	1	0	No	**4**
Filleron, 2012 (50)	0	0	0	0	0	0	0	1	0	0	1	No	**2**

Supplementary Table A.3. Continued.

First author & year	Domain 1: selection of participants		Domain 2: Study attrition			Domain 3: Prognostic factor measurement		Domain 5: Confounding	Domain 6: Model				Total[d]
	1A. Equal Source	1B. Incident vs. Prevalent	2A. Equal Source	2B. Type of Source	2C. Equal length of follow-up	3A. Equal Source	3B. Type of Source	5. Confounding	6A. Model description	6B. Censoring	6C. Event description	6D. PH assumption	
Brooks, 2012 (51)	0	1	0	0	0	0	1	0	0	0,5	0	No	**2,5**
Zhang, 2011 (22)	0	0	0	0	0	0	0	1	0	1	0	No	**2**
Vichapat, 2011 (52)	0	0	0	0	0	0	0	0	0	0	0	No	**0**
Metcalfe, 2011 (53)	1	1	0	0	0	0	0	0	0	0,5	1	No	**3,5**
Majed, 2011 (54)	0	0	0	0	0	0	0	0	0	1	0	Yes	**1**
Hackshaw, 2011 (55) [b]	0	0	0	0	0	0	0	1	0	0	1	No	**2**
Bouchardy, 2011 (56) [c]	0	0	0	0	0	0	0	0	0	0,5	0	No	**0,5**
Rubino, 2010 (57) [b]	0	0	0	0	0	0	0	0	0	1	0	No	**1**
Rondeau, 2010 (58)	0	0	0	0	0	0	0	0	0	0,5	1	No	**1,5**
Reding, 2010 (59)	0	1	0	0	0	0	0	0	0	0,5	0	No	**1,5**
Poynter, 2010 (60) [b]	0	1	0	0	0	0	1	1	0	0,5	0	No	**3,5**
Malone, 2010 (3)	0	1	0	0	0	0	0	0	0	0,5	0	No	**1,5**
Cuzick, 2010 (61) [b]	0	0	0	0	0	0	0	0	0	1	1	No	**2**
Buist, 2010 (62)	0	0	0	0	0	0	0	1	0	0,5	0	No	**1,5**
Berrington de Gonzalez, 2010 (63)	0	0	0	0	0,5	0	0	0	0	1	0	No	**1,5**
Li, 2009 (64) **A**	0	0	0	0	0	0	0	0	0	1	0	No	**1**
Li, 2009 (65) **B** [a]	0	0	0	0	0	0	1	1	0	1	0	No	**3**
Graeser, 2009 (13) [b]	0	0,5	0	0	0	0	0	1	0	0,5	1	No	**3**
Bertelsen, 2009 (66)	0	0	0	0	0	0	0	0	0	0,5	0	No	**0,5**
Alkner, 2009 (67)	0	0	0	0	0	0	0	0	0	0	0	Yes	**0**
Stovall, 2008 (68) [a]	0	1	0	0	0	0	0	0	0	0,5	0	No	**1,5**
Schaapveld, 2008 (69)	0	0	0	0	0	0	0	1	0	0,5	0	No	**1,5**
Mellemkjaer, 2008 (70)	0	1	0	0	0	0	0	0	0	0,5	0	No	**1,5**
Hooning, 2008 (71)	0	0	0	0	1	0	0	0	0	0	0	No	**1**
Bertelsen, 2008 (72)	0	1	0	0	0	0	1	0	0	0,5	0	No	**2,5**
van der Leest, 2007 (73) [b]	0	0	0	0	0	0	0	1	0	0	1	No	**2**
Trentham-Dietz, 2007 (74) [c]	0	0	0	0	0	0	1	0	0	0,5	0	No	**1,5**
Schmidt, 2007 (75) [a,c]	0	0	0	0	0	0	0	0	0	0,5	0	No	**0,5**
Rutqvist, 2007 (76)	0	0	0	0	0	0	0	1	0	0	1	No	**2**
Largent, 2007 (77) [b]	0	1	0	0	0	0	0	0	0	0,5	0	No	**1,5**

Supplementary Table A.3. Continued.

First author & year	Domain 1: selection of participants		Domain 2: Study attrition			Domain 3: Prognostic factor measurement		Domain 5: Confounding	Domain 6: Model				Total[d]
	1A. Equal Source	1B. Incident vs. Prevalent	2A. Equal Source	2B. Type of Source	2C. Equal length of follow-up	3A. Equal Source	3B. Type of Source	5. Confounding	6A. Model description	6B. Censoring	6C. Event description	6D. PH assumption	
Kirova, 2007 (78)	0	0	0	0	0,5	0	0	1	1	1	1	No	**4,5**
Hemminki, 2007 (79)	0	0	0	0	0	0	0	0	0	0,5	1	No	**1,5**
Broeks, 2007 (80)	0	1	0	0	0	0	0	0	0	0,5	0	No	**1,5**
Brekelmans, 2007 (81) [b,c]	1	0,5	0	0	0	0	0	1	0	0	1	No	**3,5**
Tilanus-Linthorst, 2006 (82) [b]	1	0,5	0	0	0	0	0	1	0	0	0	No	**2,5**
Pierce, 2006 (83) [b]	1	1	0	0	1	0	0	1	0	0,5	1	No	**5,5**
Levi, 2006 (84)	0	0	0	0	0	0	0	0	0	1	0	No	**1**
Gronwald, 2006 (85) [a]	1	1	0	0	0	0	0	1	0	0,5	0	No	**3,5**
Dignam, 2006 (86)	0	0	0	0	0	0	1	0	0	0	1	No	**2**
Brekelmans, 2006 (87) [b,c]	1	0,5	0	0	0	0	0	1	0	0	1	No	**3,5**
Nordenskjold, 2005 (88) [b]	0	0	0	0	0	0	0	1	0	1	0	Yes	**2**
Roychoudhuri, 2004 (89)	0	0	0	0	0	0	0	0	0	0,5	0	No	**0,5**
McCaskill-Stevens, 2004 (90) [a]	0	0	0	0	0,5	0	0	0	0	1	1	Yes	**2,5**
Coombes, 2004 (91) [b]	0	0	0	0	0	0	0	0	0	0,5	1	No	**1,5**
Li, 2003 (92) [c]	0	1	0	0	0	0	1	1	0	1	0	No	**4**
Gao, 2003 (21) [a]	0	0	0	0	0	0	0	1	0	1	0	No	**2**
Dignam, 2003 (93)	0	0	0	0	0	0	0	0	0	0	1	Yes	**1**
Fisher, 2002 (94)	0	0	0	0	0	0	0	0	0	1	1	No	**2**
Li, 2001 (95) [a]	0	0	0	0	0	0	0	0	0	0,5	0	No	**0,5**
Fisher, 2001 (96)	0	0	0	0	0	0	0	0	0	0,5	1	Yes	**1,5**
Vaittinen, 2000 (97)	0	0	0	0	0	0	0	1	0	1	0	No	**2**
Narod, 2000 (98) [a]	1	1	0	0	0	0	0	1	0	0,5	0	No	**3,5**
Matsuyama, 2000 (99)	0	0	0	0	0	0	0	1	0	1	1	No	**3**
Robson, 1999 (100) [b]	0	0	0	0	0	0	0	1	0	0	1	No	**2**
Newcomb, 1999 (101)	0	0	0	0	1	0	0	1	0	1	0	No	**3**
Kollias, 1999 (102)	0	0	0	0	0	0	0	0	0	0	1	No	**1**
Broet, 1999 (103)	0	0	0	0	0	0	0	0	0	0	0	No	**0**
Early Breast Cancer Trialists' Collaborative, 1998 (104) [a]	0	0	0	0	0	0	0	0	0	0	1	No	**1**

Supplementary Table A.3. Continued.

First author & year	Domain 1: selection of participants		Domain 2: Study attrition			Domain 3: Prognostic factor measurement		Domain 5: Confounding	Domain 6: Model				Total[d]
	1A. Equal Source	1B. Incident vs. Prevalent	2A. Equal Source	2B. Type of Source	2C. Equal length of follow-up	3A. Equal Source	3B. Type of Source	5. Confounding	6A. Model description	6B. Censoring	6C. Event description	6D. PH assumption	
Swedish Breast Cancer Cooperative Group, 1996 (105) [b]	0	0	0	0	0	0	0	1	0	0,5	0	No	**1,5**
Cook, 1996 (106)	0	0	0	0	0	0	0	1	0	0	0	No	**1**
Cook, 1995 (107) [a]	0	0	0	0	0	0	0	0	0	0,5	0	No	**0,5**
Broet, 1995 (108)	0	0	0	0	0	0	0	0	0	0	0	No	**0**
Healey, 1993 (109)	0	0	0	0	0	0	0	0	0	0	0	No	**0**
Storm, 1992 (110) [c]	1	0	0	0	0	0	0	0	0	0,5	0	No	**1,5**
Boice, 1992 (111) [c]	0	0	0	0	0	0	0	0	0	0,5	0	No	**0,5**
Bernstein, 1992 (112) **A** [c]	0	0	0	0	0	0	1	1	0	0,5	0	No	**2,5**
Bernstein, 1992 (113) **B**	0	0	0	0	1	0	0	0	0	0,5	0	No	**1,5**
Baum, 1992 (114)	0	0	0	0	0	0	0	1	1	1	1	No	**4**
Andersson, 1991 (115) [a]	0	0	0	0	0	0	0	1	0	1	1	No	**3**
Total	**12**	**23**	**1**	**2**	**6,5**	**1,5**	**9**	**40,5**	**3**	**46,5**	**36**		**183**

Each paper was scored on every five of the domains and their corresponding items depicted in Table 2A; the lower the total score, the lower the likelihood of bias; papers scoring less than two points in total were included in the sensitivity analyses.

[a] Studies not used for the analyses due to overlap in patients.

[b] Studies not used for the analyses due to reporting on subgroups that could not be combined with another estimate for the meta-analyses.

[c] Studies that included patients with metastatic disease at primary breast cancer diagnosis as well.

[d] if the total number of points assigned was < 2, the paper was included for the sensitivity analysis.

Supplementary Table A.4. Number of papers included in the systematic review and between brackets the number of papers used in meta-analysis per factor of interest; separately for crude and adjusted relative risk estimates and different genetic risk (sub)groups

	Population- based cohort		*BRCA1*		*BRCA2*		*CHEK2* c.1100delC		Familial	
	Number of papers included from the systematic review (number papers used in meta-analysis)									
	Crude[a,d]	**Adj[b]**	**Crude[a]**	**Adj[b]**	**Crude[a,d]**	**Adj[b]**	**Crude[a,d]**	**Adj[b]**	**Crude[a]**	**Adj[b]**
Family history of breast cancer	4 (4)	13 (10)	1	2 (2)	1	1	-	-	-	2 (-[c])
First degree	2 (2)	8 (7)	1	1	1	1	-	-	-	1
Second degree	1	4 (4)	-	-	-	-	-	-	-	1
Age at primary breast cancer diagnosis	7 (7)	21 (16)	1	2 (2)	1	1	-	-	-	-
Breast density	-	3 (3)	-	-	-	-	-	-	-	-
Menopausal status	2 (2)	7 (6)	-	-	-	-	-	-	-	1
BMI >=25 vs. <25 kg/m²	1	11 (7)	-	-	-	-	-	-	-	-
25-29 vs. <25 kg/m²	-	8 (5)								
>=30 vs. <25 kg/m²	-	8 (5)								
Tumor size: T2/3 vs. T1	4 (4)	8 (8)	-	-	-	-	-	-	-	-
T2 vs. T1[d]	4 (4)	7 (7)	-	-	-	-	-	-	-	-
T3 vs. T1[d]	3 (3)	6 (6)	-	-	-	-	-	-	-	-
Nodal status	4 (4)	8 (8)	1	1	1	1	-	-	-	-
Tumor Grade	2 (2)	4 (4)	1		1	1	-	-	-	-
ER receptor status	2 (2)	6 (5)	1	2	1	2	-	-	-	-
PR receptor status	2 (2)	6 (5)	-	-	-	-	-	-	-	-
HER2 receptor status	1	4 (4)	-	-	-	-	-	-	-	-

Supplementary Table A.4. Continued.

	Population- based cohort		*BRCA1*		*BRCA2*		*CHEK2* c.1100delC		Familial	
	Number of papers included from the systematic review (number papers used in meta-analysis)									
	Crude[a,d]	**Adj[b]**	**Crude[a]**	**Adj[b]**	**Crude[a,d]**	**Adj[b]**	**Crude[a,d]**	**Adj[b]**	**Crude[a]**	**Adj[b]**
Lobular histology	3 (3)	7 (6)	-	-	-	-	-	-	-	-
Chemotherapy	2 (2)	13 (10)	1	3 (3)	1	2 (2)	-	-	1	2 (-[c])
Age <50 years/premenopausal[d]	-	5 (3)	-	-	-	-	-	-	-	-
Age >=50 years/postmenopausal[d]	-	5 (3)	-	-	-	-	-	-	-	-
Endocrine treatment	6 (5)	20 (15)	4 (-[c])	5 (3)	4 (-[c])	5 (3)	-	-	-	1
Age <50 years/premenopausal[d]	-	6 (5)	-	-	-	-	-	-	-	-
Age >=50 years/postmenopausal[d]	-	6 (5)	-	-	-	-	-	-	-	-
Targeted therapy	-	1	-	-	-	-	-	-	-	-
Radiotherapy	9 (8)	14 (7)	2 (2)[d]	2 (2)[d]	2 (2)	2 (2)	1	-	1	2 (-[c])
Age <40 years	-	6 (3)	-	-	-	-	-	-	-	-
Age >=40 years[d]	2 (2)	6 (3)								
Follow-up >5 years	3 (3)	7 (5)	-	-	-	-	-	-	-	-
Follow-up >10 years	3 (3)	3 (2)	-	-	-	-	-	-	-	-
RRSO	-	-	2 (2)	1	2 (2)	1	-	-	-	-

[a]Crude estimates; [b]Adjusted estimates; [c]All papers were overlapping, no meta-analysis could be performed; [d]Figures not shown in supplementary material. BMI: Body mass index per kg/m²; ER: Estrogen hormone receptor; PR: Progesterone hormone receptor; RRSO: risk-reducing salpingo-oophorectomy; T1: tumor size ≤2cm; T2: tumor size 2.1-5.0cm; T3: tumor size >5.0cm; treatment-related characteristics concern primary breast cancer treatment.

Supplementary Material B

Figures

General information concerning supplementary figures: meta-analyses are conducted using a random-effects model; adjusted estimates (i.e. using multivariable estimates that were published in the included papers) are combined for the meta-analysis on all factors, unless stated otherwise.

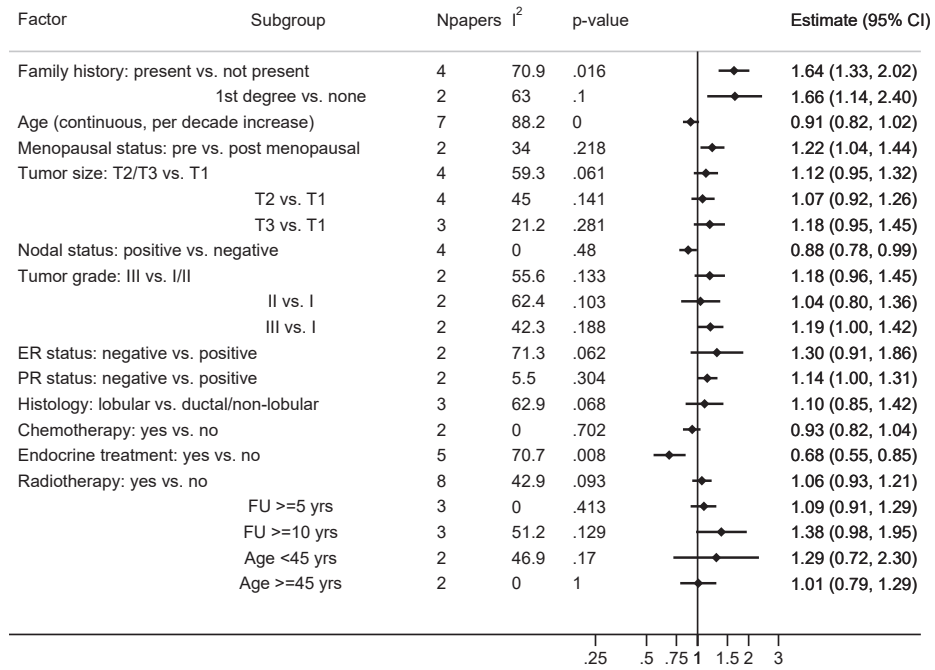
Abbreviations: Author_Year: the first author and the year of publication; HR: hazard ratio; OR: odds ratio; RCT: randomized controlled trial; RR: relative risk (could be based on standardized incidence ratio's [SIR]; RR_estimate: relative-risk estimate: is based on combining the different types of relative risks provided in column Estimate_type; Weight: value assigned by random-effects analysis using the inverse of the study variance, this variance includes the within-study variance plus the between-study variance;

I^2: test for heterogeneity; *p*-value for heterogeneity: $p<0.05$ considered significant; treatment concerns primary breast cancer treatment; clinicopathological factors are assessed at primary breast cancer diagnosis;

*Combined estimate, i.e. if only subgroup estimates were given in a paper, we combined the subgroup estimates to get an estimate for the total group.

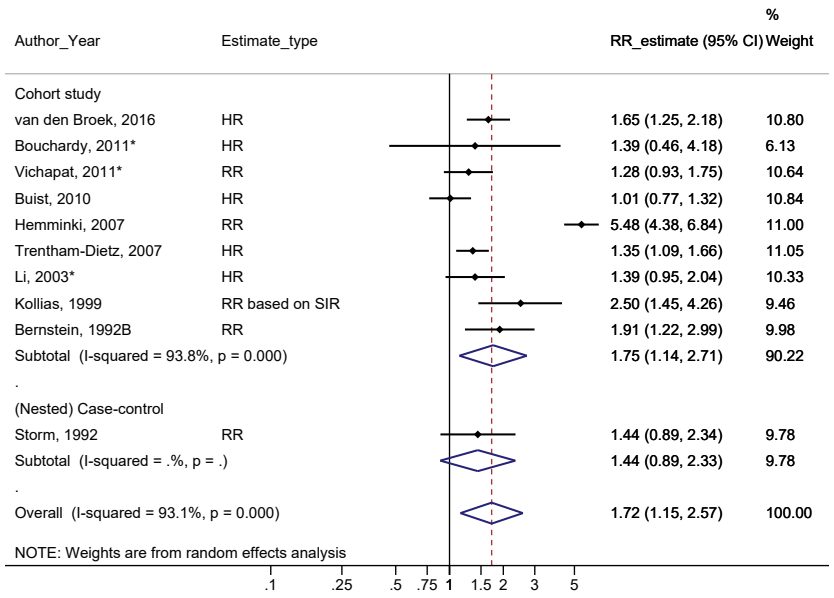
** Combined and/or reversed estimate, i.e. if only subgroup estimates were given in a paper, we combined the subgroups to get an estimate for the total group. In addition, if estimates used a different reference group, we reversed the estimate and the corresponding confidence interval (1/estimate; 1/upper limit to get the lower limit; and 1/lower limit to get the upper limit) to obtain equal reference groups and make estimates comparable.

More specific information is provided under the headings of the Supplementary Figures listed below.

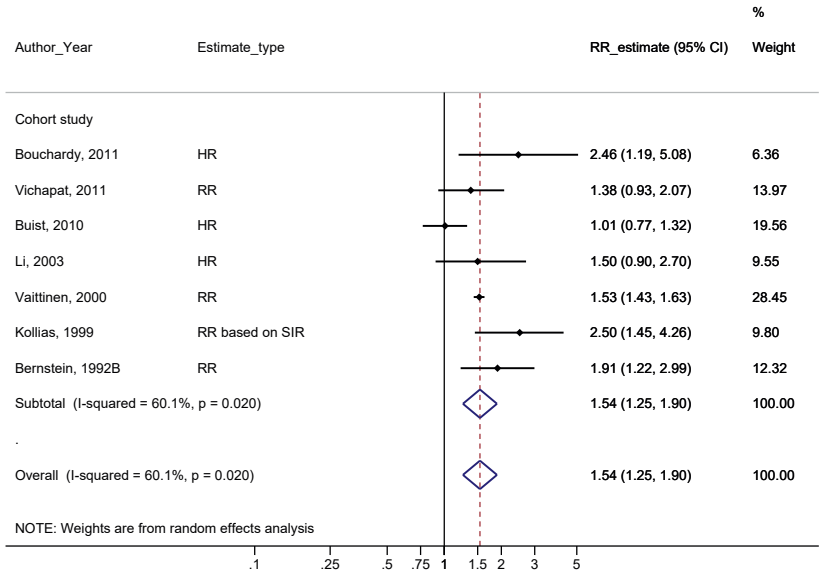


Supplementary Figure B.1. Forest plot of the overall crude (i.e. using univariable estimates provided in the included papers only) meta-analyses per clinicopathological or treatment-related characteristic on the risk of developing contralateral breast cancer in population-based cohorts

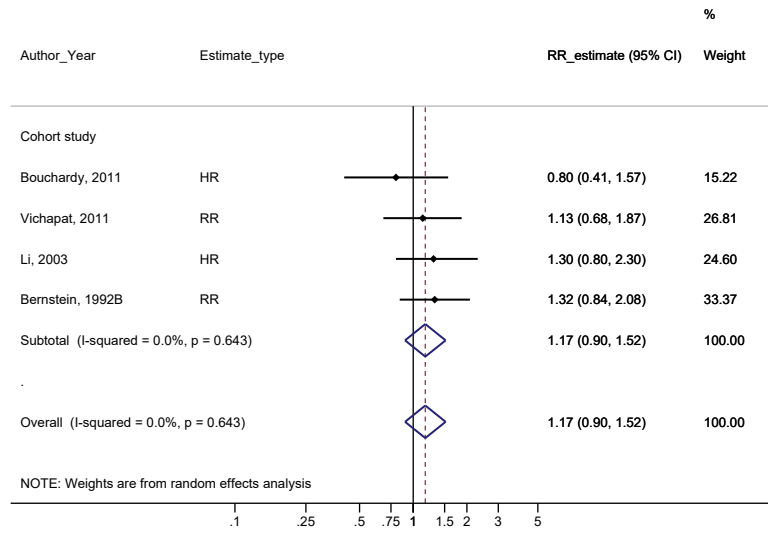
ER: Estrogen hormone receptor; PR: Progesterone hormone receptor; T1: tumor size ≤2cm; T2: tumor size 2.1-5.0cm; T3: tumor size >5.0cm; Total_N: the number of papers used for the analysis. Age concerns the age at primary breast cancer diagnosis; family history concerns the family history of breast cancer; estimate is a relative risk estimate combining hazard ratios, odds ratios and relative risks; clinicopathological factors are assessed at primary breast cancer diagnosis; I^2: test for heterogeneity; *p*-value for heterogeneity: $p<0.05$ considered significant; treatment-related characteristics concern primary breast cancer treatment. For the factor radiotherapy, age 45 years at primary breast cancer diagnosis was used as a cut-off since we had insufficient information available to use 40 years as a cut-off.



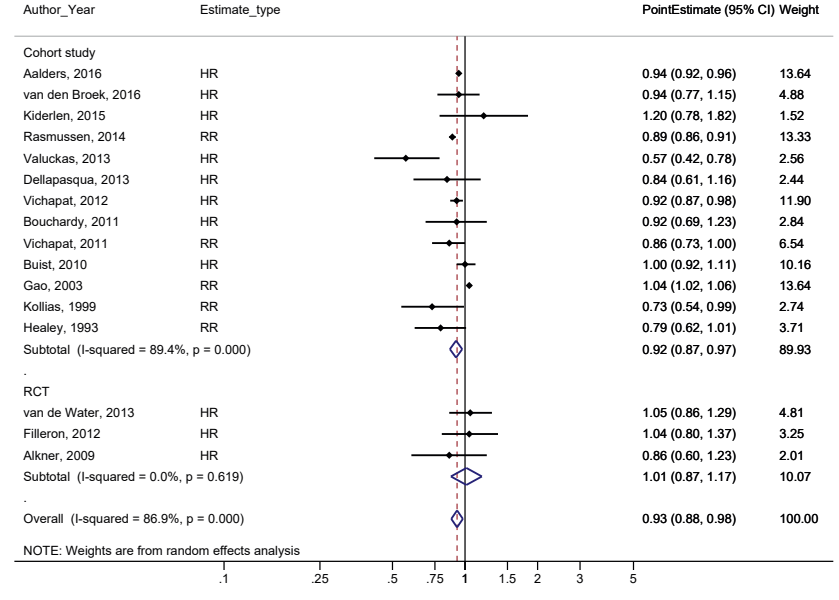
Supplementary Figure B.2. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing patients with a positive family history for breast cancer with patients with no family history of breast cancer



Supplementary Figure B.3. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing patients with first degree relatives with breast cancer with patients with no family history of breast cancer

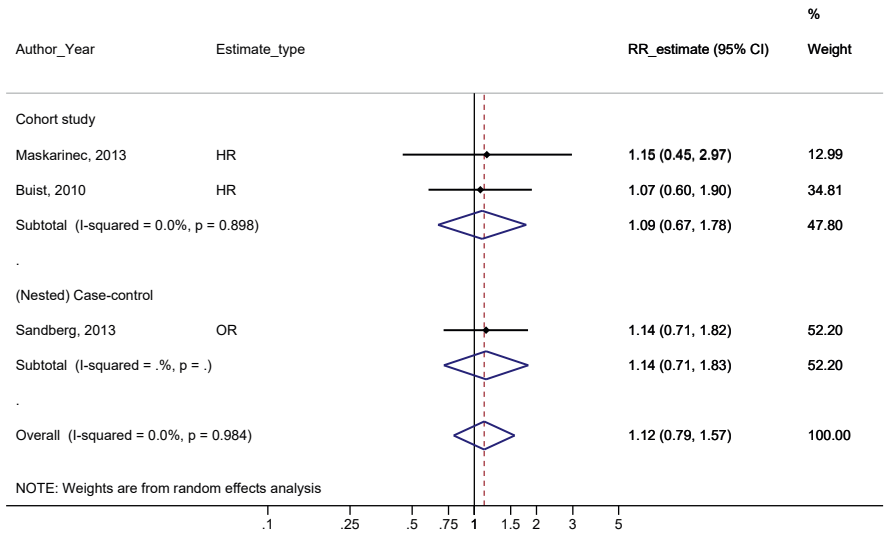


Supplementary Figure B.4. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing patients with second degree relatives with breast cancer with patients with no family history of breast cancer

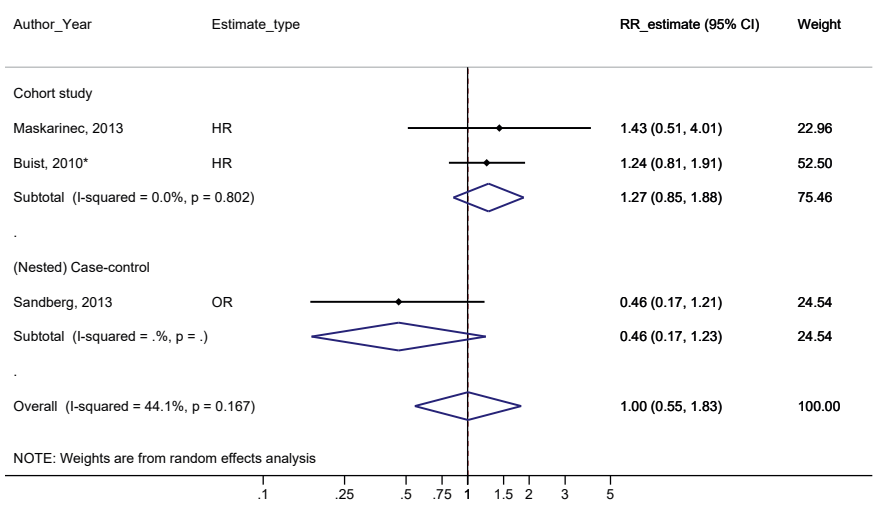


Supplementary Figure B.5. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts investigating increasing age at primary breast cancer (continuous per decade increase)

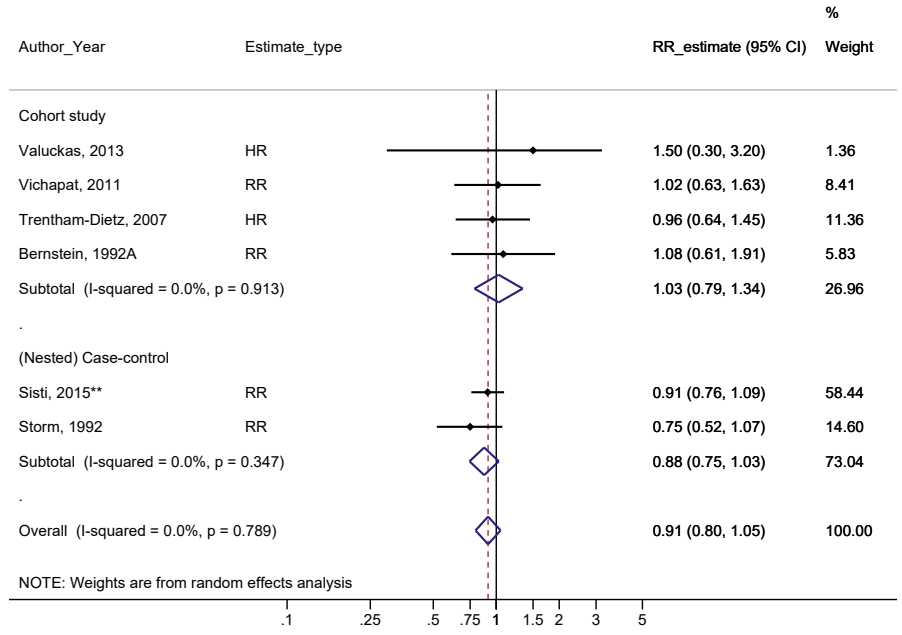
For this analysis the dose-response method proposed by Greenland et al. or a crude linear regression model was used (see methods section).



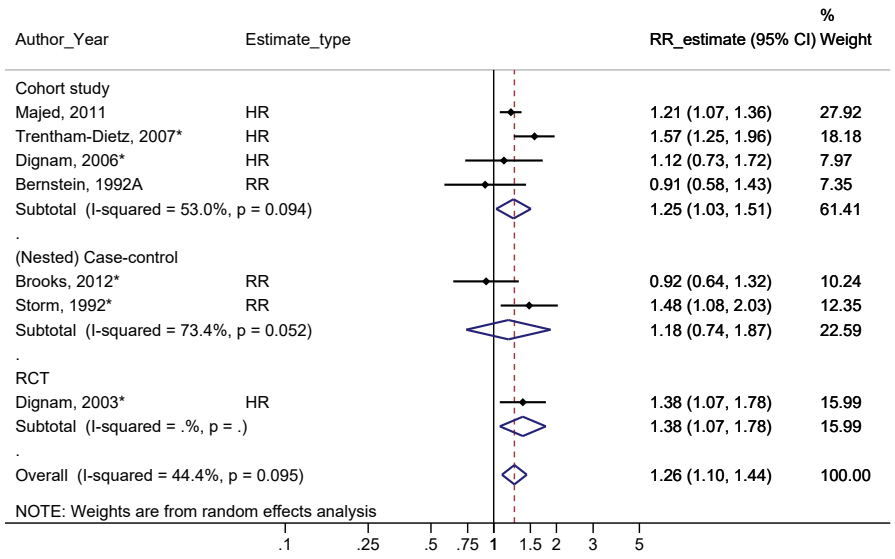
Supplementary Figure B.6. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing patients having scattered breast tissue with patients having almost entirely fatty breast tissue on a mammogram at primary breast cancer diagnosis



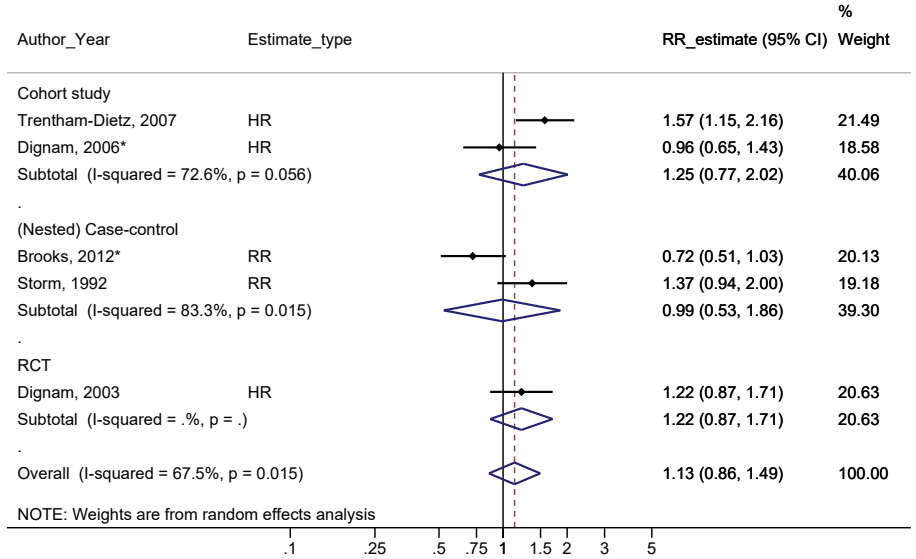
Supplementary Figure B.7. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing patients having heterogeneously/extreme dense breast tissue with patients having almost entirely fatty breast tissue on a mammogram at primary breast cancer diagnosis



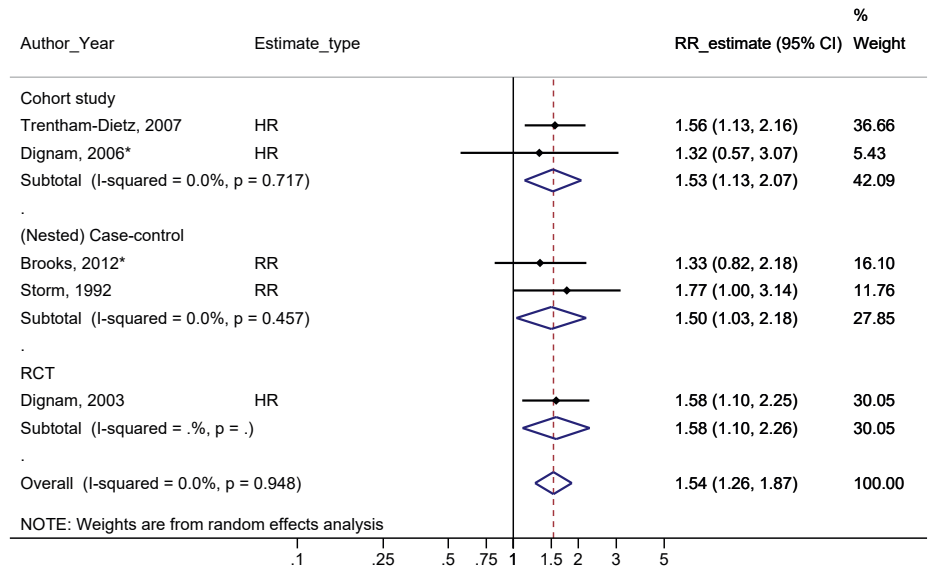
Supplementary Figure B.8. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing premenopausal status with postmenopausal status at primary breast cancer diagnosis



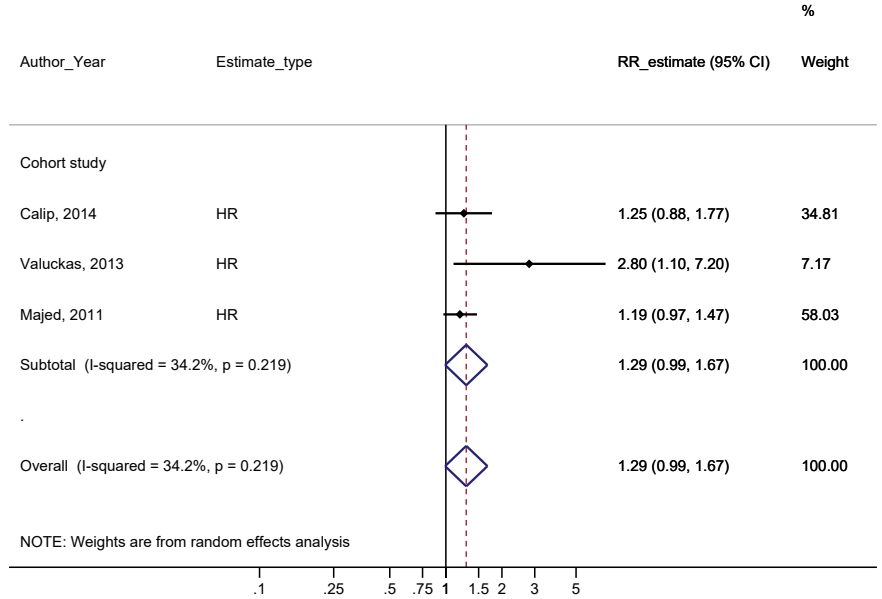
Supplementary Figure B.9. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing body mass index of ≥25 kg/m² with <25 kg/m² at primary breast cancer diagnosis



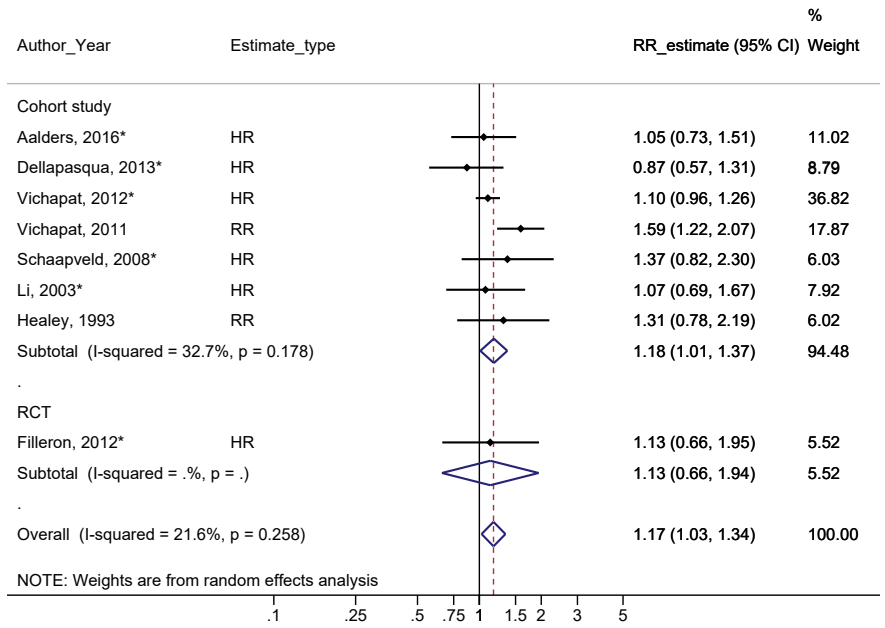
Supplementary Figure B.10. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing body mass index of 25-29.9 kg/m² with <25 kg/m² at primary breast cancer diagnosis



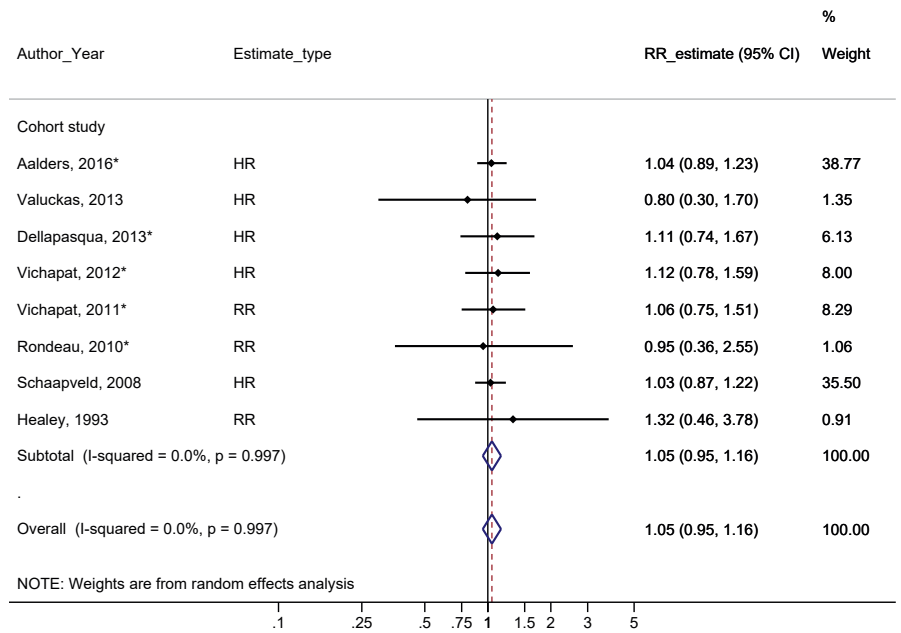
Supplementary Figure B.11. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing body mass index of ≥30 kg/m² with <25 kg/m² at primary breast cancer diagnosis



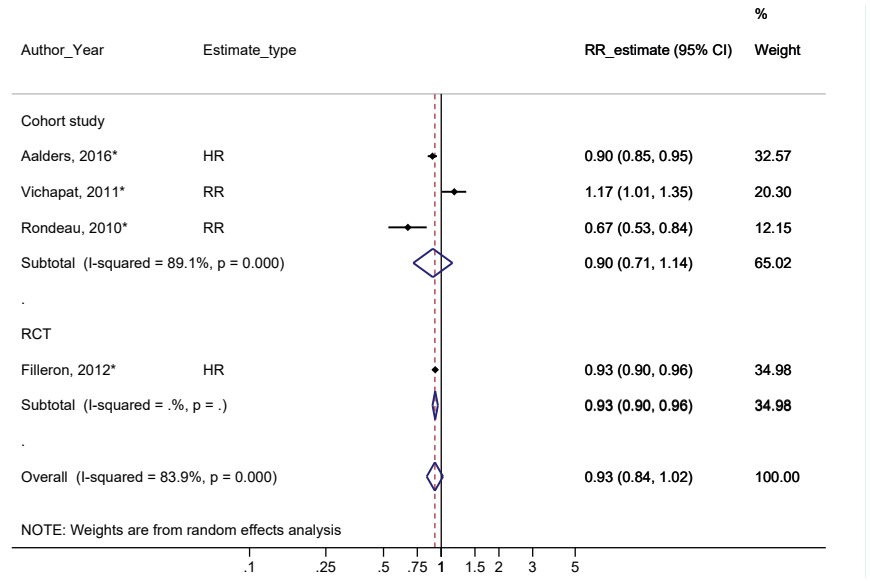
Supplementary Figure B.12. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing body mass index of ≥30 kg/m² with <30 kg/m² at primary breast cancer diagnosis



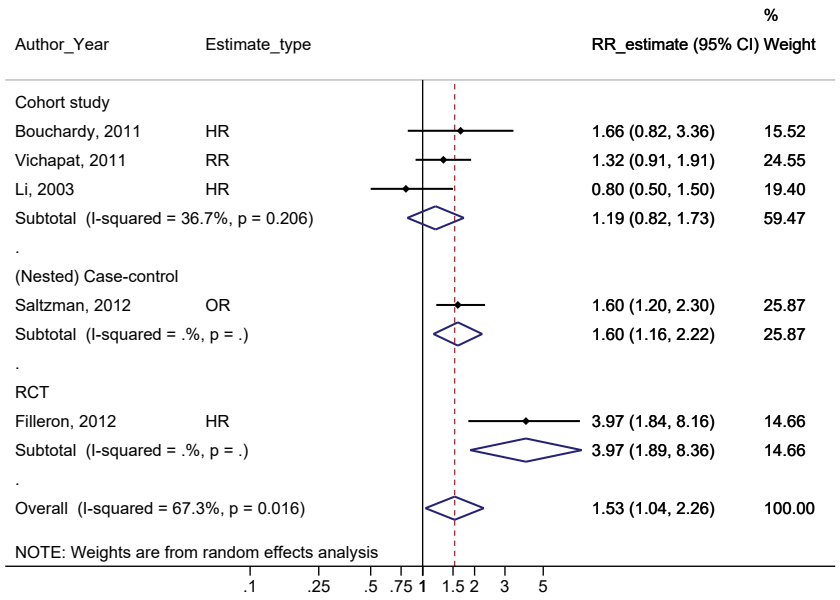
Supplementary Figure B.13. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing primary breast tumor size larger than 2.0cm with smaller than 2.0cm



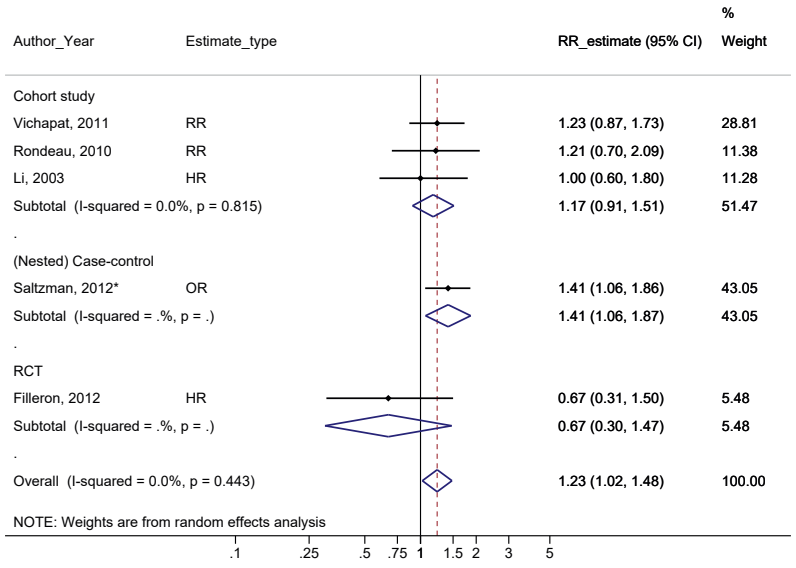
Supplementary Figure B.14. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing having a positive nodal status with having a negative nodal status in primary breast cancer



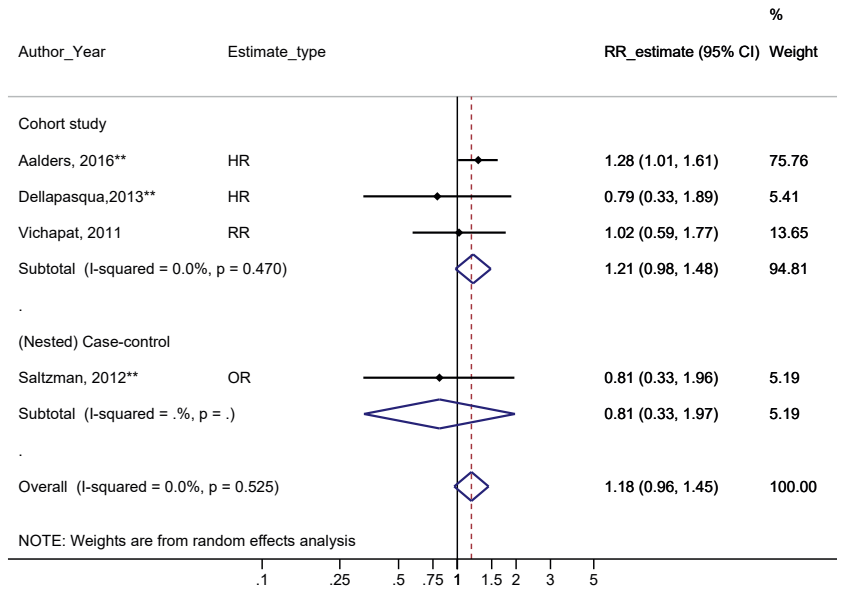
Supplementary Figure B.15. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing tumor grade of the primary breast cancer (grade III versus I/II)



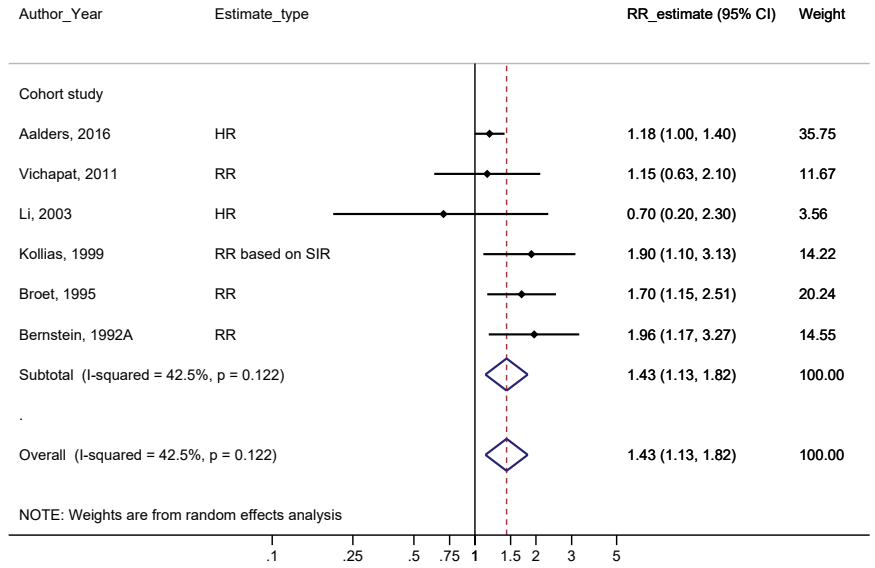
Supplementary Figure B.16. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing negative with positive Estrogen hormone receptor status of the primary breast cancer



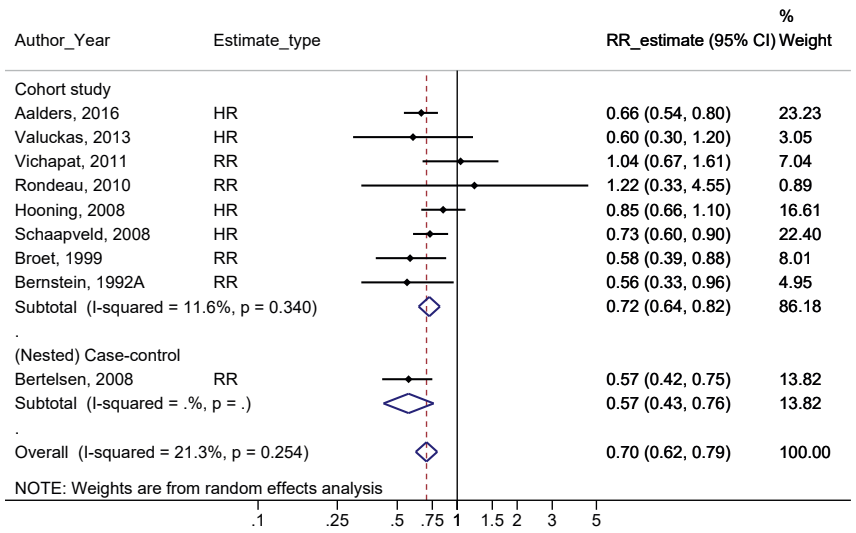
Supplementary Figure B.17. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing negative with positive Progesterone hormone receptor status of the primary breast cancer



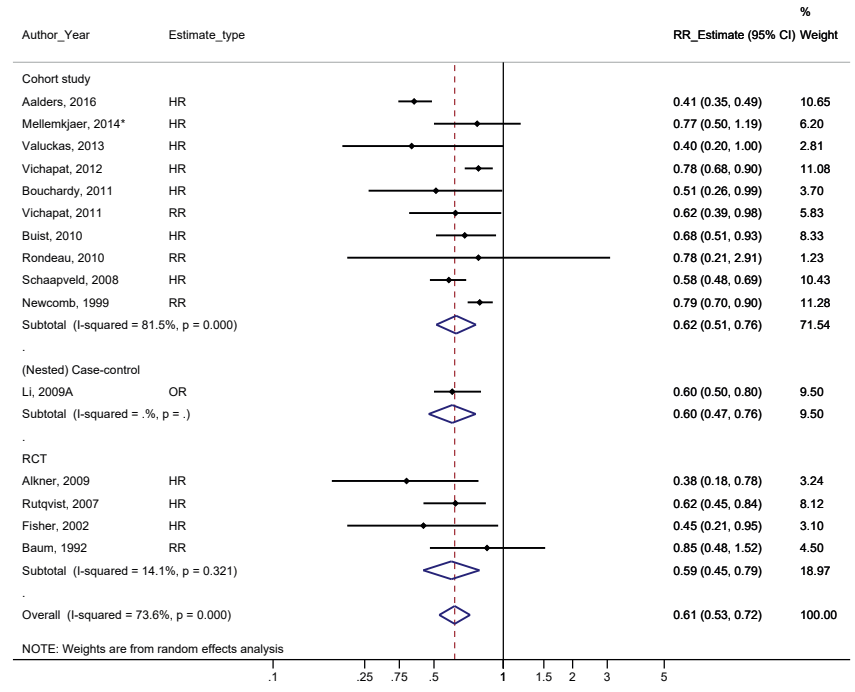
Supplementary Figure B.18. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing negative with positive HER2 status of the primary breast cancer



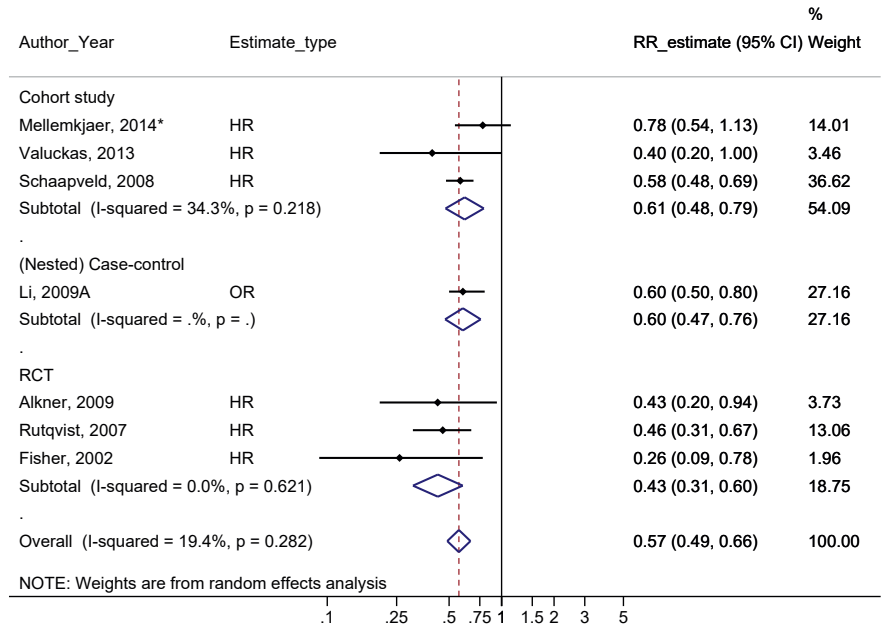
Supplementary Figure B.19. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing lobular with non-lobular histology of the primary breast cancer



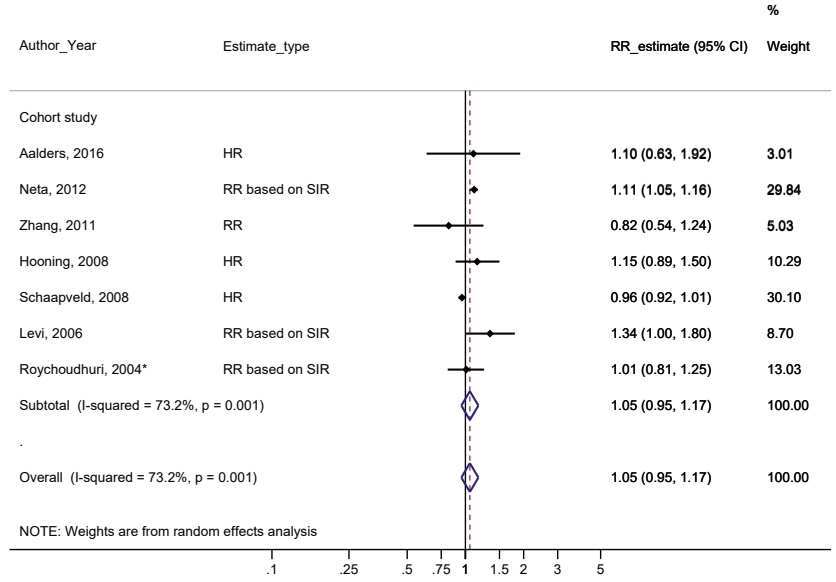
Supplementary Figure B.20. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing receiving adjuvant chemotherapy for primary breast cancer versus not.



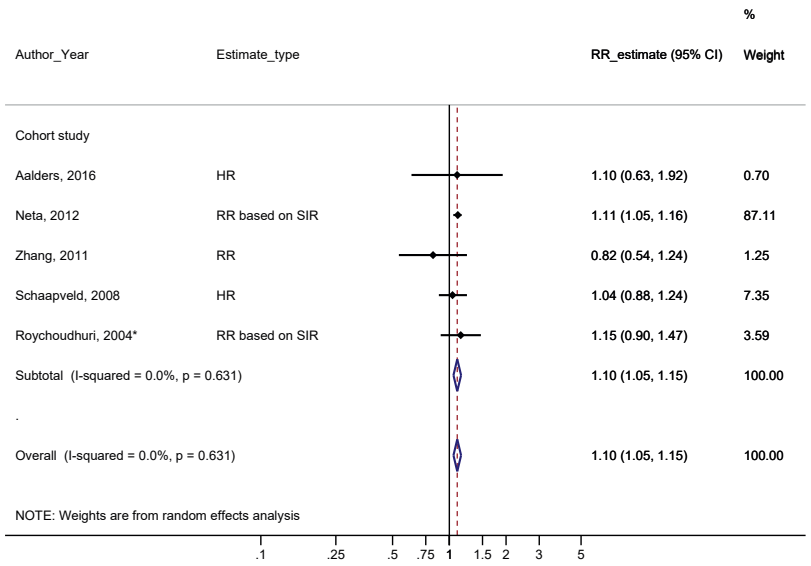
Supplementary Figure B.21. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing receiving adjuvant endocrine therapy for primary breast cancer versus not



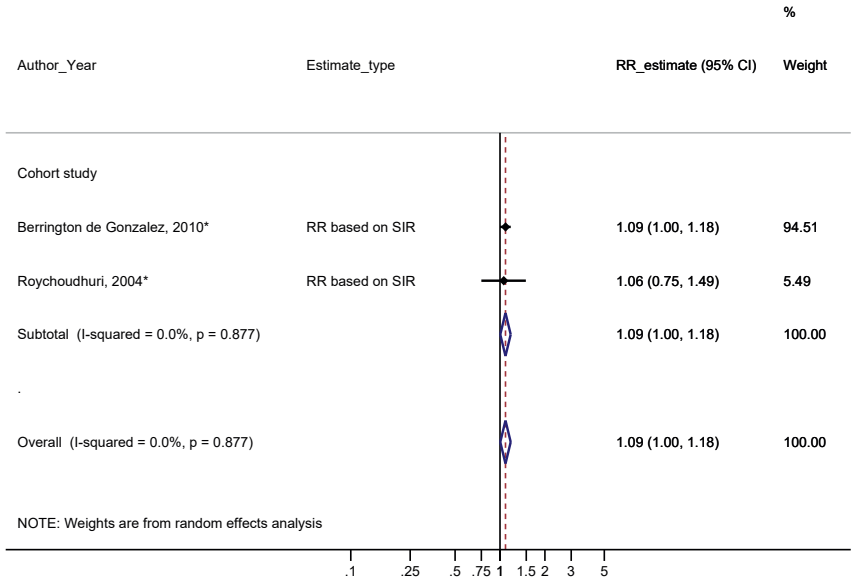
Supplementary Figure B.22. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing receiving adjuvant endocrine therapy for primary breast cancer versus not, restricted to patients with Estrogen receptor positive primary breast cancer



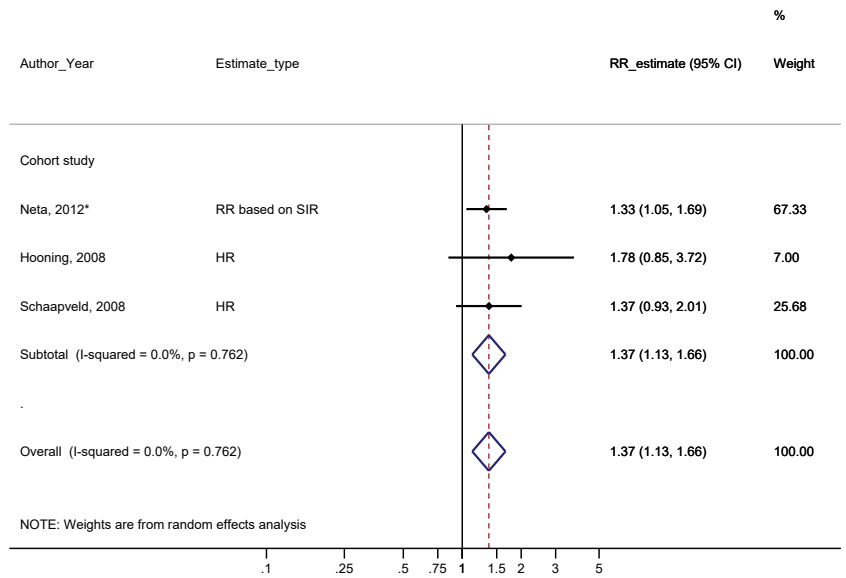
Supplementary Figure B.23. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing receiving radiotherapy for primary breast cancer versus not



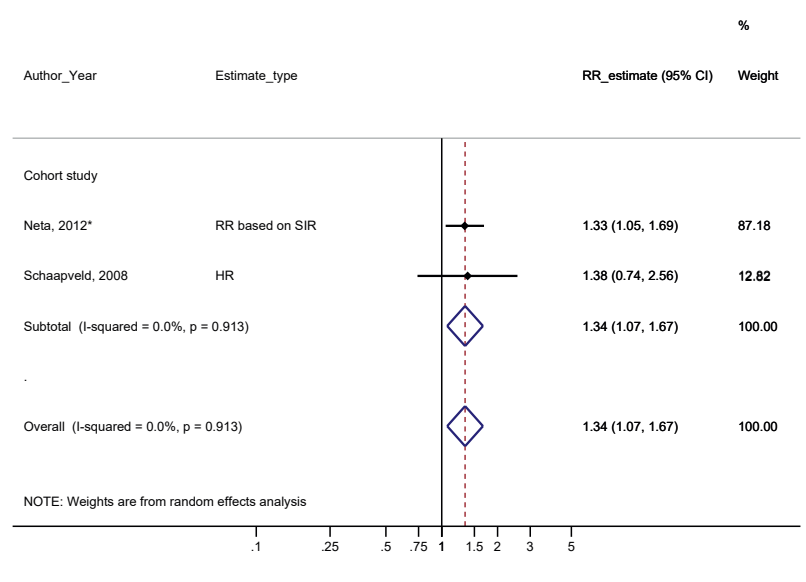
Supplementary Figure B.24. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing receiving radiotherapy for primary breast cancer versus not, restricted to patients with at least 5 years of follow-up



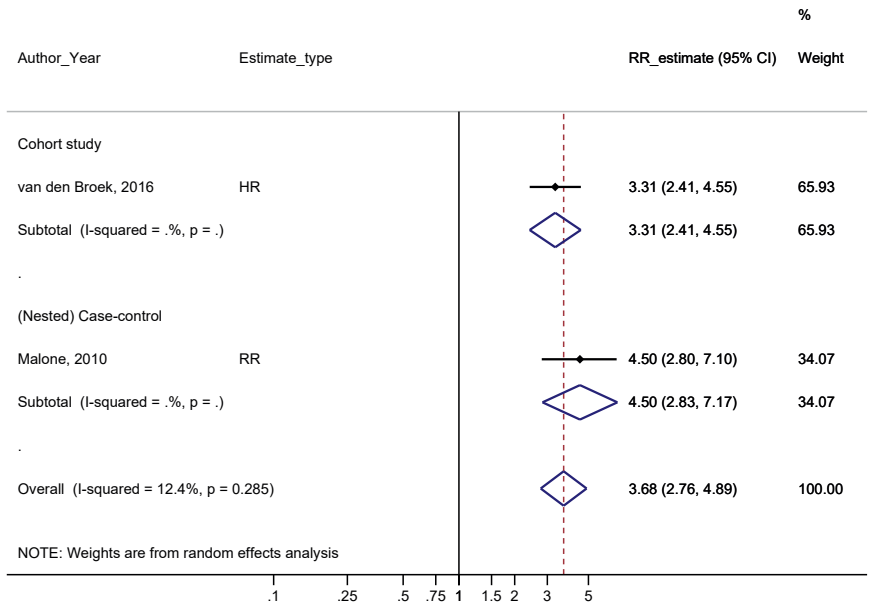
Supplementary Figure B.25. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing receiving radiotherapy for primary breast cancer versus not, restricted to patients with at least 10 years of follow-up



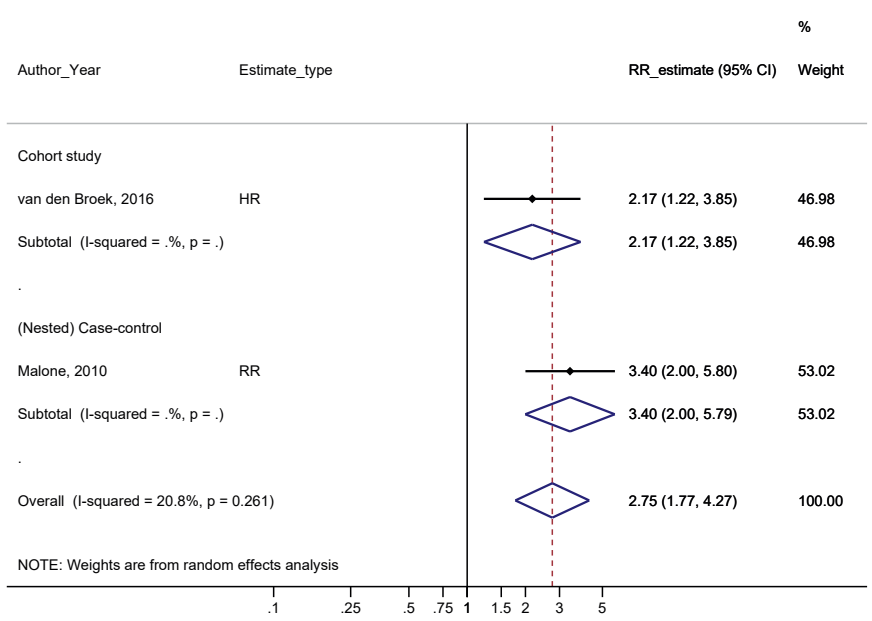
Supplementary Figure B.26. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing receiving radiotherapy for primary breast cancer versus not, restricted to patients being below 40 years of age at primary breast cancer diagnosis



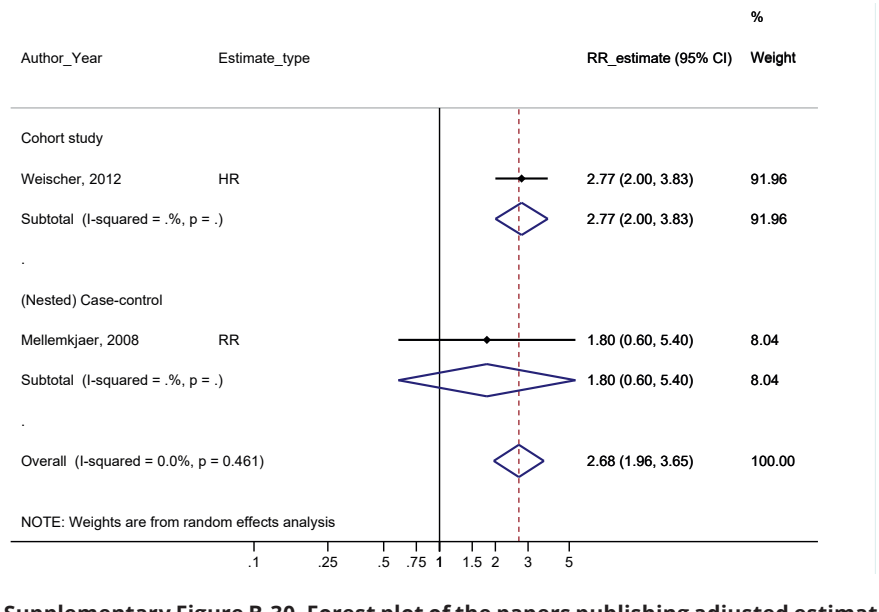
Supplementary Figure B.27. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing receiving radiotherapy for primary breast cancer versus not, restricted to patients being below 40 years of age at primary breast cancer diagnosis and having at least 5 years of follow-up



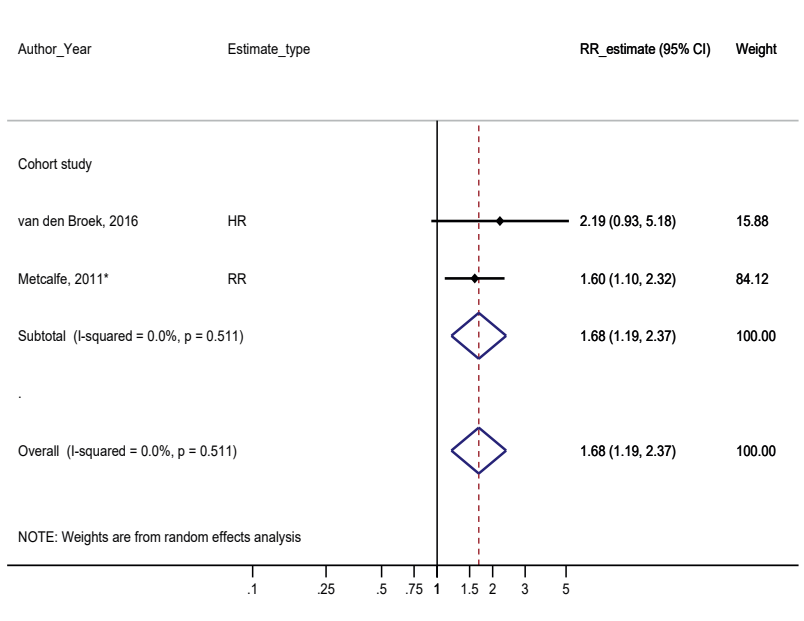
Supplementary Figure B.28. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in *BRCA1* mutation carriers versus non-carriers



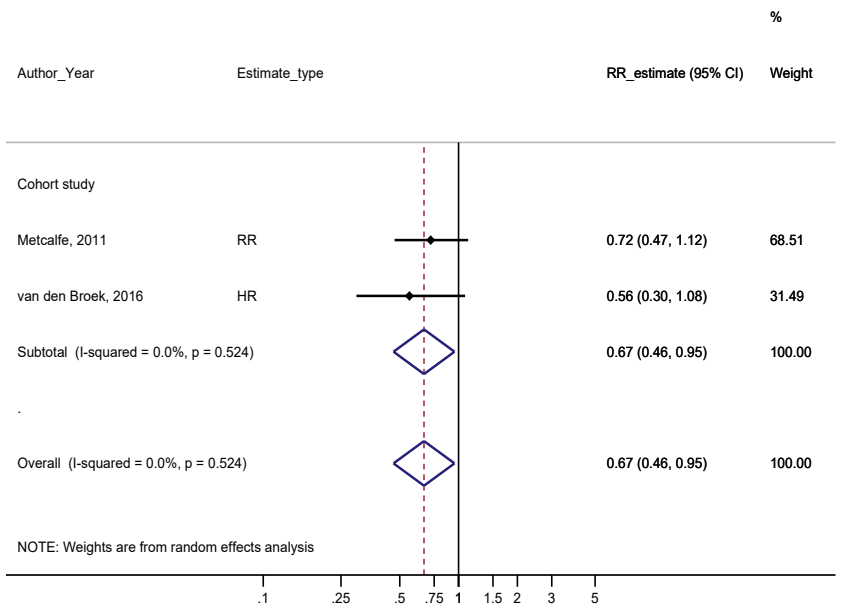
Supplementary Figure B.29. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in *BRCA2* mutation carriers versus non-carriers



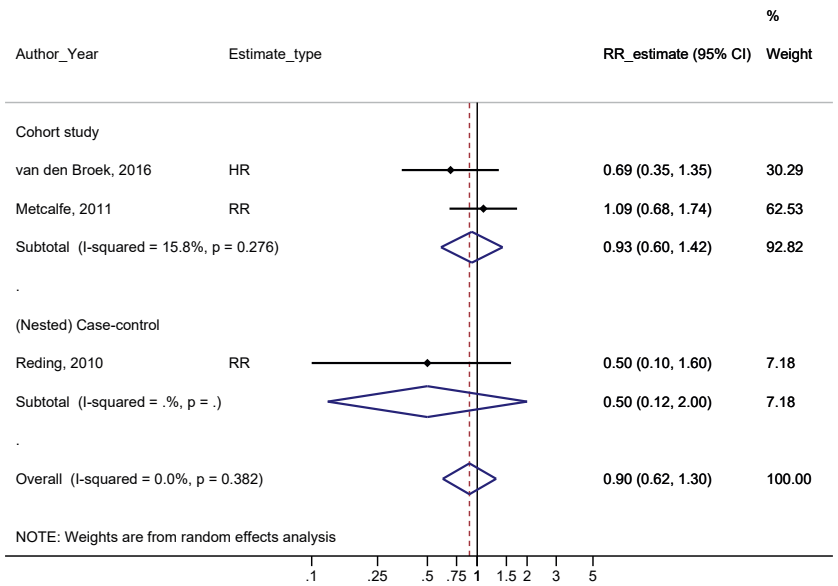
Supplementary Figure B.30. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in *CHEK21100delC mutation carriers versus non-carriers**



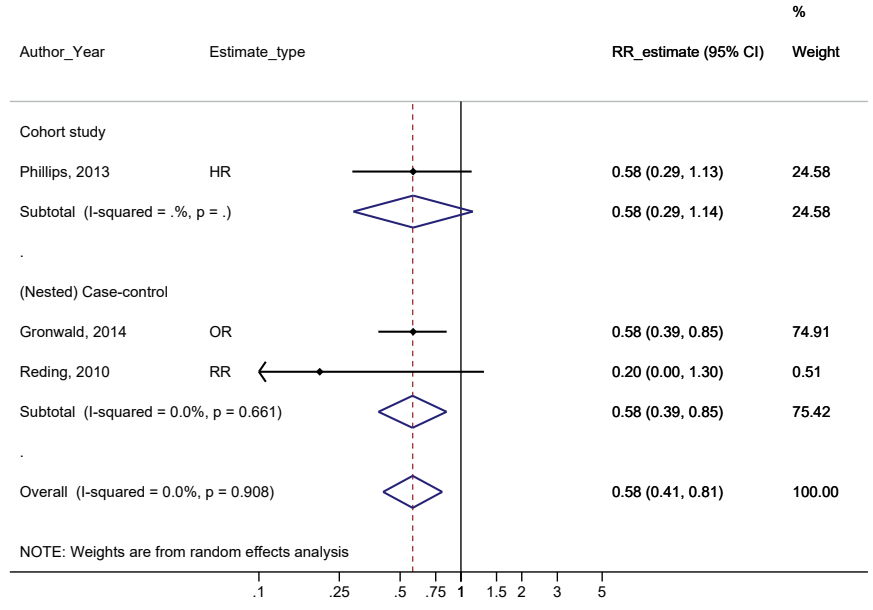
Supplementary Figure B.31. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in *BRCA1* mutation carriers comparing patients with a positive family history for breast cancer with patients with no family history of breast cancer



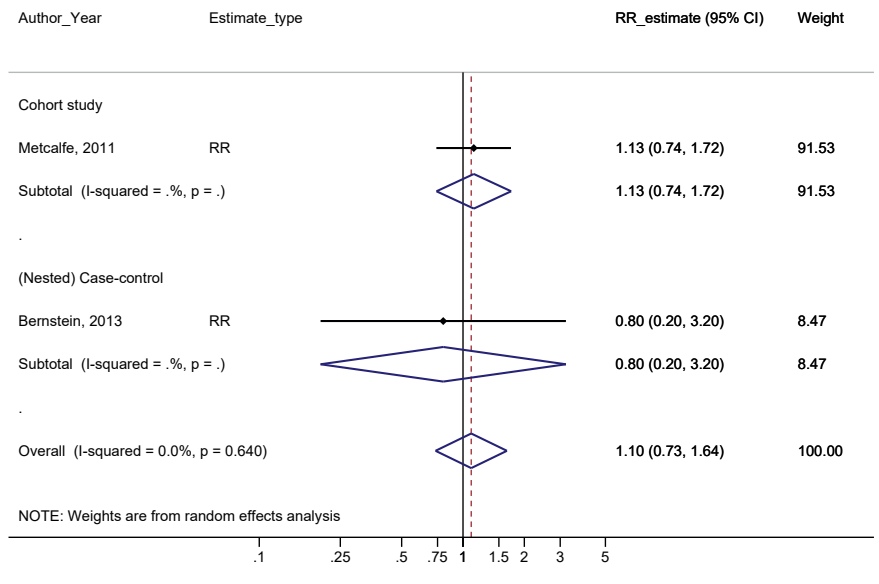
Supplementary Figure B.32. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in *BRCA1* mutation carriers comparing patients aged 41-49 years at primary breast cancer diagnosis with patients aged ≤ 40 years



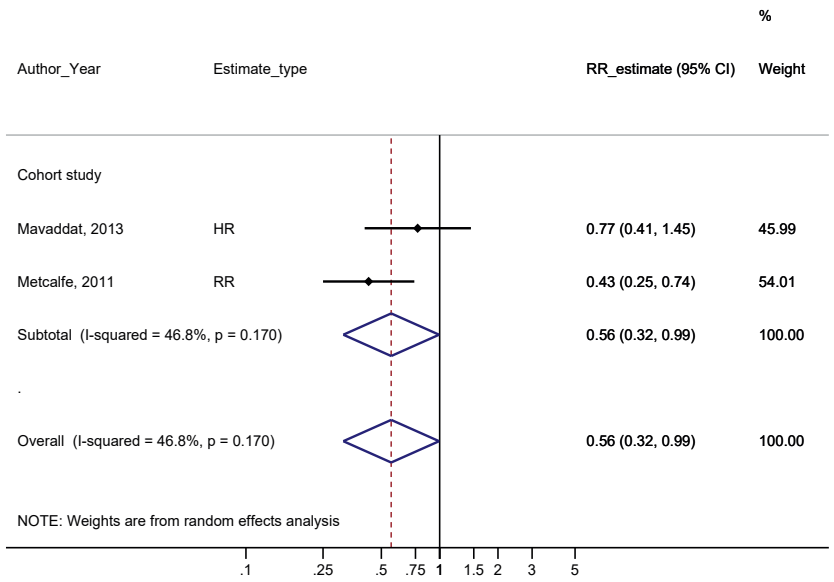
Supplementary Figure B.33. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in *BRCA1* mutation carriers comparing receiving chemotherapy for primary breast cancer versus not



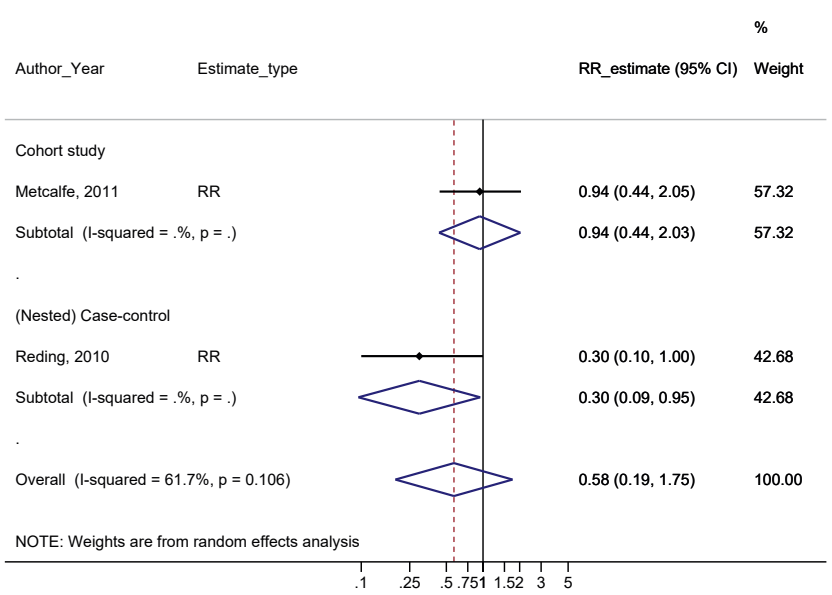
Supplementary Figure B.34. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in *BRCA1* mutation carriers comparing receiving endocrine therapy for primary breast cancer versus not



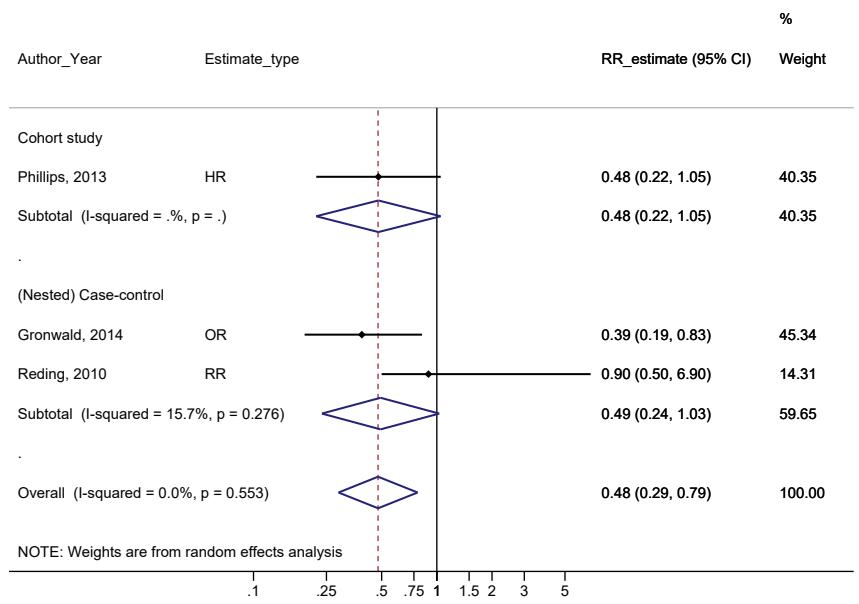
Supplementary Figure B.35. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in *BRCA1* mutation carriers comparing receiving radiotherapy for primary breast cancer versus not



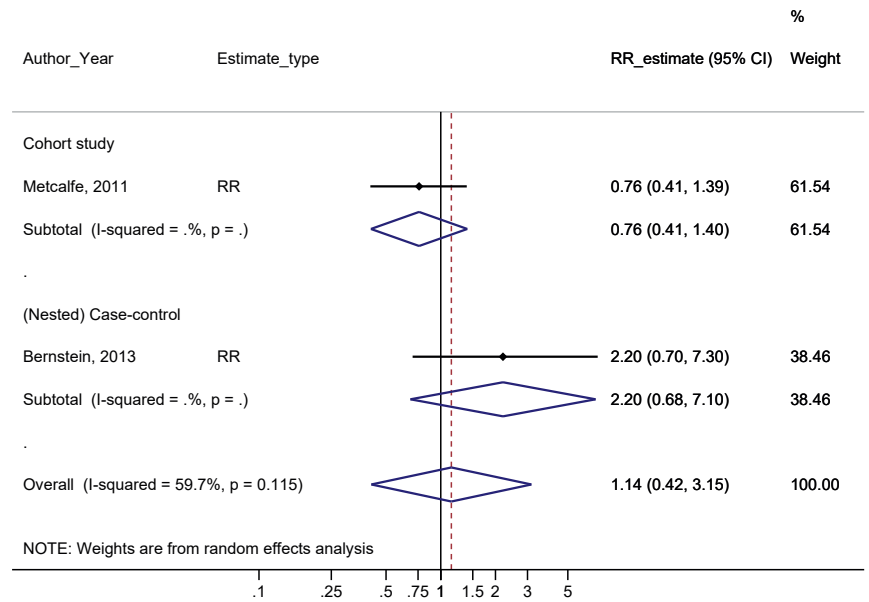
Supplementary Figure B.36. Forest plot of the papers publishing crude estimates on the risk of developing contralateral breast cancer in *BRCA1* mutation carriers comparing having had a risk-reducing salpingo-oophorectomy prior to contralateral breast cancer diagnosis versus not



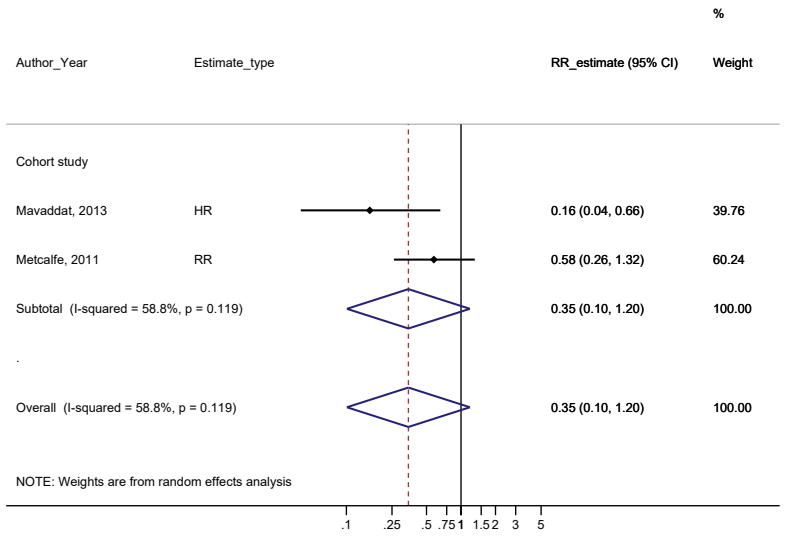
Supplementary Figure B.37. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in *BRCA2* mutation carriers comparing receiving adjuvant chemotherapy for primary breast cancer versus not



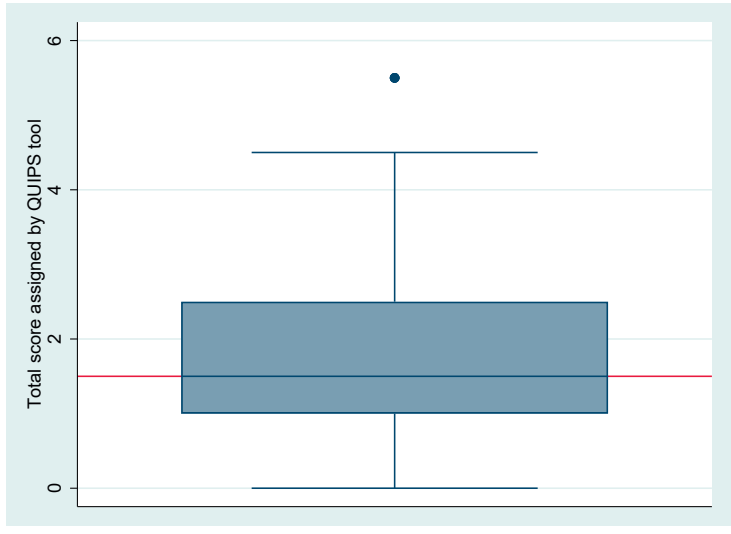
Supplementary Figure B.38. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in *BRCA2* mutation carriers comparing receiving adjuvant endocrine therapy for primary breast cancer versus not



Supplementary Figure B.39. Forest plot of the papers publishing adjusted estimates on the risk of developing contralateral breast cancer in *BRCA2* mutation carriers comparing receiving radiotherapy for primary breast cancer versus not



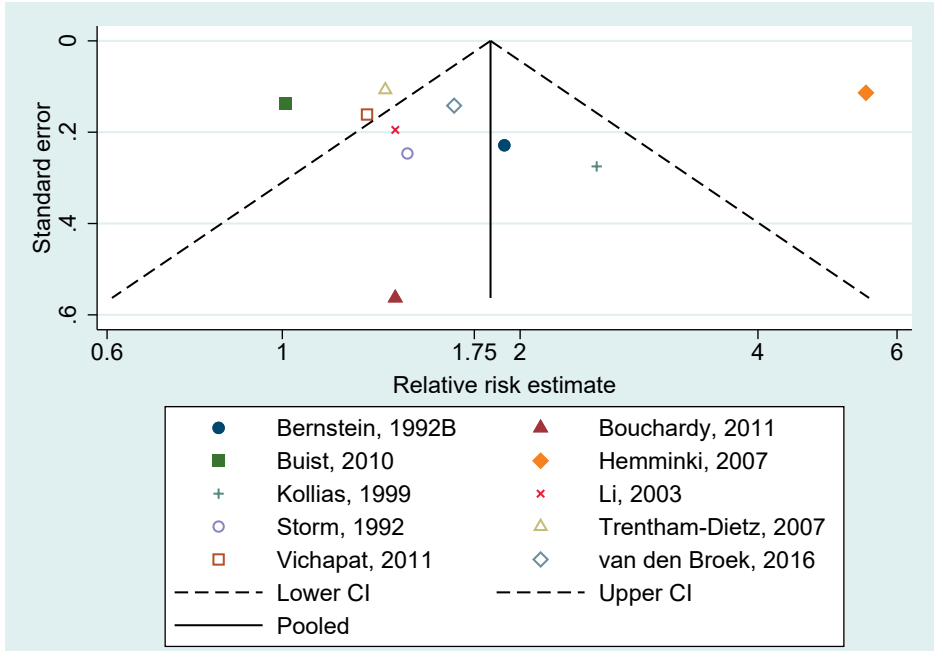
Supplementary Figure B.40. Forest plot of the papers publishing crude estimates on the risk of developing contralateral breast cancer in *BRCA2* mutation carriers comparing having had a risk-reducing salpingo-oophorectomy prior to contralateral breast cancer diagnosis versus not



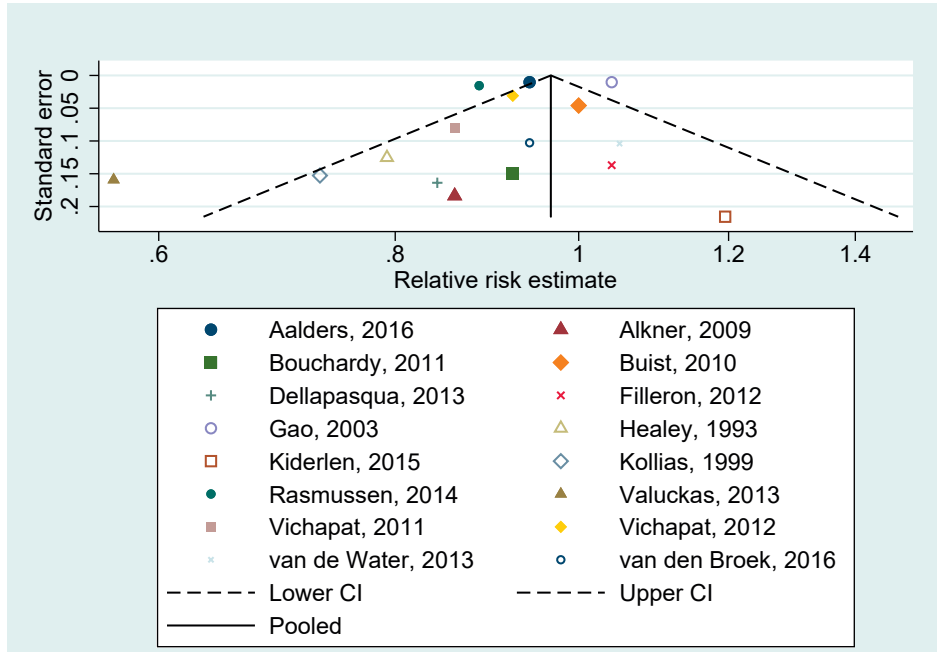
Supplementary Figure B.41. Boxplot of the scores assigned by the QUIPS tool to assess bias

The red line concerns the median value of the score (1.5); the mean score was 1.81; the standard deviation was 1.22. Papers which had a score <2 were considered high-quality papers and were used for the sensitivity analysis.

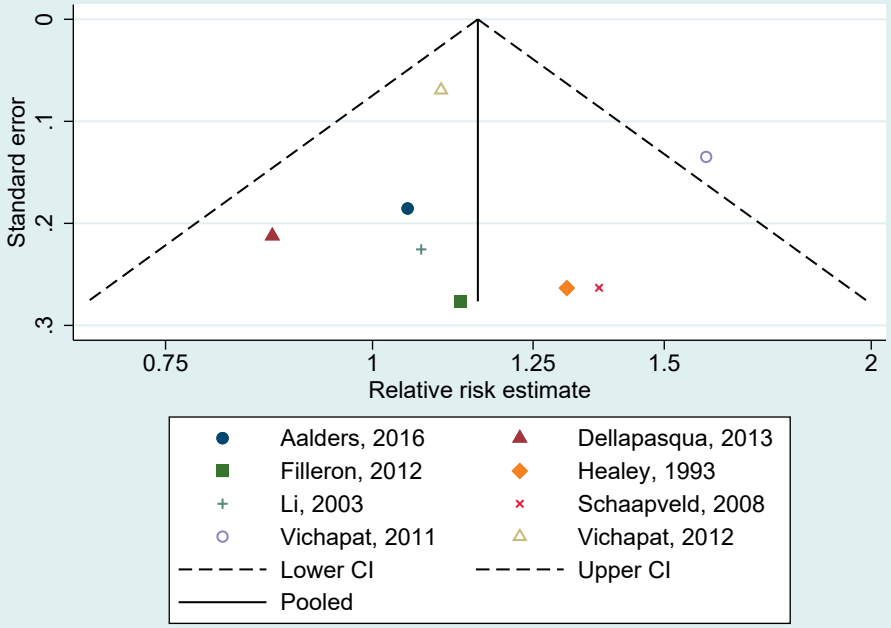
Funnel plots



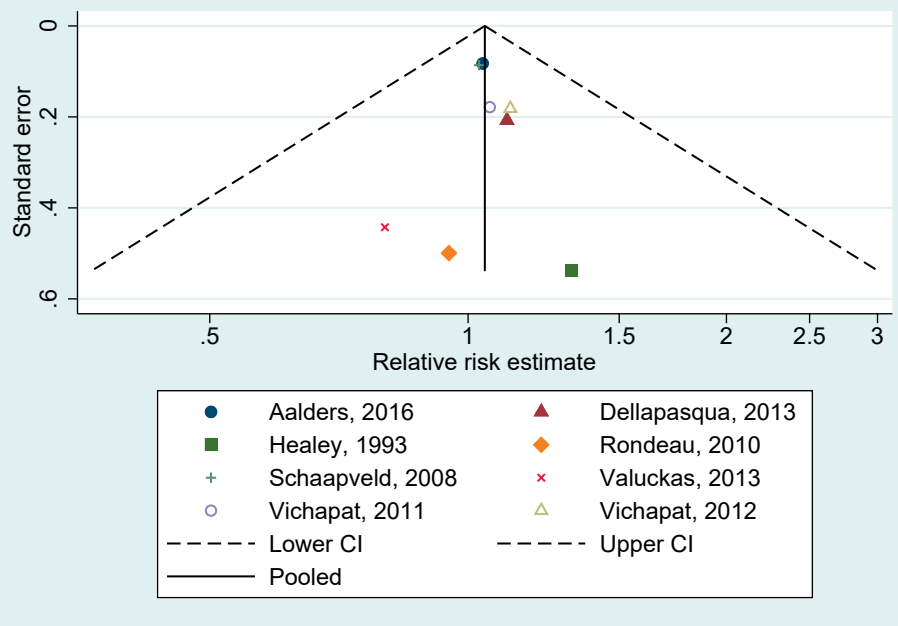
Supplementary Figure B.42. Funnel plot with pseudo 95% confidence limits (CI) for estimates published on the association of breast cancer family history with contralateral breast cancer risk in the unselected breast cancer population



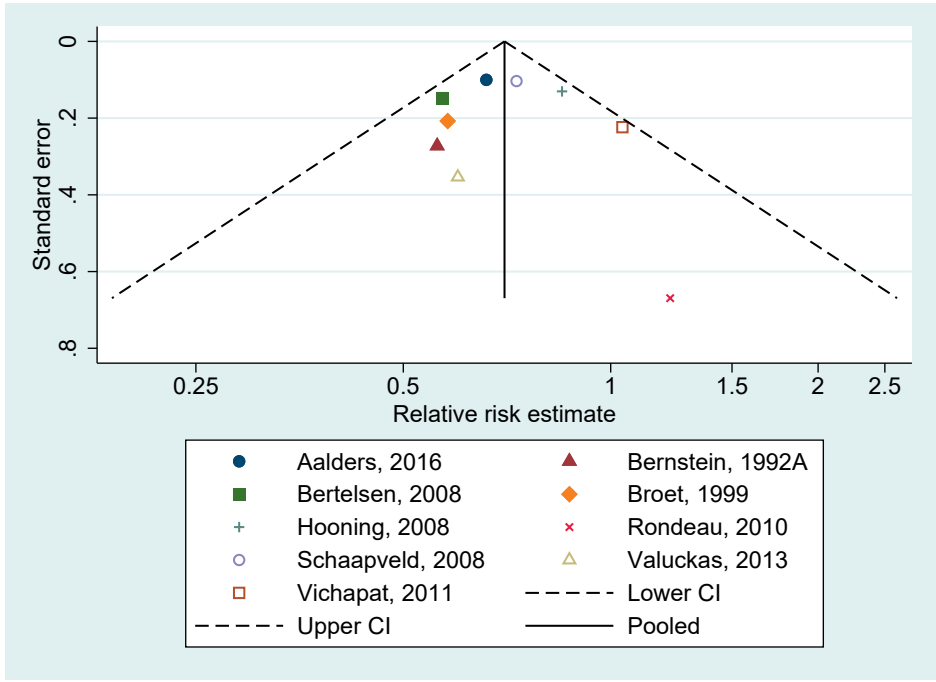
Supplementary Figure B.43. Funnel plot with pseudo 95% confidence limits (CI) for estimates published on the association of age at primary breast cancer diagnosis with contralateral breast cancer risk in the unselected breast cancer population



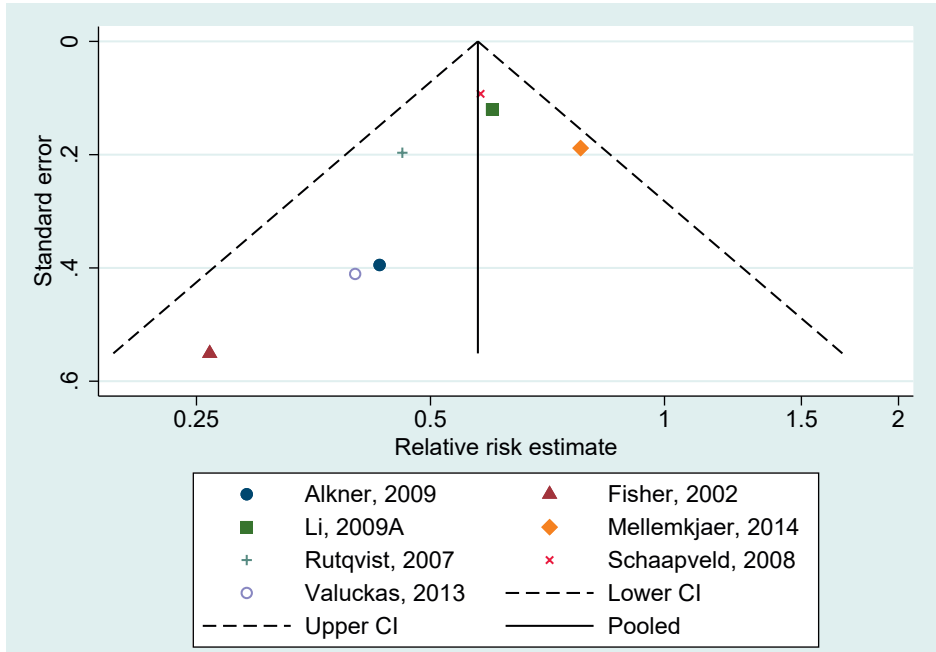
Supplementary Figure B.44. Funnel plot with pseudo 95% confidence limits (CI) for estimates published on the association of primary breast tumor size (>2cm vs. =<2cm) with contralateral breast cancer risk in the unselected breast cancer population



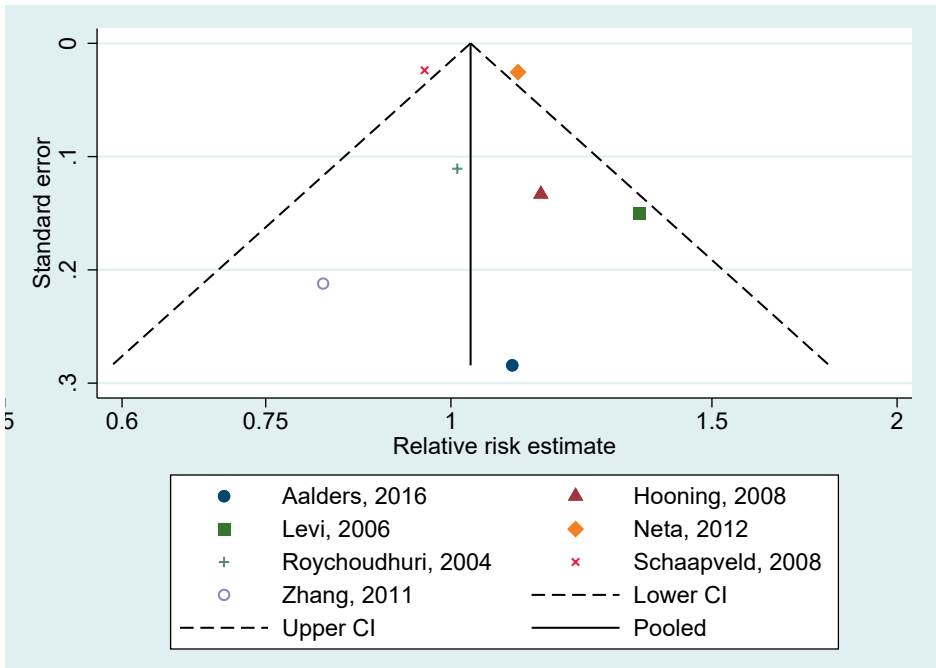
Supplementary Figure B.45. Funnel plot with pseudo 95% confidence limits (CI) for estimates published on the association of primary breast cancer nodal status (positive vs. negative) with contralateral breast cancer risk in the unselected breast cancer population



Supplementary Figure B.46. Funnel plot with pseudo 95% confidence limits (CI) for estimates published on the effects of chemotherapy on contralateral breast cancer risk in the unselected breast cancer population



Supplementary Figure B.47. Funnel plot with pseudo 95% confidence limits (CI) for estimates published on the effects of endocrine therapy on contralateral breast cancer risk in estrogen receptor positive unselected breast cancer population



Supplementary Figure B.48. Funnel plot with pseudo 95% confidence limits (CI) for estimates published on the effects of radiotherapy on contralateral breast cancer risk in unselected breast cancer population

CHAPTER 3

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

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LB Koppert, MJ Hooning
*both authors contributed equally.

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ABSTRACT

Purpose: The risk of being diagnosed with contralateral breast cancer (CBC) is an important health issue among breast cancer survivors. There is an increasing interest in the effect of lifestyle and reproductive factors on CBC risk, since these factors may partly be modifiable. We performed a systematic review and meta-analysis and aimed to evaluate the impact of lifestyle and reproductive factors on CBC risk in population-based breast cancer studies.

Methods: The PubMed electronic database was searched up to November 2nd 2019 for relevant publications. Of the included studies, a meta-analysis per lifestyle or reproductive factor was performed.

Results: Thirteen out of 784 publications were used for the meta-analysis. Body mass index (≥25 vs. <25 kg/m^2; RR=1.22; 95%CI:1.01-1.47) was associated with increased CBC risk. The estimates for alcohol use (ever vs. never; RR=1.15; 95%CI:1.02-1.31) and age at primiparity (≥25 vs. <25 years; RR=1.06; 95%CI:1.02-1.10) also showed an association with increased CBC risk. For parity (≥4 vs. nulliparous; RR=0.56; 95%CI:0.42-0.76) and age at menopause (<45 vs ≥45 years; RR=0.79; 95%CI:0.67-0.93), results from two studies suggested a decreased CBC risk. We observed no association between CBC and smoking, age at menarche, oral contraceptive use, gravidity, breastfeeding or menopausal status. Overall, the number of studies per risk factor was limited (*n*=2-5).

Conclusions: BMI is a modifiable risk factor for CBC. Data on the effect of other modifiable lifestyle and reproductive factors is limited. For better counseling of patients on lifestyle effects, more studies are urgently needed.

INTRODUCTION

Over the last decades, the survival rate of breast cancer patients has been improving as a result of earlier diagnosis and better treatment.[1,2] This leads to an increasing number of women who have been previously diagnosed with breast cancer, and are at risk for developing a second new malignancy in the opposite breast over time, i.e. metachronous contralateral breast cancer (CBC). Ten-year cumulative risk of CBC is around 4-5% in the general population.[3,4]

The risk of being diagnosed with CBC is therefore an important health issue among breast cancer survivors and often a recurring subject during follow-up at the outpatient clinic. This can also be observed from the increasing number of breast cancer survivors choosing for prophylactic removal of the contralateral breast. However, in a majority of the women with breast cancer, no survival benefit has been reported following this procedure.[5,6]

For this reason, it is important to evaluate the risk of developing CBC in individual breast cancer patients in a tailored fashion to provide them with an accurate follow-up strategy. Not only genetic and breast cancer treatment-related factors, but also lifestyle and reproductive factors should be assessed for this purpose.

Nowadays there is an increasing interest in the impact of lifestyle and reproductive factors on CBC risk among health care professionals and breast cancer survivors, since these factors may partly be modifiable. Current available estimates on lifestyle and reproductive factors need to be combined to get estimates that are based on the highest level of evidence.

We therefore conducted a systematic review with meta-analysis and aimed to evaluate the impact of lifestyle and reproductive factors on metachronous CBC risk in population-based breast cancer cohorts.

METHODS

This systematic review with meta-analysis is conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[7]

Literature search

We searched the PubMed electronic database for publications on November 2nd 2019 by using search terms related to CBC in combination with lifestyle or reproductive factors that are potentially associated with CBC risk. More specific, we were interested in the impact of dietary habits, exercise, body mass index (BMI), alcohol use, smoking, age at menarche, oral contraceptive use, gravidity, age at primiparity, parity, breastfeeding, menopausal status and age at menopause on metachronous CBC risk in population-based studies, i.e. studies with general, unselected breast cancer populations, without any germline mutation being tested on. Metachronous CBC is defined as a second primary breast cancer developed in the contralateral breast over time, from now onwards referred to as CBC.

Metachronous refers to a certain time lapse between the first primary breast cancer (PBC) diagnosis and the CBC diagnosis, but in literature this has not been clearly defined yet; mainly a time lapse of 3-12 months is being used. Our literature search included search terms for second breast cancer, thereby potentially including publications that studied the risks of developing ipsilateral second PBC, along with CBC. However, literature states that only 5% of the second PBCs develops in the ipsilateral breast.[8]

The literature search was limited to publications written in English from the 1st of January 1990 onwards. A description of the full search strategy is given in Supplementary Table 1.

Study selection

Two reviewers (MMK, CZAS) identified potentially eligible publications by reading each title and abstract. From the selected publications, the full text was read. Publications were subsequently excluded if they met at least one of the exclusion criteria, which were defined as publications without pooled data (e.g. narrative review, research report, guideline, comment editorial), publications without relative risk (RR) estimates (i.e. no relative risk, odds ratio or hazard ratio) or publications with less than 20 CBC events reported. Further exclusion concerned publications on high-risk breast cancer patients (e.g. *BRCA1/2*-related breast cancer, familial/hereditary breast cancer), publications on non-invasive CBC, publications on CBC analyzed in the context of recurrent disease or publications on topics not related to the effect of lifestyle or reproductive factors on CBC risk. When in doubt about including a paper (*n*=17), a third reviewer was consulted (DA).

In addition, references of the eligible publications were checked for additional records missed by the initial literature search.

Data extraction

The main study characteristics from the included publications were extracted and collected in an overview (Table 1). These study characteristics included the author, year of publication, origin of cancer or hospital registry, study design, date of first PBC diagnosis, selection criteria, number of first PBC and CBC patients, mean/median years of follow-up (including range) and age (including range) of women with first PBC, required time lapse between first PBC and CBC diagnosis, lifestyle and/or reproductive factors of interest and time of assessment of the risk factor status (e.g. at first PBC or CBC diagnosis).

Additionally, we extracted the relative risk estimates with the corresponding confidence interval (CI) and factors that were adjusted for in the analysis.

Statistical analyses

Relative risk estimates with the corresponding CI were collected, log transformed and pooled per lifestyle or reproductive factor. A random effects model was used for the meta-analyses. If a study did not report an overall risk estimate for a specific factor, subgroup estimates were combined to create an overall risk estimate with the use of a random effects model.

Relative risk estimates from univariable and multivariable analyses were analyzed separately. If both an adjusted (i.e. using multivariable risk estimates) and a crude meta-analysis (i.e. using univariable risk estimates) could be conducted for a factor, we only selected the papers eligible for the adjusted meta-analysis.

Subsequently, we evaluated whether there was potential overlap in patients from the different papers. In case of overlap, we selected either the most recent or the most relevant (i.e. on topic) paper.

To examine the continuous effects (trend analysis) of BMI and number of full-term pregnancies (FTP), we used the dose-response method described by Greenland et al.[9] Results from this analysis were subsequently pooled using a random-effects meta-analysis and the p-value for trend was extracted from the confidence interval of the pooled estimate.

Additionally, we tested for heterogeneity using the I^2-statistics and reported the p-value for heterogeneity for each lifestyle or reproductive factor in the figures.

We used the METAN package of Stata Statistical Software version 14.0 to conduct the statistical analyses.

RESULTS

Search results and study selection

Our PubMed literature search identified 784 publications, of which 707 met the inclusion criteria (Figure 1). Based on relevance, 41 of the remaining publications were selected for further review. Hereof, 20 publications were eligible for inclusion after applying the exclusion criteria. No additional publications were found by checking the references of the eligible publications. In addition, 7 out of these 20 publications were ineligible for the (adjusted) meta-analyses due to non-preferable risk estimates (i.e. solely reporting univariable risk estimates[10]) or patient overlap[11-16] and were therefore excluded.

From the 13 papers finally used for the meta-analysis, there were between 424-72,096 first PBC and 24-2,515 CBC patients available for the analyses. A majority of the studies (9 out of 13) were at least partially performed in the USA.

Meta-analyses

The adjusted estimates for lifestyle and reproductive factors are presented in an overall plot in Figures 2 and 3, respectively, and per lifestyle or reproductive factor in Supplementary Figures 1-16.

Heterogeneity will only be reported for risk factor estimates in case of moderate or high heterogeneity (i.e. I^2 >50%, p<0.05 as reported in the figures).

Lifestyle factors (Figure 2; Supplementary Figures S1-5)

Eight publications studied the impact of potentially modifiable lifestyle factors (BMI, alcohol use, and smoking) on CBC risk (Table 1).[11,17-24]

Being overweight and obese (BMI ≥25 kg/m^2) compared to having a normal weight (BMI <25 kg/m^2) assessed at first PBC diagnosis, was associated with an increased CBC risk (RR=1.22; 95%CI: 1.01-1.47), however, heterogeneity was high (I^2=75.4%, p=0.003; Supplementary Figure 1).[17-19,21,22]

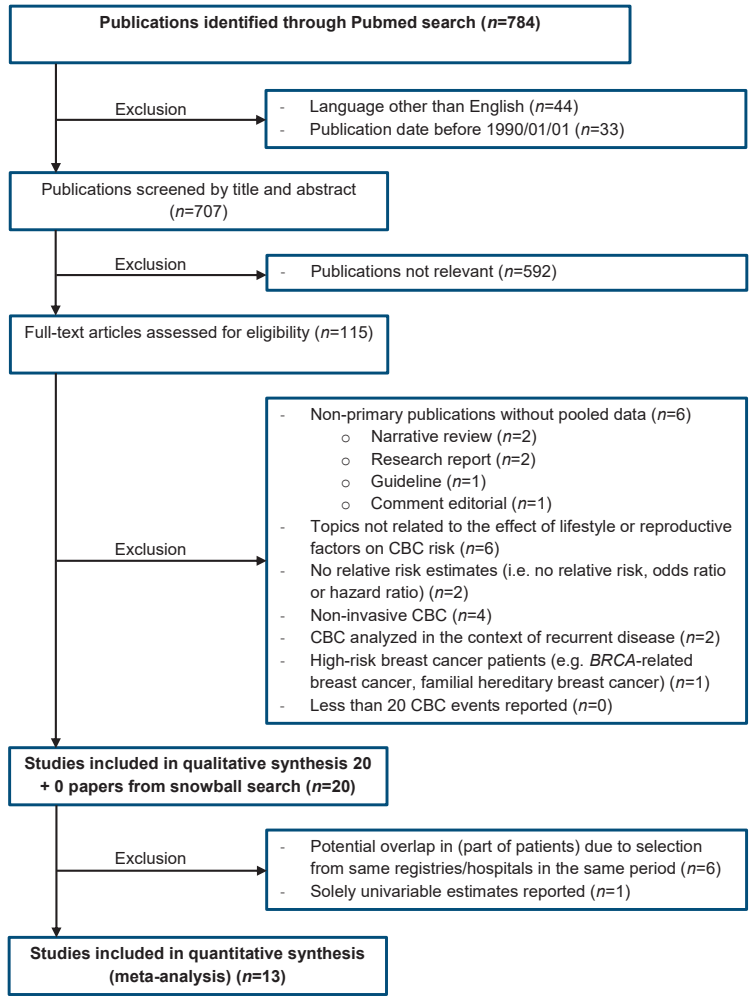

Figure 1. Flowchart of the literature search for a systematic review with meta-analysis assessing lifestyle and reproductive risk factors for contralateral breast cancer

CBC: contralateral breast cancer.

The main outlier was the study performed by Brooks et al.[17], possibly due to inclusion of mainly young, premenopausal women at first PBC diagnosis (further elaborated on in discussion section). Excluding this study resulted in a decrease in heterogeneity and a slight increase in CBC risk (RR=1.31; 95%CI: 1.15-1.50; I^2=35%). Trend analysis on BMI showed a significant increased CBC risk with increasing BMI (p-trend<0.0001).

The meta-analysis on three studies concerning alcohol use (ever vs. never; assessed at first PBC diagnosis), was suggestive of increased CBC risk (RR=1.15; 95%CI: 1.02-1.31; Supplementary Figure 4).[19,23,24] Four studies on smoking did not result in an association with CBC risk (ever vs. never; assessed at first PBC diagnosis; Supplementary Figure 5).[19,20,23,24]

There was no data available on the association between dietary habits or physical exercise and CBC risk.

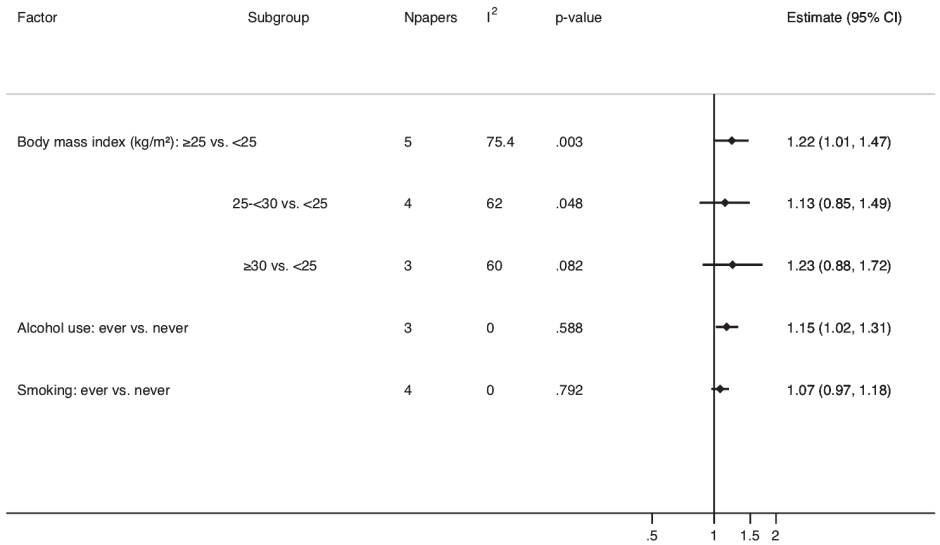

Figure 2. Forest plot of the overall adjusted meta-analyses per lifestyle factor on the risk of developing contralateral breast cancer in population-based cohorts

Npapers: number of papers used for the analysis; I^2: test for heterogeneity; p-value: p-value for heterogeneity: p<0.05 considered significant; estimate: relative risk estimate combining relative risks, odds ratios and hazard ratios.

Reproductive factors (Figure 3; Supplementary Figures 6-16)

Eight publications studied the impact of reproductive factors (age at menarche, oral contraceptive use, gravidity, age at primiparity, parity, breastfeeding, menopausal status, age at menopause) on CBC risk (Table 1).[19,20,23,25-29]

Older age at primiparity (≥25 years vs. <25 years; assessed at/before CBC diagnosis) was investigated in four studies and was associated with increased CBC risk (RR=1.06; 95%CI: 1.02-1.10; Supplementary Figure 9).[19,23,25,29]

The two studies on age at menopause (<45 years vs. ≥45 years; assessed at/before CBC diagnosis) suggested a decreased CBC risk association for this factor (RR=0.79; 95%CI: 0.67-0.93; Supplementary Figure 16).[19,26]

Three studies on parity (≥1 FTPs vs. nulliparous; assessed at/before CBC diagnosis) showed no significant association with CBC risk (Supplementary Figure 10)[19,25,29], although trend-analysis resulted in a decreasing risk with increasing numbers of FTPs (p-trend <0.0001). Moreover, subgroup analysis on two papers suggested that having ≥4 FTPs compared to being nulliparous (assessed at/before CBC diagnosis) was protective for CBC risk (RR=0.56; 95%CI: 0.42-0.76; Supplementary Figure 12).[19,28] Having ≥2 FTPs compared to 1 FTP (assessed at first PBC diagnosis), which was investigated in 3 papers, showed a protective effect for CBC risk as well (RR=0.86; 95%CI: 0.79-0.94; Supplementary Figure 13).[23,25,29] The association between breastfeeding (ever vs. never; assessed at/before CBC diagnosis) and CBC risk was borderline significant, but the meta-analysis was based on two papers only (RR=0.87; 95%CI: 0.74-1.01; Supplementary Figure 14).[23,25]

No significant association was found for age at menarche (≥13 years vs. <13 years; Supplementary Figure 6)[19,23,27], oral contraceptive use (ever vs. never; assessed before CBC diagnosis; Supplementary Figure 7)[19,23,27], gravidity (ever pregnant vs. never pregnant; assessed at first PBC diagnosis; Supplementary Figure 8)[23,25] or menopausal status (postmenopausal vs. premenopausal; assessed at/before first PBC diagnosis; Supplementary Figure 15) and CBC risk.[19,20,23,25] However, for all these factors the number of papers was limited (*n*=2-4).

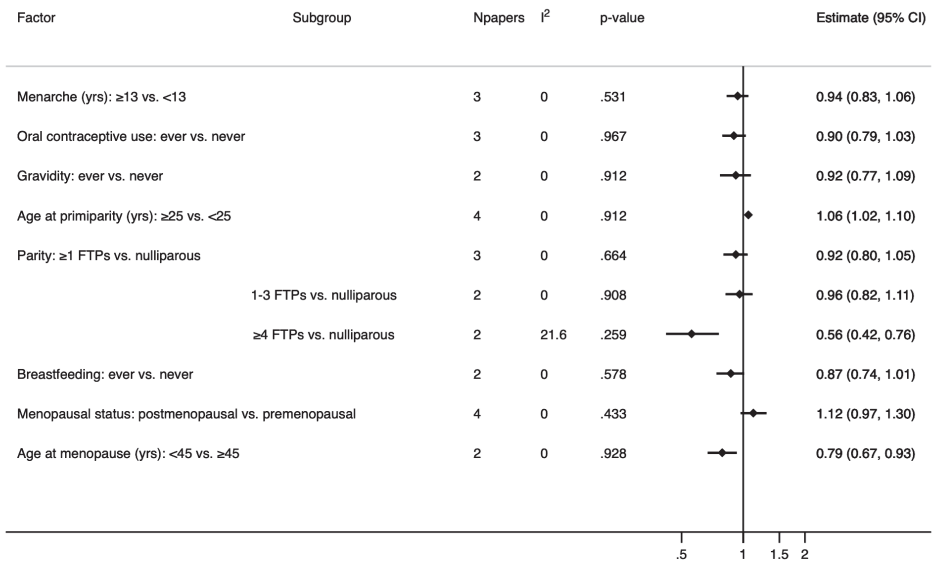

Figure 3. Forest plot of the overall adjusted meta-analyses per reproductive factor on the risk of developing contralateral breast cancer in population-based cohorts

Npapers: number of papers used for the analysis; I^2: test for heterogeneity; p-value: p-value for heterogeneity: p<0.05 considered significant; estimate: relative risk estimate combining relative risks, odds ratios and hazard ratios. yrs: years; FTP(s): full-term pregnancy/pregnancies.

DISCUSSION

In this systematic review and meta-analysis, we studied the impact of lifestyle and reproductive factors on CBC risk in population-based breast cancer cohorts.

We observed a moderately increased CBC risk in women being overweight. Further, alcohol use and older age at primiparity were suggestive of increased CBC risk, whereas a high number of full-term pregnancies and younger age at menopause seemed associated with decreased CBC risk. Overall, the number of papers available for the meta-analyses was limited.

We observed high heterogeneity in the meta-analysis concerning BMI. Difference in menopausal status at first PBC diagnosis could have led hereto, since most women of the lower risk outliers[17,21] were pre/perimenopausal as opposed to the other studies[18,19,22] in which the majority of women was postmenopausal at first PBC

diagnosis. It is sometimes hypothesized that high BMI in premenopausal women may lead to anovulation and reduction of circulating estrogen and progesterone and thereby reducing PBC risk.[30] Contrarily, in postmenopausal women, a high BMI is observed to be a risk factor for first PBC[31,32], likely due to increasing estrogen concentration through adipose tissue production and reduction of sex hormone-binding globulins.[33] These mechanisms may also be applied to the association between BMI and CBC. Still, literature supporting the inverse association between BMI and premenopausal first PBC is scarce and lacks strong evidence.[31,32]

In contrast to the systematic review of Simapivapan et al.[34] in which no conclusive association between alcohol consumption and second PBC was found, we did observe a positive association between alcohol consumption and CBC risk. Simapivapan et al. included the same publications as we did[12,14,16,19,23], but did not exclude the publications of Li et al.[12,14] which we considered to overlap in patient cohort with Knight et al.[24] Consequently, because of the limited number of available publications, we had to use a dichotomous outcome (i.e. ever vs. never), whereas Simapivapan et al. gave a narrative overview of the frequency and/or period of exposure (e.g. pre or post breast cancer diagnosis) to alcohol consumption.

Despite contradicting evidence relating to the impact of parity on CBC risk, it seems that multiple FTPs are protective for developing a CBC as shown by the trend-analysis for an increasing number of FTPs and subgroup analyses for parity (≥4 FTPs vs. nulliparous and ≥2 FTPs vs. 1 FTP). The fact that some analyses did not show a significantly decreased CBC risk for parity (≥1 FTPs vs. nulliparous and 1-3 FTPs vs. nulliparous) could be explained by a lack of power from small contrast in numbers of FTP and from including a small number of studies (*n*=2-3).

Just as for first PBC[35], having multiple full-term pregnancies seemed protective for developing CBC, whereas primiparity at an older age showed an increased risk of CBC. Pregnancy induces terminal differentiation of mammary luminal cells through exposure to human chorionic gonadotrophin (hCG). This results in changing gene expression in the mammary stem cells, becoming more refractory to carcinogenesis through increased DNA repair pathway and apoptosis control. Older age at primiparity delays the formation of this protective 'genomic signature' and extends the exposure time to carcinogens, thereby making the breast more

susceptible to carcinogenesis.[36] We assume that the underlying mechanisms for parity and age at primiparity on first PBC risk may apply for CBC as well.

We observed a decreased CBC risk in women with younger age at menopause (i.e. early/premature menopause), but the number of papers was limited (*n*=2). Nonetheless, older age at menopause has previously been described as a risk factor for first PBC[37], possibly due to a higher number of menstrual cycles experienced[38], thereby having longer exposure to high estrogen levels.[39] Therefore, it makes sense that a younger age at menopause is associated with a decreased CBC risk. Moreover, first PBC risk increases less for every year older at menopause than for every year younger at menarche, implying that not only the number of menstrual cycles plays a role in the relationship between childbearing years and first PBC risk (and possibly CBC risk)[37]; perhaps the number of reproductive years before the first FTP is even more important. Nonetheless, we did not find any association between age at menarche and CBC risk.

There are several limitations to our study that need to be considered. First, there were only few studies available per studied risk factor (two to five per meta-analysis), underlining that little is known about the impact of lifestyle and reproductive factors on CBC risk. For example, only two publications were included in the meta-analyses for gravidity, subgroups of parity (1-3 or ≥4 full-term pregnancies vs. nulliparous), breastfeeding and age at menopause. In addition, we had to use a dichotomous outcome (i.e. ever vs. never) for the meta-analyses concerning smoking, alcohol use, oral contraceptive use, gravidity and breastfeeding. The outcomes of these analyses should therefore be interpreted with caution. In addition, the small number of papers that was available per factor inhibited us from being able to inspect the presence of publication bias.

Second, the analyzed data is heterogeneous regarding the timing of assessment of the lifestyle and reproductive factors. All lifestyle factors (BMI, alcohol use and smoking) were assessed at first PBC diagnosis, whereas most reproductive factors were assessed at or before CBC diagnosis. Factors assessed at first PBC may be useful for risk prediction but are not that helpful for the prediction of risk modification. Although several modifiable factors were included in this meta-analysis, the potential effect of actual changes in lifestyle factors after first PBC diagnosis has not been addressed.

Third, our literature search included search terms for second breast cancer, thereby including publications that studied the risks of developing ipsilateral second breast cancers as well. However, considering that the large majority (95%) of second PBCs is contralateral as compared to ipsilateral[8], we do not expect large risk alterations.

Fourth, we did not perform a quality assessment of the included studies; instead, we applied our own selection criteria (e.g. selecting only papers with a minimum number of events and evaluating the statistical methods that were used). Moreover, we know from literature that quality assessment tools in meta-analyses do not prevent nor resolve potential bias.[40,41]

Many breast cancer survivors express their concern on developing breast cancer in the other breast during follow-up at the outpatient clinic. In a previous meta-analysis we assessed the impact of genetic and clinical factors (i.e. pathological characteristics and treatment) on CBC risk.[42] For example, breast cancer patients with a positive mutation status (e.g. *BRCA1*, *BRCA2* or *CHEK2* c.1100delC) have a two to four times higher relative risk of developing a CBC.[42] The contribution of lifestyle and reproductive factors on CBC risk are compared hereto relatively small. Nonetheless, there is a specific interest from breast cancer survivors in factors that can be modified after first PBC diagnosis (e.g. weight, alcohol use), thereby potentially decreasing the risk of developing a CBC.

To our knowledge, this is the first meta-analysis that studied the impact of multiple lifestyle and reproductive factors on CBC risk, thereby seeking for the best possible evidence on this topic. Healthy BMI seems to be associated with a lower risk of developing a CBC as compared to high BMI. However, we could not prove that losing weight after the first PBC actually has a risk reducing effect on developing a CBC. More research on the impact of weight loss after the first PBC on CBC risk is therefore necessary. Nonetheless, losing weight is considered beneficial for breast cancer patients who are overweight or obese, if not for decreasing CBC risk, then either for other health outcomes. Weight loss intervention programs could be considered as part of the rehabilitation program for breast cancer survivors and have already gained some success in weight loss in breast cancer patients.[43,44] In addition, breast cancer survivors in general may be advised to maintain a healthy lifestyle.

Most importantly, this systematic review and meta-analysis highlighted the current gaps in our knowledge and stresses the importance of further investigations that are needed to improve CBC risk management in breast cancer survivors. The results pointed in a specific direction for alcohol use, number of FTPs, age at primiparity and age at menopause, but to provide strong conclusions, more research is definitely needed.

Moreover, more research on the impact of modifiable lifestyle factors (e.g. exercise, dietary habits, extent and timing of alcohol use) and known reproductive risk factors for a first PBC (e.g. parity, menopausal status) on CBC risk is necessary to offer breast cancer patients personalized evidence-based CBC risk estimates.

Table 1: Main characteristics of the included studies, ranked by descending year of publication.

First author, year of publication	Origin of cancer/hospital registry (study name)	Study design	Date[a]	Selection criteria	Number of 1st (P)BC patients[b]	Number of CBC patients available for analysis	Median years of FU (range)	Mean age (range)[c]	Time lapse required between 1st PBC and CBC (months)	Lifestyle and/or reproductive factors[d]	Assess-ment risk factor status	Factors adjusted for
Knight, 2017 [24]	USA, CAN, DNK (WECARE)	Case-control	1985-2008	Age <55 years at 1st PBC diagnosis; Stage I-III 1st PBC	2212	1521	N/A	Med. 46 (23-54)	≥12 (when PBC diagnosed 1985-1999); ≥24 (when PBC diagnosed 1990-2008)	Alcohol use Smoking	1st PBC diagnosis	FH1; Men; BMI; Age; FTP; His; Stage; ER; Ctx; Rtx; Etx; Smok (in analysis for alcohol use); Alc (in analysis for smoking)
Brooks, 2016 [17]	USA, CAN, DNK (WECARE)	Case-control	1985-2008	Age ≤55 years at 1st PBC diagnosis; Stage I-III 1st PBC	2,045	1,386	N/A	Med. 46 (23-55)	≥12	BMI	1st PBC diagnosis	FH1; Men; FTP; Age; Stage, ER; Ctx; Etx; Rtx
Shankar, 2015 [10]	IND	Cohort	1997-2006	Stage I-IV 1st PBC	532	24	N/A	47 (30-69)	>6	Menopausal status	1st PBC diagnosis	N/A
Sisti, 2015 [25]	USA, CAN, DNK (WECARE)	Case-control	1985-2009	Age ≤55 years at 1st PBC diagnosis; Stage I-III 1st PBC	2,212	1,521	N/A	Med. 46 (23-55)	≥12	Menarche Gravidity Age at primiparity[e] Parity[e] Breastfeeding[e] Menopausal status	N/A 1st PBC diagnosis CBC diagnosis 1st PBC diagnosis CBC diagnosis 2 years before 1st PBC diagnosis	FH; Par; Men; Meno; Ht; Age; Stage; His; Ctx; Etx. Additionally, Prim, FTP and BF were mutually adjusted for.
Brooks, 2012 [11]	USA, DNK (WECARE)	Case-control	1985-2000	Age ≤55 years at 1st PBC diagnosis; Stage I-III 1st PBC	999	511	N/A	Med. 45 (23-55)	≥12	BMI	1st PBC diagnosis	FH1; FTP; Men; Age; Stage; His; ER; Ctx; Etx; Rtx
Majed, 2011 [18]	FRA	Cohort	1981-1999	Stage I-III 1st PBC	15,166	1,370	10 (≤24)	54 (≥18)	>6	BMI	1st PBC diagnosis	FH; Menopausal; Age; Per; N; His; HR, Tx
Poynter, 2010 [26]	USA, DNK (WECARE)	Case-control	1985-1999	Only information from non-carriers was used; Age ≤55 years at 1st PBC diagnosis; Stage I-III 1st PBC	1,325 (non-carriers)	597	N/A	NR (<55)	≥12	Menarche Age at primiparity[e] Parity Breastfeeding[e] Menopausal status Age at menopause	N/A CBC diagnosis CBC diagnosis CBC diagnosis CBC diagnosis CBC diagnosis	FTP; Men; Menopausal; Age; Stage, Ctx, Etx

Table 1: Continued.

First author, year of publication	Origin of cancer/hospital registry (study name)	Study design	Date[a]	Selection criteria	Number of 1st (P)BC patients[b]	Number of CBC patients available for analysis	Median years of FU (range)	Mean age (range)[c]	Time lapse required between 1st PBC and CBC (months)	Lifestyle and/or reproductive factors[d]	Assess-ment risk factor status	Factors adjusted for
Figueiredo, 2010 [27]	USA, DNK (WECARE)	Case-control	1985-1999	Age ≤55 years at 1st PBC diagnosis; Stage I-III 1st PBC	1,325	597	N/A	46 (20-55)	≥12	Oral contraceptive use	Before CBC diagnosis	Age. (Tested for other potential confounders as well, but no significant influence was observed. Factors tested: FH1; FTP; Men; Meno; Menopausal; Stage; His; Ctx; Etx).
Li, 2009 [12]	USA (CSS)	Case-control	1990-2005	Stage I-IIIB 1st PBC, ER-positive 1st PBC	726	365	N/A	NR (40-79)	≥6	BMI Alcohol use Smoking	1st PBC diagnosis	Ctx, Etx. Matching variables (implicitly adjusted for): County, Race; Age; Per; Stage; ST. Additionally, BMI was adjusted for use of Ht at 1st PBC diagnosis; Alc for BMI at reference date; Smok for FH1.
Figueiredo, 2008 [13]	USA, DNK (WECARE)	Case-control	1985-2001	Age ≤55 years at 1st PBC diagnosis; Stage I-III 1st PBC	1,399	708	N/A	46 (23-55)	≥12	Oral contraceptive use	Before CBC diagnosis	FH1; FTP; Men; Menopausal at reference age; Age; Stage; His; Ctx; Etx; Rtx
Trentham-Dietz, 2007 [19]	USA	Cohort	1987-2000	Age 18-79 years at 1st PBC diagnosis; Stage I-IV 1st PBC;	10,953	488	Mean 7.1 (1-19)	59 (18-79)	>12	BMI[h] Alcohol use Smoking Menarche Oral contraceptive use Age at primiparity[e] Parity Menopausal status Age at menopause[e]	1st PBC diagnosis	FH, BMI; pack-years of cigarette smoking; recent alcohol intake. FTP; Menopausal; Ht; Per; Stage. Regression model was conditional on age.

Table 1: Continued.

First author, year of publication	Origin of cancer/hospital registry (study name)	Study design	Date[a]	Selection criteria	Number of 1st (P)BC patients[b]	Number of CBC patients available for analysis	Median years of FU (range)	Mean age (range)[c]	Time lapse required between 1st PBC and CBC (months)	Lifestyle and/or reproductive factors[d]	Assess-ment risk factor status	Factors adjusted for
Largent, 2007 [28]	USA, DNK (WECARE)	Case-control	1985-1999	Age ≤55 years at 1st PBC diagnosis; Stage I-III 1st PBC	1,399	708	N/A	46 (23-55)	≥12	Menarche Gravidity Age at primiparity Parity Breastfeeding Menopausal status Age at menopause	N/A Before CBC diagnosis CBC diagnosis CBC diagnosis CBC diagnosis CBC diagnosis CBC diagnosis	FH; FTP; Men; Meno; Age; His; Stage; Tx; Rtx
Kuo, 2006 [20]	TAI	Cohort	1990-1999	Frequently diagnosed with PBC <50 years	2,022	120[i]	3.21 (NR)	50 (NR)	>6	Smoking Menopausal status	1st PBC diagnosis	Time to event. Additionally significant factors from the univariable analysis were taken into account. Factors taken into account: Meno; lobular His; Ctx; Etx; Rtx
Dignam, 2006 [21]	USA (NSABP)	Cohort	1981-1998	Stage I-III 1st PBC; ER-negative and lymph node-negative 1st PBC	4,077	242	NR	NR	NR[k]	BMI	1st PBC diagnosis	Race; Age ;T; Tx
Dignam, 2003 [22]	USA (NSABP)	Cohort	1982-1987	Stage I-III 1st PBC; ER-positive and lymph node-negative 1st PBC	3,385	193	13.8 (NR)	NR	NR[l]	BMI	1st PBC diagnosis	Race; Menopausal; Age; T; ER; PR; Tx
Li, 2003 [14]	USA (CSS)	Cohort	1983-1992	Age ≤45 years at 1st PBC diagnosis; Stage I-IV 1st PBC	1,285	77	Mean 9.0 (NR)	38 (≤45)	>6	BMI Alcohol use Menarche Oral contraceptive use Gravidity Age at primiparity Parity	1st PBC diagnosis	Study; Age; Per; Stage; Ctx
Vaittinen, Hemminki 2000 [29]	SWE	Cohort	1970-1996	Age 20-89 years at 1st PBC diagnosis	72,096	1,675	NR	NR (20-89)	≥6	Age at primiparity Parity	NR	FH; FTP; Primi; Age; Yr; Time

Table 1: Continued.

First author, year of publication	Origin of cancer/hospital registry (study name)	Study design	Date[a]	Selection criteria	Number of 1st (P)BC patients[b]	Number of CBC patients available for analysis	Median years of FU (range)	Mean age (range)[c]	Time lapse required between 1st PBC and CBC (months)	Lifestyle and/or reproductive factors[d]	Assess-ment risk factor status	Factors adjusted for
Cook, 1996 [15]	USA (CSS)	Case-control	1978-1990	Age <85 years at 1st PBC diagnosis; Stage I-III 1st PBC	424	216	N/A	NR (<85)	≥6	BMI Gravidity Parity Menopausal status	1st PBC diagnosis	FH1; Menopausal; His. Matched factors: Age; Stage; Time.
Bernstein, 1992 [23]	USA	Cohort	1980-1982	Age 20-54 years at 1st PBC diagnosis; Stage I-IV 1st PBC	4,550	136	Mean 4.3 (NR)	44 (20-54)	>6	Alcohol use Smoking Menarche Oral contraceptive use Gravidity Age at primiparity Parity Breastfeeding Menopausal status	1st PBC diagnosis	FH; Edu; BMI; FTP; Prim; Men; Meno; Menopausal; Age; Stage; Lobular His; History of benign disease.

Duplicate studies (i.e. with the same selection of patients) excluded from the meta-analyses are highlighted in dark grey;
Factors excluded from the meta-analyses because only a univariable estimate was provided (6) are highlighted in light grey.
[a] Date of first PBC diagnosis.
[b] Number of first PBC patients or BC patients (i.e. first PBC and CBC patients) for case-control and cohort studies, respectively.
[c] Mean age (range) of women with first PBC.
[d] Selected lifestyle and reproductive factors per study.
[e] Among parous women only.
[f] Only the data of non-carrier cases and controls were used for the meta-analysis.
[g] The underlying cohort of this study was composed of women with only oestrogen receptor-positive first PBC cancer; PR: Progesterone receptor status of primary breast cancer; HR: hormonal receptor status, not specified; Tx: treatment, not specified; Ctx: chemotherapy; Etx: endocrine therapy; Rtx: Radiotherapy; Time: Time between primary breast cancer and CBC diagnosis; ST: survival time; N/A: not applicable; NR: not reported.
[h] Among postmenopausal women only.
[i] Bilateral BC cases of which 44 synchronous BC, 75 metachronous BC and 1 unknown type of BC.
[j] The underlying cohort of this study was composed of women with only oestrogen receptor-negative first PBC.
[k] No definition for metachronous CBC was provided, but the paper focussed on events occurring over time, therefore assumed that the focus was on metachronous CBC events.
[l] No definition for metachronous CBC was provided, but from the figures we observed the first CBC event to appear at least 3 months after first PBC diagnosis.

Abbreviations: BC: breast cancer; PBC: primary breast cancer; CBC: contralateral breast cancer; BMI: body mass index; FU: follow-up; USA: United States of America; CAN: Canada; DNK: Denmark; FRA: France; IND: India; TAI: Taiwan; SWE: Sweden; Med: median; Race: race/ethnicity; FH: family history of breast cancer; FH1: first degree family history of breast cancer; Edu: education; Smok: smoking; Alc: alcohol use; BF: breast feeding; FTP: number of Full term pregnancies; Prim: age at primiparity; Par: age at parity; Men: age at menarche; Meno: age at menopause; Menopausal: menopausal status; Ht: postmenopausal hormone therapy; Age: age at primary breast cancer diagnosis; Yr: year of birth; Per: Period of recruitment/year of first PBC diagnosis; Stage: stage of primary breast cancer; N: number of positive lymph nodes; T: Tumor size; His: histology of primary breast cancer; ER: Estrogen receptor status of primary breast.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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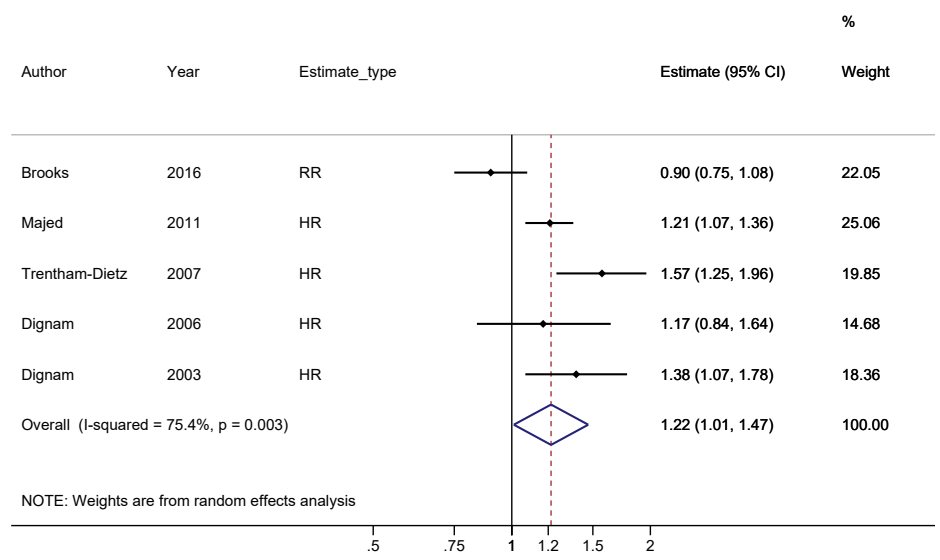
SUPPLEMENTARY MATERIAL

Supplementary Table 1. Search strategy used to identify publications publishing on lifestyle and/or reproductive risk factors for contralateral breast cancer in the PubMed electronic database

Number	Search term
1	"(Contralateral Breast Cancer* [tiab] OR Contralateral Breast Tumor* [tiab] OR Contralateral Breast Tumour* [tiab] OR Contralateral Breast Neoplasm* [tiab] OR CBC [tiab] OR (Breast Neoplasms [MeSH] AND Contralateral [tiab]) OR (Breast Neoplasms [MeSH] AND Neoplasms, Second Primary [MeSH]) OR Second Primary Breast Cancer [tiab] OR Second Breast Cancer [tiab]) AND
2	("Health Behavior"[MeSH] OR "Food Habits"[MeSH] OR "Exercise"[MeSH] OR "Smoking"[MeSH] OR Smoking [tiab] OR "Alcohol Drinking"[MeSH] OR "Body Mass Index"[MeSH] OR "Obesity"[MeSH] OR Obes* [tiab] OR "Life Style"[MeSH] OR Life Style [tiab] OR Lifestyl* [tiab]
3	OR "Parity"[MeSH] OR Parity [tiab] OR Parities [tiab] OR Primiparit* [tiab] OR Multiparit* [tiab] OR "Menarche"[MeSH] OR Menarche [tiab] OR "Menopause"[MeSH] OR Menopaus* [tiab] OR "Contraceptives, Oral, Hormonal"[MeSH] OR Contracepti* [tiab] OR "Breast Feeding"[Mesh] OR Breast feeding [tiab] OR "Reproductive History"[MeSH] OR Reproductive Histor* [tiab])".

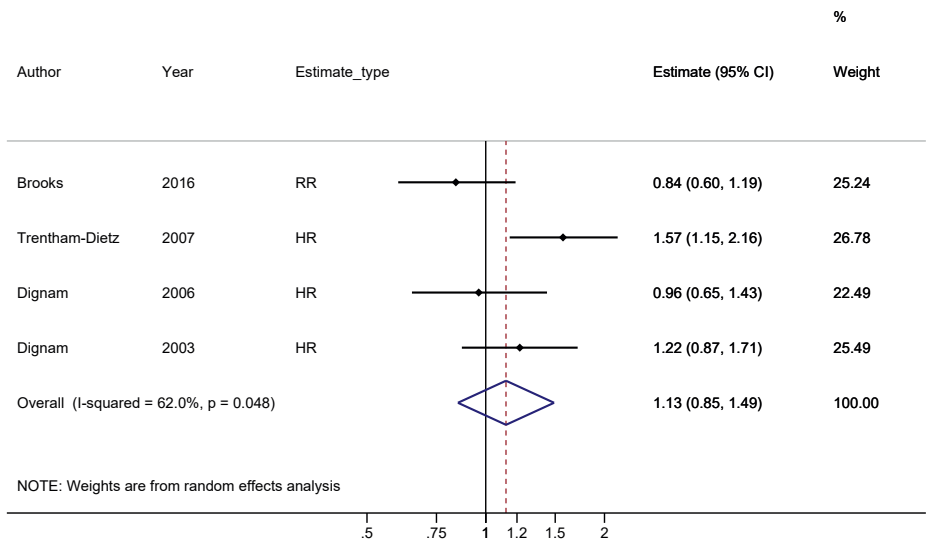
Search terms for contralateral breast cancer (number 1) were combined with search terms for lifestyle factors (number 2) and reproductive factors (number 3) .
Restrictions: publication date from 01/01/1990 onwards, papers published in English.

SUPPLEMENTARY FIGURES



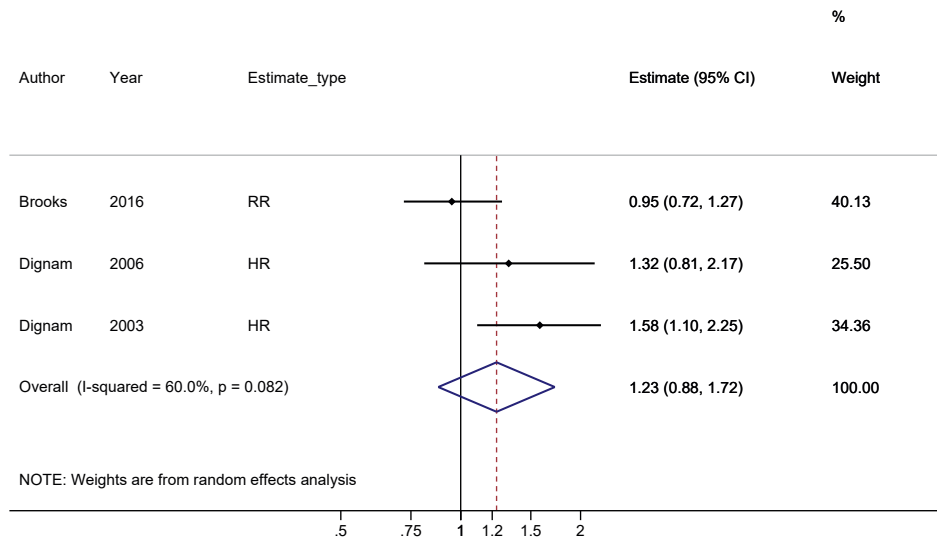
Supplementary Figure 1. Forest plot of the included publications publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing body mass index (kg/m²): ≥25 vs <25

Author: first author; Year: year of publication; Estimate_type: type of risk estimate provided, which can be a relative risk (RR), odds ratio (OR), hazard ratio (HR); Estimate: reported adjusted estimate (i.e. relative risk, odds ratio or hazard ratio); Weight: value assigned by random-effects analysis using the inverse of the study variance (variance includes within-study variance plus the between-study variance); Overall: relative risk estimate combining relative risks, odds ratios and hazard ratios; I-squared: measure of heterogeneity; p-value: p-value for heterogeneity, p<0.05 considered significant.



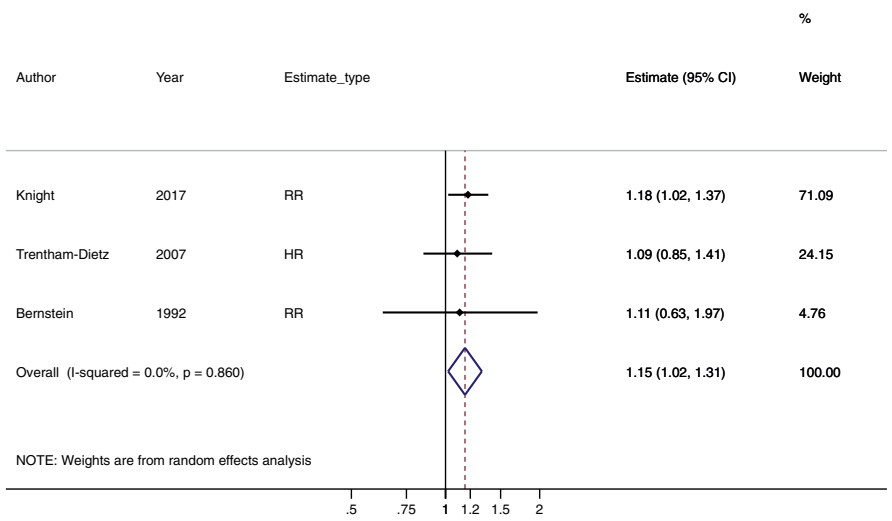
Supplementary Figure 2. Forest plot of the included publications publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing body mass index (kg/m²): 25-<30 vs <25

Author: first author; Year: year of publication; Estimate_type: type of risk estimate provided, which can be a relative risk (RR), odds ratio (OR), hazard ratio (HR); Estimate: reported adjusted estimate (i.e. relative risk, odds ratio or hazard ratio); Weight: value assigned by random-effects analysis using the inverse of the study variance (variance includes within-study variance plus the between-study variance); Overall: relative risk estimate combining relative risks, odds ratios and hazard ratios; I-squared: measure of heterogeneity; p-value: p-value for heterogeneity, p<0.05 considered significant.



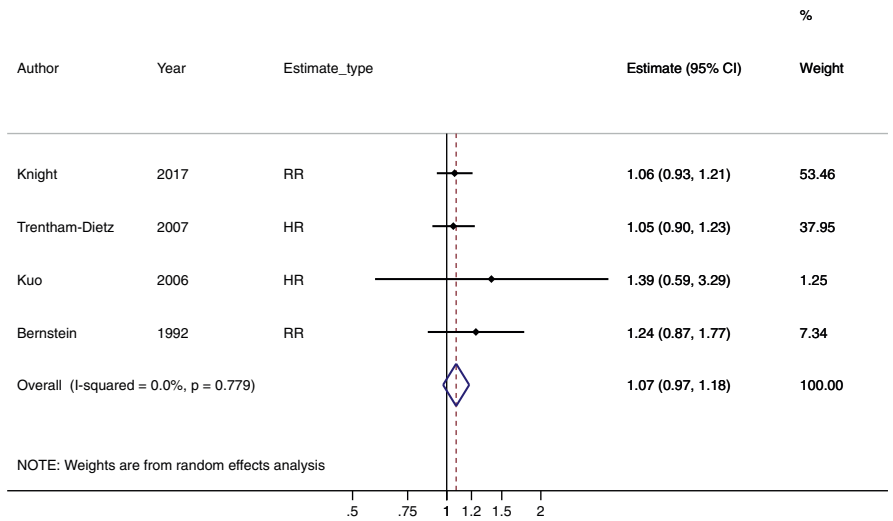
Supplementary Figure 3. Forest plot of the included publications publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing body mass index (kg/m²): ≥30 vs <25

Author: first author; Year: year of publication; Estimate_type: type of risk estimate provided, which can be a relative risk (RR), odds ratio (OR), hazard ratio (HR); Estimate: reported adjusted estimate (i.e. relative risk, odds ratio or hazard ratio); Weight: value assigned by random-effects analysis using the inverse of the study variance (variance includes within-study variance plus the between-study variance); Overall: relative risk estimate combining relative risks, odds ratios and hazard ratios; I-squared: measure of heterogeneity; p-value: p-value for heterogeneity, p<0.05 considered significant.



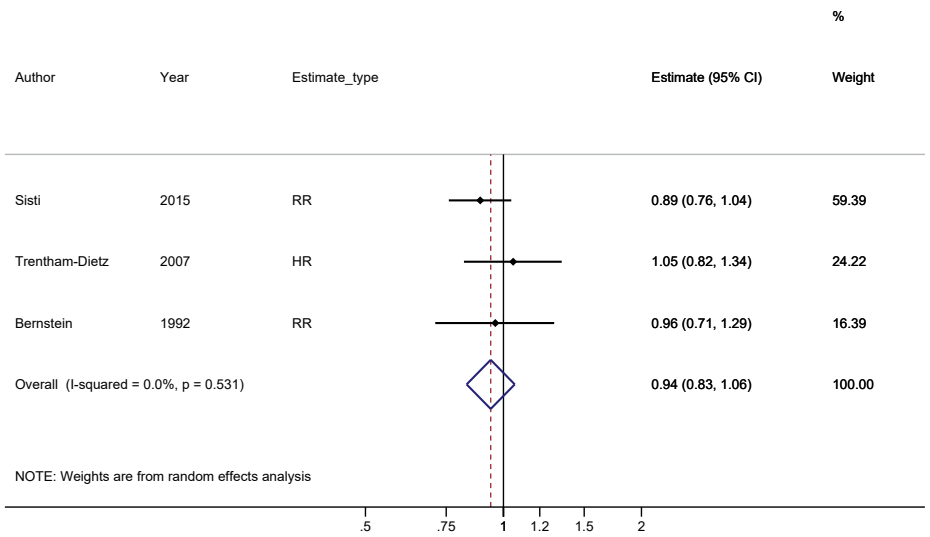
Supplementary Figure 4. Forest plot of the included publications publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing alcohol use: ever vs never

Author: first author; Year: year of publication; Estimate_type: type of risk estimate provided, which can be a relative risk (RR), odds ratio (OR), hazard ratio (HR); Estimate: reported adjusted estimate (i.e. relative risk, odds ratio or hazard ratio); Weight: value assigned by random-effects analysis using the inverse of the study variance (variance includes within-study variance plus the between-study variance); Overall: relative risk estimate combining relative risks, odds ratios and hazard ratios; I-squared: measure of heterogeneity; p-value: p-value for heterogeneity, p<0.05 considered significant.



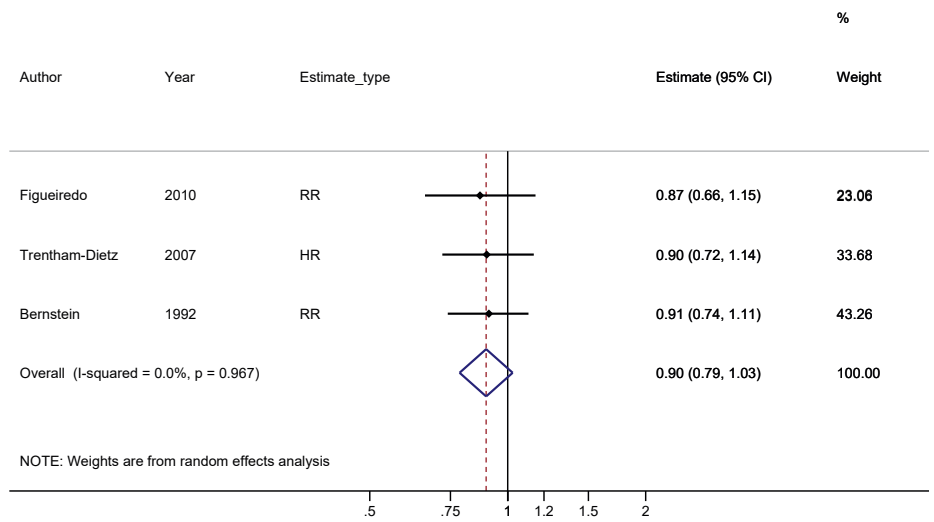
Supplementary Figure 5. Forest plot of the included publications publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing smoking status: ever vs never

Author: first author; Year: year of publication; Estimate_type: type of risk estimate provided, which can be a relative risk (RR), odds ratio (OR), hazard ratio (HR); Estimate: reported adjusted estimate (i.e. relative risk, odds ratio or hazard ratio); Weight: value assigned by random-effects analysis using the inverse of the study variance (variance includes within-study variance plus the between-study variance); Overall: relative risk estimate combining relative risks, odds ratios and hazard ratios; I-squared: measure of heterogeneity; p-value: p-value for heterogeneity, p<0.05 considered significant.



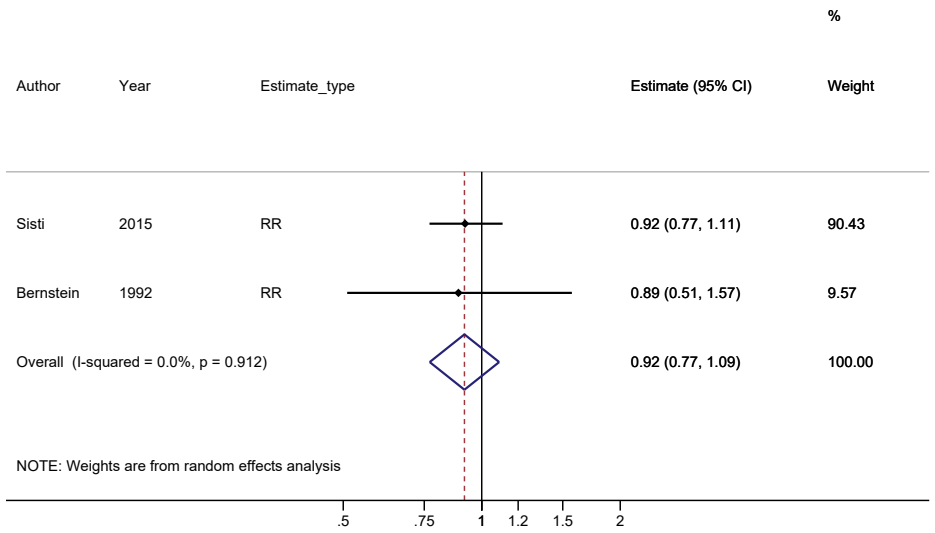
Supplementary Figure 6. Forest plot of the included publications publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing menarche (years): ≥13 vs <13

Author: first author; Year: year of publication; Estimate_type: type of risk estimate provided, which can be a relative risk (RR), odds ratio (OR), hazard ratio (HR); Estimate: reported adjusted estimate (i.e. relative risk, odds ratio or hazard ratio); Weight: value assigned by random-effects analysis using the inverse of the study variance (variance includes within-study variance plus the between-study variance); Overall: relative risk estimate combining relative risks, odds ratios and hazard ratios; I-squared: measure of heterogeneity; p-value: p-value for heterogeneity, p<0.05 considered significant.



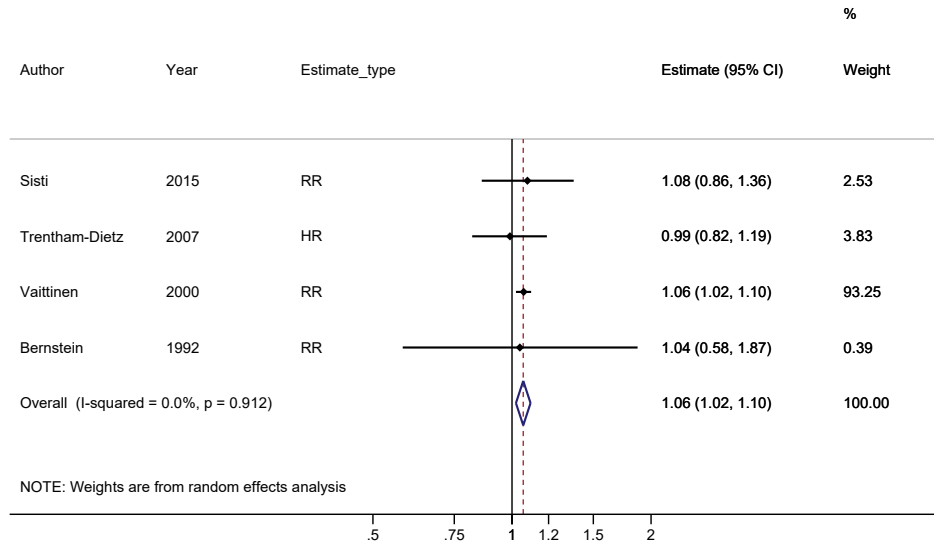
Supplementary Figure 7. Forest plot of the included publications publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing oral contraceptive use: ever vs never

Author: first author; Year: year of publication; Estimate_type: type of risk estimate provided, which can be a relative risk (RR), odds ratio (OR), hazard ratio (HR); Estimate: reported adjusted estimate (i.e. relative risk, odds ratio or hazard ratio); Weight: value assigned by random-effects analysis using the inverse of the study variance (variance includes within-study variance plus the between-study variance); Overall: relative risk estimate combining relative risks, odds ratios and hazard ratios; I-squared: measure of heterogeneity; p-value:p-value for heterogeneity, p<0.05 considered significant.



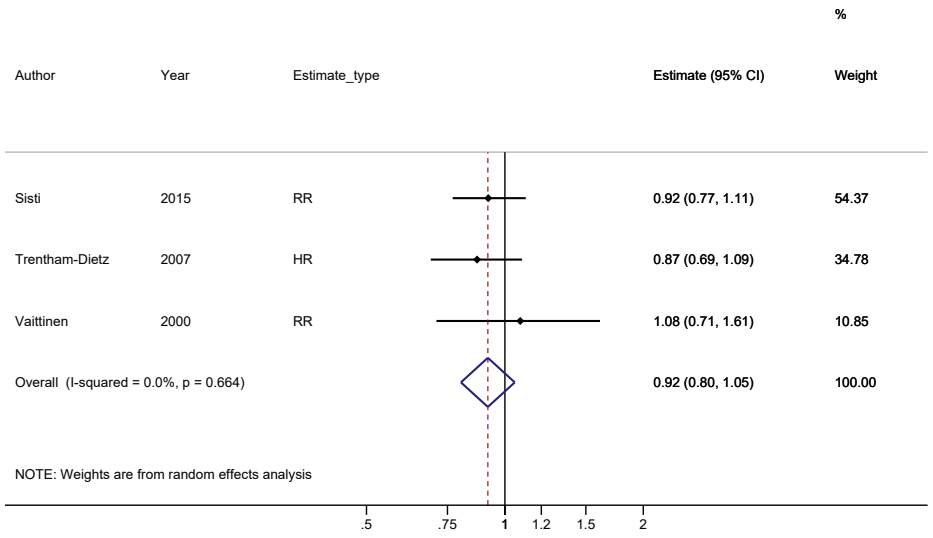
Supplementary Figure 8. Forest plot of the included publications publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing gravidity: ever vs never

Author: first author; Year: year of publication; Estimate_type: type of risk estimate provided, which can be a relative risk (RR), odds ratio (OR), hazard ratio (HR); Estimate: reported adjusted estimate (i.e. relative risk, odds ratio or hazard ratio); Weight: value assigned by random-effects analysis using the inverse of the study variance (variance includes within-study variance plus the between-study variance); Overall: relative risk estimate combining relative risks, odds ratios and hazard ratios; I-squared: measure of heterogeneity; p-value: p-value for heterogeneity, p<0.05 considered significant.



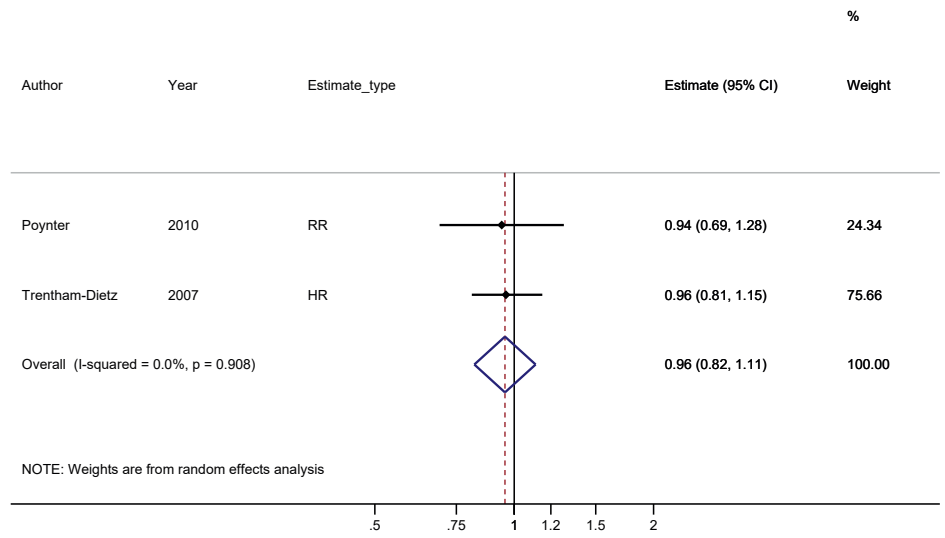
Supplementary Figure 9. Forest plot of the included publications publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing age at primiparity (years): ≥25 vs <25

Author: first author; Year: year of publication; Estimate_type: type of risk estimate provided, which can be a relative risk (RR), odds ratio (OR), hazard ratio (HR); Estimate: reported adjusted estimate (i.e. relative risk, odds ratio or hazard ratio); Weight: value assigned by random-effects analysis using the inverse of the study variance (variance includes within-study variance plus the between-study variance); Overall: relative risk estimate combining relative risks, odds ratios and hazard ratios; I-squared: measure of heterogeneity; p-value: p-value for heterogeneity, p<0.05 considered significant.



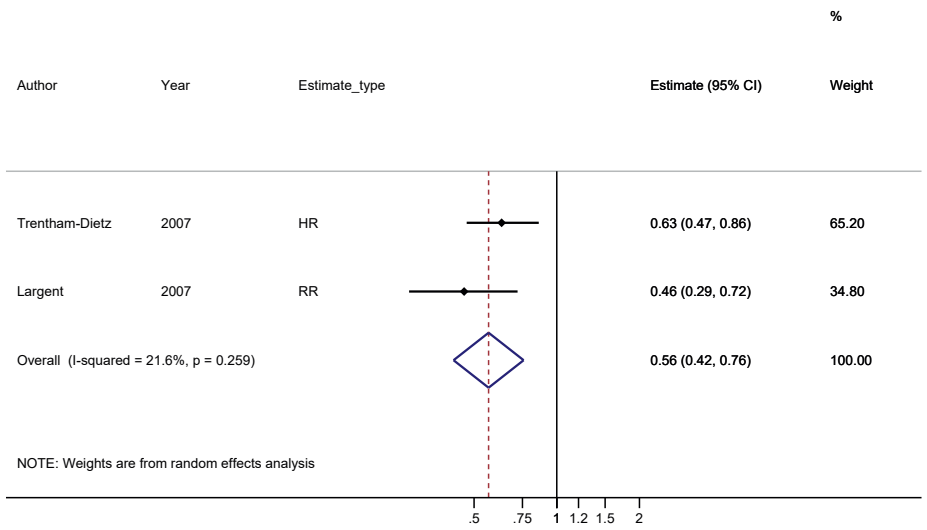
Supplementary Figure 10. Forest plot of the included publications publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing parity: ≥1 full-term pregnancies vs nulliparous

Author: first author; Year: year of publication; Estimate_type: type of risk estimate provided, which can be a relative risk (RR), odds ratio (OR), hazard ratio (HR); Estimate: reported adjusted estimate (i.e. relative risk, odds ratio or hazard ratio); Weight: value assigned by random-effects analysis using the inverse of the study variance (variance includes within-study variance plus the between-study variance); Overall: relative risk estimate combining relative risks, odds ratios and hazard ratios; I-squared: measure of heterogeneity; p-value: p-value for heterogeneity, p<0.05 considered significant.



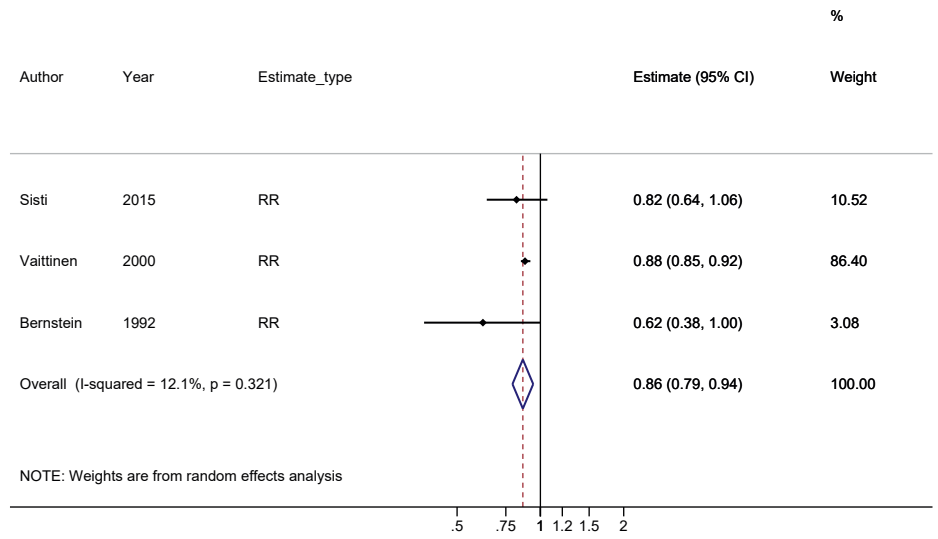
Supplementary Figure 11. Forest plot of the included publications publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing parity: 1-3 full-term pregnancies vs nulliparous.

Author: first author; Year: year of publication; Estimate_type: type of risk estimate provided, which can be a relative risk (RR), odds ratio (OR), hazard ratio (HR); Estimate: reported adjusted estimate (i.e. relative risk, odds ratio or hazard ratio); Weight: value assigned by random-effects analysis using the inverse of the study variance (variance includes within-study variance plus the between-study variance); Overall: relative risk estimate combining relative risks, odds ratios and hazard ratios; I-squared: measure of heterogeneity; p-value: p-value for heterogeneity, p<0.05 considered significant.



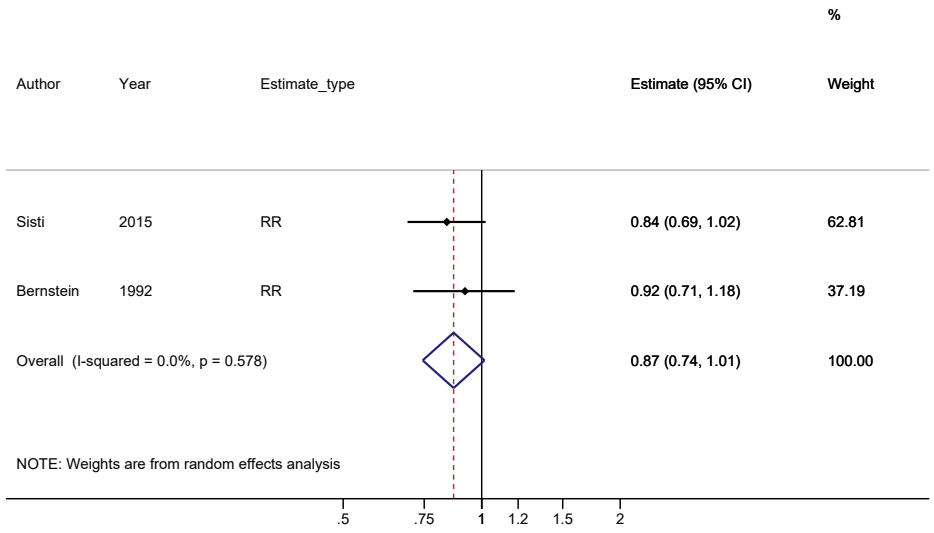
Supplementary Figure 12. Forest plot of the included publications publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing parity: ≥4 full-term pregnancies vs nulliparous

Author: first author; Year: year of publication; Estimate_type: type of risk estimate provided, which can be a relative risk (RR), odds ratio (OR), hazard ratio (HR); Estimate: reported adjusted estimate (i.e. relative risk, odds ratio or hazard ratio); Weight: value assigned by random-effects analysis using the inverse of the study variance (variance includes within-study variance plus the between-study variance); Overall: relative risk estimate combining relative risks, odds ratios and hazard ratios; I-squared: measure of heterogeneity; p-value: p-value for heterogeneity, p<0.05 considered significant.



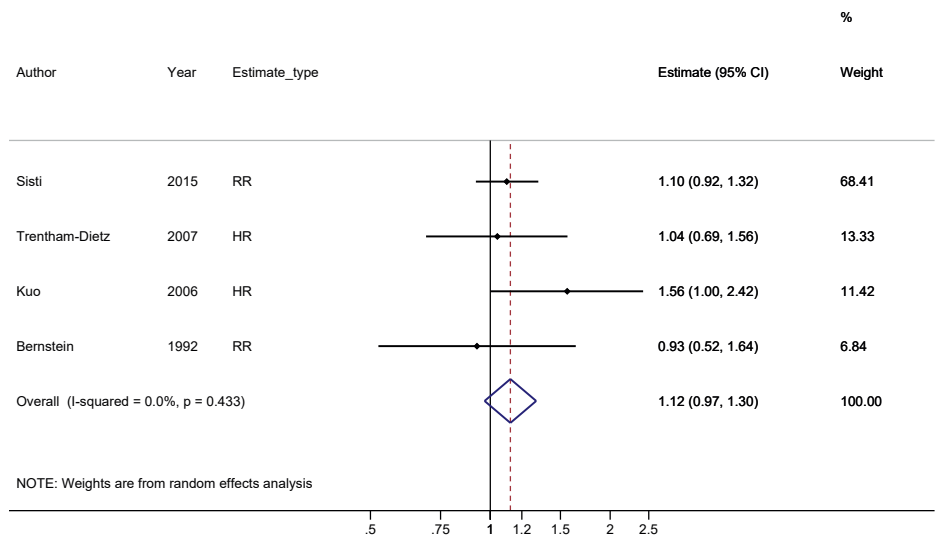
Supplementary Figure 13. Forest plot of the included publications publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing parity: ≥2 full-term pregnancies vs 1 full-term pregnancy

Author: first author; Year: year of publication; Estimate_type: type of risk estimate provided, which can be a relative risk (RR), odds ratio (OR), hazard ratio (HR); Estimate: reported adjusted estimate (i.e. relative risk, odds ratio or hazard ratio); Weight: value assigned by random-effects analysis using the inverse of the study variance (variance includes within-study variance plus the between-study variance); Overall: relative risk estimate combining relative risks, odds ratios and hazard ratios; I-squared: measure of heterogeneity; p-value: p-value for heterogeneity, p<0.05 considered significant.



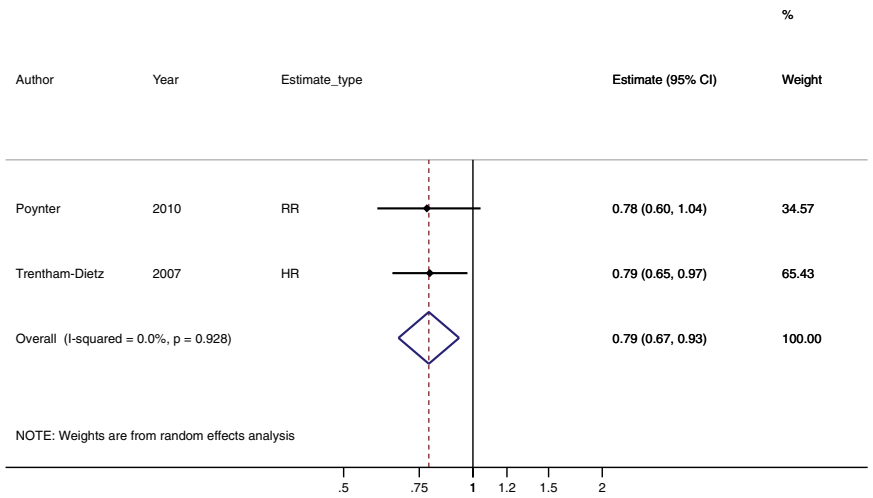
Supplementary Figure 14. Forest plot of the included publications publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing breastfeeding: ever vs never

Author: first author; Year: year of publication; Estimate_type: type of risk estimate provided, which can be a relative risk (RR), odds ratio (OR), hazard ratio (HR); Estimate: reported adjusted estimate (i.e. relative risk, odds ratio or hazard ratio); Weight: value assigned by random-effects analysis using the inverse of the study variance (variance includes within-study variance plus the between-study variance); Overall: relative risk estimate combining relative risks, odds ratios and hazard ratios; I-squared: measure of heterogeneity; p-value:p-value for heterogeneity, p<0.05 considered significant.



Supplementary Figure 15. Forest plot of the included publications publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing postmenopausal women with premenopausal women

Author: first author; Year: year of publication; Estimate_type: type of risk estimate provided, which can be a relative risk (RR), odds ratio (OR), hazard ratio (HR); Estimate: reported adjusted estimate (i.e. relative risk, odds ratio or hazard ratio); Weight: value assigned by random-effects analysis using the inverse of the study variance (variance includes within-study variance plus the between-study variance); Overall: relative risk estimate combining relative risks, odds ratios and hazard ratios; I-squared: measure of heterogeneity; p-value: p-value for heterogeneity, p<0.05 considered significant.



Supplementary Figure 16. Forest plot of the included publications publishing adjusted estimates on the risk of developing contralateral breast cancer in population-based cohorts comparing age at menopause (years): <45 vs ≥45

Author: first author; Year: year of publication; Estimate_type: type of risk estimate provided, which can be a relative risk (RR), odds ratio (OR), hazard ratio (HR); Estimate: reported adjusted estimate (i.e. relative risk, odds ratio or hazard ratio); Weight: value assigned by random-effects analysis using the inverse of the study variance (variance includes within-study variance plus the between-study variance); Overall: relative risk estimate combining relative risks, odds ratios and hazard ratios; I-squared: measure of heterogeneity; p-value:p-value for heterogeneity, $p<0.05$ considered significant.

CHAPTER 4

EFFECTS OF CHEMOTHERAPY ON CONTRALATERAL BREAST CANCER RISK IN *BRCA1* AND *BRCA2* MUTATION CARRIERS: A NATIONWIDE COHORT STUDY

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ABSTRACT

Aim: *BRCA1/2* mutation carriers with primary breast cancer (PBC) are at high risk of contralateral breast cancer (CBC). In a nationwide cohort, we investigated the effects of chemotherapeutic agents given for PBC on CBC risk separately in *BRCA1* and *BRCA2* mutation carriers**.**

Patients and Methods: *BRCA1* or *BRCA2* mutation carriers with an invasive PBC diagnosis from 1990-2017 were selected from a Dutch cohort. We estimated cumulative CBC incidence using competing risks analysis. Hazard ratios (HR) for the effect of neo-adjuvant or adjuvant chemotherapy and different chemotherapeutic agents on CBC risk were estimated using Cox regression.

Results: We included 1,090 *BRCA1* and 568 *BRCA2* mutation carriers; median follow-up was 8.9 and 8.4 years, respectively. Ten-year cumulative CBC incidence for treatment with and without chemotherapy was 6.7% [95%CI: 5.1-8.6] and 16.7% [95%CI: 10.8-23.7] in *BRCA1* and 4.8% [95%CI: 2.7-7.8] and 16.0% [95%CI: 9.3-24.4] in *BRCA2* mutation carriers, respectively.

Chemotherapy was associated with reduced CBC risk in *BRCA1* (multivariable HR: 0.46; 95%CI: 0.29-0.74); a similar trend was observed in *BRCA2* mutation carriers (HR: 0.63; 95%CI: 0.29-1.39). In *BRCA1*, risk reduction was most pronounced in the first 5 years (HR: 0.32; 95%CI: 0.17-0.61). Anthracyclines and the combination of anthracyclines with taxanes were associated with substantial CBC risk reduction in *BRCA1* carriers (HR: 0.34; 95%CI: 0.17-0.68 and HR: 0.22; 95%CI: 0.08-0.62, respectively).

Conclusion: Risk-reducing effects of chemotherapy are substantial for at least 5 years and may be used in personalized CBC risk prediction in any case for *BRCA1* mutation carriers.

INTRODUCTION

Women with a primary breast cancer (PBC) diagnosis and a pathogenic germline mutation in the *BRCA1* or *BRCA2* gene are at increased risk of developing metachronous contralateral breast cancer (CBC). The annual risk of CBC is around 1-3%, with young *BRCA1* mutation carriers having the highest risk.[1,2] *BRCA1/2* mutation carriers with PBC may opt for a contralateral risk-reducing mastectomy to reduce the risk of CBC, potentially improving survival.[3]

In sporadic PBC patients a reduction in CBC risk is found after treatment with adjuvant endocrine treatment and/or adjuvant chemotherapy for PBC.[4,5] In *BRCA*-associated breast cancer the ability to repair double-strand DNA breaks is impaired because of insufficient homologous recombination repair function of the BRCA protein.[6-9] Therefore, chemotherapeutics that cause double-strand DNA breaks (i.e. platinum salts, anthracyclines) are considered to be more effective. By eliminating precancerous cells or preclinical cancers, double-strand DNA breaks-inducing chemotherapeutics may reduce the occurrence of CBC in *BRCA1/2* mutation carriers.

So far, the effects of chemotherapy on CBC risk in *BRCA1/2* mutation carriers have been investigated only in a limited number of studies[1,10,11]; in only one study the effects of different chemotherapeutic agents on CBC risk were investigated, though with *BRCA1* and *BRCA2* mutation carriers combined.[11] *BRCA1*-associated tumours are however biologically different from *BRCA2*-associated breast tumours, and should therefore be studied separately.[1,12,13] Investigating the effects of different chemotherapy agents could prove useful for personalised CBC risk prediction and management.

In a large Dutch cohort, we therefore aimed to investigate the effects of chemotherapy overall and for various agents on CBC risk, separately for *BRCA1* and *BRCA2* mutation carriers.

PATIENTS AND METHODS

Eligible patients were selected from the Hereditary Breast and Ovarian cancer research Netherlands (HEBON) cohort.[14] The HEBON study is an ongoing Dutch nationwide collaboration that aims to include all members from breast and/or ovarian cancer families tested for a *BRCA1/2* mutation, recently extended for pathogenic mutations in *CHEK2, PALB2* and *ATM*. These women have been identified through all eight Clinical Genetics centres in the Dutch University Medical Centres and the Netherlands Cancer Institute. Approval from the Medical Ethics Committees of all participating centres was obtained. Written informed consent was provided by all participating women, or either a close relative or proxy in case of a deceased individual. From January 1999 onwards, data on patient, tumour, (preventive) treatment, and follow-up characteristics are collected and updated by linkage to the Netherlands Cancer Registry (NCR) and the Dutch Pathology Database (PALGA). In addition, regular linkage with the Municipal Administrative Database provides updated information on vital status**.** The latest follow-up date in this study is December 31, 2017.

We selected women with a proven pathogenic germline *BRCA1* or *BRCA2* mutation, diagnosed with invasive stage I-III PBC between 1990 and July 2017 (Figure 1). Information on patient, tumour, treatment and follow-up characteristics was obtained. Patients were excluded if they had a history of invasive cancer prior to their PBC (except non-melanoma skin cancer) or if data were missing regarding PBC diagnosis, chemotherapy (yes vs. no) or follow-up (i.e. dates of cancer diagnosis, DNA test results, risk-reducing surgeries, or death).

Statistical analysis

The primary endpoint was the development of a metachronous CBC, defined as the development of a new invasive or in situ tumour in the contralateral breast at least 3 months after PBC diagnosis. We assessed the effect of neo-adjuvant or adjuvant chemotherapy overall, and of different chemotherapeutic agents, compared to no chemotherapy, on metachronous CBC risk. The secondary outcome was exclusively invasive CBC.

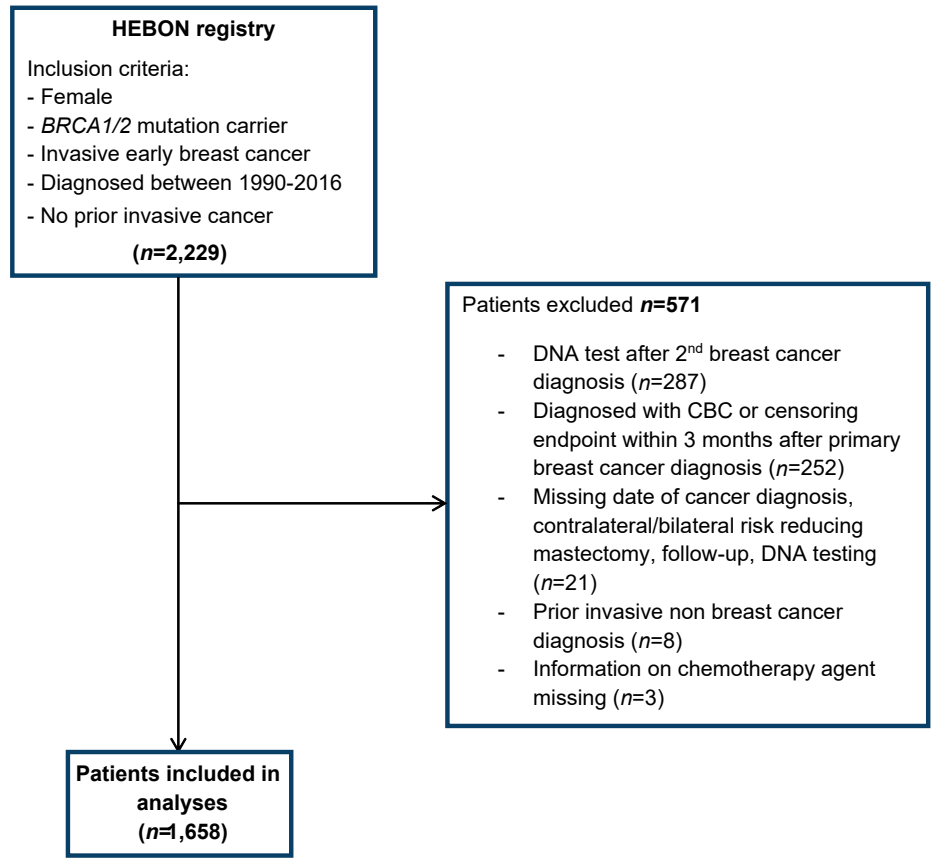


Figure 1. Inclusion of participants

CBC: contralateral breast cancer; HEBON: Hereditary Breast and Ovarian Cancer Research Netherlands

We performed two separate analyses to determine CBC risk: 1.competing risk analysis was used to determine cumulative incidence for CBC with death and contralateral or bilateral risk-reducing mastectomy as competing risks; 2. the Cox proportional hazards model was used to estimate cause-specific hazard ratios (HRs) and 95% confidence intervals (95% CI) for the association of chemotherapy with CBC risk with death and contralateral or bilateral risk-reducing mastectomy as censoring endpoints. In both the competing risk and the cause-specific analyses, additional censoring endpoints were secondary invasive cancer diagnosis (except non-melanoma skin cancer), ipsilateral secondary invasive/non-invasive breast cancer diagnosis or end of study (12/31/2017).

Age at PBC, radiotherapy, adjuvant endocrine therapy, risk-reducing salpingo-oophorectomy (time-dependent) and TNM-stage were considered as potential confounders based on published literature. Since metachronous CBC was defined as the development of a tumour in the contralateral breast at least 3 months following a PBC diagnosis, follow-up started from 3 months onwards for all patients (i.e., patients with an endpoint within 3 months were excluded). To account for prevalent cases, we applied left truncation; follow-up started 3 months after PBC diagnosis or at DNA test result, whichever came last.

For the overall analysis on chemotherapy vs. no chemotherapy, 10-year HRs were provided (i.e., patients were censored at 10 years). This cut-off was set to take into account the median follow-up. Time-dependency was explored by comparing HR estimates for the first 5 years versus 5-10 years of follow-up.

For the different chemotherapy agents, 5-year HRs were provided in order to account for the shorter median follow-up of the patients who received more recent types of treatment. Chemotherapy was categorized into 3 mutually exclusive groups: 1. CMF: cyclophosphamide, methotrexate and 5-fluorouracil; 2. anthracyclines and/or platinum-based agents; 3. combinations of anthracyclines and taxanes, with or without platinum-based agents. Chemotherapeutic agents were unknown in 40% of the cases (Eq. (A.4-A.5)). We imputed unknown agents as we know from literature that imputation can provide more reliable results than performing a complete case analysis.[15-17] Because agents depended strongly on year of PBC diagnosis, age at PBC diagnosis, PBC hormone receptor status, tumour grade and TNM-stage (according to the Dutch guidelines[18]), we performed mode imputation stratified by these variables as well as hospital of treatment and the distribution of different chemotherapy agents over the years. Patients were categorized as having received CMF if PBC diagnosis was before 01/01/1994; anthracyclines if PBC diagnosis was between 12/31/1997 and 01/01/2007; and anthracyclines in combination with taxanes if PBC diagnosis was from 01/01/2009 onwards. We additionally confirmed whether imputed agents were equal to known agents of comparable patients from the same hospital, i.e. diagnosed with PBC in the same year and with comparable TNM-stage and age at PBC diagnosis. A sensitivity analysis without imputation of chemotherapeutic agents (i.e. complete case analysis) was performed and compared with the main analysis.

For radiotherapy and endocrine therapy, missing values (28 patients in total) were imputed for the Cox model, based on other treatment determining characteristics or, if not possible, using cold deck imputation.

For the purpose of comparison with previous studies, we also obtained combined *BRCA1* and *BRCA2* estimates (Supplementary Tables A.1-A.3).

The proportional hazards assumption was evaluated both visually and, if proportional hazards violation of a variable was suspected, through adding an interaction term with time. Interaction testing was performed between chemotherapy and *BRCA* carrier status and between chemotherapy as categorized into 3 groups and *BRCA* carrier status to check for formal evidence of differential effect. Statistical analyses were performed using Stata (version 16).

RESULTS

In total, 1,090 *BRCA1* and 568 *BRCA2* mutation carriers were included (Table 1). Median follow-up was 8.9 years for *BRCA1* and 8.5 years for *BRCA2* mutation carriers.

CBC was observed as the first event in 116 *BRCA1* and 44 *BRCA2* mutation carriers, of which 23 and 18 were non-invasive, respectively. In 757 patients, risk-reducing mastectomy was performed prior to another event. Death was observed in 244 patients as the first event.

Table 1. Characteristics of *BRCA1* and *BRCA2* PBC patients: chemotherapy versus no chemotherapy

	BRCA1					***BRCA2***						
	No Chemotherapy		**Chemotherapy**[b]		**p-value**	**No chemotherapy**		**Chemotherapy**[b]		**p-value**	**Total Group**	
	N	***%***	***N***	***%***		***N***	***%***	***N***	***%***		***N***	***%***
Total	276	25.3	814	74.7		191	33.6	377	66.4		1,658	100
Median FU in years [range]	13.8 [0.3-27.9]		10.0 [0.4-27.7]		<0.001	10.4 [0.5-26.8]		9.7 [0.8-26.3]		0.4043	10.3 [0.3-27.9]	
FU in years after left truncation [range]	10.5 [0.3-26.5]		8.5 [0.4-23.6]		<0.001	8.5 [0.5-24.2]		8.4 [0.8-25.6]		0.3037	8.8 [0.3-26.5]	
Age at PBC												
Median age, years [range]	46.5 [22-85]		39.4 [19-70]		<0.001	52.5 [24-87]		43.3 [20-70]		<0.001	42.2 [19-87]	
					<0.001					<0.001		
<30	15	5.5	84	10.3		4	2.1	13	3.5		116	7.0
30-34	26	9.5	163	20.0		8	4.2	47	12.5		244	14.7
35-39	40	14.6	189	23.2		23	12.0	76	20.2		328	19.8
40-44	41	14.9	153	18.8		17	8.9	82	21.8		293	17.7
45-49	48	17.5	107	13.1		24	12.6	68	18.0		247	14.9
50-54	35	12.7	67	8.2		38	19.9	46	12.2		186	11.2
55-59	23	8.4	29	3.6		21	11.0	27	7.2		100	6.0
60+	47	17.1	22	2.7		56	29.3	18	4.8		143	8.6
Unknown	1		0			0		0			1	
Year of PBC diagnosis					<0.001					<0.001		
1990-1994	87	31.5	61	7.5		30	15.7	21	5.6		199	12.0
1995-1999	90	32.6	122	15.0		36	18.9	44	11.7		292	17.6
2000-2004	37	13.4	223	27.4		49	25.7	115	30.5		424	25.6
2005-2009	42	15.2	284	34.9		50	26.2	143	37.9		519	31.3
2010-2017	20	7.3	124	15.2		26	13.6	54	14.3		224	13.5
Stage[a]					<0.001					<0.001		
IA	153	64.8	223	31.0		114	65.1	66	19.6		556	37.9
IB	4	1.7	20	2.8		5	2.9	9	2.7		38	2.6
IIA	64	27.1	264	36.7		35	20.0	88	26.2		451	30.7
IIB	8	3.4	130	18.1		14	8.0	82	24.4		234	16.0
IIIA	2	0.9	54	7.5		4	2.3	48	14.3		108	7.4
IIIB	3	1.3	9	1.3		0	0	9	2.7		21	1.4
IIIC	2	0.9	20	2.8		3	1.7	34	10.1		59	4.0
Unknown	40		94			16		41			191	
Histological B&R grade					<0.001					<0.001		
Grade I	7	3.5	8	1.1		14	9.0	8	2.5		37	2.7
Grade II	57	28.6	77	10.8		74	47.7	113	34.8		321	23.0
Grade III	135	67.8	630	88.1		67	43.2	204	62.8		1,036	74.3
Unknown	77		99			36		52			264	

Table 1. Continued.

	BRCA1					*BRCA2*					
	No Chemotherapy		**Chemotherapy**[b]		**p-value**	**No chemotherapy**		**Chemotherapy**[b]		**p-value**	**Total Group**	
	N	***%***	***N***	***%***		***N***	***%***	***N***	***%***		***N***	***%***
Oestrogen receptor status					<0.001					0.083		
Positive	57	36.5	133	18.9		115	80.4	240	72.7		545	40.9
Negative	99	63.5	572	81.1		28	19.6	90	27.3		789	59.2
Unknown	120		109			48		47			324	
Progesterone receptor status					0.017					0.603		
Positive	37	25.3	112	16.5		80	59.7	179	56.8		408	32.1
Negative	109	74.7	565	83.5		54	40.3	136	43.2		864	67.9
Unknown	130		137			57		62			386	
HER2 receptor status					0.197					0.204		
Positive	7	9.5	28	5.6		5	5.5	26	10.9		66	7.3
Negative	67	90.5	469	94.4		86	94.5	213	89.1		835	92.7
Unknown	202		317			100		138			757	
Surgery					0.022					<0.001		
None/biopsy	4	1.6	11	1.4		7	3.9	12	3.2		34	2.1
Lumpectomy	135	54.4	359	44.9		102	56.4	121	32.3		717	44.7
Mastectomy	109	44.0	429	53.7		72	39.8	242	64.5		852	53.2
Unknown	28		15			10		2			55	
Radiotherapy					0.165					0.516		
Yes	150	57.5	507	62.4		108	59.3	234	62.4		999	61.3
No	111	42.5	305	37.6		74	40.7	141	37.6		631	38.7
Unknown	15		2			9		2			28	
Endocrine therapy					<0.001					<0.001		
Yes	31	11.9	178	21.9		49	26.9	237	62.9		495	30.3
No	230	88.1	634	78.1		133	73.1	140	37.1		1,137	69.7
Unknown	15		2			9		0			26	
Targeted therapy					[c]					[c]		
Yes	0	0	27	3.3		0	0	24	6.4		51	3.1
No	261	100	785	96.4		182	100	353	93.6		1,581	96.9
Unknown	15		2			9		0			26	
CRRM/BRRM					<0.001					<0.001		
Yes	94	34.1	457	56.1		55	28.8	215	57.0		821	49.5
No	182	65.9	357	43.9		136	71.2	162	43.0		837	50.5
RRSO					<0.001					<0.001		
Yes	173	63.1	634	78.7		122	64.2	306	81.4		1,235	75.0
No	101	36.9	172	21.3		68	35.8	70	18.6		411	25.0
Other/Unknown	2		8			1		1			12	

B&R: Bloom & Richardson; BRRM: bilateral risk-reducing mastectomy; CRRM: contralateral risk-reducing mastectomy; FU:follow-up; PBC: primary breast cancer; RRSO: risk-reducing salpingo oophorectomy.
Differentiation grade: grade I: well differentiated; grade II: moderately differentiated; grade III: poorly differentiated/ undifferentiated. Missing values were excluded for the Chi-square/ Kruskal-Wallis significance testing of the variables.
[a] Pathological TNM was used to determine stage, except for patients who received neo-adjuvant chemotherapy, clinical TNM-stage was used. Stages: IA: T1 N0 M0; IB: T0-1 N1mi M0; IIA: T0-1 N1 M0 or T2 N0 M0; IIB: T2 N1 M0 or T3 N0 M0; IIIA: T0-2 N2 M0 or T3 N1-2 M0; IIIB: T4 N0-2 M0; IIIC: Any T N3 M0.
[b] Neo-adjuvant or adjuvant chemotherapy (93 vs. 748 in *BRCA1* and 57 vs. 320 in *BRCA2*, respectively).
[c] No significance testing was performed since targeted therapy was always provided in combination with chemotherapy.

Cumulative CBC risk

Ten-year cumulative CBC risk for *BRCA1* mutation carriers was 6.7% [95% CI: 5.1-8.6] after treatment with chemotherapy and 16.7% [95% CI: 10.8-23.7] without chemotherapy. In *BRCA2* mutation carriers, the 10-year cumulative incidence rates were 4.8% [95% CI: 2.7-7.8] and 16.0% [9.3-24.4], respectively (Table 2 and Figure 2). All subtypes of chemotherapy were associated with reduced CBC risk in *BRCA1* mutation carriers, although CMF appears less effective than anthracyclines and taxanes. For *BRCA2* mutation carriers similar trends were observed when comparing the different agents (Figure 3B).

Table 2. Five- and ten-year cumulative incidence of metachronous CBC in *BRCA1* and *BRCA2 mutation carriers*: chemotherapy vs. no chemotherapy

	***N* CBC / *N* PBC**	**5-year CBC risk % [95% CI]**	**10-year CBC risk % [95% CI]**
***BRCA1* mutation carriers**			
Total	116/963	**5.2** [3.8-7.0]	**8.2** [6.5-10.1]
Chemotherapy	79/749	**3.9** [2.7-5.6]	**6.7** [5.1-8.6]
No chemotherapy	37/214	**12.6** [7.3-19.4]	**16.7** [10.8-23.7]
***BRCA2* mutation carriers**			
Total	44/506	**6.3** [3.9-9.7]	**8.1** [5.4-11.4]
Chemotherapy	23/344	**3.7** [1.8-6.6]	**4.8** [2.7-7.8]
No chemotherapy	21/162	**12.5** [6.4-20.7]	**16.0** [9.3-24.4]

CBC: contralateral breast cancer, either invasive or non-invasive; CI: confidence interval; PBC: primary breast cancer.
Competing risk analysis was used to determine cumulative incidence for invasive CBC.

Chemotherapy vs. no chemotherapy

For *BRCA1* mutation carriers, treatment with neo-adjuvant or adjuvant chemotherapy compared to no chemotherapy was associated with decreased CBC risk (multivariable HR: 0.46; 95% CI: 0.29-0.74; Table 3). We mainly observed a risk-reducing effect of chemotherapy in the first five years after PBC (HR: 0.32; 95% CI: 0.17-0.61 for the first five years after PBC diagnosis and HR: 0.69; 95% CI: 0.35-1.37 for five years onwards; p-value= 0.27 for trend; Figure 2). For *BRCA2* mutation carriers, a similar trend in 10-year risk reduction was observed (multivariable HR: 0.63; 95% CI: 0.29-1.39; Table 3; p-value=0.44 for interaction for differences in associations between *BRCA1* and *BRCA2* patients).

Chemotherapy agents

For *BRCA1* mutation carriers, treatment with anthracyclines was specifically associated with reduced CBC risk (multivariable HR: 0.34; 95% CI: 0.17-0.67; Table 4). We observed similar effects for combinations of anthracyclines and taxanes (multivariable HR: 0.22; 95% CI: 0.08-0.62; Table 4 and Figure 3A). We had insufficient power (as indicated by the wide confidence interval) to prove or refute a significant difference between the combination of anthracyclines and taxanes versus treatment with anthracyclines alone (multivariable HR: 0.65; 95% CI: 0.24-1.65).

For *BRCA2* mutation carriers similar trends for the chemotherapeutic agents were observed (Table 4).

Risk estimates for invasive CBC are presented in Supplementary Tables, B.1-B.3. For both *BRCA1* and *BRCA2* mutation carriers, cumulative incidences and hazard ratios for invasive CBC were comparable with the combined invasive and non-invasive CBC risk estimates.

Complete case analysis revealed similar results as the main analysis (Supplementary Material A.4-A.6).

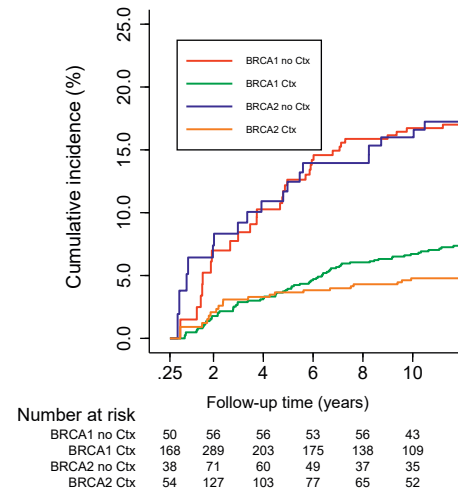
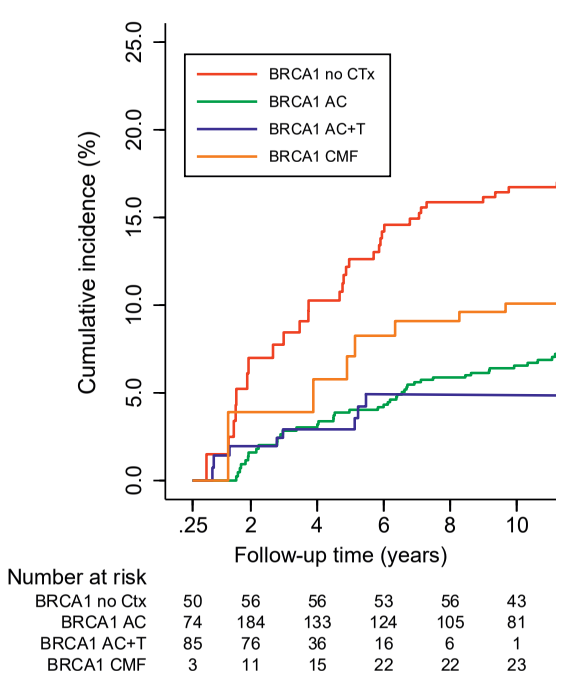


Figure 2. Cumulative incidence of developing CBC in *BRCA1* and *BRCA2* mutation carriers (%); chemotherapy vs. no chemotherapy

Abbreviations: CBC: contralateral breast cancer; Ctx: chemotherapy.
Competing risk analysis were applied for this figure.



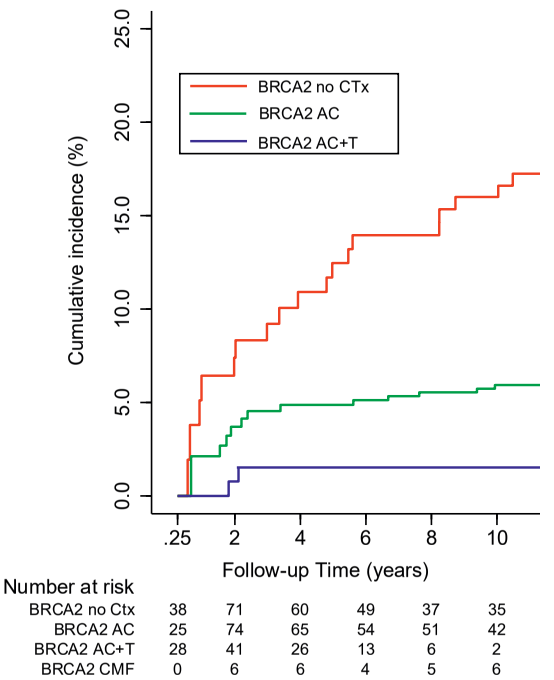


Figure 3A (left) and 3B (right). Cumulative incidence of developing CBC in *BRCA1* (Fig. 3A) and *BRCA2* (Fig. 3B) mutation carriers (%); Anthracyclines vs. Anthracyclines + Taxanes vs. CMF vs. no chemotherapy

AC: Anthracyclines; AC+T: Anthracyclines + Taxanes; CMF: Cyclophosphamide Methotrexate and 5-FU; CBC: contralateral breast cancer; Ctx: chemotherapy. Competing risk analysis were applied for this figure.

[a]CMF was left out because limited events (n=1).

Table 3. Univariable and multivariable Cox regression analyses for 10-year risk of metachronous CBC, stratified by *BRCA1* and *BRCA2* mutation

	PYO	*N* CBC	Rate Per 1000 PYO	uHR [95% CI]	mHR [95% CI]
***BRCA1* mutation carriers**					
Chemotherapy	1,939	59	30.4	0.56 [0.36-0.88]	0.46 [0.29-0.74]
No chemotherapy	538	29	53.9	**Ref.**	**Ref.**
Endocrine therapy	540	14	25.9	0.68 [0.38-1.20]	0.78 [0.44-1.40]
No endocrine therapy	1,937	74	38.2	**Ref.**	**Ref.**
Radiotherapy	1,716	64	37.3	1.04 [0.65-1.67]	1.10 [0.68-1.77]
No Radiotherapy	760	24	31.6	**Ref.**	**Ref.**
Age (continuous)	2,477	88	35.5	0.98 [0.96-1.00]	0.97 [0.95-0.99]
***BRCA2* mutation carriers**					
Chemotherapy	869	19	21.9	0.70 [0.36-1.37]	0.63 [0.29-1.39]
No chemotherapy	512	16	31.2	**Ref.**	**Ref.**
Endocrine therapy	772	13	16.8	0.48 [0.24-0.95]	0.53 [0.25-1.12]
No endocrine therapy	610	22	36.1	**Ref.**	**Ref.**
Radiotherapy	925	24	26.0	1.11 [0.54-2.28]	1.17 [0.57-2.42]
No radiotherapy	457	11	24.1	**Ref.**	**Ref.**
Age (continuous)	1,381	35	25.3	0.97 [0.94-1.00]	0.96 [0.93-0.99]

PYO: person-years of observation; *N* CBC: number of contralateral breast cancer events, either invasive or non-invasive; uHR: univariable hazard ratios; mHR: multivariable hazard ratios, with adjustment for all other variables in the model (e.g. chemotherapy was adjusted for endocrine therapy, radiotherapy and age; age was adjusted for chemotherapy, endocrine therapy and radiotherapy).

Adjusting for risk-reducing salpingo oophorectomy (time-dependent) did not lead to a substantial change in the hazard ratio and was therefore not included the multivariable model.

Age concerns age at primary breast cancer diagnosis.

Table 4. Univariable and multivariable Cox regression analyses for 5-year risk of metachronous CBC according to different partly imputed chemotherapy agents, stratified by *BRCA1* and *BRCA2* mutation

	PYO	*N* CBC	Rate Per 1000 PYO	uHR [95% CI]	mHR [95% CI]
***BRCA1* mutation carriers**					
Anthracyclines	724	20	27.6	0.42 [0.22-0.81]	0.34 [0.17-0.68]
Anthracyclines + Taxanes	319	5	15.7	0.28 [0.10-0.76]	0.22 [0.08-0.62]
CMF	69	3	43.6	0.65 [0.19-2.22]	0.57 [0.16-1.95]
No chemotherapy	274	17	62.1	**Ref.**	**Ref.**
Endocrine therapy	332	10	30.1	0.93 [0.46-1.87]	1.12 [0.54-2.30]
No endocrine therapy	1,140	37	32.4	**Ref.**	**Ref.**
Age (continuous)	1,472	47	31.9	0.99 [0.96-1.02]	0.98 [0.95-1.00]
***BRCA2* mutation carriers**					
Anthracyclines	294	7	23.8	0.68 [0.26-1.76]	0.64 [0.22-1.86]
Anthracyclines + Taxanes	177	2	11.3	0.30 [0.07-1.36]	0.30 [0.06-1.51]
CMF	21	1	47.1	1.32 [0.17-10.30]	0.80 [0.10-6.56]
No chemotherapy	304	11	36.2	**Ref.**	**Ref.**
Endocrine therapy	472	8	17.0	0.41 [0.17-0.96]	0.49 [0.19-1.26]
No endocrine therapy	353	15	42.5	**Ref.**	**Ref.**
Age (continuous)	825	23	27.9	0.97 [0.94-1.01]	0.96 [0.92-1.00]

CMF: Cyclophosphamide Methotrexate and 5-FU; PYO: Person-years of observation; *N* CBC : number of contralateral breast cancer events, either invasive or non-invasive; uHR: univariable hazard ratios; mHR: multivariable hazard ratios, with adjustment for all other variables included in the model (e.g. chemotherapeutic agents was adjusted for endocrine therapy and age; age was adjusted for chemotherapeutic agents and endocrine therapy).
Adjusting for risk-reducing salpingo oophorectomy (time-dependent) did not lead to a substantial change in the hazard ratio and was therefore not included the multivariable model.
For the missing chemotherapeutic agents, patients were categorized as CMF if the primary breast cancer diagnosis was <01/01/1994, Anthracyclines if the primary breast cancer diagnosis was between 12/31/1997 and 01/01/2007, and Anthracyclines + Taxanes if the primary breast cancer diagnosis was >12/31/2008.

DISCUSSION

We observed a reduced risk of metachronous CBC in *BRCA1* mutation carriers who received chemotherapy compared to those who did not. For *BRCA2* mutation carriers, we observed a similar trend (HR: 0.63; 95% CI: 0.29-1.39). In both groups, there was a large difference in cumulative incidence of CBC by chemotherapy. We are the first to study the effects of different chemotherapeutic agents on CBC risk, separately for *BRCA1* and *BRCA2* mutation carriers. The risk-reducing effects were the largest in *BRCA1* mutation carriers who were treated with anthracyclines alone or in combination with taxanes, though these effects only concern the first 5 years after PBC diagnosis.

In earlier studies[1,11,18], CBC risk reduction after chemotherapy was already described, which is in line with our study. However, only in the study by Reding et al.[11], the effects of different agents were examined. Reding et al. observed a decreased CBC risk, though in a combined cohort of *BRCA1* and *BRCA2* mutation carriers who were treated with anthracyclines versus those who received no chemotherapy. We also observed a risk-reducing effect when we combined *BRCA1* and *BRCA2* mutation carriers. However, in our study the effects were especially prominent among *BRCA1* mutation carriers. The limited number of patients and/or events in *BRCA2* mutation carriers though, preclude strong claims on the impact of chemotherapy in *BRCA2* mutation carriers. Also, in *BRCA2* mutation carriers the impact of endocrine therapy most likely played a more important role. Moreover, while both *BRCA1* and *BRCA2* associated tumours have a homologous recombination repair deficiency, there are phenotypical characteristics which could lead to a different chemotherapeutic response.[1,12,13] In our study for example, *BRCA1* mutation carriers were more often aged under 35 years at PBC diagnosis than *BRCA2* mutation carriers (29.8% vs. 16.7% respectively), more often had grade III PBC (83.7% vs. 56.5%), and more often had ER-negative PBC (78.2% vs. 24.7%). These features are all associated with more aggressive tumour growth and worse prognosis[19-22], and therefore chemotherapy is likely more effective in *BRCA1* mutation carriers (and by extension in the prevention of secondary breast tumours, having similar characteristics, at least in our dataset).

Double-strand DNA breaks-inducing chemotherapeutics, e.g. anthracyclines, are more effective in homologous recombination repair deficient (pre-)cancerous cells of *BRCA1/2* mutation carriers, eliminating (pre-)cancerous lesions.[23] Indeed, our

limited data suggests that there was a stronger risk-reducing effect of anthracycline-based chemotherapeutics.

In earlier studies, tumours in *BRCA1* mutation carriers were found to be less sensitive to taxane-based chemotherapy than tumours of sporadic breast cancer patients.[24,25] Taxanes do not cause double-strand DNA breaks, but act through stabilization of microtubules, resulting in cell-cycle arrest and apoptosis.[26] In a recent study however, no resistance to taxane agents was observed.[6] Taxanes may provide an additional benefit, although in our study numbers were too small to draw a definite conclusion. Further, there have been important developments in treatment over the years, i.e. better dosage of anthracyclines (e.g. dose-dense scheduling), better monitoring and better support during treatment, increasing therapy adherence, which may affect the results.

The cumulative CBC incidences we observed in *BRCA1* and *BRCA2* mutation carriers were comparable with the results from earlier studies.[1,2] We aimed to reduce survival bias by left-truncating the analysis, i.e. person-time prior to DNA testing was not taken into account. This automatically led to exclusion of patients with CBC diagnosis prior to *BRCA1/2* DNA mutation testing (*n*=287; Fig. 1), preventing an overrepresentation of CBC patients who may have undergone a DNA test because of the CBC diagnosis (i.e. limiting testing bias). This could lead to an overcorrection lowering CBC risk. On the other hand, a proportion of the mutation carriers with breast cancer who did not develop a CBC may not have been identified as a mutation carrier and are therefore not included in the study. The exclusion of these low-risk women will likely have caused an overestimation of the CBC risk in our study population, balancing a potential overcorrection. Further, although the number of exclusions was high, 25% of these patients (n=73) would still have been excluded for other reasons eventually (mainly synchronous CBC development).

The strengths of our study are the use of a cohort with nationwide coverage and generally long follow-up, as well as being the first study aiming to estimate the effect of different chemotherapeutic agents on CBC risk separately in *BRCA1* and *BRCA2* mutation carriers.

Still, there are some limitations to our study. Ideally a randomized trial would be performed to investigate the effect of systemic treatment on CBC risk. However,

it would be unethical to withhold chemotherapy from patients who are presumed to benefit from it. Therefore, we made use of existing data from an observational study. By taking into account selection and survival bias we attempted to approach a prospective study design as much as possible.

Finally, around 40% of the data on chemotherapy agents was initially missing, which could have influenced the results. However, after imputation, missing data was limited to 8%, and we observed no relevant differences when comparing the results including versus excluding the imputed agents.

Another potential limitation was the imbalance in the risk-reducing mastectomy rates between patients who were treated with chemotherapy vs. without chemotherapy (higher in the chemotherapy group). This could potentially lead to a bias. We observed that in *BRCA1* mutation carriers the median time from primary breast cancer diagnosis until a DNA test, was much shorter in the chemotherapy group (1.0 year) than in the non-chemotherapy group (3.4 years). Both the earlier DNA testing and the increased RRM rates are suggestive of a stronger family history with an even higher CBC incidence rate within these families, indicating that the baseline risk of CBC was higher in this group. Consequently, the actual CBC rate in the chemotherapy group should have been higher than we described, suggesting the protective effect of chemotherapy on CBC risk we observed is an underestimation.

Clinical implications

The primary goal of chemotherapy is to eliminate micro metastases and reducing the risk of distant and loco-regional recurrences (by extension, this may also eliminate preclinical/precancerous lesions in the contralateral breast). In that case, the effect will likely be transient. Indeed, in this study we now showed also a strong risk-reducing effect of chemotherapy in the first 5 years after PBC diagnosis on the development of new primary cancers in the contralateral breast, most notably in *BRCA1* mutation carriers. Since patients with *BRCA1*- and *BRCA2*-associated PBC have a high baseline risk of developing CBC, the relative benefit of chemotherapy leads to a high absolute reduction in CBC risk. The results of our study can be used to further personalise CBC risk management. In combination with other factors that influence CBC risk [4], we aim to identify patients at high and low risk of CBC.[27,28] Based on the results of this study, the frequency of screening and choices regarding

risk-reducing surgeries cannot be tailored to the different risk-profiles yet, though this would be the subsequent goal. Hereto, long-term effects of chemotherapy on CBC risk (i.e. beyond our median follow-up of ten years), should be investigated first, in particular in young *BRCA1/2* mutation carriers with long life expectancy. After all, if after ten years, the annual CBC risk normalises to the level of those without chemotherapy (i.e., 1.5-3.0%), overall lifetime CBC risk would not be lowered enough to change decision-making regarding screening or risk-reducing surgery. Furthermore, in future studies, the long-term effects of more recent developments in drug treatment (e.g. PARP-inhibitors), should also be taken into account.

Conclusions

Chemotherapy is associated with reduced CBC risk in *BRCA1* mutation carriers at least for the first 5 years. Anthracyclines, either alone or in combination with taxanes, may result in the largest risk reduction. For *BRCA2* mutation carriers, results pointed in the same direction. The risk-reducing effects of chemotherapy can be used to further personalise CBC risk assessment.

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Supplementary Material A

Supplementary Table A.1. Univariable and multivariable Cox regression analyses for 10-year risk of metachronous CBC in *BRCA1* and *BRCA2* mutation carriers combined

	PYO	*N* CBC	Rate Per 1000 PYO	uHR [95% CI]	mHR [95% CI]
Total group	3,858	123	31.9	-	-
BRCA1 mutation	2,477	88	35.5	1.43 [0.96-2.11]	1.20 [0.78-1.85]
BRCA2 mutation	1,381	35	25.3	**Ref.**	**Ref.**
Chemotherapy	2,808	78	27.8	0.65 [0.45-0.93]	0.49 [0.33-0.74]
No chemotherapy	1,050	45	42.9	**Ref.**	**Ref.**
Endocrine therapy	1,312	27	20.6	0.54 [0.35-0.83]	0.70 [0.44-1.11]
No endocrine therapy	2,546	96	37.7	**Ref.**	**Ref.**
Radiotherapy	2,641	88	33.3	1.08 [0.73-1.60]	1.12 [0.75-1.66]
No Radiotherapy	1,217	35	28.8	**Ref.**	**Ref.**
Age (continuous)	3,858	123	31.9	0.97 [0.96-0.99]	0.97 [0.95-0.98]

PYO: person-years of observation; N *CBC*: number of contralateral breast cancer events; uHR: univariable hazard ratios; mHR: multivariable hazard ratios, with adjustment for all other variables included in the model (e.g. chemotherapy was adjusted for *BRCA* status, endocrine therapy, radiotherapy and age; age was adjusted for *BRCA* status, chemotherapy, endocrine therapy and radiotherapy).
Adjusting for risk-reducing salpingo oophorectomy (time-dependent) did not lead to a substantial change in the hazard ratio and was therefore not included the multivariable model.
Age concerns age at primary breast cancer diagnosis.
For the missing chemotherapeutic agents, patients were categorized as CMF if the primary breast cancer diagnosis was <12/31/1994, Anthracyclines if the primary breast cancer diagnosis was between 12/31/1997 and 12/31/2006, and Anthracyclines + Taxanes if the primary breast cancer diagnosis was >12/31/2008.

Supplementary Table A.2. Univariable and multivariable Cox regression analyses for 5-year risk of metachronous CBC according to different chemotherapy agents in *BRCA1* and *BRCA2* mutation carriers combined

	PYO	*N* CBC	Rate Per 1000 PYO	uHR [95% CI]	mHR [95% CI]
Total group	2,297	70	30.5	-	-
***BRCA1* mutation**	1,472	47	31.9	1.17 [0.71-1.93]	1.13 [0.66-1.96]
BRCA2 mutation	825	23	27.9	**Ref.**	**Ref.**
No chemotherapy	577	28	48.5	**Ref.**	**Ref.**
Anthracyclines	1,018	27	26.5	0.53 [0.31-0.90]	0.40 [0.22-0.71]
Anthracyclines + Taxanes	496	7	14.1	0.30 [0.13-0.70]	0.23 [0.10-0.55]
CMF	90	4	44.5	0.88 [0.31-2.50]	0.65 [0.22-1.89]
Endocrine therapy	803	18	22.4	0.64 [0.37-1.09]	0.85 [0.48-1.52]
No endocrine therapy	1,493	52	34.8	**Ref.**	**Ref.**
Age (continuous)	2,297	70	30.5	0.98 [0.96-1.01]	0.97 [0.95-0.99]

CMF: Cyclophosphamide Methotrexate and 5-FU; PYO: person-years of observation; N *CBC*: number of contralateral breast cancer events; uHR: univariable hazard ratios; mHR: multivariable hazard ratios, with adjustment for all other variables included in the model (e.g. chemotherapeutic agents was adjusted for *BRCA* status, endocrine therapy and age; age was adjusted for *BRCA* status, chemotherapeutic agents and endocrine therapy).
Adjusting for risk-reducing salpingo oophorectomy (time-dependent) did not lead to a substantial change in the hazard ratio and was therefore not included the multivariable model.
Age concerns age at primary breast cancer diagnosis.

Supplementary Table A.3. Univariable and multivariable Cox regression analyses for 5-year risk of metachronous CBC in *BRCA1* and *BRCA*2 mutation carriers combined, according to known chemotherapy agents

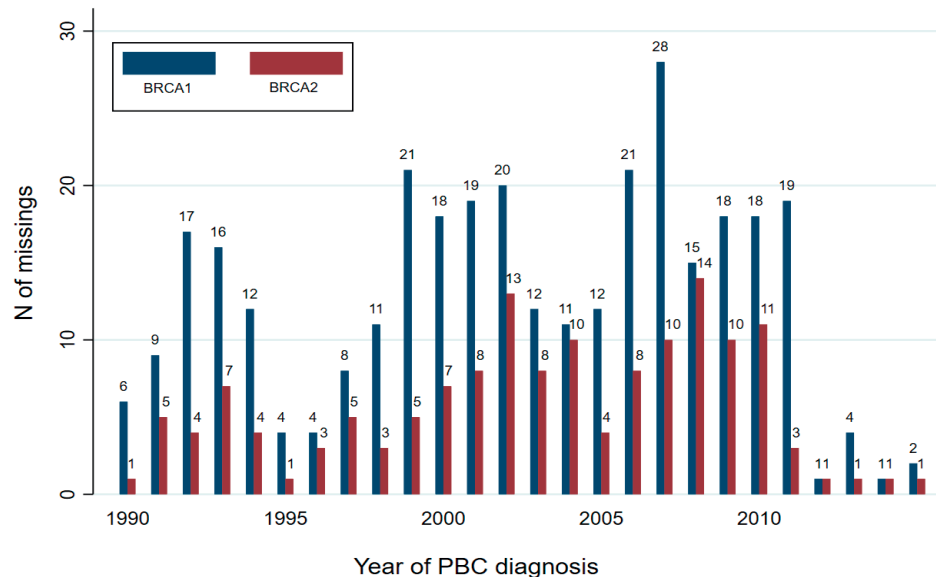
	PYO	*N* CBC	Rate Per 1000 PYO	uHR [95% CI]	mHR [95% CI]
Total group	2,297	70	30.5	-	-
***BRCA1* mutation**	1,472	47	31.9	1.17 [0.71-1.93]	1.09 [0.63-1.89]
BRCA2 mutation	825	23	27.9	**Ref.**	**Ref.**
No chemotherapy	577	28	48.5	**Ref.**	**Ref.**
Anthracyclines	644	23	35.7	0.72 [0.41-1.25]	0.55 [0.30-1.00]
Anthracyclines + Taxanes	338	5	14.8	0.32 [0.12-0.82]	0.24 [0.09-0.66]
CMF	72	4	55.4	1.10 [0.38-3.13]	0.81 [0.28-2.34]
Endocrine therapy	803	18	22.4	0.64 [0.37-1.09]	0.84 [0.47-1.50]
No endocrine therapy	1,493	52	34.8	**Ref.**	**Ref.**
Age (continuous)	2,297	70	30.5	0.98 [0.96-1.01]	0.97 [0.95-0.99]

CMF: Cyclophosphamide Methotrexate and 5-FU; PYO: person-years of observation; *N* CBC: number of contralateral breast cancer events; uHR: univariable hazard ratios; mHR: multivariable hazard ratios, with adjustment for all other variables included in the model (e.g. chemotherapeutic agents was adjusted for *BRCA* status, endocrine therapy and age; age was adjusted for *BRCA* status, chemotherapeutic agents and endocrine therapy).
Adjusting for risk-reducing salpingo oophorectomy (time-dependent) did not lead to a substantial change in the hazard ratio and was therefore not included the multivariable model.
Age concerns age at primary breast cancer diagnosis.

Supplementary Table A.4. Number of initially missing chemotherapy agents (i.e. prior to imputation) in relation to the total number of patients who received chemotherapy in *BRCA1* and *BRCA2* mutation carriers, per period of primary breast cancer diagnosis used for imputing unknown types

Period of PBC diagnosis	BRCA1			BRCA2		
	n/N	**%**	***N* missings after imputing (%)**	***n/N***	**%**	***N* missings after imputing**
1990-1993	48/48	100	0	17/17	100	0
1994-1997	28/63	44.4	23 (36.5)	13/23	56.5	13 (56.5)
1998-2006	145/400	36.3	0	66/193	34.2	0
2007-2008	43/119	36.1	40 (33.6)	24/55	43.6	18 (32.7)
2009-2017	63/184	34.2	0	28/89	31.5	0

PBC: primary breast cancer; *n/N*: number of missings/number of patients who received chemotherapy.



Supplementary Figure A.5. Number of initially missing chemotherapy agents (prior to imputation) per year of primary breast cancer (PBC) diagnosis in *BRCA1* and *BRCA2* mutation carriers

For 2015 no missings were reported.

Supplementary Table A.6. Univariable and multivariable Cox regression analyses for 5-year risk of metachronous CBC for different known chemotherapy agents, stratified by *BRCA1* and *BRCA2* mutation carriers

	PYO	*N* CBC	Rate per 1000 PYO	uHR [95% CI]	mHR [95% CI]
***BRCA1* mutation carriers**					
No chemotherapy	274	17	62.1	**Ref.**	**Ref.**
Anthracyclines	480	18	37.5	0.58 [0.30-1.13]	0.47 [0.23-0.95]
Anthracyclines + Taxanes	212	3	14.2	0.25 [0.07-0.85]	0.20 [0.06-0.69]
CMF	52	3	57.4	0.87 [0.25-2.96]	0.74 [0.21-2.54]
Endocrine therapy	332	10	30.1	0.93 [0.46-1.87]	1.11 [0.54-2.28]
No endocrine therapy	1,140	37	32.4	**Ref.**	**Ref.**
Age (continuous)	1,472	47	31.9	0.99 [0.96-1.02]	0.97 [0.95-1.00]
***BRCA2* mutation carriers**					
No chemotherapy	304	11	36.2	**Ref.**	**Ref.**
Anthracyclines	163	5	30.6	0.88 [0.31-2.53]	0.92 [0.29-2.90]
Anthracyclines + Taxanes	126	2	15.8	0.42 [0.09-1.88]	0.44 [0.09-2.26]
CMF	20	1	50.1	1.39 [0.18-10.78]	0.79 [0.10-6.45]
Endocrine therapy	472	8	17.0	0.41 [0.17-0.96]	0.45 [0.17-1.17]
No endocrine therapy	353	15	42.5	**Ref.**	**Ref.**
Age (continuous)	825	23	27.9	0.97 [0.94-1.01]	0.96 [0.92-1.00]

CMF: Cyclophosphamide Methotrexate and 5-FU; PYO: person-years of observation; *N* CBC: number of contralateral breast cancer events; uHR: univariable hazard ratios; mHR: multivariable hazard ratios, adjusted for all other variables included in the model (e.g. chemotherapeutic agents was adjusted for endocrine therapy and age; age was adjusted for chemotherapeutic agents and endocrine therapy).
Adjusting for risk-reducing salpingo oophorectomy (time-dependent) did not lead to a substantial change in the hazard ratio and was therefore not included the multivariable model.
Age concerns age at primary breast cancer diagnosis.

SUPPLEMENTARY MATERIAL B

Supplementary Table B.1. Five- and ten-year cumulative incidence of metachronous invasive CBC in *BRCA1* and *BRCA2* mutation carriers: chemotherapy vs. no chemotherapy

	N CBC / *N* PBC	5-year CBC risk % [95% CI]	10-year CBC risk % [95% CI]
***BRCA1* mutation carriers**			
Total	93/963	**4.0** [2.8-5.4]	**6.4** [5.0-8.1]
Chemotherapy	63/749	**2.9** [1.9-4.3]	**5.2** [3.8-6.9]
No chemotherapy	30/214	**10.3** [5.5-16.8]	**13.9** [8.5-20.6]
***BRCA2* mutation carriers**			
Total	26/506	**2.9** [1.4-5.3]	**4.1** [2.4-6.5]
Chemotherapy	14/344	**1.1** [0.4-2.5]	**1.9** [0.9-3.5]
No chemotherapy	12/162	**7.9** [3.3-15.1]	**10.2** [5.0-17.6]

CBC: contralateral breast cancer, only invasive; CI: confidence interval; PBC: primary breast cancer.
Competing risk analysis was used to determine cumulative incidence for invasive CBC.

Supplementary Table B.2. Univariable and multivariable Cox regression analyses for 10-year risk of metachronous invasive CBC, stratified by *BRCA1* and *BRCA2* mutation

	PYO	*N* CBC	Rate Per 1000 PYO	uHR [95% CI]	mHR [95% CI]
***BRCA1* mutation carriers**					
Chemotherapy	1,939	48	24.8	0.59 [0.36-0.97]	0.50 [0.29-0.84]
No chemotherapy	538	23	42.8	**Ref.**	**Ref.**
Endocrine therapy	540	11	20.4	0.66 [0.35-1.26]	0.73 [0.38-1.41]
No endocrine therapy	1,937	60	31.0	**Ref.**	**Ref.**
Radiotherapy	1,716	53	30.9	1.12 [0.66-1.93]	1.20 [0.70-2.07]
No Radiotherapy	760	18	23.7	**Ref.**	**Ref.**
Age (continuous)	2,477	71	28.7	0.98 [0.96-1.01]	0.98 [0.95-1.00]
***BRCA2* mutation carriers**					
Chemotherapy	869	10	11.5	0.60 [0.25-1.44]	0.61 [0.22-1.70]
No chemotherapy	512	10	19.5	**Ref.**	**Ref.**
Endocrine therapy	772	6	7.8	0.35 [0.13-0.91]	0.40 [0.14-1.15]
No endocrine therapy	610	14	23.0	**Ref.**	**Ref.**

Supplementary Table B.2. Continued.

	PYO	*N* CBC	Rate Per 1000 PYO	uHR [95% CI]	mHR [95% CI]
Radiotherapy	925	13	14.1	0.90 [0.36-2.27]	0.96 [0.38-2.43]
No radiotherapy	457	7	15.3	**Ref.**	**Ref.**
Age (continuous)	1,381	20	14.5	0.97 [0.93-1.01]	0.96 [0.92-1.00]

PYO: person-years of observation; *N* CBC: number of invasive contralateral breast cancer events; uHR: univariable hazard ratios; mHR: multivariable hazard ratios, with adjustment for all other variables in the model (e.g. chemotherapy was adjusted for endocrine therapy, radiotherapy and age; age was adjusted for chemotherapy, endocrine therapy and radiotherapy).
Adjusting for risk-reducing salpingo oophorectomy (time-dependent) did not lead to a substantial change in the hazard ratio and was therefore not included the multivariable model.
Age concerns age at primary breast cancer diagnosis.

Supplementary Table B.3. Univariable and multivariable Cox regression analyses for 5-year risk of metachronous invasive CBC according to different partly imputed chemotherapy agents, stratified by *BRCA1* and *BRCA2* mutation

	PYO	*N* CBC	Rate Per 1000 PYO	uHR [95% CI]	mHR [95% CI]
***BRCA1* mutation carriers**					
Anthracyclines	724	19	26.2	0.52 [0.26-1.08]	0.45 [0.21-0.96]
Anthracyclines + Taxanes	319	2	6.3	0.15 [0.03-0.69]	0.13 [0.03-0.59]
CMF	69	2	29.1	0.57 [0.13-2.51]	0.51 [0.11-2.27]
No chemotherapy	274	13	47.5	**Ref.**	**Ref.**
Endocrine therapy	332	8	24.1	0.95 [0.44-2.09]	1.08 [0.48-2.41]
No endocrine therapy	1,140	29	25.4	**Ref.**	**Ref.**
Age (continuous)	1,472	37	25.1	0.99 [0.97-1.03]	0.98 [0.95-1.01]
***BRCA2* mutation carriers**					
Anthracyclines	294	1	3.4	0.15 [0.02-1.21]	0.25 [0.03-2.16]
Anthracyclines + Taxanes	177	2	11.3	0.53 [0.11-2.56]	1.10 [0.20-6.06]
CMF	21.2	0	0	-[a]	-[a]
No chemotherapy	304	7	23.0	**Ref.**	**Ref.**
Endocrine therapy	472	1	2.1	0.07 [0.01-0.54]	0.08 [0.01-0.65]
No endocrine therapy	353	11	31.2	**Ref.**	**Ref.**
Age (continuous)	825	12	14.6	0.97 [0.92-1.03]	0.97 [0.92-1.03]

CMF: Cyclophosphamide Methotrexate and 5-FU; PYO: person-years of observation; *N* CBC: number of contralateral breast cancer events; uHR: univariable hazard ratios; mHR: multivariable hazard ratios, with adjustment for all other variables in the model (e.g. chemotherapeutic agents was adjusted for endocrine therapy and age; age was adjusted for chemotherapeutic agents and endocrine therapy).
Adjusting for risk-reducing salpingo oophorectomy (time-dependent) did not lead to a substantial change in the hazard ratio and was therefore not included the multivariable model.
[a]No estimates available.
Age concerns age at primary breast cancer diagnosis.
For the missing chemotherapeutic agents, patients were categorized as CMF if the primary breast cancer diagnosis was <01/01/1994, Anthracyclines if the primary breast cancer diagnosis was between 12/31/1997 and 01/01/2007, and Anthracyclines + Taxanes if the primary breast cancer diagnosis was >12/31/2008.

CHAPTER 5

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

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ABSTRACT

Purposes: To estimate the influence of adjuvant radiotherapy for primary breast cancer (BC) on the risk of contralateral BC (CBC) in *BRCA1* or *BRCA2 (BRCA1/2)* mutation carriers, with special attention to patients irradiated at age younger than 40 years. Additionally tendencies in locoregional treatments and rates of contralateral risk reducing mastectomy over time were explored.

Patients and methods: In this retrospective cohort study, 691 *BRCA1/2*-associated BC patients treated between 1980 and 2013 were followed from diagnosis until CBC or censoring event including ipsilateral BC recurrence, distant metastasis, contralateral risk-reducing mastectomy, other invasive cancer diagnosis, death or loss to follow-up. Hazard ratios (HR) for CBC associated with radiotherapy were estimated using Cox regression.

Results: Median follow-up time was 8.6 years [range 0.3-34.3 years]. No association between radiotherapy for primary BC and risk of CBC was found, neither in the total population (HR 0.82, 95% CI 0.45-1.49) nor in the subgroup of patients younger than 40 years at primary diagnosis (HR 1.36, 95% CI 0.60-3.09). During follow-up, the number of patients at risk decreased substantially since a large proportion of patients were censored after contralateral risk-reducing mastectomy or BC recurrence. Over the years, increasing preference for mastectomy without radiotherapy compared to breast-conserving surgery with radiotherapy was found ranging from less than 30% in 1995 to almost 50% after 2010. The rate of contralateral risk-reducing mastectomy increased over the years from less than 40% in 1995 to more than 60% after 2010.

Conclusion: In this cohort of *BRCA1/2*-associated BC patients no association between radiotherapy for primary BC and risk of CBC was observed in the total group, nor in the patients irradiated before the age of 40 years. The number of patients at risk after 10 and 15 years of follow-up, however, was too small to definitively exclude harmful effects of adjuvant radiotherapy.

INTRODUCTION

Both normal breast tissue and breast cancer cells are sensitive to ionizing radiation. Although adjuvant radiotherapy for early breast cancer (BC) reduces the risk of local recurrence and improves BC specific survival[1,2], it also leads to a low dose scatter radiation to the surrounding healthy tissue with potentially carcinogenic effects. In sporadic BC patients, adjuvant radiotherapy has been associated with an increased risk of contralateral breast cancer (CBC), although only among women younger than 45 years at primary BC diagnosis and after a latency period of at least 10-15 years.[3-6]

The vulnerability of cells for ionizing radiation largely depends on the rate of cell proliferation, the total dose of radiation, the fractionation scheme and the capability of the cells to repair DNA damage.[7] Younger patients have higher breast cell proliferation (in particular during puberty, adolescence and pregnancy) and thus increased DNA synthesis that might render breast tissue particularly susceptible to the carcinogenic effects of radiation.[8,9] The capacity to repair DNA damage might substantially differ between BC patients, in particular when considering patients with or without a *BRCA1* or *BRCA2* (*BRCA1/2*) mutation.

BRCA1/2-associated BC is characterized by homologous recombination deficiency, leading to inadequate repair of double strand DNA breaks.[10,11] Ionizing radiation can cause cell damage by induction of double strand DNA breaks. This has led to the hypothesis that adjuvant radiotherapy administered for *BRCA1/2*-associated BC might be more effective than radiotherapy administered for sporadic BC. On the contrary, surrounding healthy breast tissue among BC patients with a *BRCA1/2* mutation might be more vulnerable to the deleterious effects of adjuvant radiotherapy, including the development of a CBC, compared to those without a *BRCA1/2* mutation.

In unaffected *BRCA1/2* mutation carriers, exposure to low cumulative doses of diagnostic radiation (including screening mammography) at young age (<30 years) has been reported to be associated with an increased risk of BC, with a clear dose-effect relationship[12] compared to no exposure to diagnostic radiation. The possible carcinogenic effect of scatter ionizing radiation after adjuvant radiotherapy on the contralateral breast in *BRCA1/2*-associated BC patients, however, is not clear. Although a number of studies addressed this question, all these studies are

compromised by a short duration of follow-up and the lack of subgroup analyses regarding young BC patients.[13-15] Knowledge about the possibly increased risk of CBC by radiotherapy might be of great importance for optimal shared decision-making regarding mastectomy without radiotherapy versus breast conserving surgery including radiotherapy at primary BC diagnosis.

We therefore studied the impact of radiotherapy on the risk of CBC among *BRCA1/2*-associated BC patients in a retrospective cohort study, with special attention to patients younger than 40 years at primary BC diagnosis. Since over the years, an increasing proportion of *BRCA1/2* mutation carriers after developing BC seems to opt for bilateral mastectomy instead of unilateral mastectomy or breast conserving treatment with radiotherapy[16], we also explored potential tendencies in loco-regional treatments and the rates of contralateral risk-reducing mastectomy over the past decades.

METHODS

Patient selection

From the Rotterdam Family Cancer Clinic database we extracted all female patients with early stage BC (n=2,268). From this population we selected proven or obligate *BRCA1* or *BRCA2* mutation carriers, treated at the Erasmus MC Cancer Institute. Patients diagnosed from January 1st 1980, corresponding to the start of linear accelerators use for adjuvant breast radiotherapy at the Erasmus MC, to January 1st 2013 were included (n=790). Time of observation ended at April 1st 2014. Patients with less than three months of follow-up were excluded (n=52; see statistical analysis). Patients who were treated with breast/chest wall radiotherapy or systemic anticancer therapy because of a previous invasive malignancy, either prior or synchronous to the primary BC, were excluded (n=16). Patients who had synchronous bilateral BC and received bilateral radiation therapy or mastectomy (n=31) were also excluded, leaving a total of 691 patients available for the analyses.

For the eligible patients, data on primary BC and CBC characteristics (type of histology, differentiation grade, estrogen receptor (ER) status, progesterone receptor (PR) status, HER2 status and stage) and primary BC therapy (surgery, radiotherapy, chemotherapy and/or endocrine therapy) were retrieved. We also collected data on type of mutation (i.e. *BRCA1* or *BRCA2*), date of birth, primary and contralateral BC diagnoses, dates of and findings at contralateral risk-reducing mastectomy and salpingo-oophorectomy, and dates of disease recurrence and death or date of last follow-up if no event occurred.

Statistical analysis

The primary endpoint was the development of CBC defined as the occurrence of carcinoma in situ or invasive BC in the contralateral breast at least three months after primary BC diagnosis and no signs of metastatic disease. CBC diagnosis within three months was considered as synchronous bilateral BC and assumed to be unrelated to the delivery of radiotherapy for the first BC.[3-5] For this reason, patients with less than three months of follow-up were excluded.

For comparisons of patient, tumor and treatment characteristics between subgroups we used Pearson's Chi-square tests. Differences in age at primary BC diagnosis and follow-up time were analyzed using the Wilcoxon rank-sum test (Mann-Whitney).

In the Cox analyses we applied left truncation of analysis time and so considered outcome data from prospective follow-up only. Hereby we aimed to correct for potential selection bias, possibly arising due to inclusion of patients undergoing genetic testing after primary BC or CBC diagnosis.[17,18] Censoring events were: ipsilateral BC recurrence for which radiotherapy or systemic therapy was applied, distant metastasis, contralateral risk-reducing mastectomy, other (non-breast) invasive cancer for which radiotherapy or systemic therapy was applied, death and loss to follow-up.

We estimated hazard ratios (HR) and 95% confidence intervals (CI) for radiotherapy (after lumpectomy vs. after mastectomy vs. none), adjuvant chemotherapy (yes vs no), adjuvant endocrine therapy (yes vs. no), salpingo-oophorectomy (treated as time dependent variable), age at primary BC and *BRCA* mutation type (*BRCA1* vs. *BRCA2*) using Cox regression in univariable and multivariable analysis. The cumulative 5-, 10- and 15-year risks of CBC were calculated using Kaplan-Meier analysis including only patients who underwent DNA testing for *BRCA1/2* mutation before the diagnosis of CBC, to correct for potential selection bias.

Analyses were performed for the total group, and for patients younger than 40 years at primary BC, as it has been previously reported that younger patients are more susceptible for radiation-induced BC.[3-6]

The proportion of patients undergoing different locoregional treatments over time, including breast conserving treatment and mastectomy with or without radiotherapy, was estimated with a regression line of best fit and 95% CI based on the proportion per year. The same was performed for the proportion of patients undergoing contralateral prophylactic mastectomy over time. For statistical analysis STATA, version 13.0, was used. For computing the figures R, version 3.2.2 (released on 2015-08-14) and the package GGplot version 1.0.1. were used.

RESULTS

A total of 691 *BRCA1/2*-associated BC patients, consisting of 517 *BRCA1* and 174 *BRCA2* mutation carriers, were eligible for data-analysis (Table 1 and 2). Median time of follow-up of the entire cohort was 8.6 years with a range from 0.3 to 34.3 years. A total of 439 patients were treated with radiotherapy either after lumpectomy (n=349) or after mastectomy (n=85). A total of 325 patients were younger than 40 years at primary BC diagnosis (Table 2). Further details on patient, tumor and treatment characteristics are presented in Table 1 and 2.

Of all patients, 161 (23%) developed CBC, of whom 87 were younger than 40 years at BC onset. The cumulative 5-, 10-, and 15-year risks of CBC for the total cohort were 8%, 19% and 32%, respectively. Among the patients younger than 40 years the cumulative 5-, 10- and 15-year CBC risks were 11%, 32% and 40%, respectively. Cumulative risks for age and *BRCA*-specific subgroups suggest a higher cumulative risk for *BRCA1*-associated patients compared to *BRCA2*-associated patients (Table 3). Median time interval between primary BC and CBC was 4.8 years [range 0.5-29.0] for the entire cohort, and 5.5 years [range 0.5-29.0 years] for patients diagnosed before the age of 40.

Table 1. Characteristics of the patients, radiotherapy vs. no radiotherapy

	Total (*N*=691)*	**RT after lumpectomy** (*N* =349)	**No RT after mastectomy** (*N*=252)	**RT after mastectomy** (*N*=85)	**p-value**
	N(%)	*N(%)*	*N(%)*	*N(%)*	
Age at primary BC					0.943
< 30 years	55 (8.0)	29 (8.3)	19 (7.5)	7 (8.2)	
30-34 years	115 (16.6)	59 (16.9)	39 (15.5)	15 (17.0)	
35-39 years	155 (22.4)	78 (22.3)	57 (22.6)	20 (23.5)	
40-44 years	129 (18.7)	64 (18.3)	49 (19.4)	16 (18.8)	
45-50 years	100 (14.5)	48 (13.8	35 (13.9)	16 (18.8)	
> 50 years	137 (19.8)	71 (20.3)	53 (21.0)	11 (12.9)	
Mutation status					<0.001
BRCA1	517 (74.8)	277 (79.4)	186 (73.8)	50 (58.8)	
BRCA2	174 (25.2)	72 (20.6)	66 (26.2)	35 (41.2)	
Period of primary BC					0.017
1980-1989	105 (15.2)	64 (18.3)	27 (10.7)	14 (16.5)	
1990-1999	256 (37.1)	139 (39.8)	101 (35.3)	27 (31.8)	
2000-2013	330 (47.8)	146 (41.8)	164 (54.0)	44 (51.8)	
Tumor stage					<0.001
Tis	26 (4.0)	14 (4.1)	12 (5.2)	0	
T1	364 (56.0)	209 (61.8)	130 (56.5)	25 (30.9)	
T2	227 (34.9)	114 (33.7)	80 (34.8)	32 (39.5)	
T3	25 (3.9)	0	7 (3.0)	18 (22.2)	
T4	8 (1.2)	1 (0.3)	1 (0.4)	6 (7.4)	
Unknown	41	11	22	4	
Nodal status					<0.001
N0	424 (64.3)	241 (71.9)	169 (70.1)	13 (16.0)	
N1-3	235 (35.7)	94 (28.1)	72 (29.9)	68 (84.0)	
Unknown	32	14	11	4	
Histological grade					0.988
Grade 1	17 (3.3)	8 (3.1)	7 (3.6)	2 (3.0)	
Grade 2	106 (20.4)	54 (21.0)	37 (19.2)	14 (20.9)	
Grade 3	396 (76.3)	195 (75.9)	149 (77.2)	51 (76.1)	
unknown	172	92	59	18	
Hormone receptor status					0.124
Positive	227 (39.5)	108 (37.8)	80 (37.9)	39 (50.0)	
Negative	348 (60.5)	178 (62.2)	131 (62.1)	39 (50.0)	
Unknown	116	63	41	7	

Table 1. Continued.

	Total (*N*=691)*	RT after lumpectomy (*N*=349)	No RT after mastectomy (*N*=252)	RT after mastectomy (*N*=85)	p-value
	N(%)	*N(%)*	*N(%)*	*N(%)*	
HER2 status					0.646
Positive	17 (6.7)	9 (8.1)	5 (5.2)	3 (7.5)	
Negative	236 (93.3)	101 (91.8)	95 (94.8)	37 (92.5)	
Unknown	438	239	152	45	
(Contralateral) risk-reducing mastectomy					<0.001
No	424 (64.5)	243 (73.0)	127 (51.8)	54 (68.4)	
Yes	233 (35.5)	90 (27.0)	118 (46.2)	25 (31.7)	
Unknown	34	16	7	6	
Salpingo-oophorectomy					0.499
No	259 (41.2)	135 (42.5)	87 (38.2)	35 (44.3)	
Yes	370 (58.8)	183 (57.5)	141 (61.8)	44 (55.7)	
Unknown	62	31	24	6	
(Neo-) adjuvant chemotherapy					0.022
No	319 (46.6)	176 (51.0)	109 (43.6)	30 (35.7)	
Yes	365 (53.4)	169 (49.0)	141 (56.4)	54 (64.3)	
Unknown	7	4	2	1	
Adjuvant endocrine therapy					<0.001
No	555 (81.1)	300 (87.2)	203 (81.2)	48 (56.5)	
Yes	129 (18.9)	44 (12.8)	47 (18.9)	37 (43.5)	
Unknown	7	5	2	0	

RT: radiotherapy; BC: breast cancer.
* Data on type of surgery (either lumpectomy or mastectomy) was missing in 5 patients who were treated with radiotherapy.

Table 2. Characteristics of the patients with age at primary breast cancer diagnose <40 years, radiotherapy vs. no radiotherapy

	Total (*N*=325)*	RT after lumpectomy (*N*=166)	No RT after mastectomy (*N*=115)	RT after mastectomy (*N*=42)	p-value
	N(%)	*N(%)*	*N(%)*	*N(%)*	
Age at primary BC					0.996
< 30 years	55 (16.9)	29 (17.5)	19 (16.5)	7 (16.7)	
30-34 years	115 (35.4)	59 (35.5)	39 (33.9)	15 (35.7)	
35-39 years	155 (47.7)	78 (47.0)	57 (49.6)	20 (47.6)	
Mutation status					0.004
BRCA1	261 (80.3)	143 (86.1)	89 (77.4)	27 (64.3)	
BRCA2	64 (19.7)	23 (13.9)	26 (22.6)	15 (35.7)	
Period of primary BC					<0.001
1980-1989	43 (13.2)	33 (19.9)	5 (4.4)	5 (11.9)	
1990-1999	114 (35.1)	68 (41.0)	35 (30.4)	10 (23.8)	
2000-2013	168 (51.7)	65 (39.2)	75 (65.2)	27 (64.3)	
Tumor stage					<0.001
Tis	9 (2.9)	4 (2.6)	5 (4.5)	0	
T1	179 (58.5)	95 (60.5)	70 (63.6)	14 (35.9)	
T2	103 (33.7)	57 (36.3)	31 (28.2)	15 (38.5)	
T3	8 (2.6)	0	3 (2.7)	5 (12.8)	
T4	7 (2.3)	1 (0.6)	1 (0.9)	5 (12.8)	
Unknown	19	9	5	3	
Nodal status					<0.001
N0	206 (66.0)	120 (74.5)	78 (70.3)	7 (17.9)	
N1-3	106 (34.0)	41 (25.5)	33 (29.7)	32 (82.1)	
Unknown	13	5	4	3	
Histological grade					0.561
Grade 1	6 (2.5)	2 (1.7)	2 (2.1)	2 (6.5)	
Grade 2	45 (18.4)	21 (17.7)	17 (18.1)	7 (22.6)	
Grade 3	193 (79.1)	96 (80.7)	75 (79.8)	22 (71.0)	
Unknown	81	47	21	11	
Hormone receptor status					0.020
Positive	93 (33.1)	41 (29.5)	31 (30.7)	21 (52.5)	
Negative	188 (66.9)	98 (70.5)	70 (69.3)	19 (47.5)	
Unknown	44	27	14	2	
HER2 status					0.592
Positive	10 (7.6)	4 (7.8)	3 (5.5)	3 (12.0)	
Negative	122 (92.4)	47 (92.2)	52 (94.5)	22 (88.0)	
Unknown	193	115	60	17	

Table 2. Continued.

	Total (*N*=325)*	RT after lumpectomy (*N*=166)	No RT after mastectomy (*N*=115)	RT after mastectomy (*N*=42)	p-value
	N(%)	*N(%)*	*N(%)*	*N(%)*	
(Neo-) adjuvant chemotherapy					0.019
No	125 (38.9)	75 (45.7)	33 (28.9)	16 (39.0)	
Yes	196 (61.1)	89 (54.3)	81 (71.1)	25 (61.0)	
Unknown	4	2	1	1	
Adjuvant endocrine therapy					<0.001
No	262 (81.4)	148 (90.2)	90 (78.9)	22 (52.4)	
Yes	60 (18.6)	16 (9.8)	24 (21.1)	20 (47.6)	
Unknown	3	0	1	0	
Contralateral risk-reducing mastectomy					<0.001
No	174 (55.8)	105 (66.0)	46 (41.1)	23 (56.1)	
Yes	138 (44.2)	54 (34.0)	66 (58.9)	18 (43.9)	
Unknown	13	7	3	1	
Salpingo-oophorectomy					0.825
No	128 (42.8)	66 (43.7)	43 (40.6)	18 (45.0)	
Yes	171 (57.2)	85 (56.3)	63 (59.4)	22 (55.0)	
Unknown	26	15	9	2	

RT: radiotherapy; BC: breast cancer.
* Data on type of surgery (either lumpectomy or mastectomy) was missing in 2 patients who were treated with radiotherapy.

Table 3. Cumulative 5- 10- and 15-year risks of contralateral breast cancer

Years after diagnosis	Overall	*BRCA1* mutation	*BRCA2* mutation	Age <40	Age ≥40
	%(*N* at risk)	%(*N* at risk)	%(*N* at risk)	%(*N* at risk)	%(*N* at risk)
5	8 (198)	9 (140)	5 (58)	11 (86)	6 (112)
10	19 (98)	21 (75)	15 (23)	32 (39)	10 (59)
15	32 (47)	35 (37)	15 (10)	40 (17)	23 (30)

Cumulative 5- 10- and 15-year risks of contralateral breast cancer in different subgroups of breast cancer patients (*BRCA1* mutation carriers vs. *BRCA2* mutation carriers and age at primary breast cancer <40 years vs. ≥40 years). Only those patients who underwent DNA testing for *BRCA1/2* mutation before the diagnosis of contralateral breast cancer were included.

Left truncation was applied to correct for survival bias that may occur in studies with patient recruitment at a variable time after diagnosis (see statistical analysis). Consequently, a considerable number of patients did not contribute person-time to the prospective follow-up, leaving 418 patients for the main analyses. In univariable analysis the risk of CBC was increased in patients younger than 40 years compared to those older than 40 years at primary BC (HR 2.42, 95% CI 1.34-4.38). Furthermore, mutation carriership of *BRCA1* was associated with increased risk of CBC as compared to *BRCA2* mutation carriership (HR 2.32, 95% CI 0.98-5.51). Both chemotherapy and endocrine therapy were significantly associated with a decreased risk of CBC (HR 0.45, 95% CI 0.25-0.81 and HR 0.27, 95% CI 0.08-0.86, respectively). For salpingo-oophorectomy no association with CBC risk was found (HR 0.73, 95% CI 0.37-1.43) (Table 4).

No deleterious effect of radiotherapy for primary BC, either after lumpectomy or after mastectomy, on CBC risk was found for the entire population (HR 0.84, 95% CI 0.46-1.55 and HR 0.62, 95% CI 0.17-2.23, respectively) (Table 4) Adjusting for age, adjuvant chemotherapy, adjuvant endocrine therapy and type of *BRCA* mutation in a multivariable analysis still showed no association of radiotherapy on CBC risk (HR 0.74, 95% CI 0.40-1.37 and HR 0.96, 95% CI 0.23-3.97, respectively).

Subgroup analyses of patient younger than 40 years at BC onset

Also in the subgroup of patients younger than 40 years at primary BC diagnosis no effect of radiotherapy for primary BC, either after lumpectomy or after mastectomy, on CBC risk was found in univariable analysis (n=211; HR 1.41, 95% CI 0.62-3.23 and HR 0.94, 95% CI 0.18-4.86, respectively) and this was maintained in multivariable analysis (HR 1.53, 95% CI 0.22-10.51 and HR 0.97, 95% CI 0.41-2.30, respectively) (Figure 1 and Table 4). Median time interval between primary BC and CBC diagnoses was not significantly different between those treated with radiotherapy for primary BC compared to those patients not receiving radiotherapy (5.5 vs. 4.9 years, p=0.88).

Table 4. Univariable and multivariable hazard ratios for risk of contralateral breast cancer associated with selected factors

	Overall	Age <40 years	
	Univariable analyses	**Univariable analyses**	**Multivariable analysis***
	N=418 Person years: 1105 years	*N*=211 Person years: 467 years	*N*=211 Person years: 467 years
	HR (95% CI)	HR (95% CI)	HR (95% CI)
Age at primary breast cancer <40 years ≥40 years	2.42 (1.34-4.38) 1		
Age at primary breast cancer Continuous	0.94 (0.90-0.97)	0.93 (0.85-1.01)	0.96 (0.88-1.06)
***BRCA* mutation** *BRCA1* *BRCA2*	2.32 (0.98-5.51) 1	3.52 (0.83-14.99) 1	2.33 (0.51-10.73) 1
Chemotherapy No Yes	1 **0.45 (0.25-0.81)**	1 0.51 (0.24-1.09)	1 0.52 (0.24-1.14)
Endocrine therapy No Yes	1 **0.27 (0.08-0.86)**	1 0.24 (0.06-1.02)	1 0.25 (0.05-1.23)
Salpingo-oophorectomy (time dependent) No Yes	1 0.73 (0.37-1.43)	1 1.22 (0.53-2.81)	
Radiotherapy No radiotherapy after mastectomy Radiotherapy after mastectomy Radiotherapy after lumpectomy	1 0.62 (0.17-2.23) 0.84 (0.46-1.55)	1 0.94 (0.18-4.86) 1.41 (0.62-3.23)	1 0.97 (0.41-2.30) 1.53 (0.22-10.51)

HR: Hazard ratio.

* The following variables were incorporated in the multivariable model: age at primary breast cancer (continuous variable), type of *BRCA* mutation (*BRCA1* vs. *BRCA2*), adjuvant chemotherapy (yes vs. no), adjuvant endocrine therapy (yes vs. no) and radiotherapy (no radiotherapy after mastectomy vs. radiotherapy after mastectomy and vs. radiotherapy after lumpectomy).

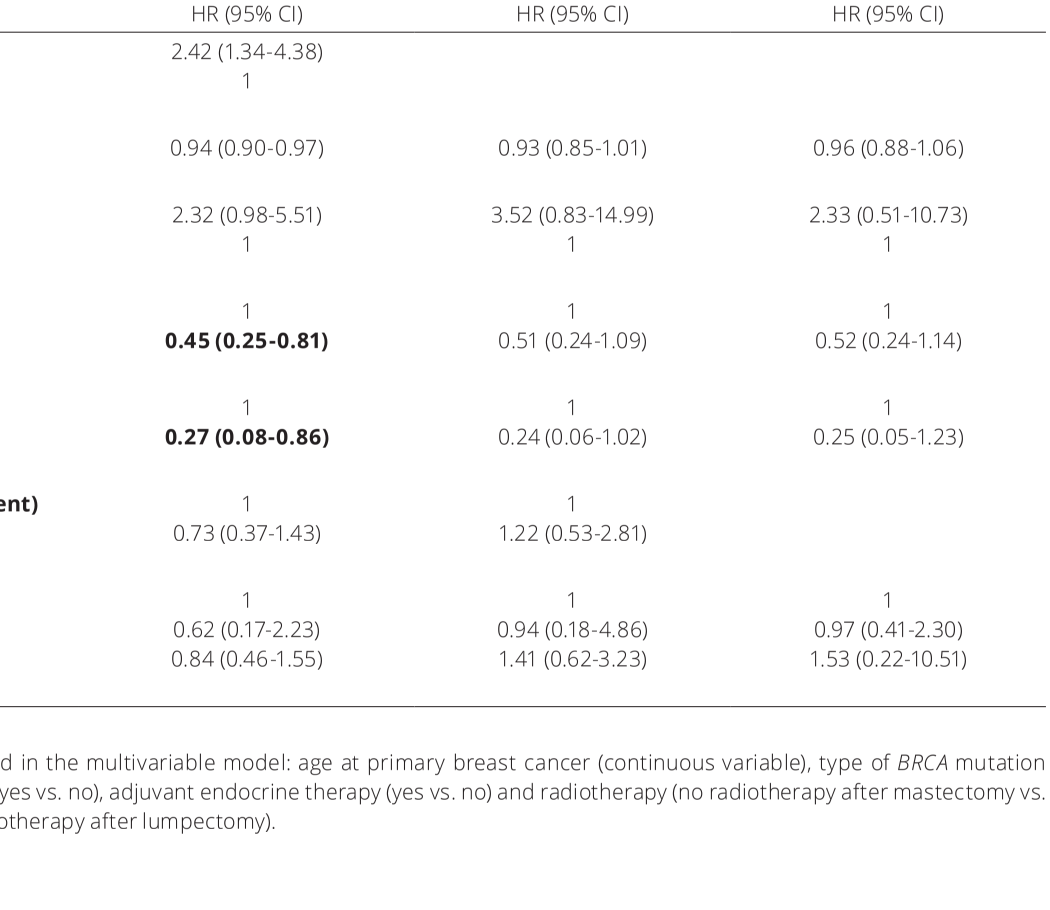

Figure 1. Kaplan Meier estimates of the contralateral breast cancer (CBC) risk in *BRCA1/2* mutation carriers, younger than 40 years of age at primary BC diagnosis

For this analysis left truncation of analysis time at the DNA test date was applied, to correct for survival bias. Patients treated with radiotherapy (either after lumpectomy or after mastectomy) were compared to patients not treated with radiotherapy at primary BC diagnosis.

During follow-up, the number of patients at risk substantially decreased because a large proportion of patients were censored as they underwent a contralateral risk-reducing mastectomy, developed a BC recurrence or a second non-breast malignancy. In the group younger than 40 years at BC onset, 165 of 325 patients (51%) were censored in the first 10 years of follow-up because of these three reasons (Figure 2). Furthermore, since a large proportion of patients had less than 10 years of follow-up time, only 29 and 14 patients were available for the prospective analyses after 10 and 15 years of follow-up in this age group, respectively.

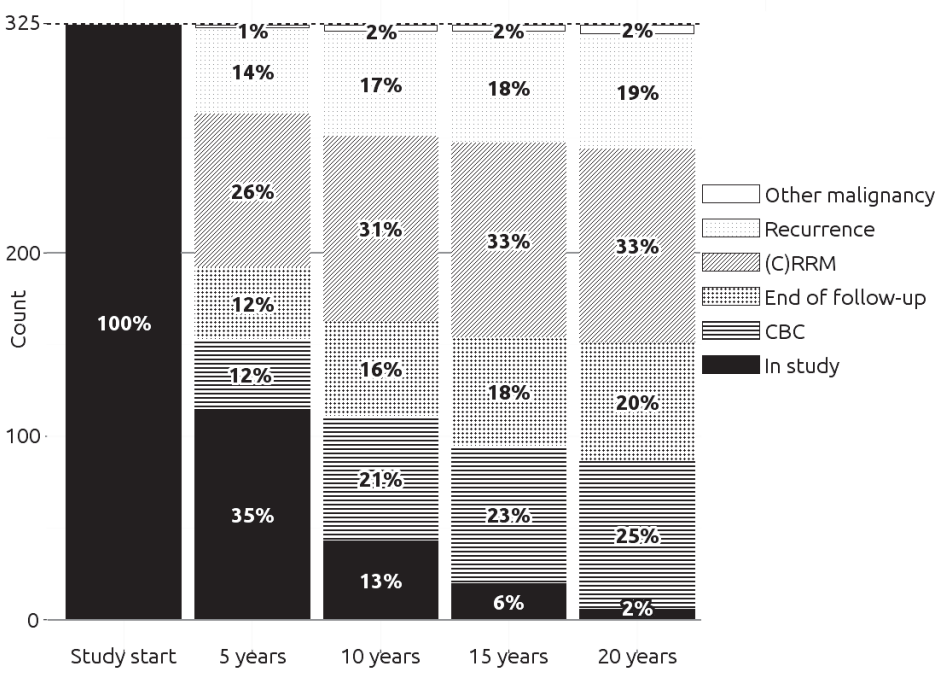

Figure 2. Cumulative frequency of contralateral breast cancer (CBC) or reasons for censoring event at study start and after 5, 10, 15 and 20 years of follow-up in all included patients who were younger than 40 years of age at primary breast cancer diagnosis

Recurrence includes both ipsilateral recurrence, a second ipsilateral primary tumor and metastatic disease. (C)RRM: (contralateral) risk-reducing mastectomy. End of FU (: follow-up) comprises patients who did not reach the primary endpoint or other censoring event at data cut-off or were lost to follow-up.

Treatment choices over time

Over the past decades, the proportion of patients at risk for radiation-induced CBC changed substantially as a result of an increased rate of mastectomy without radiotherapy instead of breast conserving therapy for primary breast cancer, and an increased rate of contralateral risk-reducing mastectomy (Figures 3 and 4). For example, patients aged younger than 40 years at diagnosis more often opted for mastectomy without radiotherapy instead of breast conserving therapy in 2010 (reaching 50%), compared to less than 30% in 1995. The proportion of patients receiving radiotherapy following mastectomy was relatively stable over time being around 10-15% (Figure 3). Since 2010, more than 60% of patients younger than 40 years at primary diagnosis opted for contralateral risk-reducing mastectomy, after primary breast cancer treatment, which was less than 40% in 1995 (Figure 4).

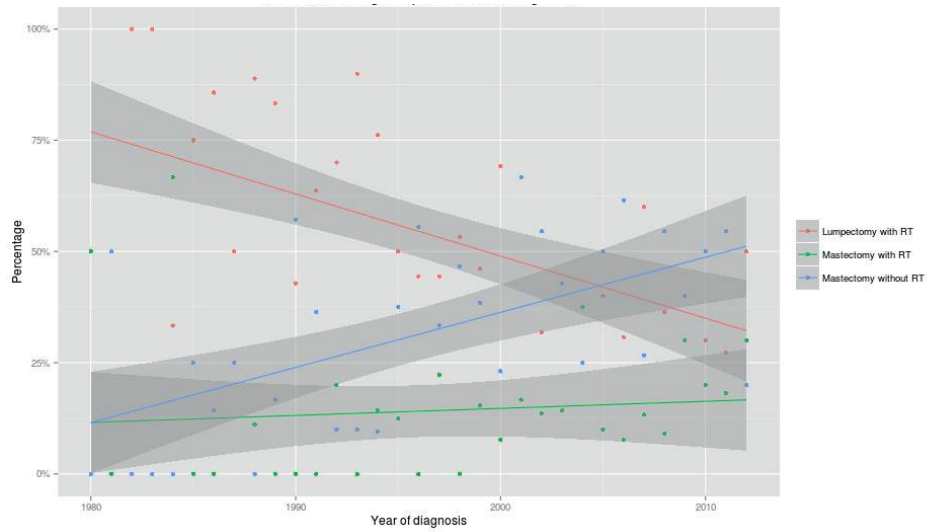

Figure 3. Distribution of the choice of local therapy at primary breast cancer diagnosis by year of diagnosis among patients younger than 40 years of age with a *BRCA1* or *BRCA2* mutation

Regression line of best fit (red, blue and green lines) and estimate of 95% confidence interval (gray). RT: Radiotherapy.

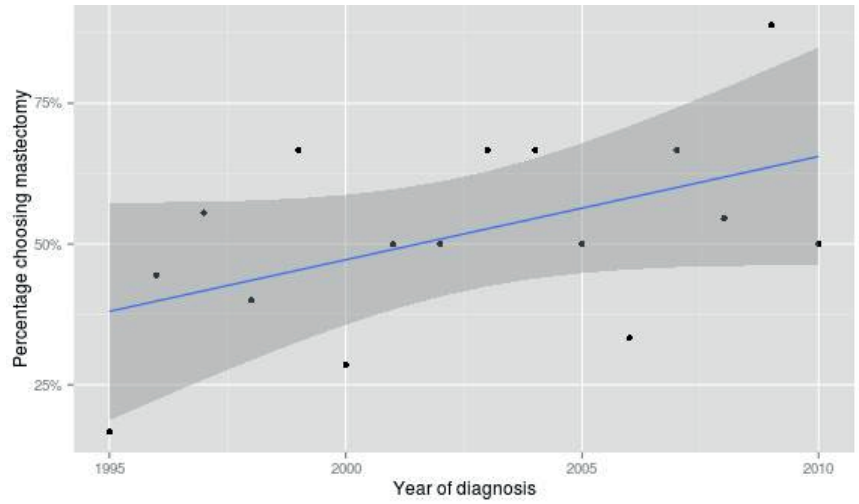

Figure 4. Proportion of patients with a *BRCA1* or *BRCA2* mutation and breast cancer diagnosis below the age of 40 opting for contralateral (or bilateral) risk-reducing mastectomy (either at primary breast cancer treatment or within the years after primary breast cancer) by year of breast cancer diagnosis.

Regression line of best fit (blue) and estimate of 95% confidence interval (gray).

DISCUSSION AND CONCLUSION

The risk of CBC among BC patients with a *BRCA1/2* mutation is high, especially for younger patients. An association between adjuvant radiotherapy and the development of CBC in *BRCA1/2*-associated BC patients was not observed, neither in the entire cohort, nor in the subgroup of patients younger than 40 years at primary diagnosis. We found in this study that during follow-up the number of patients at risk for developing CBC substantially decreased due to either contralateral risk-reducing mastectomy or BC recurrence (26% and 14%, respectively, within the first 5 years after primary BC among patients younger than 40 years). As a consequence, the number of patients at risk after 10 and 15 years of follow-up was too small to definitively exclude harmful effects of radiotherapy on the development of CBC among young *BRCA1/2* mutation carriers.

A few other studies also reported on CBC risk in *BRCA1/2*-associated BC patients treated with adjuvant radiotherapy compared to patients not treated with radiotherapy[13-15], and did not find an increased risk of CBC associated with adjuvant radiotherapy either. In the two multi-center retrospective cohort studies of breast cancer patients attending high-risk clinics[13,14] the numbers of young *BRCA1/2* mutation carriers and follow-up periods were comparable to our study (145 out of 655 patients younger than 35 years with a median follow-up of 8 years in the study of Pierce et al.[13], and 357 out of 810 patients younger than 40 years with a median follow-up of 11 years in the study of Metcalfe et al.[14] However, subgroup analyses among these younger patients were not reported. Bernstein performed a nested case-control study within the WECARE study (Women's Environmental Cancer and Radiation Epidemiology Study), which is a population-based study of patients with metachronous CBC[15], but again no results of subgroup analysis in younger patients were shown.

The main limitation of our study regarding the impact of radiotherapy on the CBC risk is the small number of patients at risk for CBC after 10-15 years of follow-up, as studies including sporadic patients suggest that a minimal latency period of 10-15 years is needed to develop radiation-induced BC.[19,20] It is, however, not known whether the latency period between exposure and development of a radiation-induced malignancy is similar for *BRCA1/2* mutation carriers compared to sporadic patients. Even, if the latency period in *BRCA1/2* mutation carriers is shorter, the number of patients at risk for CBC in our study group was too small to make definitive conclusions, especially since a large proportion of patients were already censored in the first 5 years. Given the number of events in patients younger than 40 years at primary BC diagnosis our study had 80% power to find a HR of at least 2.8 for adjuvant radiotherapy to be associated with increased risk of CBC.

In our total cohort the 10-year cumulative risk of CBC in *BRCA1/2* mutation carriers was 19%, while in the subgroup of patients younger than 40 years at BC onset this risk was 32%. These risks are comparable to the risks reported in other studies.[14,21,22] Furthermore, the CBC risk was higher in *BRCA1* compared to *BRCA2* mutation carriers. Both the increased risk in younger patients and the increased risk in *BRCA1*- compared to *BRCA2*-associated BC patients have been described in other studies.[14,21-23] Additionally, in our cohort adjuvant systemic therapy for primary BC, applying for both endocrine therapy and chemotherapy, was associated with a decreased risk of CBC. This effect, however, was only significant in the entire cohort and not in the subgroup of younger patients. Since the HR's were similar, this might be due to lack of statistical power. The risk-reductive effect of adjuvant endocrine therapy on CBC risk in *BRCA1/2* mutation carriers has been reported in previous studies.[14,24,25] Regarding chemotherapy, three studies have investigated the association between chemotherapy and CBC[14,23,26], whereby only Reding et al. found a significant association with a relative risk of 0.5. Although this latter association is biologically not totally clear, further research is certainly warranted. We did not find any impact of salpingo-oophorectomy on CBC risk, which is in contrast with previous reports[27,28], but is in line with more recent literature.[29]

In our cohort, we found a growing preference over time for mastectomy without radiotherapy instead of breast conserving therapy including radiotherapy. At the same time, the rate of contralateral risk-reducing mastectomy after primary breast cancer treatment has increased. Important reasons for the shift towards ablative breast surgery might be the improvements in and availability of (direct) breast reconstructive options, the increased awareness of the magnitude of the CBC risk and distress of screening, and the wish to avoid another treatment session for a second primary BC. Finally, the important findings of Heemskerk et al. showing that contralateral risk-reducing mastectomy improves survival, mainly in younger patients and those with favorable primary tumor characteristics[30] might lead to an even larger proportion of younger patients opting for mastectomy without

radiotherapy and contralateral risk-reducing mastectomy after primary breast cancer diagnosis in the nearby future.

These trends in locoregional treatments eventually decreased the proportion of patients at risk for radiation-induced CBC over the past few decades. Nevertheless, the question whether adjuvant radiotherapy has deleterious effect on CBC risk still remains clinically important for a significant number of patients, who want to conserve their (ipsilateral and) contralateral breast. Moreover, in the nearby future a larger proportion of patients potentially might opt for breast conserving treatment and abstain from contralateral risk-reducing mastectomy, due to an increased use of endocrine therapy as chemoprevention, improved diagnostic imaging techniques for screening and improved effectiveness of adjuvant systemic therapy (for example in combination with PARP inhibitors).[31-33]

In the current study we could not find an association between radiotherapy for primary BC and risk of CBC in (young) *BRCA1/2* mutation carriers compared to sporadic patients, however the number of patients at risk after 10 and 15 years of follow-up were too small to definitively exclude harmful effects of adjuvant radiotherapy. An increase in the percentage of young patients with *BRCA1/2* associated breast cancer choosing for conserving their (ipsilateral and) contralateral breast in not unlikely. Therefore, future research in larger study populations with minimal follow-up of 10 years is needed to achieve a better understanding of the true effect of radiotherapy on the CBC risk in *BRCA1/2*-associated BC patients. This will only be possible by combining study populations through collaborative efforts on a national, or even international level.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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CHAPTER 6

CONTRALATERAL BREAST CANCER RISK IN IRRADIATED BREAST CANCER PATIENTS WITH A GERMLINE-*BRCA1/2* PATHOGENIC VARIANT

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ABSTRACT

Background: Radiation-induced secondary breast cancer may be a concern after radiotherapy for primary breast cancer (PBC), especially in young germline (g)*BRCA*-associated breast cancer patients with already high baseline contralateral breast cancer (CBC) risk and potentially increased genetic susceptibility to radiation.

Aim: To investigate whether adjuvant radiotherapy for PBC increases the risk of CBC in g*BRCA1/2*-associated BC patients.

Methods: g*BRCA1/2* pathogenic variant carriers diagnosed with PBC were selected from the prospective International *BRCA1/2* Carrier Cohort Study. We used multivariable Cox proportional hazards models to investigate the association between radiotherapy (yes versus no) and CBC risk. We further stratified for *BRCA* status and PBC age (<40 and ≥40 years).

Results: Of 3,602 eligible patients, 2,297 (64%) received adjuvant radiotherapy. Median follow-up was 9.6 years. Patients in the radiotherapy group had more stage III PBC compared to the non-radiotherapy group (15% versus 3%, p<0.001), received more often chemotherapy (81% vs. 70%, p<0.001) and endocrine therapy (50% vs. 35%, p<0.001). The radiotherapy group had an increased risk of CBC compared to the group without radiotherapy (adjusted HR: 1.44, 95% CI: 1.12-1.86), although less pronounced in g*BRCA1* (HR: 1.29, 95% CI:0.93-1.77) than in g*BRCA2* pathogenic variant carriers (HR: 1.77, 95% CI: 1.13-2.77). In the combined *gBRCA1/2* group, patients irradiated below and above age 40 at PBC diagnosis showed a similar risk increase (HR: 1.38, 95% CI: 0.93-2.04 and HR: 1.56, 95% CI: 1.11-2.19, respectively).

Discussion/conclusion: Tailored radiotherapy regimens minimizing contralateral breast dose should be considered in g*BRCA1/2* pathogenic variant carriers.

INTRODUCTION

Breast cancer (BC) is the most common type of cancer diagnosed in women worldwide, affecting about one in seven women in industrialized countries at some point during their lifetime.[1,2] Radiotherapy is an important part of treatment, especially in the context of breast conserving therapy of invasive PBC and treatment of ductal carcinoma in situ (DCIS). Meta-analyses of randomized clinical trials have demonstrated a clear survival benefit of radiotherapy in treating BC in the general population, after both radical mastectomy and breast conserving surgery.[3,4] The prognosis of BC patients used to depend mostly on successful (local) treatment of the primary breast cancer (PBC). However, as treatments and consequently survival continue to improve, long-term effects of therapy are becoming more important. Some of the main long-term concerns of radiotherapy are the adverse effects on the heart and the risk of secondary cancer of the lung or contralateral breast.[3]

Radiotherapy utilizes ionizing radiation to achieve anti-tumor effects. Ionizing radiation induces varying types and degrees of DNA damage, but the double strand DNA breaks (DSBs), especially when clustered with other types of damage, are the most consequential in both carcinogenesis and cell death.[5] These DSBs are primarily repaired by homologous recombination, a process in which the BRCA1 and BRCA2 proteins play an essential role.[6] When homologous recombination, which is almost always error-free[6,7], is impaired, error-prone methods of DNA repair are used instead.[8] This increases the likelihood of mutations and ultimately the development of cancer.[9-11] Breast radiotherapy can lead to incidental radiation dose exposure of the contralateral breast (due to the proximity of the breast radiotherapy field/treatment volume, a concept illustrated in Figure 1).[12] This may be sufficient to increase risk of contralateral breast cancer (CBC) in BC patients.[13]

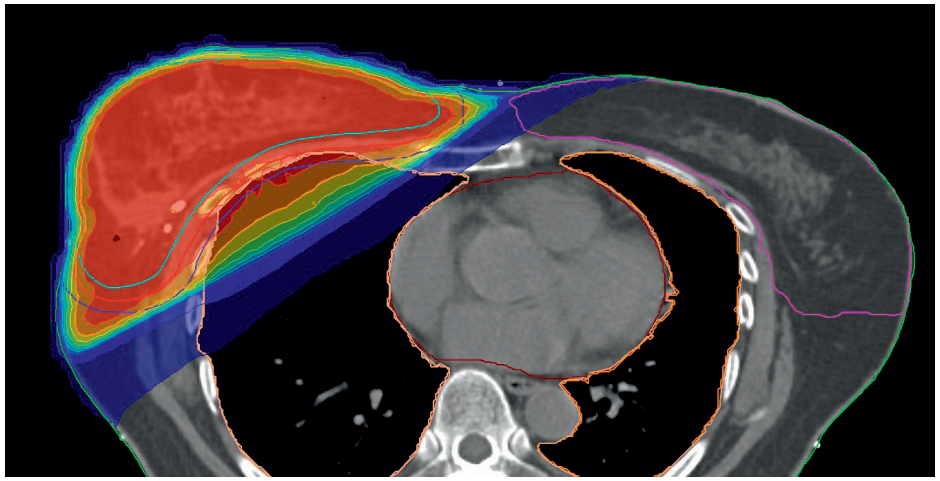


Figure 1. Example of a 3D-CRT-planning, showing 1-5% of the total prescribed dosage (dark blue area) on the contralateral breast as a result of the breast anatomy and/or tumor localization (more likely if medial).

Several breast cancer susceptibility genes have been identified which explain approximately 25% of the familial aggregation of breast cancer.[14,15] The most prominent of these are the genes encoding the aforementioned BRCA1 and BRCA2 proteins. Additionally, women carrying a pathogenic germline variant in the *BRCA1/2* genes are often younger at the time of diagnosis than women with non-hereditary BC, especially g*BRCA1* pathogenic variant carriers.[16] Breast tissue of young premenopausal women has greater density and is more actively proliferating than that of older women. In addition, breast tissue is less differentiated in nulliparous women.[17,18] These factors potentially increase vulnerability to DNA damaging agents, providing additional reasons for why this particular group could be at greater risk of CBC after radiotherapy. Moreover, the already high baseline CBC risk in g*BRCA1/2* pathogenic variant carriers further stresses the importance of identifying risk-increasing factors such as breast irradiation. Then, the risks and benefits can be weighted in clinical decision-making and preventive measures (e.g., intensive screening, prophylactic surgery, lifestyle intervention, radiotherapy techniques further minimizing mean heart or lung dose, and/or contralateral breast dose) may be taken.

However, current evidence from observational studies is inconclusive, and large studies with sufficient follow-up are lacking.[19] In order to obtain more robust evidence, we here make use of a large population from an international

collaboration. The primary research question we aim to answer is whether CBC risk in *gBRCA1/2* pathogenic variant carriers is increased after radiotherapy compared to no radiotherapy. We further investigated whether having a young age at PBC diagnosis was associated with an increased effect of radiotherapy on CBC risk. Because *BRCA1* and *BRCA2* pathogenic germline variants have distinct functional effects, we also evaluated the effects of radiotherapy separately within these groups.[20-22]

METHODS

Study population

For this study, we used data from the prospective International *BRCA1/2* Carrier Cohort Study (IBCCS). The IBCCS is described by Goldgar et al., 2000.[23] In summary, proven *gBRCA1* and g*BRCA2* pathogenic variant carriers from thirteen European countries, Australia and Canada, were eligible for inclusion, either with a history of any cancer or unaffected at time of recruitment. Other requirements were being at least 18 years of age and informed consent given for participation in a longitudinal study. Eligible subjects either entered the cohort through a hereditary cancer clinic or via previous participation in a hereditary cancer study. Upon study entry, an IBCCS-standardized questionnaire was filled out and repeated during follow-up at regular intervals, depending on country. Data on any cancer incidence, tumor characteristics and treatments were collected through the respective national/regional cancer registries and/or pathology reports. To ensure sufficient power per individual study, we only included studies with at least 10 incident CBCs.

The IBCCS includes data from Hereditary Breast and Ovarian cancer research Netherlands (HEBON).[24] For this particular study, we had a more recently updated version (i.e., more complete follow-up and larger sample size) of the data available from the HEBON database.

Medical Ethics Committee approval was obtained for all participating centers. Written informed consent was obtained from each individual participant, or from a close relative or proxy for deceased individuals.

We included patients with a proven deleterious *gBRCA1* or g*BRCA2* pathogenic variant. Further requirements for current study inclusion were a diagnosis of either in situ or invasive stage I-III PBC, diagnosed between 1990 and 2018, and without diagnosis of another cancer before PBC diagnosis. Latest follow-up was available until 2019. The flow diagram in Figure 2 provides a complete overview of the inclusion and exclusion process.

Data collection

We retrieved dates of BC diagnosis, DNA test result, birth and death, as well as information on g*BRCA* pathogenic variant, and tumor type (i.e., in situ or invasive), size and grade, lymph node status, presence of distant metastasis, estrogen receptor (ER) status, progesterone receptor (PR) status, HER2-status. Further, we collected data on type of surgery, chemotherapy, radiotherapy, endocrine therapy, HER2-targeted therapy, risk-reducing salpingo-oophorectomy, and risk-reducing mastectomy (either bilateral or contralateral). All tumor characteristics were histologically determined.

Statistical analysis

The endpoint of our study was the occurrence of a metachronous CBC, which was defined as a secondary invasive or non-invasive tumor in the contralateral breast diagnosed at least three months after PBC diagnosis. We considered a CBC within 3 months to be synchronous. Patients were therefore considered to be at risk for CBC starting from 3 months after PBC diagnosis.

To avoid cancer-induced testing bias – a serious pitfall in studies using cohorts of *gBRCA* pathogenic variant carriers – we applied left-truncation in the analyses and started the observation period either at the date of DNA test result or of PBC, whichever came last.[25] As a result, anyone with a CBC or censoring event before this moment will be left-censored and therefore excluded from analysis.

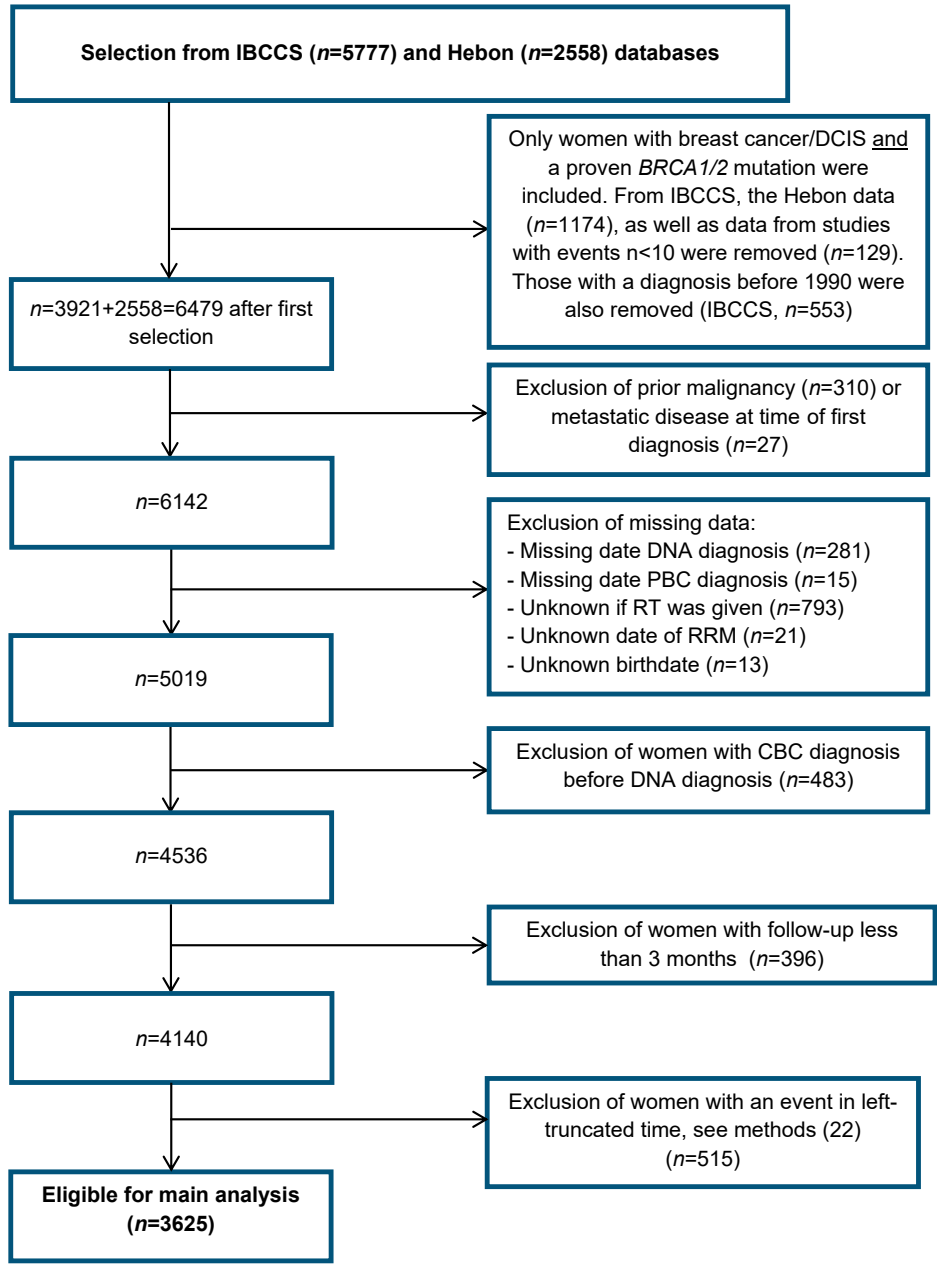


Figure 2. Flow diagram of patient inclusion

DCIS: Ductal carcinoma in situ; PBC: Primary Breast Cancer; IBCCS: International *BRCA1/2* Carrier Cohort Study; RRM: Risk-Reducing Mastectomy; CBC: Contralateral Breast Cancer.

We compared baseline characteristics between radiotherapy and non-radiotherapy groups. Differences in relative frequencies between these groups were tested for using the Chi-squared test, differences in continuous variables were tested for using the Kruskal-Wallis test.

We first fitted a Cox proportional hazards model (complete-case analysis) for radiotherapy compared to no radiotherapy, to assess the overall effect of radiotherapy for the PBC on CBC risk. We allowed for the baseline hazard to vary by country, to account for variability/heterogeneity between them. We considered age, adjuvant endocrine therapy, chemotherapy, risk reducing salpingo-oophorectomy and stage as potential confounders, based on current knowledge. Additionally, we stratified the analysis for patients aged below and above 40 years at PBC diagnosis. Further, we evaluated the effects separately for g*BRCA1* and g*BRCA2* pathogenic variant carriers. Ipsilateral second BC, any invasive cancer (except non-melanoma skin cancer and cervical intra-epithelial neoplasia), bilateral/contralateral risk-reducing mastectomy and death were considered as censoring events. Patients were also censored when they reached date of last follow-up without an event. The models were tested for interaction between covariables (age, chemotherapy, endocrine therapy, g*BRCA* pathogenic variant) and the main variable of interest (radiotherapy). We tested for satisfaction of the proportional hazard assumption, both graphically and statistically.

All analyses were performed using STATA (versions 16 and 17, StataCorp, college Station TX, USA).

RESULTS

Study population and characteristics

We selected 3,602 eligible PBC patients of whom 2,297 (64%) received radiotherapy. Median follow-up was 9.6 years. Additional patient, tumor and treatment characteristics for the different groups are displayed in Table 1. Overall, patient characteristics were similar (i.e., median age at diagnosis, type of g*BRCA* pathogenic variant). The most notable difference was stage, with 15% diagnosed with stage III PBC in the radiotherapy group, compared to 3% for the non-radiotherapy group (p<0.001). Furthermore, patients in the radiotherapy group received more often chemotherapy than patients in the non-radiotherapy group (81% vs. 70%, p<0.001), and more often endocrine therapy (50% vs. 35%, p<0.001). Similar treatment patterns were observed for g*BRCA1* and g*BRCA2* pathogenic variant carriers separately (Table 1), and for patients under and above the age of 40 at PBC diagnosis (Supplementary Table 1).

Contralateral breast cancer

CBC occurred in 252 patients in the radiotherapy group (with n=180 being invasive) and in 98 patients in the non-radiotherapy group (n=70 invasive). Risk-reducing mastectomy was the main censoring event (with n=784 in the radiotherapy group and n=564 in the non-radiotherapy group). Death was a censoring event in 235 patients in the radiotherapy group and in 95 in the non-radiotherapy group.

Associations between radiotherapy and CBC risk

Risk of invasive and in situ CBC was increased for patients receiving radiotherapy compared to patients without radiotherapy (adjusted hazard ratio (HR): 1.44, 95% CI: 1.12-1.86; Table 2, Figure 3). The risk associated with radiotherapy compared to no radiotherapy was proportional over time, and in both groups CBC risk appears to peak around 5-6 years after PBC diagnosis (data not shown). In g*BRCA2* pathogenic variant carriers, an increased risk of CBC was observed (HR: 1.77, 95% CI: 1.13-2.77). For g*BRCA1* pathogenic variant carriers we found a similar trend (HR: 1.29, 95% CI: 0.93-1.77; p-value for interaction= 0.390).

In patients younger than 40 years of age at PBC diagnosis, the HR for CBC was 1.38 (95% CI: 0.93-2.04) for radiotherapy compared to no radiotherapy. For patients 40 years of age and older, the HR was 1.56 (95% CI: 1.11-2.19; Supplementary Table 2).

The effects of radiotherapy associated with invasive CBC solely (HR: 1.44, 95%CI: 1.05-1.96) were in line with those of the main analysis and for the g*BRCA1* and g*BRCA2* pathogenic variant carriers stratified analyses (HR: 1.36, 95% CI: 0.92-1.99 for g*BRCA1* and HR: 1.63, 95% CI: 0.92-2.89 for g*BRCA2*; p-value for interaction= 0.704; Supplementary Table 3).

No interactions of chemotherapy, endocrine therapy, g*BRC*A pathogenic variant status or age at PBC diagnosis with the main variable of interest were observed.

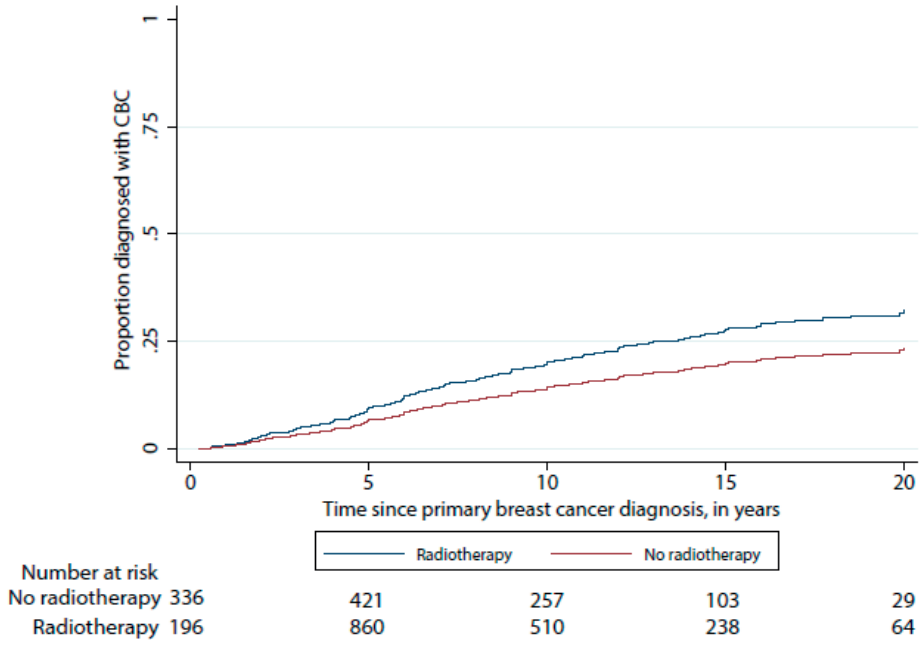
Table 1. Comparison of patient and tumor characteristics of patients, grouped by treatment with radiotherapy and g*BRCA* status

	Total *N*=3,602				*gBRCA1*[d] *N*=2,141				*gBRCA2*[d] *N*=1,457				p-value
	RTx *N*=2,297		No RTx *N*=1,305		RTx *N*=1,340		No RTx *N*=801		RTx *N*=954		No RTx *N*=503		
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	
Follow-up time in years, median (range)	10.0 (0.3-27.6)		9.0 (0.3-26.6)		10.2 (0.3-27.6)		8.8 (0.3-26.6)		9.5 (0.3-25.6)		9.2 (0.3-25.4)		<0.001[a] <0.001[b] 0.55[c]
Age at PBC diagnosis, median years (range)	42.0 (18.0-85.2)		41.4 (19.5-86.7)		40 (19.4-81.1)		40 (21.5-84.6)		45.1 (18.0-85.2)		44.0 (19.5-86.7)		0.21[a] 0.63[b] 0.046[c]
Year of PBC diagnosis, median (range)	2004 (1990-2017)		2005 (1990-2018)		2004 (1990-2017)		2005 (1990-2018)		2004 (1990-2016)		2005 (1990-2018)		<0.001[a] <0.001[b] 0.01[c]
5-year categories													<0.001[a]
1990-1994	219	9	87	7	145	11	59	7	74	8	28	5	<0.001[b]
1995-1999	413	18	207	16	259	19	138	17	153	16	69	14	0.02[c]
2000-2004	573	25	311	24	320	24	183	23	253	27	128	25	
2005-2009	666	29	364	28	388	29	205	26	276	29	159	32	
2010-2014	389	17	291	22	205	15	191	24	184	19	99	20	
2015-2020	37	2	45	3	23	2	25	3	14	1	20	4	
Timing of g*BRCA* DNA diagnosis													<0.001[a] <0.001[b]
After PBC diagnosis	2,102	92	970	74	1230	92	576	72	869	91	393	78	<0.001[c]
Before PBC diagnosis	195	8	335	26	110	8	225	28	85	9	110	22	
Stage													<0.001[a]
0 (DCIS)	37	3	71	8	9	1	26	5	28	5	45	14	<0.001[b]
1	558	40	410	46	366	43	282	50	192	35	128	40	<0.001[c]
2	593	42	378	43	384	45	244	43	208	38	134	42	
3	216	15	23	3	91	11	9	2	125	22	14	4	
unknown	893		423		490		240		401		182		
Tumor grade													0.001[a]
1	38	3	15	2	13	1	6	1	25	5	9	3	0.21[b]
2	326	22	235	30	131	15	89	18	194	35	146	48	0.001[c]
3	1,077	75	539	68	749	84	392	80	328	60	147	49	
Unknown	856		516		447		314		407		201		
ER status													0.001[a]
ER +	698	48	362	41	232	27	132	23	465	80	230	75	0.10[b]
ER -	751	52	523	59	638	73	445	77	113	20	77	25	0.06[c]
Unknown	848		420		470		224		376		196		

Table 1. Continued.

	Total *N*=3,602				*gBRCA1*[d] *N*=2,141				*gBRCA2*[d] *N*=1,457				p-value
	RTx *N*=2,297		No RTx *N*=1,305		RTx *N*=1,340		No RTx *N*=801		RTx *N*=954		No RTx *N*=503		
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	
PR status													<0.001[a]
PR +	494	38	250	31	158	20	98	18	336	66	152	58	0.24[b]
PR -	792	62	565	69	622	80	457	82	170	34	107	42	0.04[c]
Unknown	1011		490		560		246		448		244		
HER2 status													0.21[a]
HER2+	73	8	51	10	37	6	24	7	36	10	27	13	0.59[b]
HER2-	881	92	484	90	544	94	305	93	337	90	179	87	0.20[c]
Unknown	1343		770		759		472		581		297		
Chemotherapy													<0.001[a]
Yes	1,829	81	904	70	1,126	85	602	76	700	75	301	61	<0.001[b]
No	425	19	384	30	194	15	189	24	231	25	195	39	<0.001[c]
Unknown	43		17		20		10		23		7		
Endocrine therapy													<0.001[a]
Yes	1,083	50	446	35	435	35	171	22	646	70	274	56	<0.001[b]
No	1,097	50	825	65	816	65	607	78	280	30	218	44	<0.001[c]
Unknown	117		34		89		23		28		11		
Type of surgery													<0.001[a]
No surgery	12	1	12	1	9	1	3	0.4	3	0.3	9	2	<0.001[b]
Lumpectomy	1,414	65	231[e]	19	890	70	153	21	522	58	78	16	<0.001[c]
Mastectomy	753	34	956	80	375	29	567	78	377	42	389	82	
unknown	118		106		66		78		52		27		
RRM													<0.001[a]
RRM	911	40	611	47	541	40	399	50	369	39	212	42	<0.001[b]
No RRM	1,382	60	688	53	796	60	400	50	584	61	287	58	0.16[c]
unknown	4		6		3		2		1		4		
Timing RRSO													<0.001[a]
No RRSO	574	25	338	26	349	26	193	24	224	24	144	29	<0.001[b]
Before PBC	81	4	110	9	45	3	87	11	36	4	23	5	0.11[c]
After PBC	1,634	71	842	65	942	71	511	64	690	72	331	66	
At the same time	2	0.1	5	0.4	0		4	0.5	2	0.2	1	0.2	
Unknown RRSO	6		10		4		6		2		4		

PBC: Primary Breast Cancer; ER: Estrogen receptor; PR: Progesterone receptor; RRM: Risk-reducing Mastectomy; RRSO: Risk-reducing salpingo-oophorectomy; RTx: radiotherapy.
[a] comparison of radiotherapy with no radiotherapy group, overall.
[b] comparison of radiotherapy with the no radiotherapy group, in g*BRCA1* carriers only.
[c] comparison of radiotherapy with the no radiotherapy group, in g*BRCA2* carriers only.
[d]Four patients simultaneously had a g*BRCA1* and g*BRCA2* pathogenic variant, as they are not included in these columns, totals do not add up to 3602.
[e]In part, these women were initially treated with a lumpectomy, postponing radiotherapy while waiting on DNA test results to undergo risk-reducing surgery.



	Cumulative CBC risk (95% CI)*		
	5-year	**10-year**	**15-year**
Radiotherapy	9.8% (8.0-11.9)	20.0% (17.5-22.9)	28.0% (24.6-31.7)
No radiotherapy	7.3% (5.3-10.0)	15.3% (12.2-19.2)	23.3% (18.9-28.5)

Figure 3. Cox-model derived CBC incidence curve, by radiotherapy recipients and adjusted for chemotherapy, endocrine therapy and age at diagnosis

*Unadjusted Kaplan-Meier cumulative incidence estimates.

Table 2. Cox proportional hazards model for combined invasive and non-invasive CBC risk after radiotherapy overall and stratified by g*BRCA* pathogenic variant status.

	Radiotherapy	**No Radiotherapy**
Overall model		
N	2,297	1,305
N events	252	98
PYO	9,997	5,266
Incidence rate per 1000 person-years (95% CI)	25.2 (22.3-28.5)	18.6 (15.3-22.7)
	Hazard ratio (95% CI)	
Univariable analysis:		
Radiotherapy (yes vs. no)	1.35 (1.06-1.72)	
Multivariable analysis[a]:		
Radiotherapy (yes vs. no)	1.44 (1.12-1.86)	
Age (per year increase)	0.98 (0.97-0.99)	
Chemotherapy (yes vs. no)[b]	0.35 (0.22-0.54)	
Endocrine therapy (yes vs. no)	0.78 (0.61-1.00)	
***BRCA1* pathogenic variant carriers**		
N	1,340	801
N events	171	69
PYO	5,927	2,994
Incidence rate per 1000 person-years (95% CI)	28.9 (24.8-33.5)	23.0 (18.2-29.2)
	Hazard ratio (95% CI)	
Univariable analysis:		
Radiotherapy (yes vs. no)	1.25 (0.92-1.69)	
Multivariable analysis:		
Radiotherapy (yes vs. no)	1.29 (0.93-1.77)	
Age (per year increase)	0.98 (0.96-0.99)	
Chemotherapy (yes vs. no)[b]	0.34 (0.19-0.61)	
Endocrine therapy (yes vs. no)	0.99 (0.72-1.36)	
***BRCA2* pathogenic variant carriers**		
N	954	503
N events	81	29
PYO	4,058	2,269
Incidence rate per 1000 person-years (95% CI)	20.0 (16.1-24.8)	12.8 (8.9-18.4)
	Hazard ratio (95% CI)	
Univariable analysis:		
Radiotherapy (yes vs. no)	1.60 (1.04-2.47)	
Multivariable analysis:		
Radiotherapy (yes vs. no)	1.77 (1.13-2.77)	
Age (per year increase)	0.98 (0.96-1.00)	
Chemotherapy (yes vs. no)[b]	0.33 (0.16-0.69)	
Endocrine therapy (yes vs. no)	0.79 (0.50-1.23)	

PYO: person-years of observation. For all models, we allowed the baseline hazard to vary by country. Multivariable models adjusted for age at PBC diagnosis, chemotherapy and endocrine therapy. [a]Stage and Risk-reducing salpingo-oophorectomy (as a time-varying variable) were not included as a covariable, as their inclusion did not have a meaningful effect on the hazard ratio for radiotherapy nor resulted in a significant likelihood-ratio test for the model (p-value = 0.98 and p-value = 0.76, respectively). [b]The effect of chemotherapy is not constant over time, hazard ratio increases with time and reaches 1.00 between eleven and thirteen years after PBC diagnosis.

DISCUSSION

Our results show an association with moderately increased CBC risk for g*BRCA1/2* pathogenic variant carriers receiving radiotherapy after PBC diagnosis, especially for g*BRCA2* pathogenic variant carriers. The increased risk or a trend for increased risk was also observed in the combined *BRCA1/2* carrier analyses for both under the age of 40 and above the age of 40 at PBC diagnosis.

In contrast to our results, Reiner et al. observed no evidence of a direct effect of radiotherapy on CBC risk in gBRCA1/2 pathogenic variant carriers in their recent nested case-control study.[26] Moreover, several other studies did not demonstrate an increased risk of CBC after radiotherapy for PBC in g*BRCA1/2* pathogenic variant carriers.[19,27-29] For some of these studies, failure to demonstrate an increased risk may be explained by relatively small effects in combination with an insufficient sample size or follow-up.

On the other hand, results from a large randomized controlled trial-based meta-analysis did show a small but consistent effect in the general BC population.[3] In addition, other studies in sporadic BC patients have linked exposure to radiotherapy for PBC to increased risks of CBC as well. The effect was small (i.e., relative-risks 1.10-1.20)[30], unless a strong family history was present.[18] Stovall et al. (2008) found larger effects of radiotherapy on CBC risk in unselected PBC patients younger than 40 years of age, mostly for contralateral breast doses exceeding 1 Gray (mean dose) based on phantom dosimetry, which included a significant dose-effect relationship. However, there may have been a higher predominance of g*BRCA1/2* pathogenic variants in their case population (patients with CBC) than the control population (patients without CBC), especially in young age groups.[13]

Asaithamby et al. found that lower doses (i.e., 5mGy to 1Gy), unlike high (therapeutic) dose exposure, do not induce a sufficient number of DSBs to cause cell-death or apoptosis as cells have the capacity to repair them efficiently.[31] A small number of DSBs, single strand DNA breaks and other smaller DNA lesions are still, nonetheless, induced. Following faulty repair of these DSBs, e.g., due to impaired BRCA function, mutations can still accumulate, increasing the likelihood of cancer. Indeed, low dose (< 1Gy) radiation exposure from diagnostic procedures has been associated with increased PBC incidence in g*BRCA* pathogenic variant carriers.[32]

Remarkably, in the overall analysis we observed similar trends of increased risks of CBC after radiotherapy in patients both older and younger than 40 years of age at PBC diagnosis compared to those who did not receive radiotherapy. The effect of young age as a risk factor for CBC after radiotherapy has previously been reported (risk ratios: 1.5-2.5), usually with ages of 35, 40 or 45 as a cut-off between younger and older patients.[13,18,19,32] We chose the age of 40, to maintain consistency with previous studies, and to keep a large enough population for subgroup-analysis. In our study we observed that especially in carriers under the age of 40 at PBC diagnosis, more chemotherapy was administered. Chemotherapy decreases CBC risk and thus (at least partially) negates the potential side-effects of radiotherapy in this group (even though we saw no evidence for interaction in our analyses).[33,34] This can also be observed when further stratifying the analysis for chemotherapy in patients <40 years (HR: 1.13, 95% CI: 0.72-1.77 with chemotherapy versus HR: 2.63, 95% CI: 1.18-5.85 without chemotherapy; data not shown).

Increased risk of CBC following radiotherapy was more pronounced in g*BRCA2* pathogenic variant carriers than in g*BRCA1* pathogenic variant carriers. To our knowledge this was not described earlier. The effect might in part be explained by the fact we have included both invasive and non-invasive CBCs in our analysis, the latter group being more frequent within g*BRCA2* pathogenic variant carriers. Indeed, when we considered only invasive CBC as an outcome, radiotherapy as compared to no radiotherapy was no longer significantly associated with an increased risk of CBC in g*BRCA2* pathogenic variant carriers (Supplementary Table 3). However, as radiotherapy can induce new cancer growth and since DCIS is considered a precursor of an invasive tumor, it may be important to consider non-invasive CBCs as an outcome as well, even if the direct potential clinical impact is not as large as being diagnosed with invasive cancer.

The main strengths of our study are the large population size, combining several international datasets of g*BRCA1/2* pathogenic variant carriers diagnosed with breast cancer, as well as separating radiation exposure by g*BRCA* pathogenic variant status and age at PBC diagnosis. While from a biological standpoint it seems plausible that g*BRCA* pathogenic variant carriers are at increased risk of developing CBC after radiotherapy for PBC, the evidence from observational clinical studies is currently inconclusive.[19] One reason for this could be that CBC risk as a result of radiotherapy exposure is not linear, but may increase with time.[19,30] This effect may

also in part be mediated by transient protection from other treatments such as chemotherapy and endocrine therapy. Additionally, Drooger et al. noted that rates of preventive contralateral mastectomy (CPM) increased over time.[19] The resulting decrease in numbers of patients at risk for 10- and 15-year follow-up analyses impeded them in discerning a significant effect. In the current analyses we used a much larger study sample which obviated above-described limitations concerning follow-up, making our results much more robust in our opinion.

A limitation of this study was the lack of detailed information on the exact radiotherapy dose and modality (i.e., photons or electrons) that was given, treatment volumes (i.e., breast or chest wall with or without Internal Mammary Chain) and contralateral breast dose. Having this information could result in a better estimation of the association between radiotherapy and CBC risk. In addition, further evidence for a dose-effect relationship would be the finding that contralateral breast cancers are more frequent on the medial (most highly exposed) side after radiotherapy, in accordance with the results of Hooning et al. 2008 and Stovall et al. 2008.[13,18]

Further, we noted a higher uptake of CPM in the non-radiotherapy compared to the radiotherapy group. This results in earlier censoring for the non-radiotherapy group. The Cox model handles differences in censoring well, if the assumption of proportional hazards holds. The only exception would be if censoring on prophylactic mastectomy would be informative, i.e., when those who more often opt for prophylactic mastectomy are at higher risk of CBC (e.g., due to strong family history). For the context of our study, this would mean that those not receiving radiotherapy have a higher baseline risk of CBC. This decreases the difference in risk between both groups, independent from radiotherapy effects. As a result, our estimates may be an underestimation of the true effect.

Concluding, we observed an association with increased risk of CBC among g*BRCA1/2* pathogenic variant carriers who received radiotherapy compared to those who did not receive radiotherapy. Interestingly, the risk was comparable for different age groups and g*BRCA2* pathogenic variant carriers showed the highest risk. More evidence is required to conduct a proper risk-benefit analysis of tailoring radiotherapy around the contralateral breast (i.e., dosage, techniques) while maintaining oncological safety. Knowledge on the risks associated with radiotherapy can help guide decision-making for g*BRCA1/2* pathogenic variant carriers together with their physician regarding their post-treatment choices concerning surveillance and prophylactic surgery. Future studies could investigate the relationship between radiotherapy and CBC risk by looking into dose-response and localization effects (which may require individual radiation treatment plans), study other radiotherapy techniques (e.g., proton beam radiotherapy, contralateral breast sparing techniques) and factors that might affect radiation sensitivity of the contralateral breast (e.g., reproductive factors such as parity and lactation duration).

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CONFLICT OF INTEREST DISCLOSURES

All authors have completed and submitted the Form for Disclosure of Potential Conflicts of interest.

D Gareth Evans reports potential conflict of interest from AstraZeneca and AmGen; Karin Kast from Roche Pharma AG; Jacques Simard reports holding *BRCA1* and *BRCA2* patents.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Comparison/Overview of patient and tumor characteristics of patients, grouped by treatment with radiotherapy and stratified by age younger than 40 years or 40 years and older at PBC diagnosis

	RTx *N*=2,297 (64%)	No RTx *N*=1,305 (37%)	<40 years		≥40 years		p-value
			RTx *N*=917	No RTx *N*=560	RTx *N*=1,380	No RTx *N*=745	
Follow-up time in years, median (range)	10.0 (0.3-27.6)	9.0 (0.3-26.6)	10.0 (0.6-27.6)	8.7 (0.3-26.5)	9.9 (0.3-27.0)	9.2 (0.3-26.6)	<0.001[a] <0.001[b] 0.02[c]
Age at PBC diagnosis, median years (range)	42.0 (18.0-85.2)	41.4 (19.5-86.7)	34.4 (18.0-39.9)	34.7 (19.5-39.9)	48.0 (40-85.2)	47.6 (40-86.7)	0.21[a] 0.79[b] 0.72[c]
Year of PBC diagnosis, median (range)	2004 (1990-2017)	2005 (1990-2018)	2003 (1990-2016)	2006 (1990-2018)	2004 (1990-2017)	2005 (1990-2018)	<0.001[a] <0.001[b] 0.16[c]
5-year categories							
1990-1994	219 (9%)	87 (7%)	103 (11%)	33 (6%)	116 (8%)	54 (7%)	<0.001[a]
1995-1999	413 (18%)	207 (16%)	179 (20%)	83 (15%)	234 (17%)	124 (17%)	<0.001[b]
2000-2004	573 (25%)	311 (24%)	226 (25%)	125 (22%)	347 (25%)	186 (25%)	0.03[c]
2005-2009	666 (29%)	364 (28%)	258 (28%)	160 (28%)	408 (30%)	204 (27%)	
2010-2014	389 (17%)	291 (22%)	140 (15%)	146 (26%)	249 (18%)	145 (20%)	
2015-2020	37 (2%)	45 (3%)	11 (1%)	13 (2%)	26 (2%)	32 (4%)	
***BRCA* pathogenic variant**							
BRCA1	1340 (58%)	801 (61%)	653 (71%)	388 (69%)	687 (50%)	413 (55%)	0.19[a]
BRCA2	954 (41%)	503 (39%)	262 (29%)	171 (31%)	692 (50%)	332 (45%)	0.71[b]
Both	3 (0.1%)	1 (0.1%)	2 (0.2%)	1 (0.2%)	1 (0.1%)	0	0.04[c]
Timing of *BRCA* DNA diagnosis							<0.001[a]
After PBC diagnosis	2,102 (92%)	970 (74%)	868 (95%)	438 (78%)	1,234 (90%)	532 (71%)	<0.001[b]
Before PBC diagnosis	195 (8%)	335 (26%)	49 (5%)	122 (22%)	146 (11%)	213 (29%)	<0.001[c]

Supplementary Table 1. Continued.

	RTx *N*=2,297 (64%)	No RTx *N*=1,305 (37%)	<40 years		≥40 years		p-value
			RTx *N*=917	No RTx *N*=560	RTx *N*=1,380	No RTx *N*=745	
Stage							
0 (DCIS)	37 (3%)	71 (8%)	10 (2%)	28 (7%)	27 (3%)	43 (9%)	
1	558 (40%)	410 (46%)	197 (36%)	168 (44%)	361 (42%)	243 (49%)	
2	593 (42%)	378 (43%)	254 (46%)	178 (46%)	339 (40%)	200 (40%)	<0.001[a]
3	216 (15%)	23 (3%)	86 (16%)	9 (2%)	130 (15%)	14 (3%)	<0.001[b]
unknown	893	423	370	178	523	245	<0.001[c]
Tumor grade							
1	38 (3%)	15 (2%)	9 (2%)	8 (2%)	29 (3%)	7 (2%)	
2	326 (22%)	235 (30%)	84 (15%)	80 (23%)	242 (28%)	155 (35%)	0.001[a]
3	1,077 (75%)	539 (68%)	470 (83%)	258 (75%)	607 (69%)	281 (63%)	0.01[b]
Unknown	856	516	354	214	502	302	0.01[c]
ER status							
ER +	698 (48%)	362 (41%)	206 (37%)	129 (32%)	492 (56%)	233 (49%)	0.001[a]
ER -	751 (52%)	523 (59%)	357 (63%)	277 (68%)	394 (44%)	246 (51%)	0.12[b]
Unknown	848	420	354	154	494	266	0.02[c]
PR status							
PR +	494 (38%)	250 (31%)	161 (31%)	89 (23%)	333 (43%)	161 (37%)	<0.001[a]
PR -	792 (62%)	565 (69%)	351 (69%)	290 (77%)	441 (57%)	275 (63%)	0.01[b]
Unknown	1,011	490	405	181	606	309	0.04[c]
HER2 status	73 (8%)	51 (10%)	27 (7%)	16 (7%)	46 (8%)	35 (12%)	0.21[a]
HER2+	881 (92%)	484 (90%)	347 (93%)	230 (94%)	534 (92%)	254 (88%)	0.73[b]
HER2-	1343	770	543	314	800	456	0.05[c]
Unknown							
Chemotherapy							
Yes	1,829 (81%)	904 (70%)	815 (90%)	456 (82%)	1,014 (75%)	448 (61%)	<0.001[a]
No	425 (19%)	384 (30%)	89 (10%)	100 (18%)	336 (25%)	284 (39%)	<0.001[b]
Unknown	43	17	13	4	30	13	<0.001[c]

Supplementary Table 1. Continued.

	RTx *N*=2,297 (64%)	No RTx *N*=1,305 (37%)	<40 years		≥40 years		p-value
			RTx *N*=917	No RTx *N*=560	RTx *N*=1,380	No RTx *N*=745	
Endocrine therapy							
Yes	1,083 (50%)	446 (35%)	356 (42%)	160 (29%)	727 (55%)	286 (40%)	<0.001[a]
No	1,097 (50%)	825 (65%)	499 (58%)	387 (71%)	598 (45%)	438 (60%)	<0.001[b]
Unknown	117	34	62	13	55	21	<0.001[c]
Type of surgery							
No surgery	12 (1%)	12 (1%)	5 (1%)	7 (1%)	7 (1%)	5 (1%)	<0.001[a]
Lumpectomy	1,414 (65%)	231 (19%)[d]	532 (61%)	116 (23%)	882 (68%)	115 (17%)	<0.001[b]
Mastectomy	753 (34%)	956 (80%)	338 (38%)	385 (76%)	415 (32%)	571 (83%)	<0.001[c]
unknown	118	106	42	52	76	54	
RRM							
RRM	911 (40%)	611 (47%)	425 (46%)	312 (56%)	486 (35%)	299 (40%)	<0.001[a]
No RRM	1,382 (60%)	688 (53%)	490 (54%)	244 (44%)	892 (65%)	444 (60%)	<0.001[b]
unknown	4	6	2	4	2	2	0.02[c]
Timing RRSO							
No RRSO	574 (25%)	338 (26%)	306 (33%)	197 (36%)	268 (19%)	141 (19%)	<0.001[a]
Before PBC	81 (4%)	110 (9%)	2 (0.2%)	11 (2%)	79 (6%)	99 (13%)	0.002[b]
After PBC	1,634 (71%)	842 (65%)	605 (66%)	344 (62%)	1,029 (75%)	498 (67%)	<0.001[c]
At the same time	2 (0%)	5 (0%)	1 (0.1%)	2 (0.4%)	1 (0.1%)	3 (0.4%)	
Unknown RRSO	6	10	3	6	3	4	

ER: Estrogen receptor, PR: Progesterone receptor, RRM: Risk-reducing Mastectomy, RRSO: Risk-reducing salpingo-oophorectomy, RTx: Radiotherapy.
[a] comparison of radiotherapy with no radiotherapy group, overall.
[b] comparison of radiotherapy with the no radiotherapy group, in patients <40 years at PBC diagnosis.
[c] comparison of radiotherapy with the no radiotherapy group, in patients ≥40 years at PBC diagnosis.
[d] In part, these patients were initially treated with a lumpectomy, postponing radiotherapy while waiting on DNA test results to undergo risk-reducing surgery.

Supplementary Table 2. Cox proportional hazards model for combined invasive and non-invasive CBC risk after radiotherapy stratified by age at primary breast cancer diagnosis (younger or older than 40 years of age)

	Radiotherapy	No Radiotherapy
Age <40 years at PBC diagnosis		
N	917	560
N events	100	45
PYO	3,790	2,006
Incidence rate per 1000 person-years (95% CI)	26.4 (21.7-32.1)	22.4 (16.7-30.0)
	Hazard ratio (95% CI)	
Univariable analysis:		
Radiotherapy (yes vs. no)	1.24 (0.86-1.79)	
Multivariable analysis:		
Radiotherapy (yes vs. no)	1.38 (0.93-2.04)	
Age (per year increase)	0.96 (0.92-1.00)	
Chemotherapy (yes vs. no)[a]	0.24 (0.11-0.50)	
Endocrine therapy (yes vs. no)	0.78 (0.52-1.18)	
Age ≥40 years at PBC diagnosis		
N	1,380	745
N events	152	53
PYO	6,207	3,260
Incidence rate per 1000 person-years (95% CI)	24.5 (20.9-28.7)	16.3 (12.4-21.3)
	Hazard ratio (95% CI)	
Univariable analysis:		
Radiotherapy (yes vs. no)	1.49 (1.07-2.06)	
Multivariable analysis:		
Radiotherapy (yes vs. no)	1.56 (1.11-2.19)	
Age (per year increase)	0.97 (0.95-0.99)	
Chemotherapy (yes vs. no)[a]	0.43 (0.25-0.74)	
Endocrine therapy (yes vs. no)	0.80 (0.58-1.10)	

CBC: contralateral breast cancer; PYO: person-years of observation; PBC: primary breast cancer. Multivariable models adjusted for age at PBC diagnosis, chemotherapy and endocrine therapy.
[a]The effect of chemotherapy is not constant over time, hazard ratio increases with time and reaches 1.00 between ten and eleven years after PBC diagnosis.

Supplementary Table 3. Cox proportional hazards model for invasive only CBC risk after radiotherapy overall, and stratification by g*BRCA* pathogenic variant status and age at primary breast cancer diagnosis

	Total group		BRCA1		BRCA2		< 40 years at PBC		≥ 40 years at PBC	
	RTx	No RTx	RTx	No RTx	RTx	No RTx	RTx	No RTx	RTx	No RTx
N	2,297	1,305	1,340	801	954	503	917	560	1,380	745
***N* events**	180	70	130	48	50	20	69	33	111	37
PYO	9,997	5,266	5,927	2,994	4,058	2,269	3,790	2,006	6,207	3,260
Incidence rate per 1000 PYO	18.0	13.3	21.9	16.7	12.3	8.8	18.2	16.4	17.9	11.4
(95% CI)	(15.6-20.8)	(10.5-16.8)	(18.5-26.0)	(12.7-22.0)	(9.3-16.3)	(5.7-13.7)	(14.4-23.1)	(11.7-23.1)	(14.8-21.5)	(8.2-15.7)
	Multivariable hazard ratios (95% CI)									
Radiotherapy (yes vs. no)	1.51 (1.12-2.04)		1.41 (0.97-2.03)		1.72 (0.99-2.98)		1.29 (0.81-2.04)		1.74 (1.17-2.59)	
Age (per year increase)	0.98 (0.97-0.99)		0.98 (0.97-1.00)		0.99 (0.96-1.01)		0.94 (0.89-0.98)		0.97 (0.95-1.00)	
Chemotherapy (yes vs. no)	0.38 (0.18-0.56)*		0.36 (0.180.70)*		0.26 (0.10-0.67)*		0.79 (0.47-1.35)		0.40 (0.21-0.75)*	
Endocrine therapy (yes vs. no)	0.39 (0.20-0.69)*		0.95 (0.66-1.37)		0.78 (0.45-0.1.35)		0.71 (0.44-1.16)		0.36 (0.18-0.72)*	

CBC: Contralateral breast cancer; PBC: primary breast cancer; PYO: person-years of observation; RTx: radiotherapy. For all models, we allowed the baseline hazard to vary between countries. All models adjusted for age at PBC diagnosis, chemotherapy and endocrine therapy.
*time-dependent hazard ratio.

CHAPTER 7

RISK OF METACHRONOUS CONTRALATERAL BREAST CANCER IN PATIENTS WITH PRIMARY INVASIVE LOBULAR BREAST CANCER: RESULTS FROM A NATIONWIDE COHORT

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ABSTRACT

Lobular primary breast cancer (PBC) histology has been proposed as a risk factor for contralateral breast cancer (CBC), but results have been inconsistent. We investigated CBC risk and the impact of systemic therapy in lobular versus ductal PBC. Further, CBC characteristics following these histologic subtypes were explored.

We selected 74,373 women diagnosed between 2003 and 2010 with stage I-III invasive PBC from the nationwide Netherlands Cancer Registry. We assessed absolute risk of CBC taking into account competing risks among those with lobular (*n*=8,903), lobular mixed with other types (*n*=3,512), versus ductal (*n*=62,230) histology. Hazard ratios (HRs) for CBC were estimated in a cause-specific Cox model, adjusting for age at PBC diagnosis, radiotherapy, chemotherapy and/or endocrine therapy.

Multivariable HRs for CBC were 1.18 (95% CI: 1.04-1.33) for lobular and 1.37 (95% CI: 1.16-1.63) for lobular mixed versus ductal PBC. Ten-year cumulative CBC incidences in patients with lobular, lobular mixed versus ductal PBC were 3.2%, 3.6% versus 2.8% when treated with systemic therapy and 6.6%, 7.7% versus 5.6% in patients without systemic therapy, respectively. Metachronous CBCs were diagnosed in a less favourable stage in 19%, 26% and 23% and less favourable differentiation grade in 22%, 33% and 27% than the PBCs of patients with lobular, lobular mixed and ductal PBC, respectively.

In conclusion, lobular and lobular mixed PBC histology are associated with modestly increased CBC risk. Personalized CBC risk assessment needs to consider PBC histology, including systemic treatment administration. The impact on prognosis of CBCs with unfavourable characteristics warrants further evaluation.

INTRODUCTION

Advances in treatment and in screening methods have led to improved breast cancer survival over the last 2-3 decades.[1] Estimating and preventing long-term risks, in particular the risk of contralateral breast cancer (CBC), have therefore become more relevant. Identifying risk factors for CBC can contribute to personalised risk management.

Around 10-15% of primary breast cancers (PBC) have a lobular histology. Lobular PBC histology has been proposed as a risk factor for CBC but results of previous studies have been inconsistent.[2-7] Differences in study design (i.e. small and heterogeneous study populations) could explain this inconsistency. Also, part of the lobular PBCs have a mixed or a mixed non-classic type lobular histology rather than a classic type, but have not always been analysed as a separate group. Especially the mixed non-classic types seem to entail a different entity.[8] Also, the increased use of neo-adjuvant and adjuvant systemic treatment in more recent years may have led to a decrease in CBC risk following a lobular PBC, but this warrants further investigation.

Further, little is known about the tumour characteristics of CBC following a lobular or lobular mixed PBC. If we can distinguish lobular or lobular mixed subtypes that are associated with more aggressive types of CBC, these patients might benefit from earlier detection.

In a large nation-wide cohort, we aimed to assess metachronous CBC risk in patients with lobular or lobular mixed type PBC as compared to ductal PBC. In addition, we assessed the associations with systemic treatment (i.e., chemotherapy and/or endocrine therapy) and compared characteristics of CBCs following a lobular, lobular mixed or ductal PBC.

METHODS

Data collection

From the Netherlands Cancer Registry (NCR), we requested all patient, tumour, treatment and follow-up data from women, diagnosed with invasive PBC at age

≥18 years between 2003 and 2010, without a previous diagnosis of invasive cancer (except basal cell cancer or squamous cell cancer of the skin). The NCR receives notifications of all new malignancies from the Dutch nationwide network and registry of histopathology and cytopathology (PALGA), and from the national hospital discharge databank containing the discharge diagnosis of all patients from Dutch hospitals. Trained research assistants collect patient, tumour and treatment information from pathology reports and medical files within the hospitals. In addition, vital status is assessed yearly by linkage with the Nationwide Municipal Administrative Database. The review boards of the NCR and PALGA approved the proposal and the data were handled in accordance to the privacy regulations for medical research.[9] All data were anonymous to the researchers involved.

Of the 94,600 potentially eligible patients, we selected all women diagnosed with pathologically confirmed stage I-III PBC, having either lobular histology, lobular histology mixed with other subtypes (mainly mixed with ductal histology (92%); from now on referred to as "lobular mixed") or ductal histology (Figure 1).

Statistical analysis

We compared the lobular and lobular mixed histologic groups with ductal using chi-square statistics for categorical characteristics and the Kruskal-Wallis test for continuous characteristics. We used the Fine and Gray competing risk model to determine cumulative CBC risk, with death and non-invasive CBCs as competing risks. The Cox proportional hazards model was used to estimate univariable and multivariable cause-specific hazard ratios (HR) for CBC risk by histological PBC subtype; also the impact of neo-adjuvant or adjuvant treatment was evaluated, which we defined as treatment with chemotherapy and/or endocrine therapy. Potential confounders added to the overall model were age at PBC, application of neo-adjuvant or adjuvant systemic therapy (i.e., none, only chemotherapy, only endocrine therapy or both) and peri-operative radiotherapy. Patients were followed from PBC diagnosis until the development of an invasive metachronous CBC and were censored at diagnosis of an ipsilateral invasive second breast tumour, a second invasive non-breast tumour, occurrence of in situ CBC, death, or at last follow-up (31/12/2015). We performed a subgroup analysis in PBC patients with the combination of positive oestrogen receptor (ER) status (irrespective of progesterone receptor (PR) status) or positive PR status (if ER status was unknown) and negative HER2-status PBC, to estimate the hazard ratios for CBC in a more homogeneous patient population. Analyses were performed using Stata version 15.0.

To determine the cumulative incidence by CBC subtype, the other CBC subtypes were taken into account as competing events, and patients were censored at diagnosis of an ipsilateral second breast tumour, a second non-breast tumour, non-invasive CBC, death, or at last follow-up (31/12/2015). R software (version 3.5.1) was used for this analysis.

Since metachronous CBC was defined as the development of a new PBC in the opposite breast at least 3 months after the PBC diagnosis, follow-up started from 3 months onwards for all patients. Consequently, patients who developed an event within the first 3 months following PBC diagnosis were excluded (*n*=1,833).

The proportional hazards assumption was inspected using Schoenfeld residuals. Subsequently, we added interaction terms to investigate whether effect modification was present between the histologic groups and other variables included in the overall Cox model. In addition, we explored the presence of effect modification between the systemic therapy subgroups and the other variables in the stratified models within the histologic groups.

Five-year follow-up information on recurrent disease (local and distant) was available for all patients diagnosed with PBC between 2003 and 2006 (*n*=35,512) and for 56% of the patients diagnosed between 2007-2008 (*n*=11,103).

A sensitivity analysis was performed to investigate whether taking into account recurrent disease as a censoring event could lead to different results. For this analysis, only patients with complete 5-year information on recurrent disease (i.e., local, regional and distant recurrence) were included. Censoring endpoints were diagnosis of recurrent disease, an ipsilateral invasive second breast tumour, a second invasive non-breast tumour, occurrence of in situ CBC, death, or at last follow-up (31/12/2015). The results were subsequently compared to the same group of patients ignoring recurrent disease as a censoring endpoint.

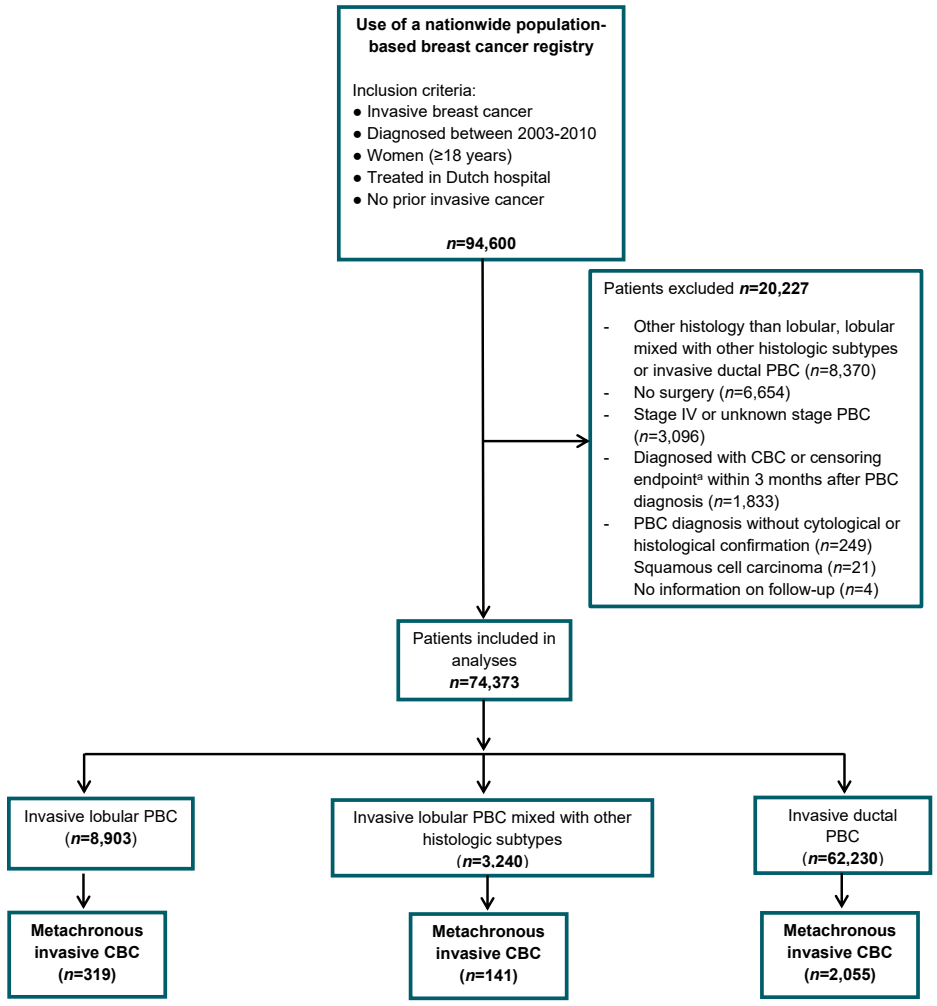


Figure 1. Overview of the cohort

PBC: primary breast cancer, CBC: contralateral breast cancer.
[a] endpoints: second breast or invasive non-breast tumour (except non-melanoma skin cancer); DCIS ipsilateral or contralateral; death; end of follow-up (31/12/2015).

RESULTS

Clinical characteristics of the 74,373 included patients are presented in Table 1. During a median follow-up time of 7.8 years, 2,515 patients were diagnosed with a metachronous invasive CBC. Patients with lobular PBC were more often above 60 years of age at PBC diagnosis (54.0%) than the lobular mixed (45.4%) and the ductal (44.6%) group (p<0.05). Patients with lobular mixed PBC were most often treated with both modalities (i.e., chemotherapy and endocrine therapy): 32% vs. 35% vs. 28% in lobular, lobular mixed and ductal PBC, respectively (p<0.001), while the majority of the lobular and ductal PBC patients received no systemic treatment (33% vs. 35% vs. 37%, respectively).

Table 1. Characteristics of patients included comparing lobular vs. ductal and lobular mixed vs. ductal groups

	Total group		Lobular		Lobular mixed		Ductal		*p*-value[a]
	N	***%***	***N***	***%***	***N***	***%***	***N***	***%***	
Total	***74,373***	100	***8,903***	12.0	***3,240***	4.4	***62,230***	83.7	
Median follow-up in years [IQR]	7.8 [5.9-10.1]		7.8 [5.9-10.1]		8.2 [6.2-10.3]		7.8 [5.9-10.1]		<0.001[b]
Age at PBC									
Median age, years [range]	58.4 [19-101]		61.4 [20-98]		58.2 [21-95]		58.0 [19-101]		<0.05
									<0.001
<30	387	0.5	7	0.1	8	0.3	372	0.6	
30-39	4,164	5.6	180	2.0	134	4.1	3,850	6.2	
40-49	15,076	20.3	1,612	18.1	667	20.6	12,797	20.6	
50-59	20,772	27.9	2,331	26.2	957	29.5	17,484	28.1	
60-69	17,841	24.0	2,307	25.9	766	23.6	14,768	23.7	
70-79	10,968	14.8	1,642	18.4	512	15.8	8,814	14.2	
80-89	4,828	6.5	778	8.7	185	5.7	3,865	6.2	
90+	337	0.5	46	0.5	11	0.3	280	0.5	
Stage									<0.001
IA	32,201	43.3	3,157	35.5	1,269	39.2	27,775	44.6	
IB	2,943	4.0	233	2.6	119	3.7	2,591	4.2	
IIA	19,191	25.8	2,369	26.6	832	25.7	15,990	25.7	

Table 1. Continued.

	Total group		Lobular		Lobular mixed		Ductal		*p*-value[a]
	N	***%***	***N***	***%***	***N***	***%***	***N***	***%***	
IIB	9,693	13.0	1,408	15.8	469	14.5	7,816	12.6	
IIIA	6,255	8.4	994	11.2	342	10.6	4,916	7.9	
IIIB	852	1.2	88	1.0	30	0.9	734	1.2	
IIIC	3,238	4.4	654	7.4	179	5.5	2,405	3.9	
Differentiation grade									<0.001
Grade I: well differentiated	14,655	21.3	1,635	21.8	626	21.3	12,394	21.3	
Grade II: moderately differentiated	31,560	46.0	5,056	67.3	1,742	59.4	24,762	42.5	
Grade III: poorly differentiated/ undifferentiated	22,456	32.7	827	11.0	566	19.3	21,063	36.2	
Unknown	5,702		1,385		306		4,011		
Oestrogen receptor status									<0.001
Positive	58,521	82.2	8,214	95.7	2,909	94.1	47,398	79.6	
Negative	12,694	17.8	366	4.3	182	5.9	12,146	20.4	
Unknown/not determined	3,158		323		149		2,686		
Progesterone receptor status									<0.001
Positive	45,727	66.5	6,288	76.6	2,321	77.2	37,118	64.6	
Negative	22,997	33.5	1,926	23.5	684	22.8	20,387	35.5	
Unknown/not determined	5,649		689		235		4,725		
HER2 receptor status									<0.001
Positive	8,530	15.2	277	4.2	211	8.8	8,042	17.1	
Negative	47,573	84.8	6,400	95.9	2,190	91.2	38,983	82.9	
Unknown/not determined	18,270		2,226		839		15,205		

Table 1. Continued.

	Total group		Lobular		Lobular mixed		Ductal		*p*-value[a]
	N	***%***	***N***	***%***	***N***	***%***	***N***	***%***	
Surgery									<0.001
Lumpectomy	40,993	55.1	3,638	40.9	1,444	44.6	35,911	57.7	
Mastectomy	33,380	44.9	5,265	59.1	1,796	55.4	26,319	42.3	
Radiotherapy									<0.001
Yes	50,241	67.6	5,486	61.6	1,981	61.1	42,774	68.7	
No	24,132	32.5	3,417	38.4	1,259	38.9	19,456	31.3	
Chemotherapy									<0.005
Yes	31,417	42.2	3,219	36.2	1,312	40.5	26,886	43.2	
No	42,956	57.8	5,684	63.8	1,928	59.5	35,344	56.8	
Endocrine therapy									<0.001
Yes	37,047	49.8	5,582	62.7	1,949	60.2	29,516	47.4	
No	37,326	50.2	3,321	37.3	1,291	39.9	32,714	52.6	
Targeted therapy									<0.001
Yes	5,181	7.0	137	1.5	119	3.7	4,925	7.9	
No	69,192	93.0	8,766	98.5	3,121	96.3	57,305	92.1	
Systemic therapy									<0.001
No chemotherapy or endocrine therapy	27,180	36.6	2,950	33.1	1,120	34.6	23,110	37.1	
Only chemotherapy	10,146	13.6	371	4.2	171	5.3	9,604	15.4	
Only endocrine therapy	15,776	21.2	2,734	30.7	808	24.9	12,234	19.7	
Both chemotherapy and endocrine therapy	21,271	28.6	2,848	32.0	1,141	35.2	17,282	27.8	

PBC: primary breast cancer; Stage**:** Stage I: T1N0M0 and T0-1N1mi M0; Stage II: T0-1N1M0, T2N0M0, T2N1M0, or T3N0M0; Stage III: T0-2N2M0, T3N1-2M0, T4N0-2M0, or any T N3M0 breast cancer.

[a] *p*-values account for comparison lobular vs. ductal and lobular mixed vs. ductal PBC.

[b] *p*=0.7229 for lobular vs. ductal.

Table 2. Five- and ten-year cumulative metachronous CBC incidence according to histologic subtypes and systemic treatment, with death and non-invasive CBC as competing risk

	All patients		Lobular		Lobular (mixed)		Ductal	
	N PBC	*N* CBC	*N* PBC	*N* CBC	*N* PBC	*N* CBC	*N* PBC	*N* CBC
Total	*74,373*	*2,515*	*8,903*	*319*	*3,240*	*141*	*62,230*	*2,055*
5-year CBC risk % [95%CI]	**1.9** [1.8-2.0]		**2.1** [1.8-2.4]		**2.6** [2.1-3.2]		**1.8** [1.7-1.9]	
10-year CBC risk % [95%CI]	**4.0** [3.8-4.2]		**4.4** [3.9-4.9]		**5.1** [4.3-6.0]		**3.9** [3.7-4.0]	
No systemic therapy received	*27,180*	*1,393*	*2,950*	*174*	*1,120*	*80*	*23,110*	*1,139*
5-year CBC risk % [95%CI]	**3.0** [2.8-3.2]		**3.6** [2.9-4.3]		**3.8** [2.8-5.0]		**2.8** [2.6-3.1]	
10-year CBC risk % [95%CI]	**5.8** [5.5-6.1]		**6.6** [5.6-7.6]		**7.7** [6.1-9.6]		**5.6** [5.2-5.9]	
Only chemotherapy received	*10,146*	*354*	*371*	*9*	*171*	*8*	*9,604*	*337*
5-year CBC risk % [95%CI]	**2.0** [1.7-2.3]		**2.2** [1.0-4.1][a]		**3.6** [1.5-7.2][c]		**1.9** [1.7-2.2]	
10-year CBC risk % [95%CI]	**4.1** [3.7-4.6]		**NA**[b]		**NA**[d]		**4.2** [3.7-4.7]	
Only endocrine therapy received	*15,776*	*345*	*2,734*	*61*	*808*	*24*	*12,234*	*260*
5-year CBC risk % [95%CI]	**1.3** [1.1-1.5]		**1.3** [0.9-1.8]		**2.3** [1.4-3.5]		**1.2** [1.1-1.5]	
10-year CBC risk % [95%CI]	**2.7** [2.4-3.0]		**3.0** [2.2-3.9]		**3.6** [2.3-5.3][e]		**2.5** [2.2-2.9]	
Chemotherapy and endocrine therapy received	*21,271*	*423*	*2,848*	*75*	*1,141*	*29*	*17,282*	*319*
5-year CBC risk % [95%CI]	**0.9** [0.8-1.1]		**1.3** [0.9-1.8]		**1.4** [0.9-2.3]		**0.8** [0.7-1.0]	
10-year CBC risk % [95%CI]	**2.5** [2.2-2.8]		**3.5** [2.7-4.4]		**3.5** [2.3-5.1]		**2.3** [2.0-2.6]	

N PBC: number of PBC patients; *N* CBC: number of contralateral breast cancer events; NA: not applicable.
Time starts from 3 months after primary breast cancer diagnosis until metachronous CBC; Censoring events: ipsilateral recurrence including second breast cancer (either invasive or DCIS); second invasive non-breast tumour (except non-melanoma skin cancer); loss to follow-up; end of follow-up (31/12/2015); competing events: death and non-invasive contralateral BC.
[a] Time point of observation available was 4.5 years after PBC diagnosis.
[b] Last time point of observation was at 7.5 years after PBC diagnosis.
[c] Time point of observation available was 3.8 years after PBC diagnosis.
[d] Only 2 events remained (i.e. at time points 6.6 and 11.4 years after PBC diagnosis).
[e] Last time point of observation was at 8.9 years after PBC diagnosis.

CBC risk: overall and by systemic treatment

Ten-year cumulative CBC incidence was 4.4% (95% CI: 3.9-4.9%) in the lobular group, 5.1% (95% CI: 4.3-6.0%) in the lobular mixed group vs. 3.9% (95% CI: 3.7-4.0%) in the ductal group (Table 2; Figure 2).

In all patients who were treated with peri-operative systemic therapy, i.e. either chemotherapy and/or endocrine therapy, ten-year cumulative CBC incidences were 3.2% (95% CI: 2.7-3.8%) and 3.6% (95% CI: 2.7-4.7%) vs. 2.8% (95% CI: 2.6-3.0%) for the lobular, lobular mixed versus the ductal group, respectively. Ten-year cumulative CBC incidences were 6.6% (95% CI: 5.6-7.6%) and 7.7% (95% CI: 6.1.7-9.6%) vs. 5.6% (95% CI: 5.2-5.9%), respectively, if no systemic therapy was given.

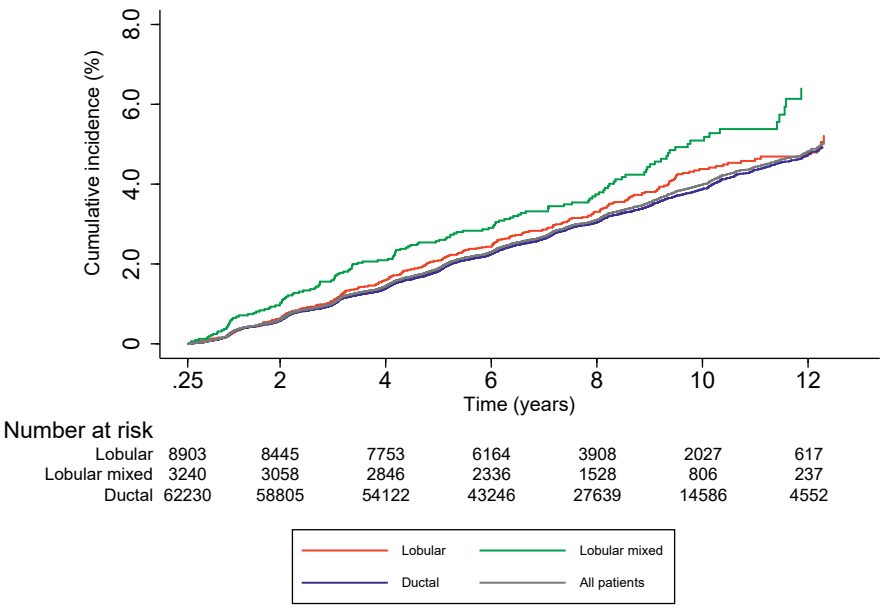


Figure 2. Cumulative incidence of developing metachronous invasive CBC according to PBC histology using competing risk analysis (%)
PBC: primary breast cancer, CBC: contralateral breast cancer.

There was no evidence for effect modification of CBC risk, not between the three histologic subgroups and the systemic therapy categories (Table 3), nor between the systemic therapy categories and the other variables included in the multivariable model stratified for the histologic subgroups (Supplementary Tables 1A-1B).

The multivariable hazard ratios for CBC risk were increased for both the lobular (multivariable HR: 1.18, 95% CI: 1.04-1.33) and lobular mixed group (multivariable HR 1.37, 95% CI: 1.16-1.63), as compared to ductal PBC patients (Table 3).

In the subset of patients with hormone receptor positive disease, 6,170 lobular, 2,094 lobular mixed and 32,393 ductal PBCs were included (of which 98.8% had ER+/HER2- PBC and 1.2% had ER unknown/PR+/HER2- PBC). Multivariable hazard ratios in this subset were similar to those in the total group, both for lobular as for lobular mixed compared to ductal PBC patients (HR:1.12, 95% CI: 0.96-1.31, and 1.36, 95% CI: 1.10-1.70, respectively; Table 3).

The lowest HRs were seen for patients treated with both chemotherapy and endocrine therapy in all three subtypes (multivariable HR: 0.43, 95% CI: 0.32-0.57 for lobular, HR: 0.36, 95% CI: 0.23-0.56 for lobular mixed and HR: 0.37, 95% CI: 0.32-0.42 for ductal PBC patients; Supplementary Table 1A). In the subselection of hormone receptor positive PBC patients, hazard ratios were 0.39 (95% CI: 0.27-0.57), 0.35 (95% CI: 0.20-0.62) and 0.34 (95% CI: 0.28-0.40), respectively (Supplementary Table 1B).

We observed no substantial alterations in the sensitivity analysis when censoring for recurrent disease versus ignoring recurrent disease; negligible differences were observed between the multivariable hazard ratios (Supplementary Table 3).

Comparisons of CBC characteristics

The cumulative CBC subtype incidence curves are shown in Figure 3, separately for patients with a lobular, lobular mixed or ductal PBC. The majority of CBCs had favourable tumour characteristics (i.e. stage I, ER-positive, grade I/II), with a similar distribution between the three groups.

CBC versus PBC characteristics

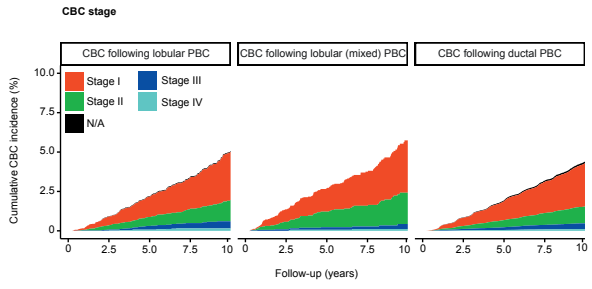
In 59 (19%), 36 (26%) and 470 (23%) of the lobular, lobular mixed and ductal PBC patients who developed a CBC, respectively, a more advanced stage than in the primary tumour was observed (Supplementary Tables 2A-2C). The CBC differentiation grade was higher than the PBC differentiation grade in 52 (22%), 38 (33%) and 459 (27%), respectively.

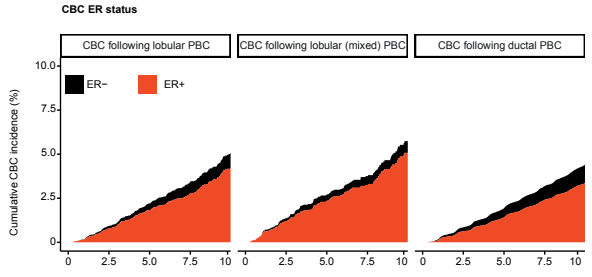
Further, all PBC patients mainly developed ductal CBCs (179 (56%), 78 (55%) and 1,541 (75.0%) , respectively). Patients with lobular (36%) or lobular mixed PBC (26%) more often developed a lobular/lobular mixed CBC than in ductal patients (14.3%).

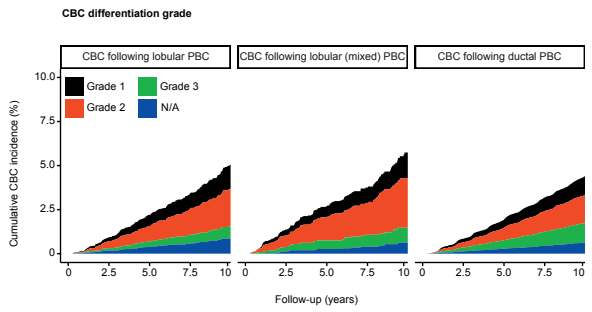
Table 3. Univariable and multivariable Cox regression analyses for invasive metachronous CBC risk in the total group and the subset of ER-positive with HER2-negative PBC

	Total group				*Subset analysis*			
	PYO	***N* CBC**	**uHR [95% CI]**	**mHR [95% CI]**	**PYO**	***N* CBC**	**uHR [95% CI]**	**mHR [95% CI]**
Total group	560,314	2,515	-	-	288,186	1,306	-	-
Lobular	66,754	319	1.09 [0.97-1.23]	1.18 [1.04-1.33]	43,255	199	1.04 [0.89-1.21]	1.12 [0.96-1.31]
Lobular (mixed)	24,866	141	1.29 [1.09-1.52]	1.37 [1.16-1.63]	15,117	87	1.29 [1.04-1.61]	1.36 [1.10-1.70]
Ductal	468,693	2,055	**Ref.**	**Ref.**	229,813	1,020	**Ref.**	**Ref.**
Systemic therapy								
None	212,195	1,393	**Ref.**	**Ref.**	108,157	793	**Ref.**	**Ref.**
Only chemotherapy	71,773	354	0.76 [0.68-0.86]	0.76 [0.67-0.86]	6,178	13	0.29 [0.17-0.50]	0.29 [0.17-0.51]
Only endocrine therapy	110,189	345	0.48 [0.43-0.55]	0.49 [0.43-0.55]	70,579	240	0.47 [0.41-0.54]	0.46 [0.39-0.53]
Both chemotherapy and endocrine therapy	166,157	423	0.39 [0.35-0.43]	0.38 [0.34-0.43]	103,272	260	0.35 [0.30-0.40]	0.35 [0.30-0.41]
Radiotherapy	385,424	1,744	1.02 [0.94-1.11]	1.01 [0.92-1.10]	202,469	913	0.98 [0.87-1.10]	0.94 [0.84-1.06]
No Radiotherapy	174,889	771	**Ref.**	**Ref.**	85,717	393	**Ref.**	**Ref.**
Age (10-year increase)	560,314	2,515	1.02 [1.01-1.03]	0.98 [0.94-1.02]	288,186	1,306	1.12 [1.07-1.17]	1.02 [0.97-1.07]

ER: estrogen receptor status; PR: progesterone receptor status; PYO: person-years of observation; *N* CBC: number of contralateral breast cancer events; uHR: univariable hazard ratio; mHR: multivariable hazard ratio. Time starts from 3 months after primary breast cancer diagnosis until metachronous CBC; Censoring events: ipsilateral recurrence including second breast cancer (either invasive or in situ); non-invasive contralateral breast cancer; second invasive non-breast tumour (except non-melanoma skin cancer); loss to follow-up; end of follow-up (31/12/2015); death.







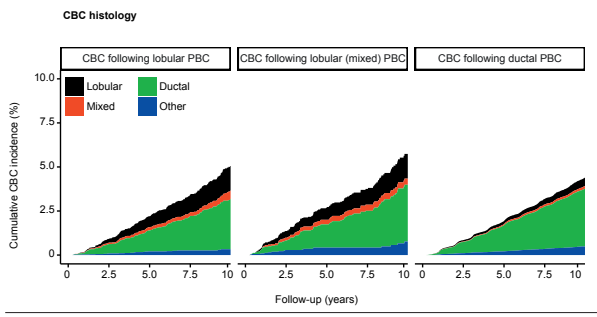


Figure 3. Stacked 10-year cumulative metachronous invasive CBC subtype incidence according to PBC histology (%)

10-year cumulative incidence of CBC	CBC incidence following lobular PBC	CBC incidence following lobular mixed PBC	CBC incidence following ductal PBC
	[95% CI]	[95% CI]	[95% CI]
CBC stage			
Stage I CBC	3.4 [2.8-4.0]	3.7 [2.8-4.8]	3.5 [3.2-3.7]
Stage II CBC	1.5 [1.2-2.0]	3.1 [2.1-4.6]	1.3 [1.2-1.5]
Stage III CBC	0.5 [0.3-0.7]	0.3 [0.1-0.7]	0.5 [0.4-0.6]
Stage IV CBC	0.2 [0.1-0.5]	0.1 [0.0-0.4]	0.1 [0.1-0.2]
CBC ER status			
ER+ CBC	4.7 [4.1-5.5]	6.3 [5.0-7.9]	4.3 [4.1-4.6]
ER- CBC	0.9 [0.7-1.2]	1.2 [0.6-2.3]	1.2 [1.1-1.4]
CBC differentiation grade			
Grade I CBC	1.5 [1.1-2.0]	1.8 [1.2-2.8]	1.3 [1.2-1.5]
Grade II CBC	2.3 [1.9-2.8]	2.9 [2.3-3.8]	2.1 [1.9-2.3]
Grade III CBC	0.8 [0.6-1.1]	1.2 [0.7-2.1]	1.4 [1.2-1.6]
CBC histology			
Lobular CBC	1.4 [1.1-1.8]	2.1 [1.3-3.4]	0.6 [0.5-0.8]
Lobular Mixed CBC	0.5 [0.3-0.9]	0.3 [0.2-0.7]	0.2 [0.2-0.3]
Ductal CBC	3.2 [2.7-3.8]	4.3 [3.2-5.7]	4.1 [3.9-4.4]
Other CBC	0.5 [0.2-0.9]	0.8 [0.5-1.3]	0.6 [0.5-0.7]

CBC: contralateral breast cancer; PBC: primary breast cancer; ER: Oestrogen receptor status; 95%CI: 95% Confidence Interval.
Stage: Stage I: T1N0M0 and T0-1N1mi M0; Stage II: T0-1N1M0, T2N0M0, T2N1M0, or T3N0M0; Stage III: T0-2N2M0, T3N1-2M0, T4N0-2M0, or any T N3M0 breast cancer; stage IV: metastatic breast cancer.

DISCUSSION

In this nationwide cohort study with over 74,000 patients, we observed that lobular and lobular mixed type PBC was associated with a higher risk of CBC than ductal PBC. The application of neo-adjuvant or adjuvant systemic therapy was associated with a decreased CBC risk among patients, irrespective of PBC histology. Further, in about a quarter of the CBC patients with a lobular mixed or ductal PBC, and in approximately a fifth of CBC patients with lobular PBC, the characteristics of the CBCs were worse, i.e., higher stage and grade, than their primary tumour.

The lobular mixed type PBCs tended to be associated with the highest CBC risk increase (HR: 1.37 compared to ductal), which is in line with the study published by Peiro et al.[10] They investigated lobular mixed with ductal histology subtypes. The majority of the mixed types in our dataset concerned lobular mixed with ductal histology subtypes as well (92%); for the remaining 8%, we had no information on the accompanying subtypes.

We also found an increased risk of CBC for patients with lobular PBC in comparison to ductal PBC. This is in line with multiple other studies[2-4,6,7,11], although in the older studies (mainly studies published prior to 2000), the effect sizes were larger, with relative risks ranging from 1.7-2.0. The attenuation might be explained by the fact that in recent studies the more extensive use of peri-operative systemic therapy resulted in a decreased risk association, as has been shown for CBC risk in general.[12] Since the introduction of adjuvant endocrine therapy for ER-positive BC, lobular PBCs, in which ER is expressed more frequently than in ductal PBCs, have probably been treated with endocrine treatment more often. We therefore assume that absolute CBC risk decreased more in lobular compared to in ductal PBCs, resulting in a lower relative CBC risk. Indeed in our study, ER-positivity was observed in 96% of lobular and 94% of lobular mixed and 80% in ductal PBC, respectively. Patients with lobular and lobular mixed PBCs were treated with endocrine therapy more often than patients with ductal PBCs in our study (65%, 64% and 59%, respectively, when restricted to ER-positive BC patients; Table 1).

Within the group of lobular PBC patients, the associations of chemotherapy with CBC risk were comparable to the associations of endocrine therapy alone and of endocrine therapy in combination with chemotherapy. However, the observational nature of our study and the small number of events in the chemotherapy group within lobular PBC patients prohibit us from drawing strong conclusions on the impact of different systemic therapy types on CBC risk.

The histology of the PBC and the CBC was more often similar in patients with lobular or lobular mixed PBCs than in patients with ductal PBC. The similarity in histology between the primary and secondary breast cancer might suggest that part of these tumours could be a metastatic spread of the primary tumour, rather than a new entity. From literature it is known that lobular breast cancers have a diffuse growth pattern and metastasize more often, perhaps also affecting the contralateral breast.[6,13,14] Since lobular BCs have been difficult to visualize on mammography[15], these metastatic spreads might have been missed initially and may have been classified as new primary tumors later on. Genetic analysis investigating clonality between a primary and second primary tumour may shed light on CBC being a true primary tumour or a metastatic disease in these cases. Lifestyle factors (e.g. hormone replacement therapy use) and germline pathogenic variants in the *CDH1* gene are also associated with the development of multiple lobular breast cancers.[16,17,18]

This study used a large and comprehensive population-based dataset to evaluate metachronous CBC risk in lobular and lobular mixed PBC patients. In addition, complete information on second breast cancer occurrence was present. This makes our results generalizable, although a limitation to our study is that we did not have complete follow-up information concerning recurrent disease for patients diagnosed between 2007 and 2010. Especially the occurrence of metastatic disease might be of importance, because in that case, patients will be mainly treated with systemic therapy, potentially lowering their risk of CBC. The sensitivity analysis in the group with complete information on recurrent disease which was censored for at occurrence confirmed our initial findings, suggesting negligible bias. Further research is needed though, especially in ER-positive BCs, which have a tendency to develop metastases after longer time periods than 5 years following PBC diagnosis.

Another potential limitation of our study is the lack of information on the presence of germline pathogenic mutations. This could have resulted in an overestimation of CBC risk in the present study. However, no association of lobular histology with known breast cancer gene mutations (*BRCA1/2*, *CHEK2*, *PALB2* and *ATM*) has

been reported in literature so far.[17] Therefore, we do not expect that this will be a confounding factor in our study.

In conclusion, lobular and lobular mixed histology of PBC are associated with increased risks of CBC as compared to ductal PBC. Personalized CBC risk assessment needs to consider PBC histology, including the administration of peri-operative systemic treatment. The impact on prognosis of CBCs with unfavourable characteristics warrants further evaluation.

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ETHICAL APPROVAL STATEMENT

The review boards of the Netherlands Cancer Registry and the Dutch nationwide network and registry of histopathology and cytopathology (PALGA) approved the proposal and the data were handled in accordance to the privacy regulations for medical research (Federa_code_of_conduct_english.pdf (bbmri.nl)).

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DISCLOSURES

The authors have declared no conflicts of interest

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the Netherlands Cancer Registry. Restrictions apply to the availability of these data, which were used under license for this study. Data are available at www.iknl.nl with the permission of the Netherlands Comprehensive Cancer Organisation.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1A. Univariable and multivariable Cox regression analyses of invasive metachronous CBC risk stratified by histologic subtype

	Lobular			*Lobular mixed*			*Ductal*		
	PYO (*N* CBC)	**uHR [95% CI]**	**mHR [95% CI]**	**PYO (*N* CBC)**	**uHR [95% CI]**	**mHR [95% CI]**	**PYO (*N* CBC)**	**uHR [95% CI]**	**mHR [95% CI]**
Total group	66,754 (319)	-	-	24,866 (141)	-	-	468,693 (2,055)	-	-
Systemic therapy									
No chemotherapy or endocrine therapy	23,682 (174)	**Ref.**	**Ref.**	9,131 (80)	**Ref.**	**Ref.**	179,382 (1,139)	**Ref.**	**Ref.**
Only chemotherapy	2,557 (9)	0.49 [0.25-0.95]	0.45 [0.23-0.88]	1,183 (8)	0.78 [0.38-1.61]	0.73 [0.35-1.54]	68,033 (337)	0.73 [0.64-0.82]	0.78 [0.69-0.89]
Only endocrine therapy	18,852 (61)	0.45 [0.33-0.60]	0.48 [0.36-0.65]	5,697 (24)	0.49 [0.31-0.77]	0.51 [0.32-0.82]	85,639 (260)	0.45 [0.39-0.51]	0.49 [0.42-0.56]
Both chemotherapy and endocrine therapy	21,664 (75)	0.47 [0.36-0.62]	0.43 [0.32-0.57]	8,856 (29)	0.38 [0.25-0.58]	0.36 [0.23-0.56]	135,638 (319)	0.38 [0.34-0.43]	0.37 [0.32-0.42]
Radiotherapy	41,582 (209)	1.15 [0.91-1.45]	1.09 [0.86-1.38]	15,312 (92)	1.17 [0.83-1.65]	1.16 [0.81-1.64]	328,530 (1,443)	1.07 [0.98-1.18]	0.98 [0.89-1.08]
No radiotherapy	25,172 (110)	**Ref.**	**Ref.**	9,554 (49)	**Ref.**	**Ref.**	140,163 (612)	**Ref.**	**Ref.**
Age (10-year increase)	66,754 (319)	0.9 [0.94-1.02]	0.91 [0.82-1.01]	24,866 (141)	1.01 [0.96-1.08]	0.95 [0.81-1.12]	468,693 (2,055)	1.00 [0.997-1.003]	0.99 [0.95-1.03]

PYO: Person-years of observation; *N* CBC: Number of contralateral breast cancer events; uHR: univariable hazard ratio; mHR: multivariable hazard ratio. Estimates in the multivariable model were adjusted for all variables from the univariable model.
Time starts from 3 months after primary breast cancer diagnosis until metachronous contralateral breast cancer; Censoring events: ipsilateral recurrence including second breast cancer (either invasive or in situ); in situ contralateral breast cancer; second invasive non-breast tumour (except non-melanoma skin cancer); loss to follow-up; end of follow-up (31/12/2015); death.

Supplementary Table 1B. Univariable and multivariable Cox regression analyses of invasive metachronous CBC risk for ER/PR-positive with HER2- negative PBC stratified by histologic subtype

	Lobular			*Lobular mixed*			*Ductal*		
	PYO (*N* CBC)	**uHR [95% CI]**	**mHR [95% CI]**	**PYO (*N* CBC)**	**uHR [95% CI]**	**mHR [95% CI]**	**PYO (*N* CBC)**	**uHR [95% CI]**	**mHR [95% CI]**
Total group	43,255 (199)	-	-	15,117 (87)	-	-	229,813 (1,020)	-	-
Systemic therapy									
No chemotherapy or endocrine therapy	13,073 (99)	**Ref.**	**Ref.**	5,041 (48)	**Ref.**	**Ref.**	90,043 (646)	**Ref.**	**Ref.**
Only chemotherapy	1,008 (2)	0.26 [0.06-1.06]	0.24 [0.06-0.99]	313 (0)	*	*	4,857 (11)	0.32 [0.18-0.58]	0.33 [0.18-0.60]
Only endocrine therapy	13,003 (46)	0.47 [0.33-0.67]	0.50 [0.35-0.71]	3,735 (19)	0.54 [0.32-0.93]	0.55 [0.31-0.95]	53,840 (175)	0.46 [0.39-0.54]	0.44 [0.37-0.53]
Both chemotherapy and endocrine therapy	16,171 (52)	0.43 [0.30-0.60]	0.39 [0.27-0.57]	6,029 (20)	0.35 [0.21-0.60]	0.35 [0.20-0.62]	81,073 (188)	0.32 [0.28-0.38]	0.34 [0.28-0.40]
Radiotherapy	27,343 (130)	1.09 [0.81-1.46]	1.04 [0.77-1.40]	9,345 (55)	1.06 [0.69-1.65]	1.06 [0.68-1.64]	165,781 (728)	0.95 [0.83-1.09]	0.91 [0.79-1.04]
No radiotherapy	15,912 (69)	**Ref.**	**Ref.**	5,773 (32)	**Ref.**	**Ref.**	64,032 (292)	**Ref.**	**Ref.**
Age (10-year increase)	43,255 (199)	1.01 [0.90-1.13]	0.93 [0.80-1.07]	15,117 (87)	1.13 [0.94-1.35]	1.00 [0.82-1.24]	229,813 (1,020)	1.14 [1.08-1.20]	1.04 [0.98-1.10]

ER: estrogen receptor status; PR: progesterone receptor status; PYO: person-years of observation; *N* CBC: number of contralateral breast cancer events; uHR: univariable hazard ratio; mHR: multivariable hazard ratio. Estimates in the multivariable model were adjusted for all variables from the univariable model.

Time starts from 3 months after primary breast cancer diagnosis until metachronous contralateral breast cancer; Censoring events: ipsilateral recurrence including second breast cancer (either invasive or in situ); in situ contralateral breast cancer; second invasive non-breast tumour (except non-melanoma skin cancer); loss to follow-up; end of follow-up (31/12/2015); death.

SUPPLEMENTARY TABLES S2A-C

Metachronous invasive CBC characteristics of women with different histologic PBC subtypes.

CBC: contralateral breast cancer; *PBC*: primary breast cancer; ER: Oestrogen receptor status; PR: Progesterone receptor status.

Stage**:** Stage I: T1N0M0 and T0-1N1mi M0; Stage II: T0-1N1M0, T2N0M0, T2N1M0, or T3N0M0; Stage III: T0-2N2M0, T3N1-2M0, T4N0-2M0, or any T N3M0 breast cancer; stage IV: metastatic breast cancer.

Supplementary Table 2A. Metachronous CBC characteristics of women with lobular PBC

CBC characteristics	PBC characteristics						Total		
	N	***%***	***N***	***%***	***N***	***%***	***N***	***%***	***p*-value**
	PBC histology								
CBC histology	Lobular								
Lobular	89	*27.9*							
Lobular mixed	26	*8.2*							
Ductal	179	*56.1*							
Other	25	*7.8*							
Total	*319*	*100*							
	PBC TNM-stage								
	I		II		III				
CBC TNM-stage									<0.001
I	113	*71.5*	59	*58.4*	18	*32.1*	190	*60.3*	
II	34	*21.5*	35	*34.7*	15	*26.8*	84	*26.7*	
III	9	*5.7*	4	*4.0*	16	*28.6*	29	*9.2*	
IV	2	*1.3*	3	*3.0*	7	*12.5*	12	*3.8*	
Total	*158*	*100*	*101*	*100*	*56*	*100*	*315*	*100*	
	PBC differentiation grade								
	I		II		III				
CBC differentiation grade									0.007
I	26	*52.0*	40	*25.2*	5	*21.7*	71	*30.6*	
II	18	*36.0*	91	*57.2*	13	*56.5*	122	*52.6*	
III	6	*12.0*	28	*17.6*	5	*21.7*	39	*16.8*	
Total	50	*100*	159	*100*	23	*100*	232	*100*	
	PBC ER status								
	Positive		Negative						
CBC ER status									0.169
Positive	249	*86.2*	11	*73.3*			260	*85.5*	
Negative	40	*13.8*	4	*26.7*			44	*14.5*	
Total	289	*100*	15	*100*			304	*100*	

Supplementary Table 2A. Continued.

CBC characteristics	PBC characteristics						Total		
	N	***%***	***N***	***%***	***N***	***%***	***N***	***%***	***p*-value**
	PBC PR status								
	Positive		Negative						
CBC PR status									
Positive	142	*62.0*	30	*54.6*			172	*60.6*	
Negative	87	*38.0*	25	*45.5*			112	*39.4*	
Total	229	*100*	55	*100*			284	*100*	
	PBC HER2 status								
	Positive		Negative						
CBC HER2 status									0.015
Positive	4	*26.7*	15	*7.8*			19	*9.2*	
Negative	11	*73.3*	177	*92.2*			188	*90.8*	
Total	15	*100*	192	*100*			207	*100*	

Supplementary Table 2B. Metachronous CBC characteristics of women with lobular mixed PBC

CBC characteristics	PBC characteristics						Total		
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*p*-value
	PBC histology								
CBC histology	Lobular mixed								
Lobular	36	*25.5*							
Lobular mixed	10	*7.1*							
Ductal	78	*55.3*							
Other	17	*12.1*							
Total	*141*	*100*							
	PBC TNM-stage								
	I		II		III				
CBC TNM-stage									0.004
I	46	*59.0*	24	*57.1*	8	*40.0*	78	*55.7*	
II	30	*38.5*	16	*38.1*	6	*30.0*	52	*37.1*	
III	2	*2.6*	1	*2.4*	4	*20.0*	7	*5.0*	
IV	0	*0*	1	*2.4*	2	*10.0*	3	*2.1*	
Total	*78*	*100*	*42*	*100*	*20*	*100*	*140*	*100*	
	PBC differentiation grade								
	I		II		III				
CBC differentiation grade									0.161
I	6	*19.4*	24	*35.8*	3	*16.7*	33	*28.4*	
II	21	*67.7*	30	*44.8*	10	*55.6*	61	*52.6*	
III	4	*12.9*	13	*19.4*	5	*27.8*	22	*19.0*	
Total	31	*100*	67	*100*	18	*100*	116	*100*	
	PBC ER status								
	Positive		Negative						
CBC ER status									0.236
Positive	112	*90.3*	7	*77.8*			119	*89.5*	
Negative	12	*9.7*	2	*22.2*			14	*10.5*	
Total	124	*100*	9	*100*			133	*100*	

Supplementary Table 2B. Continued.

CBC characteristics	PBC characteristics						Total		
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*p*-value
	PBC PR status								
	Positive		Negative						
CBC PR status									0.335
Positive	65	*66.3*	17	*56.7*			82	*64.1*	
Negative	33	*33.7*	13	*43.3*			46	*35.9*	
Total	98	*100*	30	*100*			128	*100*	
	PBC HER2 status								
	Positive		Negative						
CBC HER2 status									0.291
Positive	2	*28.6*	12	*13.8*			14	*14.9*	
Negative	5	*71.4*	75	*86.2*			80	*85.1*	
Total	7	*100*	87	*100*			94	*100*	

Supplementary Table 2C. Metachronous CBC characteristics of women with ductal PBC

CBC characteristics	PBC characteristics						Total		
	N	***%***	***N***	***%***	***N***	***%***	***N***	***%***	***p*-value**
	PBC histology								
CBC histology	Ductal								
Lobular	217	*10.6*							
Lobular mixed	75	*3.7*							
Ductal	1,541	*75.0*							
Other	222	*10.8*							
Total	*2,055*	*100*							
	PBC TNM-stage								
	I		II		III				
CBC TNM-stage									<0.001
I	861	*69.7*	316	*56.7*	101	*47.9*	1,278	*63.8*	
II	280	*22.7*	164	*29.4*	48	*22.8*	420	*21.0*	
III	76	*6.2*	60	*10.8*	44	*20.9*	180	*9.0*	
IV	19	*1.5*	17	*3.1*	18	*8.5*	54	*2.7*	
Total	*1,236*	*100*	*557*	*100*	*211*	*100*	*2,004*	*100*	
	PBC differentiation grade								
	I		II		III				
CBC differentiation grade									<0.001
I	182	*39.7*	202	*27.2*	81	*17.0*	465	*27.7*	
II	201	*43.9*	357	*48.1*	171	*35.8*	729	*43.4*	
III	75	*16.4*	183	*24.7*	226	*47.3*	484	*28.8*	
Total	458	*100*	742	*100*	478	*100*	1,678	*100*	
	PBC ER status								
	Positive		Negative						
CBC ER status									<0.001
Positive	1,264	*86.8*	239	*55.6*			1,503	*79.7*	
Negative	192	*13.2*	191	*44.4*			383	*20.3*	
Total	1,456	*100*	430	*100*			1,886	*100*	

Supplementary Table 2C. Continued.

CBC characteristics	PBC characteristics						Total		
	N	***%***	***N***	***%***	***N***	***%***	***N***	***%***	***p*-value**
	PBC PR status								
	Positive		Negative						
CBC PR status									<0.001
Positive	761	*65.9*	294	*46.5*			1,055	*59.0*	
Negative	394	*34.1*	338	*53.5*			732	*41.0*	
Total	1,155	*100*	632	*100*			1,787	*100*	
	PBC HER2 status								
	Positive		Negative						
CBC HER2 status									<0.001
Positive	53	*30.6*	105	*8.7*			158	*11.4*	
Negative	120	*69.4*	1,103	*91.3*			1,223	*88.6*	
Total	173	*100*	1,208	*100*			1,381	*100*	

Sensitivity analysis

To investigate whether ignoring recurrent disease in the main analysis could lead to biased results, we performed a sensitivity analysis.

Five-year follow-up on recurrences was complete for patients diagnosed with PBC between 2003 and 2006 and for 56% of the patients diagnosed between 2007-2008. For patients diagnosed between 2009-2010 no information on recurrences was available. With the subgroup of patients with complete 5-year follow-up information on recurrent disease, we performed a sensitivity analysis in order to evaluate the impact of taking into account recurrent disease on the results. For this analysis, local, regional and distance recurrences were also considered as a censoring endpoint, next to censoring at diagnosis of an ipsilateral second breast tumour, a second non-breast tumour, non-invasive CBC, death, or last follow-up (31/12/2015).

The analyses were compared to the same subset with ignoring local, regional and distant recurrence as an endpoint.

In total, 46, 591 patients with complete 5-year follow-up information on recurrent disease were included for this analysis. HRs from the sensitivity analysis were overlapping with those from the primary analysis.

Supplementary Table 3. Sensitivity analysis: univariable and multivariable Cox regression analyses for invasive metachronous CBC risk in patients with complete follow-up on recurrent disease

	PYO		*N* CBC		uHR [95% CI]		mHR [95% CI]	
Total group	207,681	*214,921*	874	*934*	-	-	-	-
Lobular	25,242	*25,992*	126	*133*	1.24 [1.02-1.50]	*1.23 [1.02-1.48]*	1.35 [1.12-1.64]	*1.36 [1.13-1.64]*
Lobular (mixed)	9,918	*10,205*	52	*56*	1.30 [0.98-1.72]	*1.32 [1.00-1.73]*	1.40 [1.05-1.85]	*1.43 [1.09-1.88]*
Ductal	172,617	*178,724*	696	*745*	**Ref.**	***Ref.***	**Ref.**	***Ref.***
Systemic therapy								
No chemotherapy or endocrine therapy	84,404	*86,683*	534	*546*	**Ref.**	***Ref.***	**Ref.**	***Ref.***
Only chemotherapy	25,130	*26,780*	121	*144*	0.77 [0.63-0.94]	*0.86 [0.72-1.03]*	0.79 [0.64-0.98]	*0.87 [0.72-1.06]*
Only endocrine therapy	40,960	*42,139*	108	*113*	0.42 [0.34-0.52]	*0.43 [0.35-0.52]*	0.41 [0.33-0.51]	*0.42 [0.34-0.52]*
Both chemotherapy and endocrine therapy	57,284	*59,319*	111	*131*	0.31 [0.25-0.37]	*0.35 [0.29-0.42]*	0.30 [0.24-0.37]	*0.34 [0.27-0.41]*
Radiotherapy	142,209	*146,861*	594	*637*	0.97 [0.84-1.12]	*0.99 [0.86-1.14]*	0.96 [0.83-1.11]	*0.98 [0.85-1.12]*
No Radiotherapy	65,569	*68,060*	280	*297*	**Ref.**	***Ref.***	**Ref.**	***Ref.***
Age (10-year increase)	207,681	*214,921*	874	*934*	1.03 [1.00-1.05]	*1.03 [0.98-1.09]*	0.99 [0.93-1.05]	*0.98 [0.92-1.04]*

Abbreviations: PYO: Person-years of observation; *N* CBC: number of contralateral breast cancer events; uHR: univariable hazard ratio; mHR: multivariable hazard ratio. Estimates in the multivariable model were adjusted for all variables from the univariable model.
Time starts from 3 months after primary breast cancer diagnosis until metachronous contralateral breast cancer; Censoring events: local, regional or distant recurrence, ipsilateral second breast cancer (either invasive or in situ); in situ contralateral breast cancer; second invasive non-breast tumour (except non-melanoma skin cancer); loss to follow-up; end of follow-up (31/12/2015); death. Hazard ratios are given for the first 5 years since information on recurrence is only available for the first 5 years of follow-up.
Numbers in Italic depict the outcome when local, regional and metastatic disease was ignored as a censoring event.

CHAPTER 8

DISCUSSION

While the Egyptians (2600 B.C.) were probably the first to describe cancer as a disease, Hippocrates (400 B.C.) was the first to use the word cancer. He used the Greek word for crab, i.e., karkínos, as he observed a similarity between the hard body of a crab (forming the nucleus of a tumor) and its legs (looking like the tentacles of a tumor).[1] In many centuries thereafter cancer was considered a deadly disease by definition, which also accounts for breast cancer. However, especially in the last five decades more knowledge has been gathered concerning the etiology and treatment of breast cancer, which enabled patients to survive for longer periods and even being cured.[2,3] On the other hand, as a consequence the risk of developing late complications from primary breast cancer treatment have become more important, for example the risk of developing secondary tumors such as contralateral breast cancer (CBC) and cardiovascular disease. For CBC risk, we know that the yearly risk varies between 0.5% in the general population and can be up to 3% in young women carrying a *BRCA1/2* mutation.[4-8] However, which other factors, and to which extent, are contributing to CBC risk has been under debate. Also, within different risk groups, different effects of certain factors might be observed. For example, systemic treatment of the primary breast cancer has been associated with decreased CBC risk. However, whether the risk of CBC in women with a *BRCA1/2* mutation might be influenced by primary breast cancer treatment in a different way than in sporadic primary breast cancer patients, is to be investigated.

The key objective of the studies in this thesis was to identify factors associated with metachronous CBC risk. This insight is crucial since these risk factors could subsequently be integrated in a personalized CBC risk prediction model to provide accurate risk estimates and to help patients and physicians to make better, evidence-based choices concerning risk-reducing measures and screening. Eventually, we also hope that patients who experience anxiety might be reassured of their choices. In this chapter we elaborate on additional concepts and perspectives of studies on CBC risk and we will end with recommendations for future research.

Within this thesis, mainly observational cohort studies were conducted (chapters 4-7). One of the most important caveats in the adequate interpretation of the data from this type of studies is to correct for the different forms of biases that can accompany observational cohort studies. Bias is defined as any systematic error in the design, conduct or analysis of a study that results in a mistaken estimate of an exposure's association with the risk of the disease.[9] In general, three types of bias can play a role, which will be elaborated on in the context of our studies.

Bias Type	*Definition*	*Example*	*Chapters*
1.Selection bias	Differences in the condition or procedure that have been used to select patients and/or differences in factors that influence study participation that can lead to a distortion of the results. *Types of selection bias* *Survival bias*: A distortion in the selection that occurs if participants needed to have survived beyond a specific time point (in our studies until *BRCA1/2* testing became available) to be included in the study. Patients who did not survive up until this time point have not been included. *Testing bias*: when the selection of patients is being distorted as a result of different criteria that have been applied to test for the presence of a germline mutation. *Ascertainment bias*: a distortion in the selection that occurs due to differences in the date of ascertainment in families (e.g., families with higher penetrance of a gene might be identified earlier than families with a low penetrance).	Including patients with a germline *BRCA1/2* mutation.	4,5,6
2.Confounding	A factor (i.e., the confounder) distorting the estimate because it is associated with both the factor of interest (i.e., the exposure) and the outcome and is not a mediator.	Age at primary breast cancer diagnosis in the studies investigating chemotherapy and radiotherapy.	4,5,6
3.Information bias	A distortion of the results as a consequence of systematic differences in the collection, recall, recording or handling of study information. The systematic differences can be different for the exposed and non-exposed (i.e., differential misclassification) or the differences can be the same across the groups (non-differential).	Part of the CBCs might actually be a metastatic spread of the primary tumor rather than a new primary tumor.	2,3,4,5,6,7

1. Selection bias

In our studies including carriers of a pathogenic germline mutation in the *BRCA1* or *BRCA2* gene (chapters 4, 5 and 6), mainly survival, ascertainment and testing bias played a potential role, which are all part of selection bias. Selection bias is induced when there are differences in the condition or procedure that was used to select patients and/or when there are differences in factors that influence study participation.[10]

Survival bias

In our studies concerning *BRCA1/2* mutation carriers (chapters 4-6), patients were included prior to genetic testing for the presence of a *BRCA1/2* mutation became available. For example, in chapter 4 where we investigated the association of chemotherapy with CBC risk in *BRCA1/2* mutation carriers, *BRCA1/2* mutation carriers were eligible if they had a primary breast cancer diagnosis from 1990 onwards. Genetic testing for the presence of a germline *BRCA1/2* mutation became however only available around 1994-1995. Therefore, patients with a primary breast cancer diagnosis prior to this period needed to have survived until the test became available to be included in the study (situation *A*, Figure 1). Patients who died prior to that period, would not be included since they have not survived long enough to be tested (situation *B*, Figure 1). Patients were thus included conditional on survival until the test result.

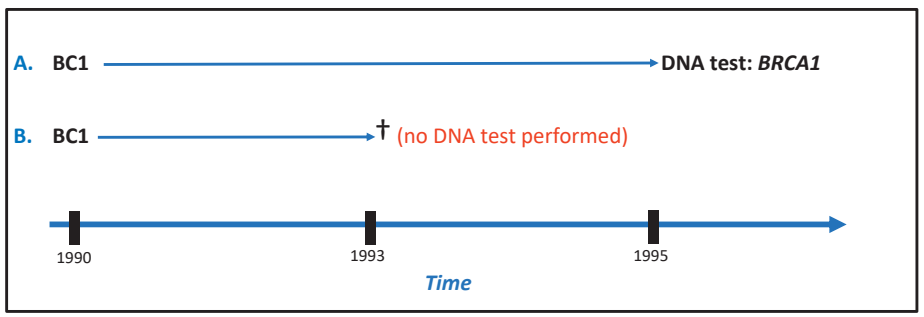

Figure 1. A graphical presentation of survival bias.

Primary breast cancer patients needed to have survived until 1995 (i.e., the year BRCA1/2 testing became known and available; situation A). Patients who have not survived (situation B) will not be identified as a carrier and will not be included in a study. This can lead to a distortion in the selection of patients.

Therefore it might seem that patients with a mutation will survive for longer periods leading to survival bias.[11] In chapter 4, *BRCA1/2* mutation carriers with a small and node negative primary breast cancer did not receive chemotherapy because of a relatively favorable prognosis. They have more likely survived long enough to be identified as a carrier in comparison to mutation carriers with primary breast cancer who did receive chemotherapy and had relatively unfavorable prognosis. As a consequence of longer survival, the *BRCA1/2* mutation carriers who did not receive chemotherapy because of their favorable prognosis have likely been longer at risk to develop a CBC. Indeed, follow-up in *BRCA1* carriers was longer in the non-chemotherapy group (13.8 years vs. 10.0 years in the chemotherapy group, respectively, p<0.001) and a similar trend was observed in *BRCA2* carriers (10.4 years vs. 9.7 years, respectively, p=0.40). In addition, *BRCA1* and *BRCA2* mutation carriers who received no chemotherapy were more often diagnosed with their primary breast cancer between 1990 and 1995 in comparison to the groups with chemotherapy (32% vs. 8% in *BRCA1* and 16% vs. 6% in *BRCA2*, respectively). In the years 1990-1995, breast cancer patients were mainly treated with cyclophosphamide, methotrexate and 5-fluorouracil (CMF), a regimen that we showed was not associated with reduced CBC risk (chapter 4). Potentially, if carriers with CMF-based chemotherapy that are currently missing from the years prior to testing becoming available, could have been included in the study, there would have been more patients in the chemotherapy group with relatively more CBCs. The difference in the number of CBCs between the chemotherapy and the non-chemotherapy group would therefore become smaller, resulting in a reduction of the size of the association. The current effect of chemotherapy on CBC risk could thus have been slightly overestimated.

Ascertainment and Testing bias

Ascertainment and testing bias are other forms of selection bias which we took into account in our studies including patients with pathogenic germline mutations (i.e., *BRCA1/2* mutation; chapters 4-6). In more recent years the criteria to being tested for the presence of a germline mutation have been broadened, and more carriers have been identified. However, overall, not all women meet the criteria to being tested, despite being a carrier. In general, if a positive test result is necessary for study entrance but not everyone is being tested because specific testing criteria are used, a distortion in the selection can occur, i.e., testing bias.[12] For example, if a woman is diagnosed with her primary breast cancer under the age of 50 and

she is subsequently being diagnosed with a CBC, this is an indication to test for the presence of a *BRCA1/2* mutation.[13] This leads to oversampling of patients with a CBC diagnosis in our studies concerning *BRCA1/2* mutation carriers, which will presumably result in a higher CBC incidence. Carriers who have not developed a CBC are less likely to be identified as a carrier and included in the study, since they had no indication to be tested. Including these non-identified carriers without a CBC would most likely result in lower CBC incidence. In addition, a strong positive family history for breast cancer is also an indication to test for the presence of a *BRCA* mutation. The stronger the family history, the sooner a mutation would be ascertained, which leads to more families with high penetrant genes being ascertained earlier and also more often than families with relatively low prevalence of breast cancer, leading to ascertainment bias.[12]

With delayed entry or left truncation, which was applied in chapters 4-6, we aimed to limit survival, ascertainment and testing bias.[12,14] In the analysis we started follow-up time from primary breast cancer diagnosis or DNA test diagnosis, whichever came last. Patients will then be included in the study but less follow-up time will be assigned.

In Figure 2 different scenarios are illustrated to elucidate on this matter. In our studies, patients similar to situation *A* developed a primary breast cancer and as for example more relatives developed cancer, an indication to test for the presence of a pathogenic germline mutation can arise. After the patient is being tested positive, she develops a CBC. Since however not all patients will meet the criteria for being tested, this can give rise to bias. For example, patients similar to situation *B*, who might come from a small family and die of metastatic disease prior to developing a CBC, might never be identified as a carrier and would therefore not be included in our study.

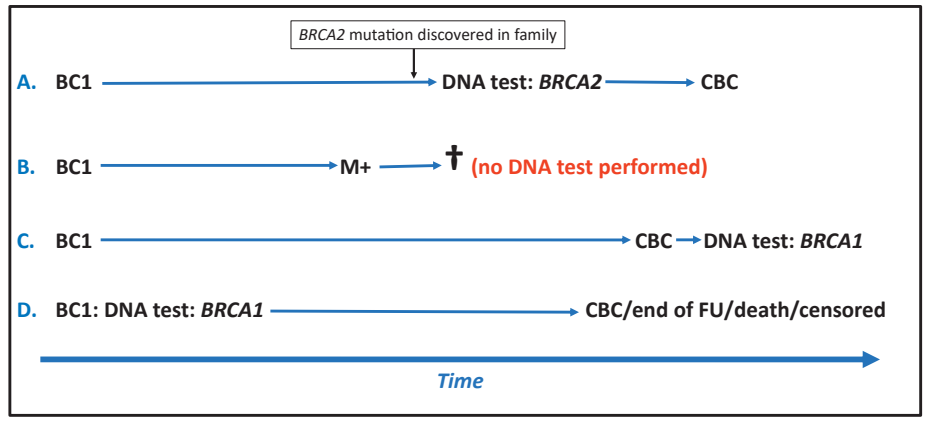

Figure 2. A graphical representation of different timelines for patients to be or not to be identified as a BRCA1/2 mutation carrier and the risk of ascertainment and testing bias that can arise

Situation A: the patient develops her primary breast cancer (BC1) and after a genetic BRCA2 mutation has been discovered in the family, this patient is also tested and identified as a carrier. The stronger the family history, the sooner a carrier will get identified, resulting in ascertainment bias. If a patient comes from a small family and the patient dies from metastatic disease (B), she might not be identified as a carrier. A patient who develops a CBC during follow-up (C), has a reason to be tested if the primary tumor was diagnosed under the age of 50. However, since developing a CBC is the reason for being tested, this can give rise to testing bias. Situation D depicts the ideal situation: every primary breast cancer patient is tested and subsequently followed over time.

To correct for the missed time from the non-included carriers who did not survive long enough or did not meet the criteria for being tested, we started follow-up time at the DNA test diagnosis for included patients comparable to situation A. The time on the left side of the DNA test diagnosis in the figure is excluded for the analysis, i.e., left truncation of the analysis.[11] As mentioned, these patients will enter the study at a later time point (as can also be observed from the numbers at risk in Figures 3A and 3B which can increase over time). Less time will thus be taken into account and this will compensate for the missed time from the non-included carriers who did not survive long enough or did not meet the criteria to being tested for the presence of a *BRCA1/2* mutation.

Further, oversampling of CBC cases may occur, which is the main problem of our studies (chapters 4, 5 and 6) and is described in situation C: patients have an indication to be tested if they develop a primary breast cancer before the age of 50 and subsequently a CBC, but if the same patient (comparable to a patient from

situation *B*) would not have developed a CBC, she might not have been tested and not be identified as a carrier. These non-identified patients have not been able to contribute any time to the analysis. Including these patients would lead to a lower CBC incidence. Therefore, in these cases too, we applied left truncation and started follow-up from the DNA test diagnosis. In situation C, it results in patients being excluded, since they developed the study endpoint (CBC) before entering the study (i.e., follow-up time starts at DNA test). This will subsequently lead to a lower CBC incidence and therefore compensate for the missed time from the non-identified carriers without CBC.

To illustrate the potential consequences of not taking into account ascertainment/testing bias on absolute CBC risk, we used our study on CBC risk after chemotherapy for primary breast cancer in *BRCA1/2* mutation carriers (chapter 4). Data hereon are presented in Figure 3A (no left truncation applied; left) and Figure 3B (left truncation applied; right) and Table 1. Higher absolute CBC risks can be observed in the analysis prior to correcting for ascertainment/testing bias. Though a small overcorrection can have been created by excluding the prevalent cases, the estimates following from Figure 3B are more in line with results from other studies in which a prospective analysis has been performed (such as demonstrated in situation D from Figure 2).[7,8]

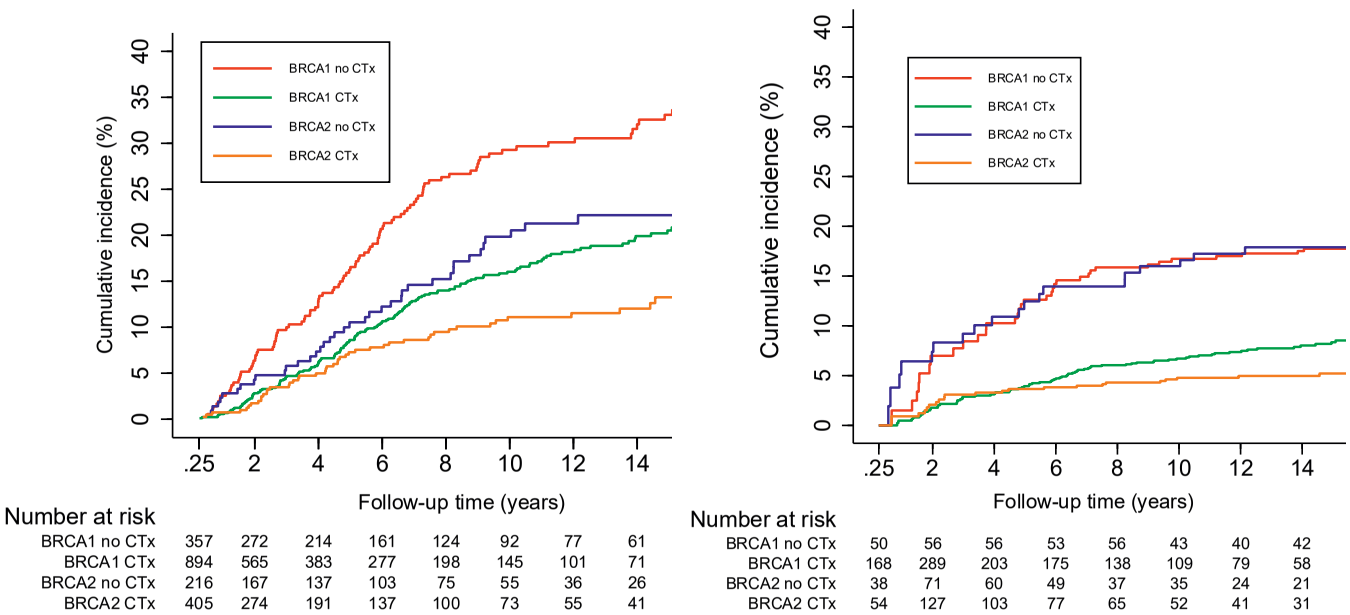

Figure 3A and 3B. Cumulative CBC risk when left truncation was not applied (left) and when left truncation was applied (right)
Patients were censored at secondary invasive cancer diagnosis (except non-melanoma skin cancer), ipsilateral secondary invasive/non-invasive breast cancer diagnosis or end of study. Death and contralateral or bilateral risk-reducing mastectomy were treated as competing risks.

Table 1. Ten-year cumulative CBC risk (%) in *BRCA1* and *BRCA2* mutation carriers when left truncation is applied and when no left truncation to correct for ascertainment/ testing bias is applied.

Ten-year cumulative CBC risk	No left truncation applied	Left truncation applied
***BRCA1* Chemotherapy**	16%	7%
***BRCA1* No chemotherapy**	30%	17%
***BRCA2* Chemotherapy**	11%	5%
***BRCA2* No chemotherapy**	21%	16%

Idealistically, to get an unbiased estimate for the absolute CBC risk, we would thus test the entire group of women who have developed a primary breast cancer for the presence of a *BRCA1/2* mutation and we would follow all carriers over time (i.e., prospective analysis; depicted in Figure 2, situation D and as has been performed in the BOSOM study).[8] Subsequently, CBC development is being monitored and risk associations can be established. In chapter 4, we also inspected absolute CBC risk estimates within a prospective setting. Though the number of events was small, results for *BRCA1* carriers were comparable to left truncating the analysis, indicating that left truncation of the analysis provides reliable estimates (for *BRCA2* mutation carriers the numbers were too small to provide reliable estimates).

2. Confounding

In our studies concerning *BRCA*-associated breast cancer (chapters 4-6), multiple factors have been suggested as a confounder and if so, we corrected for this factor in the analysis. Confounding occurs when a factor (i.e., treatment, labeled: 'exposure' in epidemiological terms; Figure 4) seems associated with the outcome, while in fact there is a another factor (i.e., the confounder) that leads to a distortion of this association.[10] A factor should be considered a confounder if it is associated with both exposure and outcome and it is not an intermediate between the exposure and outcome.

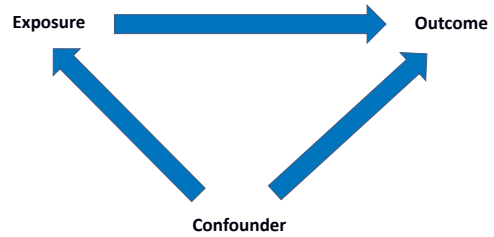

Figure 4. Confounding

An example hereof is finding an association between chemotherapy and CBC risk, while actually age at primary breast cancer diagnosis as a confounder in this matter, is associated with both receiving chemotherapy and CBC risk. This is also represented in the directed acyclic graph from Figure 5A.

Studies analyzing CBC risk in *BRCA1/2* mutation carriers have been often adjusted for the potential confounding effect of risk-reducing salpingo-oophorectomy. From Figures 5A and 5B, it however shows that since risk-reducing salpingo oophorectomy is not associated with the exposure chemotherapy, it cannot be labeled as a confounder. Instead, it should be considered as a proxy confounder as it lies between the confounder age and the outcome CBC. Through correction for the variable age, risk-reducing salpingo oophorectomy can be accounted for concurrently.[15]

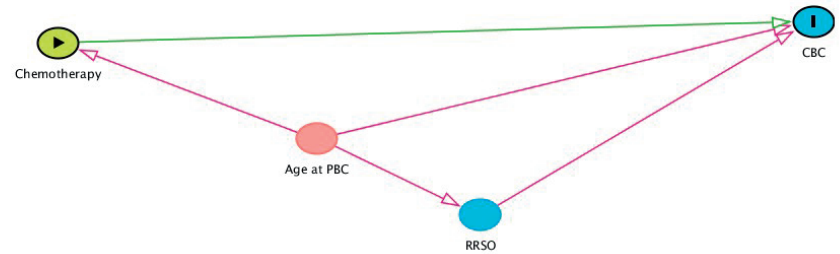

Figure 5A. Directed acyclic graph of showing the confounding effect of age at primary breast cancer (PBC) diagnosis on the exposure chemotherapy

Age is a confounder as it has an association with the exposure chemotherapy and the outcome CBC and it is not a mediator between chemotherapy and CBC. When not adjusted for age, the association between chemotherapy and CBC risk is biased. Age can also be considered a confounder for the exposure Risk-reducing salpingo oophorectomy (RRSO). RRSO is considered a proxy-confounder, i.e., a covariate that lies between the confounder and the exposure or outcome.

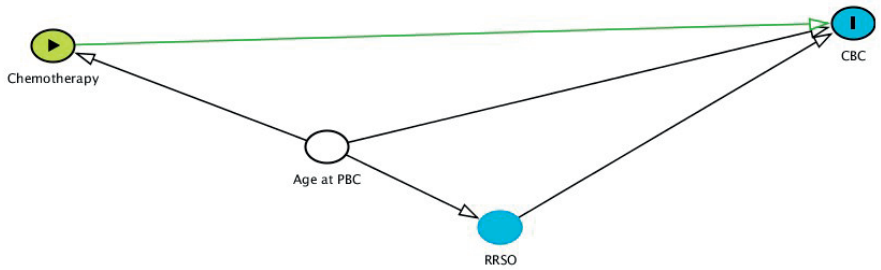

Figure 5B. Directed acyclic graph showing the effects of adjusting for the confounding effect of age at primary breast cancer (PBC) diagnosis on the exposure chemotherapy and the outcome CBC (in this figure signified by the pink lines that have now turned black, see also Figure 5A)

The effect of chemotherapy on CBC risk is no longer considered biased. By correcting for age the effect of the proxy confounder RRSO on CBC risk has also been controlled for.

In our studies where we investigated the potential confounding effect of risk-reducing salpingo-oophorectomy (chapters 4-6), we found no association with the exposure nor did we observe an alteration of the CBC risk. It should be noted that regardless of whether an association between risk-reducing salpingo oophorectomy and CBC was found, no adjustment for risk-reducing salpingo oophorectomy is necessary as it is a proxy confounder. The hypothesis is that risk-reducing salpingo-oophorectomy interacts with Estrogen hormone receptor status and is especially protective for Estrogen hormone receptor-positive breast cancers, which are predominately present in *BRCA2* mutation carriers.[16-18] In our studies concerning *BRCA1/2* mutation carriers, the group of *BRCA2* mutation carriers was often smaller and likely therefore, we did not observe an effect of risk-reducing salpingo-oophorectomy. Also, in the specific case of CBC risk association studies, the modifying effects of primary breast cancer therapy needs to be taken into account. For example, Tamoxifen, an anti-estrogen compound, has been proven a very effective drug for treatment of hormone receptor positive primary breast cancer and could conceal the effects of risk-reducing salpingo-oophorectomy. Further, it is of importance to have knowledge on the time it will take in *BRCA1/2* mutation carriers for a new tumor to develop. If (contralateral) tumors are already present (but non-detectable), the effects of risk-reducing salpingo-oophorectomy might be minimal.

3. Information bias

Throughout our research a recurring subject of discussion was misclassification of outcome. In our project, misclassification concerns the question whether a part of the CBCs are metastases of the primary tumor rather than new tumors in the contralateral breast. Misclassification is a form of information bias. Differential misclassification in cohort studies occur when there are systematic differences in the collection, recording or handling of study information for the exposed and non-exposed.[10] In our systematic review and meta-analysis including tumor characteristics (chapter 2), we noticed that especially factors that have been associated with poor survival, i.e., higher stage, worse differentiation grade and negative Estrogen hormone receptor status, were associated with higher CBC risk. This could suggest that part of the CBCs were actually metastatic spreads of the primary tumor. Moreover, in part of these studies, patients were not censored at diagnosis of metastatic disease, probably resulting in even higher numbers of misclassified cases.

On the other hand, considering the anatomy of the female thorax, there are no direct lymphatic or vessel connections between the breasts. A contralateral metastasis might therefore not be more likely than metastases at other locations in the body. This can also be concluded from literature: metastasis to the contralateral breast is observed in only 6% of the women who have been diagnosed with metastatic disease (though in these studies it was not mentioned how metastatic disease and true CBCs have been discerned).[19-21] We would assume this percentage would be higher if a direct connection would exist. (Nonetheless, a contralateral breast tumor is often considered a new secondary tumor, making it difficult to assess the true number of metastatic spreads to the contralateral breast).

However, heterogeneity in susceptibility for cancer development and/or the presence of multiple underlying risk factors might play a role in the development of multiple cancers. If a woman develops a primary tumor, a higher susceptibility for the development of a secondary tumor might be present as well. Perhaps it is even possible that when a tumor is developing in one breast, biologically a change occurs that affects the environment in both breasts. This could create a niche, making the contralateral breast more vulnerable to a new tumor and/or to metastatic spread, as has also been observed for bone metastases.[22,23]

The methods to distinguish a CBC and metastatic spread have evolved rapidly over the years: at first clinical-pathological factors were used, e.g., hormone receptors and the time window between the first and the second tumor; more

recently, biological, i.e., molecular and genetic comparisons have been made between the primary breast cancer and the CBC, first in several smaller studies[24-26], and more recently also in a few larger studies.[27,28] In a majority of these studies a relatively small percentage of the secondary tumors were actually defined as a metastatic spread (between 0-12%), except for one study in which it was 20%.[28]

If we are able to differentiate between metastatic spreads originating from the primary tumor and new independent second malignancies we might be able to better inform patients. First it would be important to be able to differentiate because of survival differences, as a secondary cancer diagnosis has better prognosis than being diagnosed with metastatic disease. Also, this differentiation might potentially provide us additional information on who will eventually develop metastatic disease elsewhere in the body and who will not, and thus, who did not have a beneficial effect of the primary breast cancer treatment (as metastatic disease developed after the given treatment). These patients might therefore benefit from other treatment options.

Perhaps differentiating can also provide us insight on how to treat patients, i.e., if the metastatic spread is only present in the contralateral breast, should we then treat a patient as metastasized disease or can we still treat it as a new entity, or should we treat it as oligo metastatic disease? In other words, in retrospect, we would like to know whether survival was different for patients with a second malignancy in the contralateral breast from tumors that were actually a metastatic spread. Subsequently, we need to identify new or perhaps a combination of new and classical clinical and pathological characteristics that will enable us to make selections of patients at higher risk of metastatic disease in the contralateral breast. In this way, we will also be able to differentiate between those who might or might not benefit from alternative treatment options.

CBC risk prediction model

The final goal of the whole Dutch Cancer Society project, which this thesis is part of, was to build a CBC risk prediction model that can provide accurate personalized CBC risk estimates based on personal and primary tumor and treatment characteristics. The risk factors that have been incorporated in the current model are *BRCA* and *CHEK2c.1100delC* mutation status, PRS-313 score, BMI, parity, first degree family history (yes/no), age at primary breast cancer diagnosis, primary breast cancer characteristics (tumor size, nodal status, morphology, tumor grade, Estrogen receptor status, HER2 status) and primary breast cancer treatment (chemotherapy, endocrine therapy, targeted therapy and radiotherapy). The current model has an observed/expected ratio of 0.92 at 10 years and an Area-Under-the-Curve of 0.65.[5,29,30] Eventually, we aim to help patients and their physicians to consider and discuss a personalized strategy concerning CBC risk management. There are several considerations that have to be made when implementing a CBC risk prediction model. Here we will mainly focus on the psychosocial aspects.

In previous studies it was shown that a considerable part of the patients without germline pathogenic variants tend to overestimate their risk of CBC.[31,32] In addition, the uptake of risk-reducing surgery has been significantly increasing over time, while no survival benefit has been shown for the majority of the patients.[33,34] If we can show, by using a personalized risk prediction model, that CBC risk is relatively low for most patients, and that survival is mainly determined by other outcomes (e.g., outcome of the primary breast cancer, cardiovascular disease or other malignancies), at least a part of the patients can be reassured.

Nonetheless, one of the main reasons for patients to opt for preventive removal of the contralateral breast is that they do not want to experience the psychological burden of a cancer diagnosis all over again.[31,35,36] Apparently, the knowledge of having a relatively low CBC risk and having a high survival probability, does not outweigh the potential adverse effects of prophylactic surgery, while up to one out of three patients can experience side-effects from prophylactic surgery.[31,37,38] Nonetheless, a recent study showed that patients who opt for a contralateral prophylactic mastectomy as a risk-reducing measure, experience more anxiety prior to surgery than women who were planning to undergo a single sided procedure, indicating that the fear of developing another cancer or recurrent disease is a major concerning issue, but probably already present and opting for prophylactic surgery was a coping strategy.[39] In addition, quality of life in women who have undergone a risk-reducing measure seems to worsen over time, mainly as a consequence of a negative body image.[39,40]

It is therefore of importance to increase awareness among counselors of taking even more into account a woman's character, perceptions and her approach to life events when counseling on risk-reducing treatment options after primary breast cancer diagnosis. This will also at least in part help to determine whether/how a woman is receptive to the risk estimate from a risk prediction model. To reduce fear, it could help to offer longer and/or more intensive psychological support after a primary breast cancer diagnosis.

Future research

To optimize the predictive ability of the current model[29], there are some other potential risk factors that have to be investigated. For example, pathogenic germline variants in genes other than *BRCA1* and *BRCA2* have been associated with a (potentially) increased risk of CBC, such as *PALB2*, *TP53*, *BARD1*, *RAD51C*, *RAD512D* and *CHEK2* (other than the 1100delC mutation) (Morra et al, under review). The sizes of the association and its potential impact on risk-reducing measures should be further investigated to improve personalized counseling in these patients.

Further, breast density has earlier been strongly associated with primary breast cancer.[41] The association between breast density and CBC risk is however only investigated in a few studies with inconclusive results.[42-44] Breast density is therefore certainly a factor that needs to be further investigated, preferably in a cohort with fully-automated measuring programs to reduce intra and especially inter observer variability.

Also, the effect of different (changes in) lifestyle and reproductive factors, that we investigated in chapter 3, should be assessed, preferably in a randomized setting.

In recent studies, PARP-inhibitors and platinum-containing chemotherapeutics have been associated with increased primary breast cancer survival in metastasized *BRCA1/2*-defective cancers.[45,46] In addition, another randomized clinical trial also showed increased disease-free survival after adjuvant treatment with PARP-inhibitors.[47] In future studies this will therefore most likely result in the implementation of PARP-inhibitors in the adjuvant treatment of breast cancer. Whether this also reduces CBC risk needs to be investigated as well.

Currently, primary breast cancer treatment is administered to eliminate micro-metastases and reduce recurrence rates. CBC risk is not taken into account when primary breast cancer patients receive their treatment. However, we did observe that both chemotherapy and endocrine therapy are associated with reduced CBC risk. In future studies it could prove useful to identify risk factors for aggressive CBCs (i.e., CBCs with higher risk of recurrence or metastatic disease) that might benefit from additional primary breast cancer systemic treatment options or additional screening. This could impact the survival of breast cancer patients.

As has been mentioned above, a small but non-negligible part of CBCs are probably a metastatic spread of the primary tumor rather than a new tumor. In future studies, methods to differentiate between metastatic disease and secondary cancers, as well as the possible considerations mentioned earlier, should be investigated.

In a CBC risk prediction model, it would be of importance to put CBC risk in perspective of other outcomes, such as (C)BC survival and death due to other causes such as cardiovascular disease. In addition, the benefit and harms of preventive removal of a healthy (contralateral) breast should be incorporated. This will provide both the patient and the treating physician a wide overview of the possibilities and can help to make better choices. In future studies it would therefore be very useful to incorporate not only CBC risk, but all other outcomes, such as survival gain of risk-reducing surgery and death due to other causes, as well.

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CHAPTER 9

GENERAL SUMMARY

Breast cancer is the leading cause of cancer in women in industrialized countries, with 1 out of 7 women being affected in her life in the Netherlands. Over the years advanced screening, treatment and surveillance options have led to increased breast cancer survival. Consequently, the number of breast cancer survivors at risk of developing contralateral breast cancer (CBC) has been increasing as well. The annual CBC risk varies between 0.5% in the general population and can be up to 3% in women with a germline *BRCA1/2* mutation.

We aimed to identify risk factors for CBC that could be used in a CBC risk prediction model to provide individualized CBC risk estimates for patients diagnosed with primary breast cancer. The main goal of this risk prediction model is to provide better insight into CBC risk for the individual patient, thereby optimizing surveillance and treatment decisions and improving quality of life in breast cancer patients.

In **Chapter 2** and **Chapter 3** a systematic review and meta-analysis were conducted to investigate the effects of confirmed and suggested risk factors for CBC development that should be added to a personalized risk prediction model. In **Chapter 2**, we focused on several genetic, patient-, primary breast tumor- and treatment- related characteristics. In total, 68 papers that were published until July 2016 were identified and used for the meta-analysis. We concluded that *BRCA1/2* pathogenic variants and the *CHEK2c*.1100delC mutation, a positive family history, high body mass index, treatment of primary breast cancer with radiotherapy and primary breast cancer characteristics (larger tumor size, lobular histology and negative Estrogen and Progesterone hormone receptor status) were associated with increased CBC risk in population-based studies. primary breast cancer treatment with endocrine therapy and chemotherapy as well as increasing age at primary breast cancer diagnosis were associated with decreased CBC risk. We also observed that for *CHEK2c*.1100delC (n=2), *BRCA1* (n=8) and *BRCA2* (n=7) mutation carriers, the number of studies concerning the effects of patient, tumor and treatment characteristics was limited and that more research is needed in these groups to determine these effects.

In **Chapter 3** we focused on lifestyle and reproductive factors and their association with CBC risk in population-based studies. We included 13 papers with publication dates up until November 2019 for the meta-analysis. We observed an increased CBC risk in primary breast cancer patients with high body mass index, patients who had been using alcohol, patients who were >25 years old at primiparity and patients who had an older age at menopause. A decreased CBC risk was observed in primary breast cancer patients who had multiple full-term pregnancies. No association was observed for the factors smoking, age at menarche, oral contraceptive use, breastfeeding and menopausal status.

We recognize that the number of papers on specific factors was limited (n=2-5). With this systematic review and meta-analysis the current gaps in our knowledge concerning lifestyle and reproductive factors were highlighted (i.e., the effect of dietary habits, exercise and change in lifestyle factors). In addition, it stressed the urgent need for studies that are necessary to improve CBC risk management in breast cancer survivors.

In **Chapter 4** we focused on the effect of chemotherapy on CBC risk in *BRCA1* and *BRCA2* mutation carriers with breast cancer. In the general population, treatment of primary breast cancer with chemotherapy has been associated with a decreased risk of metachronous CBC. In women with a *BRCA1/2* mutation these effects were yet to be investigated for *BRCA1* and *BRCA2* mutation carriers separately, as well as the effect of different chemotherapeutic agents within these groups. We therefore explored the effect of different types of chemotherapy on CBC risk, separately for *BRCA1* and *BRCA2* mutation carriers. We used the ongoing nationwide cohort study Hereditary Breast and Ovarian Cancer in the Netherlands (HEBON) and included 1,090 *BRCA1* and 568 *BRCA2* mutation carriers with an invasive primary breast cancer diagnosis with 116 and 44 CBCs, respectively. We observed a decreased CBC risk in both *BRCA1* (50% reduction) and *BRCA2* (40% reduction) mutation carriers after treatment of primary breast cancer with chemotherapy, although results were non-significant in the latter group. Also, the effects for *BRCA1* mutation carriers were mainly observed in the first five years. With respect to the types of chemotherapy, we found that specifically anthracyclines, alone or in combination with taxanes, were associated with decreased CBC risk in *BRCA1* mutation carriers.

In **Chapter 5 and chapter 6** we focused on the effects of radiotherapy given for primary breast cancer on CBC risk in *BRCA1* and *BRCA2* mutation carriers. Radiotherapy induces double strand DNA breaks in both tumor cells and healthy cells. Since the contralateral breast can be exposed to radiation when treating the primary breast cancer (depending on the target fields and the anatomy of the patient) and radiotherapy may involve scattering (i.e., deflecting radiation, radiation that spreads out into the surrounding tissue) towards the contralateral breast, primary breast cancer patients can be at risk for developing radiotherapy-

induced CBC. In addition, double-strand DNA breaks cannot be sufficiently repaired in patients with a mutation in the *BRCA1* or *BRCA2* protein, since these proteins are involved in the homologous recombination process needed to repair these breaks. Also, in young patients the DNA replication process is more active (i.e., more prone to damage), especially in the reproductive organs such as the breast tissue. It is therefore thought that especially young *BRCA1/2* mutation carriers are at increased risk of radiotherapy-induced CBC.

In **Chapter 5**, we used data (n=691) from the Family Cancer Clinic of the Erasmus MC Cancer Institute to investigate the effects of radiotherapy for primary breast cancer on CBC risk in patients with a germline mutation in the *BRCA1* or *BRCA2* gene. We did not observe an increased CBC risk, not in the total group, nor in young (<40 years at primary breast cancer diagnosis) *BRCA1/2* mutation carriers. In **Chapter 6**, data from the International *BRCA1/2* Carrier Cohort Study (IBCCS) cohort was used to perform the same analysis in a larger group of carriers (n=3,602). We observed an increased risk of CBC (hazard ratio: 1.4, 95% CI: 1.1-1.9) after radiotherapy in *BRCA1* and *BRCA2* mutation carriers combined, which was less pronounced in *BRCA1* (hazard ratio: 1.3, 95% CI: 0.9-1.8) than in *BRCA2* (hazard ratio: 1.8, 95% CI: 1.1-2.8) mutation carriers. In the total group, no differences were observed between patients below and above 40 years of age.

In both study populations we found that since 1990 there has been an increasing number of *BRCA1/2* mutation carriers who opt for preventive removal of the healthy breast tissue of the contralateral breast. Knowledge on the risks associated with radiotherapy can help guide decision-making for *BRCA1/2* mutation carriers together with their physician regarding their post-treatment choices concerning surveillance and prophylactic surgery.

Being diagnosed with invasive lobular or lobular mixed primary breast cancer, has also been associated with CBC risk, but results have been inconsistent. Especially in studies published after 2000 the association of lobular primary breast cancer with CBC risk seemed attenuated. The increased use of systemic treatment could have resulted in a relatively decreased risk of CBC, but this was not investigated yet. Further, CBC characteristics had never been described following a lobular primary breast cancer. However, if we can distinguish lobular or lobular mixed subtypes that are associated with more aggressive types of CBC, these patients might benefit from earlier detection. In **Chapter 7** we therefore focused on the effects of having either a lobular or lobular mixed versus ductal primary breast cancer and evaluated the risk of CBC. In addition, we inspected the effects of systemic therapy and described CBC tumor characteristics following the different histologic subtypes. From the Netherlands Cancer Registry, which has complete information on cancer development and vital status of all breast cancer patients in the Netherlands, we selected all women with a diagnosis of early invasive primary breast cancer of lobular (n=8,903), lobular mixed (n=3,512) or ductal (n=62,230) histology. We observed a moderately increased CBC risk in women with a lobular or lobular mixed primary breast cancer as compared to ductal primary breast cancer. However, since the introduction of adjuvant endocrine therapy for Estrogen hormone receptor-positive breast cancer, lobular primary breast cancers, in which the Estrogen receptor is expressed more frequently than in ductal primary breast cancers, have probably been treated with endocrine treatment more often, which on itself has been associated with decreased CBC risk. Indeed, in our study, Estrogen hormone receptor-positivity was observed in 96% of lobular and 94% of lobular mixed and 80% in ductal primary breast cancer, respectively. Patients with lobular and lobular mixed primary breast cancers were treated with endocrine therapy more often than patients with ductal primary breast cancer in our study (65%, 64% and 59%, respectively, when restricted to Estrogen hormone receptor-positive primary breast cancer patients). We therefore assume that absolute CBC risk decreased more in lobular compared to in ductal primary breast cancers, resulting in a lower relative CBC risk.

Furthermore, we found that systemic treatment (i.e., either chemotherapy or endocrine therapy) was associated with decreased CBC risk, separately for all three groups. Also, we observed that CBC characteristics were less favorable in around 20-25% of patients who developed a CBC, independent of primary histology. Whether this also influences survival should still be investigated. However, assuming that the tumor with the worst characteristics determines a patient's prognosis, it might be relevant to identify these patients and detect these CBCs in an earlier stage.

The results of our studies can be incorporated in a personalized CBC risk prediction model that can help patients to obtain accurate risk estimates. In future studies, the effects of other factors, such as other genetic mutations, (change in) breast density and (change in) lifestyle factors should be investigated. Further, incorporating the effects of major events such as recurrent disease and death from cardiovascular disease should be evaluated, as well as implementation studies of a risk prediction tool. Eventually, combining all this information can help in choosing the best strategy for both high and low-risk women concerning CBC risk management.

CHAPTER 10

SAMENVATTING

INLEIDING

Borstkanker is de meest voorkomende kankersoort bij vrouwen. Gemiddeld zal in Nederland 1 op de 7 vrouwen in haar leven borstkanker ontwikkelen. De laatste decennia is de overleving van borstkanker flink verbeterd als gevolg van een (verbeterde) screening en verbeterde behandeling. Daarnaast zijn er voor vrouwen met een verhoogd risico op borstkanker als gevolg van de aanwezigheid van een erfelijke mutatie in het *BRCA1/2* gen, betere preventieve maatregelen ten aanzien van borstkanker mogelijk, zoals het preventief verwijderen van gezond borstklierweefsel. In Nederland is tegenwoordig de 10-jaars overleving van borstkanker ongeveer 79%. Door de verbeterde overleving zijn er echter wel steeds meer vrouwen die het risico lopen op het ontwikkelen van een tweede nieuwe borstkanker in de andere borst, zogenaamde contralaterale borstkanker (CBC). Als we kijken naar de algehele bevolking, dan is het risico op het ontwikkelen van een CBC ongeveer 0.5% per jaar. Bij patiënten die een erfelijke vorm van borstkanker hebben kan dit risico oplopen tot 3% per jaar.

Steeds meer vrouwen willen tegenwoordig bij de diagnose van de borstkankerdiagnose de gezonde contralaterale borst preventief laten verwijderen. Vaak speelt de angst om opnieuw borstkanker te ontwikkelen een grote rol bij deze wens. Het blijkt echter dat veel van deze vrouwen hun risico op het ontwikkelen van contralaterale borstkanker overschatten en dat de overleving door een preventieve ingreep niet wordt verbeterd in deze laag-risico vrouwen. Bij een deel van de hoog-risico vrouwen bestaat er soms ook discussie over de beste strategie aangaande het CBC risico. Hoewel een preventieve verwijdering van de contralaterale borst namelijk de meeste risicoreductie zal geven, zal er bijvoorbeeld bij een gedeelte van de oudere hoog-risico patiënten met een relatief lage penetrantie van het *BRCA1/2* gen, kunnen worden volstaan met screening of de adjuvant gegeven systemische therapie voor de primaire borstkanker (bijvoorbeeld endocriene therapie en/of chemotherapie). Voor zowel de hoog- als de laag-risico vrouwen zou het helpen om gebruik te kunnen maken van een risicopredictie model om het risico op een CBC beter te kunnen schatten. Het doel van dit proefschrift was het onderzoeken van de factoren die mogelijk geassocieerd zijn met het risico op het ontwikkelen van een CBC. De resultaten hiervan zijn gebruikt om een gepersonaliseerd risicopredictie model te bouwen. Dit model zou kunnen bijdragen in de keuzes ten aanzien van de behandeling en de vervolgstrategie van de contralaterale borst in patiënten met borstkanker.

Om mogelijke risicofactoren te identificeren die een rol zouden kunnen spelen hebben wij in **hoofdstuk 2 en hoofdstuk 3** op systematische wijze door de beschikbare medische literatuur gezocht naar factoren die van invloed kunnen zijn op het ontwikkelen van een CBC. Vervolgens is een meta-analyse uitgevoerd: voor alle factoren werden de beschikbare schattingen uit de literatuur verzameld en is een overkoepelend risico per factor bepaald. Uit deze analyses is gebleken dat de volgende factoren zijn geassocieerd met een verhoogd CBC risico: een pathogene kiembaanmutatie in het *BRCA1*, *BRCA2* of *CHEK2* gen; eerste en/of tweedegraads familieleden met (bilaterale) borstkanker; obesitas; alcohol gebruik; oudere leeftijd ten tijde van de eerste zwangerschap; oudere leeftijd ten tijde van de overgang; behandeling van de primaire borstkanker met radiotherapie (in het bijzonder op jonge leeftijd); een oestrogeen of progesteron negatieve primaire borstkanker; een grote primaire borstkanker, een lobulaire primaire borstkanker.

De volgende factoren waren geassocieerd met een verlaagd CBC risico: behandeling met chemotherapie voor de primaire borstkanker, behandeling met anti-hormonale (endocriene) therapie voor de primaire borstkanker; oudere leeftijd ten tijde van de primaire borstkanker diagnose; jongere leeftijd ten tijde van de eerste zwangerschap; meerdere voltooide zwangerschappen.

Voor de leefstijlfactoren roken, leeftijd ten tijde van de eerste menstruatie, menopauzale status, orale anticonceptie gebruik en het hebben gegeven van borstvoeding werd er geen associatie met het CBC gevonden. Het aantal studies naar leefstijlfactoren is echter gelimiteerd en de associatie met een CBC zal nader moeten worden onderzocht om deze resultaten te bevestigen. Daarnaast is er binnen *BRCA1*, *BRCA2* en *CHEK2c*.1100delC mutatiedraagsters ook onderzoek nodig naar de effecten van patiënt-, tumor- en behandelingskarakteristieken en naar leefstijl en reproductieve factoren.

De lokale behandeling van primaire borstkanker wordt vaak gevolgd door medicamenteuze therapie, bijvoorbeeld in de vorm van chemotherapie en/of endocriene therapie. Chemotherapie wordt gegeven voor borstkanker om de uitgroei van eventuele niet-zichtbare uitzaaiingen te voorkomen. Binnen sporadische borstkanker patiënten is bewezen dat chemotherapie een beschermende werking heeft op het ontwikkelen van een CBC. Binnen *BRCA1* en *BRCA2* mutatiedraagsters

is er voor endocriene therapie al wel een effect gevonden en lijkt er ook een beschermend effect te zijn van chemotherapie, maar de tot dusver verrichte onderzoeken kunnen geen robuuste conclusies trekken door kleine aantallen patiënten en bepaalde selecties in de studie-opzet (selectiebias/survival bias/ testing bias). Het is tevens bekend dat anthracyclines en op platinum-gebaseerde chemotherapieën zorgen voor dubbelstrengs DNA breuken. De effectiviteit van deze soorten wordt hoger ingeschat, omdat BRCA1 en BRCA2 eiwitten zorgen voor de reparatie van dubbelstrengs DNA breuken, en deze eiwitten bij mutatiedraagsters zijn aangedaan en daarom mogelijk minder goed functioneren. Dit was tot dusver slechts in één studie onderzocht, waarbij *BRCA1* en *BRCA2* mutatiedraagsters waren samengenomen om een grotere groep te vormen en hiermee de betrouwbaarheid te vergroten. De tumorkarakteristieken van deze groepen zijn echter behoorlijk verschillend (*BRCA1* mutatiedraagsters hebben vaak ongunstigere en agressievere borstkankerkenmerken dan *BRCA2* mutatiedraagsters). Deze twee groepen zouden daarom bij voorkeur niet gecombineerd moeten worden.

In **hoofdstuk 4** hebben wij gebruikt gemaakt van een nationaal cohort van *BRCA1* en *BRCA2* mutatiedraagsters (HEBON) en hebben we apart voor *BRCA1* en *BRCA2* mutatiedraagsters de effecten van chemotherapie onderzocht op het ontwikkelen van een CBC. We hebben in deze studie aangetoond dat chemotherapie is geassocieerd met een verlaagd CBC risico in *BRCA1* mutatiedraagsters (50% reductie) en een gelijke (non-significante) trend werd gezien in *BRCA2* mutatiedraagsters (40% reductie). In *BRCA1* mutatiedraagsters zagen we dat het effect met name in de eerste 5 jaar standhield. Verder observeerden we dat anthracyclines en de combinatie van anthracyclines met taxanen de sterkste risicoreductie gaven.

Bij radiotherapie, een vorm van lokale therapie, wordt er straling gegeven op de tumor en op het gebied rondom de tumor. Hiermee wordt het risico op het ontwikkelen van een recidief in het borstkankergebied gereduceerd met 50%. De BRCA1 en BRCA2 eiwitten zijn betrokken bij het repareren van de schade die door radiotherapie wordt toegebracht. Omdat in patiënten met erfelijke borstkanker op basis van een *BRCA1* of *BRCA2* mutatie deze eiwitten niet goed functioneren is de hypothese dat in *BRCA1* en *BRCA2* mutatiedraagsters met borstkanker het risico op het ontwikkelen van een tweede tumor na radiotherapie groter is dan in patiënten zonder deze mutatie.

Daarnaast is in jonge patiënten het DNA in de cellen actiever (meer celdelingen) en zijn de melkklieren nog in ontwikkeling. Meer delingen brengt het risico met zich mee dat er vaker een fout kan optreden en er wederom ongecontroleerde celdelingen kunnen ontstaan met een eventuele maligniteit als gevolg. Verder worden, onder andere door het afbuigen van de straling, de omliggende weefsels waaronder de contralaterale borst ook blootgesteld aan radioactieve straling (strooistraling). Nog belangrijker is echter de directe blootstelling van borstweefsel dat in het radiotherapeutisch doelgebied kan liggen.

De gedachte is daarom dat het risico op het ontwikkelen van een CBC verhoogd is door de behandeling met radiotherapie, met name in jonge borstkanker patiënten met een *BRCA1* of *BRCA2* mutatie.

In **hoofdstuk 5** en **hoofdstuk 6** hebben we gekeken naar de effecten van radiotherapie op het ontwikkelen van een CBC in patiënten met een *BRCA1* of *BRCA2* mutatie ten tijde van hun primaire borstkankerdiagnose. Daarnaast hebben we ook specifiek gekeken in de groep van vrouwen jonger dan 40 jaar. In hoofdstuk 5, waarin 691 mutatiedraagsters waren geïncludeerd, toonden we aan dat er noch in de totale groep noch in de groep jonger dan 40 jaar een significant verhoogd risico op het ontwikkelen van een CBC bestond na radiotherapie. In **hoofdstuk 6** hebben we dit in een grotere internationale dataset bekeken, bestaande uit 1.955 *BRCA1* en 1.351 *BRCA2* mutatiedraagsters (International *BRCA1/2* carrier cohort study, IBCCS). In de gecombineerde groep van *BRCA1/2* mutatiedraagsters vonden we een verhoogd risico op een CBC na radiotherapie (hazard ratio: 1.4), wat voornamelijk bleek te berusten op het effect binnen *BRCA2* mutatiedraagsters (hazard ratio: 1.8). We vonden geen significant verschil tussen vrouwen onder en boven de 40.

In beide studies werd duidelijk dat er sinds de ontdekking van de *BRCA1* en *BRCA2* mutatie in 1994-1995, een stijging is ontstaan in het percentage patiënten dat kiest voor een amputatie van de aangedane borst en een preventieve verwijdering van de gezonde contralaterale borst. Het percentage dat koos voor een preventieve ingreep steeg van ruim 30% in 1995 naar ruim 60% in 2010. Kennis over het CBC risico na radiotherapie gegeven voor een primaire borstkanker kan *BRCA1/2* mutatiedraagsters met een primaire borstkankerdiagnose helpen om samen met hun behandelaar een keuze te maken over het vervolgtraject ten aanzien van de contralaterale borst (screenen dan wel preventieve verwijdering).

In **hoofdstuk 7** hebben we gekeken naar de associatie van een primaire lobulaire borstkanker met het ontwikkelen van een CBC. Er zijn verschillende soorten borstkanker, de meest voorkomende borstkankers zijn ductale borstkankers (85% van alle borstkankers). De tweede meest voorkomende vorm van borstkanker is de lobulaire borstkanker (10-15%).

Lobulaire borstkankers hebben in vergelijking met ductale tumoren een diffuse groeiwijze en zijn in eerdere onderzoeken geassocieerd met een slechte prognose. Daarnaast is in een aantal studies een verhoogd risico op een CBC na lobulaire borstkanker gevonden, maar in meer recente studies was er geen associatie meer zichtbaar. Gedacht wordt dat dit ten dele een gevolg kan zijn van de toegenomen systemische behandeling maar ook kleine studiepopulaties. In hoofdstuk 7 hebben we daarom in een groot nationaal cohort bestaande uit 74.373 patiënten uitgezocht of het hebben van een lobulaire dan wel lobulair gemixt met ductaal type borstkanker (lobulair gemixt) versus ductale borstkanker geassocieerd was met een verhoogd risico op het ontwikkelen van een CBC. Daarnaast hebben we gekeken naar de effecten van systemische behandeling en hebben we CBC karakteristieken vergeleken met de karakteristieken van de primaire borstkanker.

Wij vonden een licht verhoogd CBC risico in lobulaire en vooral de gemixte lobulaire tumoren ten opzichte van ductale borstkanker. Voor alle drie tumortypes vonden wij dat na behandeling met chemotherapie en/of endocriene therapie het risico op een CBC was gehalveerd. Daarnaast was in bijna een kwart van de CBCs, ongeacht het tumortype, het tumorstadium en de gradering hoger dan van de eerste borstkanker. Dit zou voor deze patiënten kunnen betekenen dat de tweede tumor bepalend is voor hun overleving en dat het bij deze patiënten zou kunnen helpen om wellicht intensievere follow-up aan te bieden om deze tumoren in een eerder stadium te vinden.

Concluderend, in dit proefschrift hebben we voor multipele patiënt-, tumor- en behandeling-gerelateerde factoren de associatie met het risico op een CBC onderzocht. De uitkomsten van deze onderzoeken zijn in een parallel promotietraject gebruikt om een risicopredictie model te bouwen dat inzicht kan geven in individuele CBC risico's.[1,2] In toekomstige studies zal het mogelijk bijdragend zijn om te onderzoeken wat de effecten van andere factoren, bijvoorbeeld andere genetische mutaties en borstdensiteit, zijn op het CBC risico. Het uiteindelijke doel is om vrouwen meer inzicht te geven en beter te informeren over hun CBC risico en aan de hand van deze risico's het beleid te bepalen ten aanzien van screening,

behandeling en preventieve chirurgische verwijdering van de contralaterale borst. Hierin zal het bijdragend zijn om deze risico's af te zetten tegen andere events, zoals terugkeer van de ziekte, late effecten van de primaire tumor behandeling (bijvoorbeeld het risico op cardiovasculaire events en andere tweede tumoren) en andere doodsoorzaken.

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CHAPTER 11

DANKWOORD

DANKWOORD

Misschien was dit gedeelte nog wel het leukste stuk om te schrijven, een hoofdstuk zonder woordenlimiet met als mooiste herinnering de onvoorwaardelijke steun die ik van mijn familie, vrienden, collega's en leidinggevenden heb ontvangen. Er zijn enorm veel mensen om mij heen die mij steunen en hebben gesteund, die mij vooruit hebben geholpen. Ik ben jullie oprecht zo enorm dankbaar. Het leven is een feest met jullie, dank jullie wel. In het bijzonder wil ik de onderstaande mensen bedanken.

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CHAPTER 12

CURRICULUM VITAE | LIST OF PUBLICATIONS | PHD PORTFOLIO

CURRICULUM VITAE

Delal Akdeniz werd op 14 januari 1987 geboren te Oldenzaal. In 2006 voltooide zij het VWO aan het Twents Carmel Lyceum, locatie de Thij te Oldenzaal. In hetzelfde jaar begon zij aan de studie Geneeskunde aan het Erasmus MC. In 2013 behaalde zij haar artsexamen. Aansluitend werkte zij als arts niet in opleiding tot specialist in het Ikazia Ziekenhuis te Rotterdam. In 2014 startte zij met de huisartsenopleiding via de SBOH aan het Erasmus MC te Rotterdam. In hetzelfde jaar kreeg zij de mogelijkheid om te promoveren bij de Werkgroep Kanker Epidemiologie binnen de Interne Oncologie van het Erasmus MC Kanker Instituut te Rotterdam, wat heeft geresulteerd in dit proefschrift. In 2015 startte zij tevens met de Master of Science opleiding Clinical Epidemiology aan het Netherlands Institute for Health Sciences te Rotterdam en behaalde het diploma in 2017. Vanaf juni 2022 tot 1 maart 2023 is zij werkzaam geweest als arts niet in opleiding tot specialist in een huisartsenpraktijk. Vanaf 1 maart is zij wederom gestart met de huisartsopleiding in het Erasmus MC te Rotterdam.

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PhD Portfolio

Name: Delal Akdeniz

Title project: Risk management of contralateral breast cancer: development and validation of an online decision aid for physicians and patients

Departments:
1. Erasmus MC Cancer Institute:
Department of Medical Oncology, research group: Cancer Epidemiology
2. Netherlands Cancer Institute:
Division of Molecular Pathology; Division of Psychosocial Research and Epidemiology

Promotor:
Prof. Stefan Sleijfer (Erasmus MC)
Prof. Ewout W. Steyerberg (LUMC)
Prof. Marjanka K. Schmidt (NKI)

Co-promotor:
dr. Maartje J. Hooning (Erasmus MC)

Research School: NIHES

PhD period: Oct 2014 – Jan 2023

	Workload		
	Year	**Hours**	**ECTS**
PhD Training			
General Courses			
KWF Basiscursus Oncologie	2015	40	1.5
Microsoft Access (basic & advanced)	2015	16	0.7
Integrity in Science	2016	10	0.3
Endnote	2017	8	0.2
Biomedical English Writing and Communication	2017	60	2
Specific Courses			
NIHES: Clinical Epidemiology Master Program	2015-2017		70
Common core			
• Study design			
• Biostatistical methods I: Basic Principles			
• Biostatistical methods II: Classical Regression Models			
• Clinical Epidemiology			
• Methodologic Topics in Epidemiologic Research			
• Principles of Research in Medicine and Epidemiology			
• Methods of Public Health Research			
• Clinical Trials			
• The Practice of Epidemiologic Analysis			
• Fundamentals of Medical Decision Making			
• Clinical Practice-Relevant Therapeutic Trials			
Elective courses			
• Cancer Epidemiology			
• Topics in Meta-Analysis			
• Cohort Studies			
• Case-Control Studies			
• Markers and Prognostic Research			
• Erasmus Summer Lectures			
• Survival Analysis for Clinicians			
• Introduction to Psychology in Medicine			
• Primary and Secondary Prevention Research			

Seminars and Workshops			
Hereditair Borst- en eierstokkanker Onderzoek Nederland (HEBON) congress	2014-2018	24	0.8
Borstkanker Behandeling Beter	2014-2018	20	0.7
Cochrane Symposium	2015	8	0.2
Advanced Breast Imaging	2018	8	0.3
Poster Presentations			
Poster presentation Werkgroep Epidemiologisch Onderzoek Nederland (WEON)	2016	30	1
Poster presentation San Antonio Breast Cancer Symposium	2016	60	2
Poster presentation European Breast Cancer Conference	2018	60	2
Oral presentations			
Borstkanker Behandeling Beter congres	2016	15	0.5
HEBON congress	2016-2017	15	0.5
International conferences			
European Breast Cancer Conference	2016, 2018	144	4.5
WEON Epidemiology congress	2016	48	1.5
Teaching			
Supervising Master's Thesis	2015	60	2
Supervising Minor Oncology Students	2015-2017	30	1