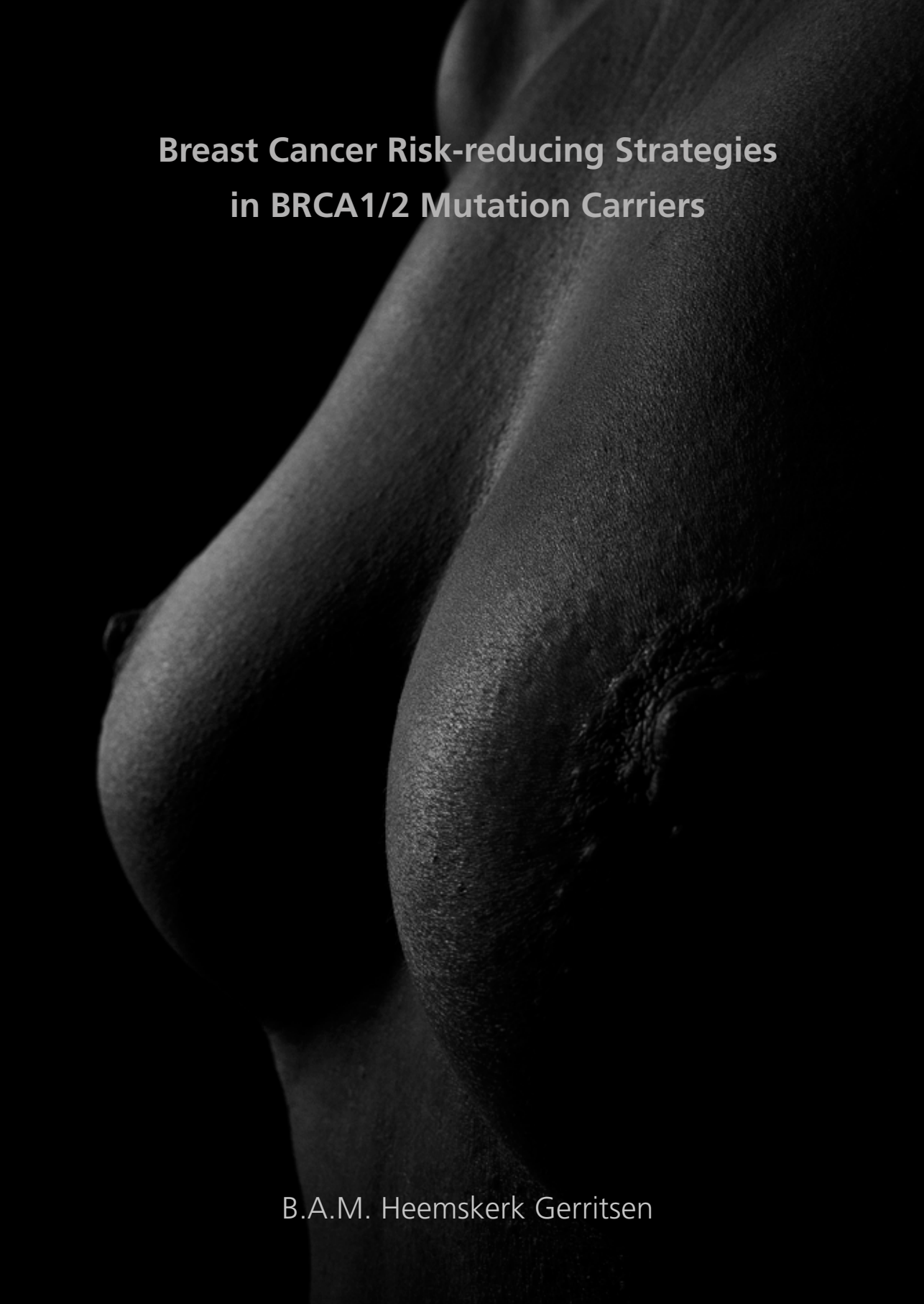
Breast Cancer Risk-reducing Strategies in BRCA1/2 Mutation Carriers



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Borstkanker risico-reducerende strategieën in BRCA1/2 mutatiedraagsters

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Chapter 1

Introduction

General introduction

Breast cancer (BC) is the most common type of cancer in women in developed countries. Currently, approximately 14,000 women are diagnosed with BC every year in the Netherlands. One out of eight Dutch women (12%-13%) will develop BC during their life, and 3% to 4% of all Dutch women will die from BC.[1]

A genetic predisposition may be responsible for about 5% to 10% of all BC cases.[2-4] Approximately 25% of these cases can be attributed to a mutation in one of the BReast CAancer (BRCA) genes, BRCA1 and BRCA2.[5] Both genes act as tumour suppressor genes and are involved in important cell functions, including cell cycle control, gene expression regulation, and DNA repair mechanisms.[6,7] Cells with deficiencies in genes involved in DNA repair are unable to repair DNA double-strand breaks, resulting in genomic instability and a predisposition to malignant transformation.[7]

BRCA1/2 mutation carriers have an increased risk of developing a first BC, estimated to range from 45% to 88% by the age of 70.[8-13] The estimated cumulative lifetime risk of developing contralateral BC is 65-87% for BRCA1 mutation carriers,[8,12,14] and 52-62% for BRCA2 mutation carriers.[12,15] Adjuvant treatment with Tamoxifen after unilateral BC may reduce the contralateral BC risk with approximately 50%.[16,17] The risk of developing a contralateral BC may also depend on age at first BC diagnosis and adjuvant treatment with chemotherapy.[18,19]

In BRCA1/2 mutation carriers, BC is diagnosed at younger age than in the general population.[20,21] Further, compared with sporadic breast tumours, BRCA1-associated breast tumours are more often poorly differentiated (i.e. grade 3) and more often have a triple-negative phenotype, i.e. estrogen receptor (ER)-negative, progesterone receptor (PR)-negative and HER2 receptor-negative.[22,23] Additionally, BRCA1-associated breast tumours exhibit higher mitotic counts, a larger proportion of the tumour with continuous pushing margins, less tubule formation, and more lymphocytic infiltration than sporadic breast tumours.[24,25] BRCA2-associated breast tumours show similar histological characteristics and hormone-receptor phenotypes as sporadic breast tumours,[22,23] but exhibit lower mitotic counts, a higher proportion of the tumour with continuous pushing margins, and a higher score for tubule formation than sporadic breast tumours.[24] Nevertheless, despite differences in tumour characteristics, no significant differences in disease-free and overall survival have been reported between BRCA1/2-associated and sporadic BC cancer patients.[22,26-28]

Besides the increased BC risks, BRCA1/2 mutation carriers also have much higher risks of developing ovarian cancer (OC) and fallopian tube cancer (FTC), again occurring at younger age than for women without a BRCA mutation. In the general Dutch population the cumulative lifetime risk of developing OC is 1-2%,[1] and the median age at onset is 62 years.[29,30] In contrast, for BRCA1 mutation carriers the risk of developing OC/FTC is 31% to 63% during life,[8,10-13] occurring at a median age of 50 years.[11,31] For BRCA2 mutation carriers

this risk varies from 6% to 35%,[9-13] and occurs at a median age of 56 years.[11,31] Surprisingly, previous studies have reported a better survival for BRCA-associated OC patients than for sporadic OC patients.[32-35] As BRCA-deficiency is associated with an impaired DNA repair mechanism, an increased sensitivity to DNA-damaging platinum-based chemotherapy may be an explanation for the observed survival benefit. However, since up to 90% of all OC cases is diagnosed at advanced stage (FIGO stage III/IV)[32-35], also for BRCA-associated OC patients, the ten-year survival rate remains low (20-35%).[32-34]

Options to reduce the risk of BC include bilateral risk-reducing mastectomy (RRM) for healthy BRCA1/2 mutation carriers, bilateral and contralateral RRM for BRCA1/2 mutation carriers with a history of first BC, and possibly chemoprevention. Data on the latter, however, are scarce, and due to the side effects, chemoprevention is not widely used. An alternative option for women who do not want to undergo RRM (yet), is regular BC surveillance aiming at early detection of BC and the prevention of subsequent BC-related death.

Regarding OC/FTC risk, gynaecological screening has not proven to be effective in early detection of OC/FTC.[36-38] Therefore, in the Netherlands the advice towards BRCA1/2 mutation carriers is to undergo risk-reducing salpingo-oophorectomy (RRSO) - removing both ovaries and fallopian tubes – once childbearing is completed, or from the age of 35-40 years for BRCA1 mutation carriers, and from the age of 40-45 years for BRCA2 mutation carriers.[39]

Breast cancer surveillance in the Netherlands

Currently, regular BC surveillance for BRCA1/2 mutation carriers in the Netherlands consists of annual imaging by magnetic resonance imaging (MRI) between 25 and 60 years, annual imaging by mammography as of 30 years of age, and annual clinical breast examination from the age of 25 years.[39] This surveillance programme results in early detection of invasive BC and of carcinoma in situ. Especially due to the introduction of MRI the sensitivity to detect invasive BC was doubled in BRCA1/2 mutation carriers.[40-42] While early detection reduces BC-specific mortality in the general population,[43] conclusive data on mortality reduction after BC screening in BRCA mutation carriers are still lacking. However, preliminary data from the Dutch MRISC study – evaluating the value of MRI in addition to mammography for BC surveillance in high-risk women – showed an improved short-term overall survival when MRI was added to the BC surveillance program. Still, not all BC deaths could be prevented by early detection.[44]

Previous studies on the psychological aspects of intensive BC surveillance programs showed that BC-specific and general distress around two successive biannual surveillance appointments remained within normal ranges,[45,46] although identified subgroups of vulnerable women may experience elevated levels of psychological distress.[46] MRI was reported to cause limited bother and in case of a favourable test result women where highly reassured about the absence of BC. In general, MRI has shown to be an acceptable screening modality for women at increased BC risk, and women preferred MRI above mammography.[47]

Risk-reducing surgery

Risk-reducing surgery is the most effective option to reduce the risk of developing BC and OC/FTC. In healthy BRCA1/2 mutation carriers, bilateral RRM reduces the risk of BC significantly, with estimates of risk-reduction even up to 100%.[48-52] However, since there will always be some residual mammary-gland tissue after surgery, RRM does not completely eliminate the risk of developing a BC.[50,52] Still, in view of the very low remaining BC risks after bilateral RRM of only 1-2%, continuing BC surveillance is not considered necessary for healthy BRCA1/2 mutation carriers who have undergone bilateral RRM.

No prospective data regarding survival after bilateral RRM in healthy BRCA1 and BRCA2 mutation carriers are available yet. Neither is it clear whether BRCA1/2 mutation carriers show better survival after bilateral RRM than BRCA1/2 mutation carriers remaining under intensive surveillance. The only available information hereon is derived from risk estimates assessed in mathematical models with simulated cohorts. These models yielded maximal survival probability for healthy BRCA1/2 mutation carriers undergoing both RRM and RRSO,[53-57] although Kurian and colleagues suggested that surveillance with both mammography and MRI in combination with RRSO might offer an almost comparable survival.[55,57] In view of recent results indicating that intensive surveillance results in early BC detection with good prognosis, and the current availability of modern neo-adjuvant and adjuvant treatment options – leading to an improved BC-specific survival –, it can indeed be hypothesized that intensive BC surveillance may lead to a similar survival as bilateral RRM.

In the general population, contralateral BCs are usually diagnosed at a more favourable stage than the first BCs,[58] i.e. at smaller size and more often with a node-negative status, suggesting no additional adverse effect on survival after unilateral BC. Nevertheless, in a large Swedish population-based study, patients with contralateral BC showed worse survival than unilateral BC patients.[59] The most effective option to reduce the risk of contralateral BC is contralateral mastectomy. In BRCA1/2 mutation carriers with a history of BC, contralateral RRM reduces the risk of contralateral BC with more than 90%.[60-62] So far, data on survival after bilateral or contralateral RRM in BRCA1/2 mutation carriers with a history of unilateral BC are scarce and inconsistent. Recent studies showed improved survival,[62,63] while another study with smaller sample sizes and shorter follow-up did not.[60]

In the Netherlands, the uptake of bilateral RRM by healthy BRCA1/2 mutation carriers was relatively high (55%) in the past,[48] but seems to have declined over the last decade, possibly due to the incorporation of MRI as a more sensitive screening method than mammography in the surveillance program. For BRCA1/2 mutation carriers with a history of BC, the uptake of contralateral RRM has been estimated to be approximately 27% worldwide, although there were large differences by country in this study. In the United States 49% of the affected BRCA1/2 mutation carriers opted for contralateral RRM, while this was only 5% in the participating European countries, i.e. 5 centres in Austria, France, Italy, Norway, and Poland, respectively.[64] The introduction of national guidelines, however, may have led to

more homogeneity in the prevalence for contralateral RRM among the different countries. In the Netherlands two previous small studies reported an uptake of contralateral RRM of 35% and 53%.[60,65]

The decision to choose for bilateral or contralateral RRM over intensive surveillance may be driven by a combination of various factors, including (perceived) cancer risk, experiences with cancer in family relatives, fear of cancer, increasing possibilities for breast reconstruction and the desire to avoid another BC treatment. Still, RRM is a drastic and irreversible intervention, and – in case of a breast reconstruction – may be complicated by necessary additional operations.[66,67] Further, the impact of RRM – with or without breast reconstruction – on body image and sexual relationship should not be underestimated. Previous psychological studies from our institute showed that 40% of the women with a breast reconstruction after RRM were not completely satisfied with the cosmetic result, and 44% of the women undergoing RRM reported adverse changes in the sexual relationship.[68] Reassuringly, 95% of the women would opt for RRM again, and the investigators found that RRM decreased psychological cancer-related distress.[68,69]

Because current screening protocols are ineffective in early detection of OC/FTC, [36-38] the uptake of RRSO after completion of childbearing is high, reported to be up to 75%.[70-75] After RRSO, the risk of OC/FTC is reduced with 80-100%.[51,76-80] Consequently, the intervention is associated with lower OC-specific mortality,[51] despite a small residual risk for peritoneal cancer after RRSO.[78] Further, RRSO has been reported to reduce the risk of developing a subsequent BC with approximately 50%.[51,77,79,81-84] However, studies on the efficacy of risk-reducing surgery in BRCA1/2 mutation carriers are confined to observational studies, thus challenging several methodological issues. Consequently, previous estimates on BC risk-reduction after RRSO may have been influenced by bias associated with selection of study subjects, bias associated with start of follow-up, or by confounding,[85] and BC risk-reduction may have been overestimated.

Data regarding the psychological impact of RRSO are scarce. Previously, in one study – characterized by a small sample size (n=96) and a short period of follow-up (mean 13.7 months) – women showed to experience less OC-specific distress after RRSO, and no adverse effects on physical and mental health-related quality of life were reported.[86]

Study population

This thesis is based on studies conducted in female BRCA1/2 mutation carriers within the framework of the Rotterdam Family Cancer Clinic, and the Hereditary Breast and Ovarian cancer study group in the Netherlands (HEBON).

The Rotterdam Family Cancer Clinic started in 1991. The purpose was to coordinate the research regarding hereditary breast/ovarian cancer as well as the multidisciplinary care that is required for the management of families at high-risk of breast and/or ovarian cancer, in view of all clinical genetic, oncological, surgical, gynaecological and psychological aspects.

Since the start of the Family Cancer Clinic, all members of genetically susceptible families are registered in the institutional database. After 1994-1995, when genetic testing for mutations in the BRCA1 and BRCA2 genes became available, the possibility of genetic testing was and still is discussed with all members of families applying for genetic counselling as well as with members of families that visited the department of Clinical Genetics before the identification of the BRCA1 and BRCA2 genes. So far, 4467 families have been registered in the Rotterdam Family Cancer Clinic database. In 584 families a pathogenic mutation in the BRCA1 gene was found, and in the BRCA2 gene in 269 families. In total, 2018 female BRCA1/2 mutation carriers – either with or without a history of breast and/or ovarian cancer – are included in the institutional registry database. All registered individuals were informed by oral and written information and asked for written consent. Throughout the years, we retrospectively as well as prospectively collected and updated information on dates of birth and death, dates of genetic counselling and DNA disclosure, type of mutation and mutation carrier status, dates and types of cancer diagnoses, tumour and treatment characteristics, dates of recurrent disease, dates and types of risk-reducing surgeries, and dates and types of screening visits.

The HEBON study is an ongoing nationwide cohort study including members of high risk breast and/or ovarian cancer families, who were tested for a BRCA1/2 mutation after genetic counselling in the Netherlands. The HEBON study was initiated in 1998 by the Departments of Epidemiology and Pathology of the Netherlands Cancer Institute – in collaboration with departments of the University Medical Centres of Rotterdam, Leiden and Nijmegen –, and was designed as a cross-sectional study with a prospective follow-up. The main aim of the study was to investigate cancer risks in familial and hereditary breast and/or ovarian cancer families. In addition, interaction between hormonal and lifestyle risk factors and cancer genes in breast and ovarian cancer development were to be examined. High-risk families were identified through the Departments of Clinical Genetics/Family Cancer Clinics of all Dutch academic centres, the Netherlands Cancer Institute, and the Foundation for the detection of Hereditary Tumours (STOET). Initially, the HEBON study resulted in a cohort of 758 BRCA1/2 mutation families available for risk analyses, representing 2546 BRCA mutation carriers and 2221 non-carriers of the familial pathogenic mutation, and 1756 non-BRCA1/2 typed families. According to protocols approved by the Medical Ethical Committees of the participating centres, all included women provided written informed consent. Linkage to the Netherlands Cancer Registry, the Netherlands Pathology Database, and municipal registries were used to retrieve and prospectively update data on the occurrence of any cancer, tumour characteristics, and recurrent disease after previous breast and/or ovarian cancer, on previous and ongoing therapy, on preventive strategies, and on deaths.

Aims and outline of this thesis

The aim of this thesis is to obtain more knowledge on risk-reducing strategies – in particular mastectomy and salpingo-oophorectomy – in BRCA1/2 mutation carriers. Accurate knowledge

on the efficacy, and the benefits and disadvantages, of the different risk-reducing strategies will enable clinical geneticists and oncologists, and oncological health care workers to offer more exact and personalised counselling with respect to risk-reduction, survival and potential adverse effects. This is especially important for BRCA1/2 mutation carriers who face the difficult decision regarding the choice between intensive surveillance and risk-reducing surgeries.

More specifically, the main research questions of this thesis are:
- What is the impact of bilateral RRM on BC incidence and survival in healthy BRCA1/2 mutation carriers?
- What is the impact of contralateral RRM on contralateral BC incidence and survival in BRCA1/2 mutation carriers with a history of unilateral BC?
- To what extent does RRSO contribute to BC risk-reduction in healthy BRCA1/2 mutation carriers?

Secondary questions were:
- What is the impact of bias on (previous) estimates regarding the efficacy of RRSO on BC risk-reduction?
- Are there differences between BCs that develop after RRSO and PBCs not preceded by RRSO with respect to tumour characteristics and tumour growth rates?

In **Chapter 2** of this thesis, we describe the long-term experiences at the Rotterdam Family Cancer Clinic with RRM in proven BRCA1/2 mutation carriers and in 50% risk carriers from hereditary breast/ovarian cancer families. In this single-centre study, we updated and extended previously reported data from our institute[48,67,87] concerning BC occurrence after RRM, and postoperative complications of RRM in combination with breast reconstruction. In **Chapter 3** we report on a single-centre, prospective cohort study, in which healthy BRCA1/2 mutation carriers opting either for RRM or for intensive surveillance were compared with respect to BC incidence and BC-specific and overall survival. **Chapter 4** addresses on a nationwide multicentre, prospective cohort study, in which we compared BRCA1/2 mutation carriers with a history of unilateral BC opting either for RRM or for intensive surveillance with respect to contralateral BC incidence and overall survival. **Chapter 5** compares tumour characteristics and tumour growth rates from first BCs that develop after RRSO and first BCs that arise without RRSO. **Chapter 6** evaluates the impact of potential biases that may have disturbed previously published estimates regarding the effect of RRSO on subsequent BC risk. To minimize bias as much as possible in observational studies on the efficacy of risk-reducing strategies in BRCA1/2 mutation carriers, a revised design was proposed in this chapter. Finally, in **Chapter 7**, the results are summarised, conclusions are drawn, and various aspects of the studies are discussed. Further, implications for clinical practice as well as recommendations for future studies will be given.

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Chapter 2

Prophylactic mastectomy in BRCA1/2 mutation carriers and women at risk of hereditary breast cancer: long-term experiences at the Rotterdam Family Cancer Clinic

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Abstract

Background

BRCA1/2 mutation carriers, and women from a hereditary breast(/ovarian) cancer family have a highly increased risk of developing breast cancer (BC). Prophylactic mastectomy (PM) results in the greatest BC risk reduction. Long-term data on the efficacy and sequels of PM are scarce.

Methods

From 358 high-risk women (including 236 BRCA1/2 carriers) undergoing PM between 1994 and 2004, relevant data on the occurrence of BC in relation to PM, complications in relation to breast reconstruction (BR), mutation status, age at PM and preoperative imaging examination results were extracted from the medical records, and analysed separately for women without (unaffected, n=177) and with a BC history (affected, n=181).

Results

No primary BCs occurred after PM (median follow-up 4.5 years). In one previously unaffected woman, metastatic BC was detected almost 4 years after PM (primary BC not found). Median age at PM was younger in unaffected women ($P < 0.001$), affected women more frequently were 50% risk carriers ($P < 0.001$). Unexpected (pre)malignant changes at PM were found in 3% of the patients (in 5 affected and 5 unaffected women, respectively). In 49.6% of the women opting for BR one or more complications were registered, totalling 215 complications, leading to 153 surgical interventions (71%). Complications were mainly related to cosmetic outcome (36%) and capsular formation (24%).

Conclusions

The risk of developing a primary BC after PM remains low after longer follow-up. Preoperative imaging and careful histological examination is warranted because of potential unexpected (pre)malignant findings. The high complication rate after breast reconstruction mainly concerns cosmetic issues.

Introduction

Women with a germline BRCA1 or BRCA2 mutation as well as 50% risk carriers from a hereditary breast(/ovarian) cancer (HB(O)C) family are at increased risk of developing breast and/or ovarian cancer compared to the general population.[1-4] Options to reduce this risk are regular surveillance, chemoprevention, or prophylactic surgery. Prophylactic surgery includes prophylactic mastectomy (PM) and/or prophylactic bilateral salpingo-oophorectomy (PBSO). PM implies either a bilateral prophylactic mastectomy (BPM) in high-risk unaffected women as well as in high-risk women with a history of breast cancer (BC) previously treated with breast conserving therapy (BCT) or a contralateral prophylactic mastectomy (CPM) after a unilateral therapeutic mastectomy. Several studies have shown that PM strongly reduces the risk of developing (contralateral) breast cancer, while PBSO reduces the risk of ovarian as well as primary breast cancer.[5-10] These strategies therefore have commonly been accepted at this moment as risk reducing strategies for women being at increased risk of HB(O)C.

PM, however, is a drastic and irreversible intervention, and in case of breast reconstruction (BR), is accompanied by a substantial complication rate.[11] Further issues of concern with respect to PM include changes in a woman's body image and self-esteem, changes of sexual function, and in psychological distress.

At the Rotterdam Family Cancer Clinic, 35 – 51% of women carrying a BRCA1 or BRCA2 mutation opt for either bilateral or contralateral PM.[12,13] For women who are contemplating this intervention, it is imperative to have reliable data on the outcomes of PM in a well-defined cohort in order to make a good informed decision and to minimize postoperative feelings of deception. At the Rotterdam Family Cancer Clinic there is ample and long-term experience with sufficient numbers of women undergoing PM. We previously reported data concerning the occurrence of breast cancer after BPM in unaffected women with a proven BRCA1/2 mutation,[5,14] complications of PM with breast reconstruction,[11,15] and psychological aspects of PM in combination with BR.[16]

In the current analysis we report on an extended series with longer follow-up of women having undergone a PM at the Rotterdam Family Cancer Clinic because of either a proven BRCA1/2 mutation or a genetic susceptibility (50% risk carriers from a HB(O)C family). Our study sample was large enough to discriminate between unaffected women and women with a history of BC (affected). Special attention is paid to the prevalence of (pre)malignant lesions in prophylactically removed mastectomy specimens. Further, we report on the postoperative complications of PM in combination with breast reconstruction.

Patients and Methods

As of the start of the Rotterdam Family Cancer Clinic in 1991, PM and/or PBSO are being discussed as risk-reducing strategies with women at increased risk of hereditary BC and/or

ovarian cancer. In early years, PM was discussed with BRCA1/2 mutation carriers as well as with women from a HB(O)C family without a proven mutation (so-called 50% risk carriers), and applied for unaffected as well as affected (with a history of breast cancer) women. Due to the development of more advanced mutation-detection methods enabling the performance of a complete gene mutation screen, there has been a shift in more recent years to only discuss the option of PM with identified mutation carriers. Before 1996, the decision to undergo a PM and/or PBSO was discussed individually by the doctor and the woman in question. As of 1996, women opting for either PM and/or PBSO are additionally discussed in the multidisciplinary Committee on Hereditary Tumours. For this purpose, institutional guidelines concerning the surveillance schedule and indications regarding PM/PBSO have been further elaborated and implemented as of 2000, which were updated as knowledge progressed and more evidence-based data became available.

Before 2000, no additional examinations were performed before PM, irrespective of the individual situation (unaffected/affected; mutation/50% risk carrier). Women were seen biannually for physical examination, while a mammography was performed annually. As of 2000, institutional guidelines from the working party on Hereditary Tumours recommended to perform clinical breast examination (CBE) and imaging examination within 3 months prior to PM, to minimize the risk of finding unexpected malignant changes at PM. At first, imaging examination consisted of either mammography or magnetic resonance imaging (MRI) scan, while more recently MRI has been preferred. Breast ultrasound (US) and, if necessary, fine needle aspiration cytology (FNAC) are additionally performed in case lesions are found at CBE or one of the imaging examinations. Further, the guidelines recommend the discussion of the case in the multidisciplinary Committee on Hereditary Tumours, and a standard visit with a psychologist. For affected women, the guidelines are extended with dissemination investigations to rule out recurrent or distant breast cancer activity (chest X-ray, liver ultrasound, bone scan, liver functions and determination of Ca15.3/Ca125). Where women with a history of ovarian cancer were previously eligible for PM, at the moment this is not discussed anymore in this setting, because the prognosis is mainly dictated by the ovarian cancer. In the sample, these women were classified as 'unaffected', unless they also had a history of BC.

To evaluate the short-term and long-term medical effects of prophylactic surgery in high-risk women, a combined retrospective and prospective, longitudinal study was activated at our institution, including all genetically susceptible women who had opted for prophylactic surgery (either PM and/or PBSO). Women were informed by oral and written information, and asked for written consent. The protocol was approved by the institutional review board (project EMC-DDHK 98-15).

Surgical technique

At our institute, the oncological and plastic surgeon perform the PM and BR as a team. During the operation the patient is under general anaesthesia in a half-supine position. A

skin-sparing mastectomy is performed through a vertical, peri-areolar incision, which extends from just above the nipple down the submammary fold. The breast tissue, including the superficial fascia (creating thin skin flaps), the axillary tail, the inframammary fold, the nipple-areolar complex, and the fascia of the pectoral muscle are removed. In case of immediate breast reconstruction, either a subpectoral silicone implant is inserted in a pocket created below the pectoral muscles in a one-stage procedure, or autologous tissue is used. Autologous reconstruction encompasses a broad range of procedures incorporating the patient's own tissues to recreate the breast. The transverse rectus abdomis myocutaneous (TRAM) flap and latissimus dorsi flap are two standard myocutaneous flaps used for breast reconstruction. More recent modifications to the traditional techniques led to the use of the deep inferior epigastric perforator (DIEP) flap. Nipple reconstruction is offered after 6 months and consists of three small transposition flaps; the areola is mimicked by tattooing the desired skin colour. Breast reconstruction is not always performed in the same operation as the mastectomy; the techniques for these delayed reconstructions, however, are as described above.

Microscopic examination of mastectomy specimens

As of 1995, a standard procedure has been followed for meticulous microscopic examination of prophylactically removed mastectomy specimens to rule out the presence of "occult" (microscopic) malignant alterations. The protocol prescribes that mastectomy specimens are cut into slices of 0.5 – 1 cm thickness, whereby each slice is carefully inspected and palpated for abnormalities. Standard, three randomly selected parenchymal tissue samples from each quadrant and a transverse section through the nipple are submitted for histology, in addition to samples of all visible or palpable abnormalities. Further, three samples from each quadrant of the mastectomy specimens are snap frozen for the tissue bank. Radiographic examination of breast tissue specimens is not performed on a routine basis.

Study design

The current study included all women at increased risk of hereditary BC, according to previously described criteria,[17] who underwent prophylactic bi- or contralateral mastectomy between January 1, 1994 until December 31, 2004. Of our study cohort 310 women (86.6%) underwent PM at our clinic, while 48 women (13.4%) were treated elsewhere, e.g. due to a waiting-list at our clinic, or the fact that previous surgery was performed elsewhere. The latter women were only eligible for this analysis if the follow-up after PM took place at our clinic, and a copy of the pathology report was available. In general, DNA testing was performed before the prophylactic surgery, although some women choose for prophylactic surgery without or irrespective of DNA testing. DNA analysis was performed according to standard procedures, as has been previously described.[18,19] DNA testing was not an inclusion criterion for participation in the study. Proven non-carriers from a family BRCA mutation were excluded from the study.

Relevant data were extracted from the hospital records. For each woman, including deceased women, the following information was obtained: date of birth, death, and PM, performance (yes/no) and type of breast reconstruction, PBSO, diagnosis of breast and/or ovarian cancer, mutation status, duration of follow-up after PM (end date being either the date of death or the date of last clinic visit in case of loss to follow-up, or the end date of this study, i.e. December 31, 2004), and type and number of complications after breast reconstruction. Regarding the latter, we distinguished between early (within 6 weeks) and late postoperative complications (after 6 weeks). Early complications consisted of infection, necrosis, bleeding, and luxation of the prosthesis. Late complications were divided in surgical complications (such as capsular formation, infection, necrosis, and luxation of the prosthesis), and complaints related to cosmetic outcome (such as poor symmetry and dog ears). Nipple reconstruction is regarded as part of the breast reconstruction, and therefore has not been registered as a cosmetic complication. A computerized database (MS-Access) was used to process the data. Data were entered retrospectively as well as prospectively after each clinic visit.

Statistical analysis

Descriptive statistics (median, range, and frequency) were computed. When appropriate, statistical significance testing between relevant subgroups was performed using the chi-

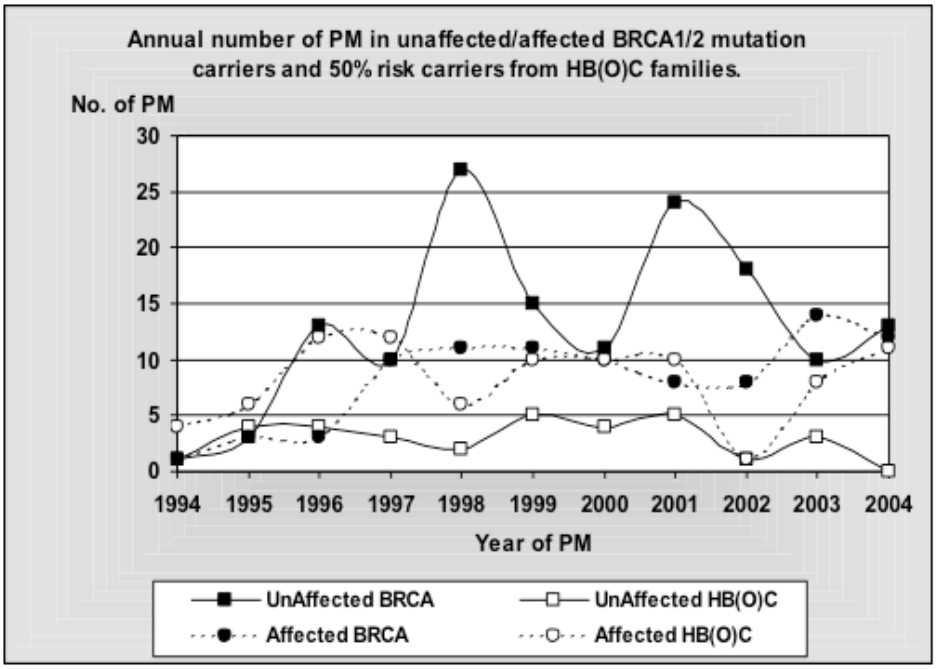

Figure 1. Annual number of PM in unaffected/affected BRCA1/2 mutation carriers and 50% risk carriers from HB(O)C families

square test for categorical variables and a t-test for continuous variables. A *P* value of less than .05 was considered statistically significant.

Results

Study population

In Table 1 the characteristics of the women who underwent a PM are shown. A total of 358 women, with a median follow-up after PM of 4.5 years, fulfilled the study eligibility criteria, consisting of 181 (50.6%) women with a history of breast cancer (affected women), and 177 (49.4%) women without a history of BC (unaffected women). A total of 236 (65.9%) women were BRCA1/2 mutation carriers, while the other 122 women (34.1%) were 50% risk carriers from a HB(O)C family. The unaffected group mainly consisted of BRCA1/2 mutation carriers as compared to 50% risk carriers (82% versus 18%, $P < .001$), whereas the affected group consisted of an equal number of mutation carriers and 50% risk carriers (91 mutation carriers vs 90 50% risk carriers, $P = .94$). This difference in distribution was highly significant ($P < .001$).

The median age at PM in the unaffected and affected group was significantly different, being 37 and 44 years, respectively (Table 2, $P < .001$). In the affected group, mutation carriers were significantly younger at PM than 50% risk women (42 vs 47 years, respectively; $P = .045$). In the unaffected group we found no significant difference in this respect (36 vs 38.5 years, respectively; $P = .102$). A history of ovarian cancer was present in 4 mutation carriers from the unaffected group (2.3%) and in 3 women (1.7%) in the affected group.

In figure 1 the number of PMs per year, separately for unaffected/affected BRCA mutation carriers, as well as for unaffected/affected 50% risk carriers is shown. In the unaffected group, the annual number of women undergoing PM was always larger in mutation carriers as compared to 50% risk carriers, except for the first two years (1994/1995). Further, the number of unaffected BRCA mutation carriers undergoing PM widely differed over the years, with two peaks in 1998 and 2001, respectively. In contrast, the number of unaffected 50% risk carriers undergoing PM was quite stable over the years.

In the affected group, however, the pattern was different, whereby a shift has taken place through the years. In the early years of the study period (1994 – 1997) mainly women without a proven mutation underwent PM in this group. Between 1997 and 2001, approximately as many mutation carriers as 50% risk carriers underwent a PM. As of 2002, more mutation carriers have undergone a PM, although the number of PMs in 2004 again was not different between mutation and 50% risk carriers.

Prophylactic bilateral salpingo-oophorectomy

A considerable part of the BRCA mutation carriers undergoing PM also opted for PBSO, being 57% in the unaffected versus 67% in the affected group, respectively (Table 1, $P = .13$). In the HB(O)C group, however, only a minority of the women underwent a PBSO,

Table 1. Characteristics of the study population

History of BC	No (Unaffected, *n* = 177)				Yes (Affected, *n* = 181)				
	BRCA		HB(O)C		BRCA		HB(O)C		
	n	(%)[a]	*n*	(%)[a]	*n*	(%)[a]	*n*	(%)[a]	*P* value[d]
No. of women (*N*)	145	(82)[b]	32	(18)[b]	91	(50)[c]	90	(50)[c]	< .001[e]
Death due to cancer	0	(0)	1	(3)	9	(10)	7	(8)	
Age at PM (years)									
Median	36.0		38.5		42.0		47.0		< .001
Range	22–65		28–55		25–65		26–68		
< 30	18	(12)	3	(9)	6	(7)	2	(2)	< .001
30 – 39	74	(51)	14	(44)	26	(29)	22	(24)	
40 – 49	36	(25)	10	(31)	38	(42)	31	(34)	
≥ 50	17	(12)	5	(16)	21	(23)	35	(39)	
Duration of follow-up (years)	4.4		4.7		3.9		4.5		
Mutation status									
BRCA1	115	(79)	-		76	(84)	-		
BRCA2	30	(21)	-		15	(16)	-		
PBSO									
Yes	83	(57)	3	(9)	61	(67)	12	(13)	.13
No	62	(43)	29	(91)	30	(33)	78	(87)	
Age at PBSO (years)									
Median	40.0		45.0		43.0		49.0		< .01
Range	29–57		35–45		32–65		37–58		
Timing of PBSO									
Before PM	18	(22)	2	(67)	14	(23)	6	(50)	.11
At PM	35	(42)	1	(33)	16	(26)	5	(42)	
After PM	30	(36)	0	(0)	31	(51)	1	(8)	
Ovarian cancer before PM	4	(3)	0	(0)	2	(2)	1	(1)	
Unexpected (p)MF at PM	3	(2)	2	(6)	4	(4)	1	(1)	
Cancer after PM									
Breast cancer	1	(1)	0	(0)	0	(0)	0	(0)	
Ovarian cancer	2	(1)	0	(0)	1	(1)	0	(0)	

Abbreviations: BC, breast cancer; HB(O)C, hereditary breast(/ovarian) cancer; PM, prophylactic mastectomy; PBSO, prophylactic bilateral salpingo-oophorectomy; (p)MF, (pre)malignant findings.
[a] Percentage of the number of women in column in question, unless stated otherwise.
[b] Percentage of unaffected women.
[c] Percentage of affected women.
[d] Difference between unaffected and affected BRCA1/2 mutation carriers, unless stated otherwise.
[e] Difference in distribution between unaffected and affected women.

being 15 of 122 50% risk carriers (12%). The median age at PBSO was younger in BRCA mutation carriers compared with the 50% risk carriers, both in the affected and the unaffected group. This difference, however, was not significant (*P* = .13, and *P* = .40, respectively).

Further, the median age at PBSO was lower in the unaffected compared with the affected group, being 40 versus 44 years, respectively (Table 2, $P < .001$). In addition, unaffected BRCA mutation carriers underwent PBSO at a younger age compared with mutation carriers with a history of BC (40 vs 43 years, respectively, $P < .01$, Table 1).

Table 2. Comparison between unaffected and affected women

	Unaffected	Affected	*P* value
No. of women (*N*)	177 (%)	181 (%)	
Age at PM (years)			
Median	37	44	< .001
Range	22–65	25–68	
< 30	21 (12)	8 (4)	< .001
30 – 39	88 (50)	48 (27)	
40 – 49	46 (26)	69 (38)	
≥ 50	22 (12)	56 (31)	
PBSO			
Yes	86 (49)	73 (40)	.12
No	91 (51)	108 (60)	
Age at PBSO (years)			
Median	40	44	< .001
Range	29–57	32–65	

Abbreviations: PM, prophylactic mastectomy; PBSO, prophylactic bilateral salpingo-oophorectomy.

(Pre)malignant findings at PM

In 10 of the 358 women (2.8%), abnormal findings were unexpectedly found in the mastectomy specimens (Table 1). Prior to intended PM there was no suspicion to justify an axillary nodal dissection in combination with the PM procedure. This occurred in five "unaffected" (2.8%) as well as in five previously affected women (2.8%), and in both mutation carriers (3%) and 50% risk carriers (2.5%).

The characteristics of the unexpected (pre)malignant findings as well as the preoperative screening results are chronologically described in detail in Table 3. In 1995, in one woman both preoperative clinical breast examination (CBE) and mammography were suspicious for a malignancy. However, additional investigation, consisting of ultrasound and cytology, did not reveal a malignancy. Nevertheless, histological examination of the mastectomy specimens revealed an invasive ductal carcinoma (IDC), eventually staged as a $pT_xN_1M_o$. The patient died of metastatic breast cancer four years after the PM. In another

two women, undergoing PM in 1996 and 1997, a lesion was found preoperatively (CBE and mammography, respectively) and classified as probably benign. No malignant abnormalities were seen at subsequent ultrasound examination, which is the reason a FNAC was not performed. However, histological examination of the mastectomy specimens revealed a small ductal carcinoma in situ (DCIS) in both women. In 2002, in one woman preoperative MRI revealed a lesion classified as probably benign. Indeed, no malignant abnormalities were found at additional ultrasound examination. Histological examination of the mastectomy specimens, however, revealed an IDC in the right, and an invasive medullar carcinoma (IMC) in the left breast. Preoperative screening in the remaining six women, performed 1 – 6 months preceding PM, did not show suspicious abnormalities. Still, another invasive carcinoma, three cases of DCIS, and two cases of lobular carcinoma in situ (LCIS) were found in the mastectomy specimens. All unexpected (pre)malignant findings in the affected women were found in the contralateral breast.

Table 3. Characteristics of unexpected (pre)malignant findings in the PM specimens

							Preoperative			
Year of PM	Genetic risk group	History of BC before PM	Histology	Grade	Tumour size (mm)	ER/PR status	CBE	Mx	MRI	
1995	HB(O)C	No	IDC	NA	NA	NA	SC	SC		a, b
1996	HB(O)C	No	DCIS	II	<2	NA	PB	nl		a
1996	BRCA1	No	LCIS	NA	NA	NA	nl	nl		
1997	HB(O)C	Yes	DCIS	I	NA	NA	nl			c
1997	BRCA1	Yes	DCIS	II	<2	NA	nl	PB		a
1997	BRCA1	Yes	IDC	II	3	Negative	nl			c
1998	BRCA1	Yes	DCIS	NA	<2	NA	nl	nl		
2000	BRCA1	Yes	LCIS	NA	NA	NA	nl	nl		
2002	BRCA1	No	IDC	III	5	Negative	nl	nl	PB	a
			IMC	III	6	positive				
2003	BRCA2	No	DCIS	II	<2	NA	nl	nl		

Abbreviations: PM, prophylactic mastectomy; BC, breast cancer; HB(O)C, hereditary breast(/ovarian) cancer; DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; IMC, invasive medullar carcinoma; CBE, clinical breast examination; Mx, mammography; MRI, magnetic resonance imaging; SC, suspicion of cancer; PB, probably benign; nl, normal; NA, not applicable.

[a] Additional investigation normal.

[b] Macroscopic palpable tumor; microscopic no clear border, therefore tumor size and grade not determinable.

[c] Treated previously for BC at another hospital. No information about preoperative imaging available.

Cancer during follow-up after PM

After PM, no incident breast cancer cases were observed in 50% risk carriers.

In BRCA mutation carriers, ovarian cancer was detected in two women in the unaffected, and in one woman in the affected group (Table 1).

One BRCA1 mutation carrier from the unaffected group presented in 2001, 3.5 years after PM (no malignant findings at histological examination), with metastatic adenocarcinoma in an axillary lymph node, morphologically and immunohistochemically consistent with breast cancer. Additional dissemination examinations also revealed metastases in bone and liver. Review of the preoperative data (physical examination and mammography) at the presentation of metastatic disease did not show malignant alterations, while meticulous reexamination of all pathology slides and additional investigation of frozen tissue material did not detect a primary breast cancer.

Reconstructive breast surgery

In Table 4, the numbers and types of reconstructive breast surgery, as well as the numbers and types of complications after breast reconstruction (BR) are shown. Of the total population (n = 358), 276 women underwent BR in combination with PM, being 60% unaffected and 40% affected women. The presence or absence of a BRCA1/2 mutation did not influence the BR rate (P = .23 for unaffected, and *P* = .10 for affected women), or the type of BR (*P* = .25 for unaffected, and *P* = .68 for affected women). Unaffected women mainly opted for BR (94%), consisting of 163 immediate and three delayed reconstructions, while a considerable part of the affected women did not opt for BR (37%) (*P* < .001). Further, unaffected women mainly opted for BR by means of (silicon) prosthesis (96%). In contrast, affected women opting for BR (102 immediate, and 8 delayed) more often had a reconstruction by means of autologous tissue compared with unaffected women, being 13% versus 3%, respectively (P = .004).

In 137 of 276 women opting for BR (49.6%) one or more complications were registered, totalling 215 complications. Surgical re-interventions were performed in 153 cases (124 for late complications).

Concerning the number of complications, this was not different between unaffected and affected women, neither for the moment of the complication (i.e., early or late) (*P* = .74), the necessity of re-intervention due to the complication (*P* = .67), nor for the type of complication (*P* = .09 for early complications and P = .33 for late complications).

Discussion

In this study we updated and extended the long-term experiences at the Rotterdam Family Cancer Clinic with prophylactic mastectomy (PM) in proven BRCA1/2 mutation carriers and in 50% risk carriers from a HB(O)C family. We compared the data of PM and breast reconstruction (if performed) in women with (affected) and without a personal history of BC (unaffected women) and further distinguished between women with a BRCA 1 or 2 mutation, and women without a proven mutation. While within the unaffected group, especially BRCA1/2 mutation carriers opted for PM, we observed that within the affected group an equal number of mutation carriers and 50% risk carriers from a HB(O)C family opted for PM. Women carrying a

BRCA 1 or 2 mutation are known to have an increased risk of developing contralateral primary BC,[20,21] which is even more apparent among women who are younger when diagnosed with a primary breast carcinoma (age < 50 yrs).[22,23] For high-risk women without a proven mutation inconsistent results on the risk of developing a contralateral breast cancer (CBC) were reported. Shahedi et al.[24] as well as Kirova et al.[25] reported an increased risk of developing CBC in non-BRCA1/2 women, while Tilanus et al.[26] concluded that the rate of CBC was only slightly and non-significantly increased in non-BRCA1/2 as compared with sporadic breast cancer patients. In view of these results, one expects that after a first diagnosis of breast cancer, especially mutation carriers will opt for prophylactic removal of the remaining breast tissue. In our study sample, however, also a considerable part of the women without a proven BRCA1/2 mutation opts for prophylactic mastectomy, especially after a history of breast cancer. It has to be mentioned that, since information on the BRCA1/2 mutation status is not always known in the latter group and genetic testing is missing a number of mutations, it is likely that some of these patients are in fact mutation carriers. Further, the group of women with a history of breast cancer, with and without a gene mutation, may partly consist of women who initially chose for surveillance, and eventually opted for PM after the diagnosis of breast cancer. This is in accordance with an earlier report indicating that women may be more likely to undergo PM after a previous diagnosis of BC.[27] This might also partly explain the higher age at the time of PM in the group with a history of BC.

We found that the age at PM was younger in unaffected women, both for mutation carriers as for 50% risk carriers. Further, we found a significant difference between unaffected and affected mutation carriers in the distribution of the numbers of PM over the various age categories, with the highest numbers of PM in the age group of 30–40 for unaffected, and 40–50 for affected carriers, respectively. Moreover, the distribution of PM over the various age categories remained completely identical to the age distribution reported in a previous study on PM from our institute,[5] indicating consistency over time.

Our data show that, despite preoperative (imaging) examination, the presence of unexpected microscopic (pre)malignant findings in this group of high-risk women is real (3%). Other studies reporting on high-risk and/or pathologic findings in prophylactically removed breast tissues, described percentages varying from 0.1–57%.[8,27-29] However, the comparison of frequencies of unexpected (pre)malignant findings between studies is hampered by differences in population selection, preoperative screening methods, pathological examination of the specimens, and definition of what is considered (pre)malignant [e.g. lobular carcinoma in situ (LCIS)]. The percentage of unexpected invasive carcinomas in these studies ranged from 0.1–7.7% (0.8% in our study). Most of these studies, however, did not provide information about the outcome of preoperative physical breast or imaging examination, which at the moment is a standard procedure at our institution. It might be that since the implementation of institutional guidelines concerning preoperative breast examination in 2000, and the introduction of magnetic resonance imaging (MRI), being

Table 4. Breast reconstruction (BR) in women undergoing PM

History of BC	No (Unaffected)		Yes (Affected)		
Number of women	177	(%)	181	(%)	*P* value
Breast reconstruction (BR)					
No	9	(5)	68	(37)	< .001
Yes	166	(94)	110	(61)	
Unknown[a]	2	(1)	3	(2)	
Type of BR					
Silicon prosthesis	159	(96)	95	(86)	.004
Autologeous tissue	6	(3)	14	(13)	
Unknown[a]	1	(1)	1	(1)	
Therapy first BC[b]					
BCT	-	-	7	(6)	
BCT/RT	-	-	33	(30)	
MAST	-	-	67	(67)	
MAST/RT	-	-	3	(3)	
Women with complications after BR					
No	84	(51)	55	(50)	.92
Yes	82	(49)	55	(50)	
Total number of complications after BR[c]	127		88		
Early (< 6 weeks after BR)	42	(33)	31	(35)	.74
Late (> 6 weeks after BR)	85	(67)	57	(65)	
Surgery due to complications					
No	38	(30)	24	(27)	.67
Yes	89	(70)	64	(73)	
Early complications					
Surgery due to early complications					
No	27	(64)	17	(55)	.42
Yes	15	(36)	14	(45)	
Type of early complication					
Infection	8	(19)	14	(45)	.09
Necrosis	11	(26)	4	(13)	
Bleeding	20	(48)	12	(39)	
Prosthesis luxation	2	(5)	0	(0)	
Poor arterial inflow	0	(0)	1	(3)	
Pneumothorax	1	(2)	0	(0)	
Late complications					
Surgery due to late complications					
No	27	(13)	7	(12)	.91
Yes	74	(87)	50	(88)	
Type of late complication					
Infection	4	(4)	0	(0)	.33
Necrosis	1	(1)	3	(5)	
Capsular formation	31	(37)	20	(35)	
Prosthesis luxation	2	(2)	3	(5)	
Poor cosmetic appearance[d]	31	(37)	19	(34)	
Dog ear	16	(19)	12	(21)	

Abbreviations: PM, prophylactic mastectomy; BC, breast cancer; BR, breast reconstruction; BCT, breast conserving therapy; BCT/RT, BCT in combination with radiotherapy, MAST: therapeutic mastectomy; MAST/RT, MAST in combination with radiotherapy.

[a] Surgery performed at another hospital, not included in *P* value calculation.

[b] Data showed for women with a BR after PM.

[c] One woman can have ≥ 1 complication.

[d] Including asymmetry.

more sensitive in detecting carcinomas in high-risk women,[30,31] as detection tool, the number of unexpected malignant findings in the PM specimens is decreasing.

With a 3% incidence of unexpected microscopic (pre)malignant findings, the potential role of sentinel node biopsy (SN) for all patients undergoing PM has been discussed. However, the majority of the (pre)malignant findings we found in this series, represents DCIS/LCIS; settings for which a sentinel node biopsy is not standardly indicated. Invasive cancer was found in only 0.8% of the patients in this series. Therefore, in our opinion, routine use of SN in all patients undergoing PM is not warranted, which is also supported in the paper by Boughey et. al.[32]

A previous study from our institution, investigating the efficacy of PM in unaffected women with a proven BRCA1/2 mutation, observed no cases of breast cancer after PM.[5] The mean follow-up in that study was 3 years. In the current cohort, one BRCA1 mutation carrier presented with metastatic disease 3.5 years after PM (no primary BC found), suggesting the presence of an occult primary tumour that was never found, despite a thorough re-examination of the specimen at the presentation of the metastatic disease. This finding emphasizes the fact that despite thorough examination of the mastectomy specimens, the presence of an occult breast cancer cannot be ruled out completely, and indicates that a form of surveillance after PM might be relevant.

The number of reconstructions after risk reducing mastectomy was lower in the affected group. This may be due to the fact that BR after previous radiotherapy and/or therapeutic mastectomy not always leads to satisfactory cosmetic results.[15,33] Some patients abandon, in consultation with and/or at the advice of their (oncological/plastic) surgeon, from BR for this reason. Other women have accepted the mutilation/alteration of body image caused by mastectomy, are reluctant to undergo renewed surgery, and prefer the use of external prosthesis.

During the follow-up period of this study, 49.6% of the women with immediate or delayed BR after PM showed complications. In total 215 complications were registered, leading to surgical re-intervention in 153 cases. These findings are consistent with several other reports,[34,35] though there are also studies reporting lower,[11,15,33] or even higher[36] complication rates after (immediate) BR. However, the literature in this area is difficult to compare, in part because not all previous series compare bilateral prophylactic mastectomy in unaffected women with risk-reducing mastectomies in women after a previous therapy for breast cancer. Furthermore, data may not be comparable because of different definitions of complications. Moreover, some studies describe the complication rate as a percentage of the total number of reconstructions,[11,15,33] while others, like our study, present the percentage of women with complications.[34-36]

We found no differences in the numbers of complications after (immediate) breast reconstruction in unaffected women compared with previously affected women in this study. This finding appears to be in contrast with earlier reports (also from our institution)

describing the occurrence of more complications after mastectomy followed by (immediate) breast reconstruction in affected women. These studies report negative effects of preoperative radiotherapy on the cosmetic outcome of the reconstruction, in particular the risk of capsular formation would be increased, having negative consequences on the symmetry of the breasts. Further, asymmetry can be expected to occur more often after previous therapeutic mastectomy. Although we have no explanation for our findings, it is possible that the experience of the surgeons at our institution is important. Indeed, where previously BR by means of silicon prosthesis after breast conserving therapy was performed, this is not done anymore.

In summary, we confirmed our previous findings that prophylactic mastectomy strongly reduces the risk of developing breast cancer in both BRCA1/2 mutation carriers and 50% risk carriers. As the frequency of unexpected cancers in this high risk group remains real, preoperative imaging and careful histological examination is warranted. Further, we found a substantial complication rate after breast reconstruction, which mainly concerned late cosmetic issues, almost always leading to additional surgery. In this respect, patients should be informed preoperatively that an optimal cosmetic effect cannot unconditionally be achieved in just one single operation. Concerning the complication rate after BR, we did not find a significant difference between affected and unaffected women. To our opinion, our data are providing additional data on this issue and may help to inform women considering prophylactic mastectomy and their physicians, in the complex process of decision-making.

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Chapter 3

Substantial breast cancer risk-reduction and potential survival benefit after bilateral mastectomy when compared with surveillance in healthy BRCA1 and BRCA2 mutation carriers: a prospective analysis

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Abstract

Background

To prospectively assess the efficacy of bilateral risk-reducing mastectomy (BRRM) when compared with surveillance on breast cancer (BC) risk and mortality in healthy BRCA1 and BRCA2 mutation carriers.

Patients and methods

Five hundred and seventy healthy female mutation carriers (405 BRCA1, 165 BRCA2) were selected from the institutional Family Cancer Clinic database. Eventually, 156 BRCA1 and 56 BRCA2 mutation carriers underwent BRRM. The effect of BRRM versus surveillance was estimated using Cox models.

Results

During 2037 person-years of observation (PYO), 57 BC cases occurred in the surveillance group versus zero cases during 1379 PYO in the BRRM group (incidence rates, 28 and 0 per 1000 PYO, respectively). In the surveillance group, four women died of BC, while one woman in the BRRM group presented with metastatic BC 3.5 years after BRRM (no primary BC), and died afterwards, yielding an HR of 0.29 (95% CI, 0.02–2.61) for BC-specific mortality.

Conclusions

In healthy BRCA1/2 mutation carriers, BRRM when compared with surveillance reduces BC risk substantially, while longer follow-up is warranted to confirm survival benefits.

Introduction

Women with a germline BRCA1 or BRCA2 mutation are at increased risk of developing breast and/or ovarian cancer compared with the general population.[1-4] Options to reduce breast cancer (BC) risk and/or subsequent mortality are regular surveillance with magnetic resonance imaging (MRI) and mammography, chemoprevention, and bilateral risk-reducing mastectomy (BRRM). Further, risk-reducing salpingo-oophorectomy (RRSO) aiming at reduction of ovarian cancer risk also reduces the risk of BC.[5]

Knowledge on the efficacy of the different risk-reducing strategies is important to both counsellors and BRCA1/2 mutation carriers, the latter facing the difficult decision of having their healthy breasts removed in order to prevent BC and possibly achieve better survival.

Previous publications, including the study by Meijers-Heijboer et al. from our institute, reported reduction of BC risk after BRRM in healthy BRCA1 or BRCA2 mutation carriers.[6-10] However, most studies were limited by small sample sizes, short follow-up periods, and retrospective study designs, thus facing methodological limitations, especially biases associated with start of follow-up such as cancer-induced testing bias and familial event bias.[11] Clearly, for ethical reasons randomized studies on risk-reducing strategies in BRCA1/2 mutation carriers are not feasible. Still, observational studies in BRCA1/2 mutation carriers can be set up in a prospective design, providing similar baseline risk for all study subjects, by starting the follow-up at the date of individual DNA diagnosis and excluding subjects having the event of interest before that date.[11]

Further, to date, no prospective data are available regarding survival in healthy BRCA1 and BRCA2 mutation carriers comparing BRRM with regular surveillance. The only available information hereon is derived from risk estimates assessed in mathematical models with simulated cohorts, yielding maximal survival probability for BRCA1/2 mutation carriers undergoing both BRRM and RRSO,[12-16] although Kurian et al. suggested that surveillance using mammography and MRI in combination with RRSO might offer almost comparable survival.[14,16] In view of surveillance aiming at early BC detection, and the current availability of modern (neo)adjuvant treatment strategies, it can indeed be hypothesized that regular surveillance may lead to similar survival as compared to BRRM.

The purpose of the current prospective study was to compare the rates of BC incidence, all-cause mortality and BC-specific mortality in healthy BRCA1 and BRCA2 mutation carriers opting for either BRRM or regular surveillance.

Methods

Study population

For this prospective cohort study, women were selected from the institutional Family Cancer Clinic registry database considering the following eligibility criteria: (i) proven BRCA1 or

BRCA2 mutation carrier, (ii) no history of cancer at the time of DNA testing, (iii) both breasts and both ovaries in situ at the time of DNA testing, and (iv) follow-up at the Family Cancer Clinic. Women with symptomatic BC before the first screening round were excluded. Written informed consent for prospective data collection was obtained from all included mutation carriers according to research protocols approved by the Medical Ethical Committee. A total of 570 women fulfilled the inclusion criteria. Eventually, 212 of these women chose to undergo BRRM before the end of the follow-up period (December 31, 2011), whereas the other 358 women remained under BC surveillance. Study follow-up started at the date of DNA diagnosis, being available as of 1994.

Data collection

Data on the following variables were retrieved from the medical files: mutation status, dates of birth, individual DNA diagnosis, breast and/or ovarian cancer diagnosis, dates of and findings at BRRM and/or RRSO, disease recurrence, and death.

Breast cancer surveillance and bilateral risk-reducing mastectomy

Regular breast cancer surveillance for BRCA1/2 mutation carriers consisted of (bi)annual clinical breast examination and annual mammography, while monthly breast self-examination was advised. As of 1998 annual MRI was added to the mammography, performed within a 6 weeks time span. From 2007, imaging was carried out biannually, alternating mammography and MRI. Ultrasound and cytological/histological examinations were carried out on indication.

Women considering BRRM were discussed prior to surgery in the multidisciplinary hereditary tumour board. Since 2000, clinical breast examination and MRI within 3 months before BRRM were recommended. In case of malignant findings detected during this screening round, events were allocated to the surveillance group.

Ninety-seven percent of the skin-sparing BRRM procedures were carried out at our clinic. Mastectomy specimens were examined thoroughly according to protocol by the pathologist.

After BRRM, patients were seen yearly at the Family Cancer Clinic including physical examination in order to investigate the long-term medical effects of prophylactic surgery and breast reconstruction. No standard imaging examination was scheduled.

Statistical analysis

For evaluation of person characteristics, the BRRM group included all women having undergone BRRM, and the surveillance group all women who did not. To estimate the efficacy of BRRM on the incidence of BC, the BC incidence rate for the group of women undergoing BRRM was compared with that of the surveillance group. To estimate the effect of BRRM on all-cause mortality and BC-specific mortality, hazard ratios (HR) with the surveillance group as the reference group, and accompanying 95% confidence intervals (CI) were provided using

Cox models, with BRRM as time-dependent covariate. Mutation status (i.e. BRCA1 or BRCA2 carrier), year of birth, age at DNA diagnosis and RRSO (as a time-dependent covariate) were considered as potential confounders.

For all women, start of follow-up was defined as the date of the individual DNA diagnosis. Women who underwent BRRM contributed person-years of observation (PYO) before surgery to the surveillance group, while PYO after surgery were contributed to the BRRM group. Of note, women with unexpected malignant findings in the mastectomy specimens contributed PYO to the surveillance group, from the date of DNA diagnosis until surgery, and the BC cases were counted as events in the surveillance group (no PYO contributed to the BRRM group). For the women not opting for BRRM, all PYO were contributed to the surveillance group.

The duration of PYO in the surveillance group ended on the date of the event of interest, being either the date of BC diagnosis for the BC incidence analysis or the date of death for the analyses on mortality rates, the date of BRRM, or date of a censoring event, whichever came first. The duration of PYO in the BRRM group ended on similar end points as described for the surveillance group. Censoring events were date of last contact, study closing date (i.e. December 31, 2011), and for BC incidence analysis also death.

All *P*-values were two-sided, and a significance level $\alpha = 0.05$ was used. Analyses were carried out with STATA (version 12.0; StataCorp, CollegeStation, TX, USA).

Results

Study population

Of the 570 unaffected BRCA1/2 mutation carriers, eventually 156 BRCA1 and 56 BRCA2 mutation carriers opted for BRRM (Table 1), at a median age of 34 and 37 years, respectively. Median follow-up was 8.5 years (range 0.6–17.8) for the women undergoing BRRM with 6.3 years after surgery (range 0.1–17.4), and 4.1 years (range 0.1–16.1) for the women under surveillance (data not shown). Compared with the surveillance group, the BRRM women underwent DNA testing in earlier years (2001 versus 2006), and at younger age (33 versus 36 years); also they more often opted for RRSO (54% versus 38%), again at younger age (40 versus 47 years; Table 1).

Breast cancer

After BRRM, no incident BC cases were observed during 1379 PYO, while during 2037 PYO 57 women in the surveillance group were diagnosed with BC. The corresponding incidence rates per 1000 PYO were 0 and 28, respectively (Table 2). Ten-year BC-free survival was 100% for the BRRM and 74% for the surveillance group (Figure 1A).

The majority of the 57 breast tumours in the surveillance group (including unexpected malignant findings detected at BRRM) were diagnosed at a favourable stage, including six

cases of ductal carcinoma *in situ* (DCIS, 10%) and 37 T1N0 cases (66%; Table 3).

BC was detected more often in BRCA1 than in BRCA2 mutation carriers (20% versus 7%; $P < 0.01$), but the median age at BC diagnosis was not different (43 versus 44 years; data not shown). In BRCA2 mutation carriers all breast tumours were detected at a favourable stage (DCIS or T1N0), while this was 72% in BRCA1 mutation carriers (Table 3).

Unexpected malignant findings in the mastectomy specimens were found in six women (Table 4). Two cases of DCIS concerned BRCA2 mutation carriers above 40 years. The invasive cases all concerned BRCA1 mutation carriers being ≤ 36 years, and in two women even being bilateral. Subsequent staging showed micrometastasis in only one patient. So far, all these women are still alive without recurrent disease.

Metastatic breast cancer

Four of the 51 women diagnosed with invasive BC in the surveillance group developed metastatic breast cancer 1.7 to 3.6 years after BC diagnosis (Table 5), all being BRCA1 mutation carriers including three (75%) having triple-negative BC. The woman with hormone sensitive BC never received systemic therapy, neither at diagnosis (not indicated according to guidelines) nor for metastatic disease due to comorbidity (renal insufficiency). Of note, all four women died within one year after detection of metastatic disease.

In the BRRM group, one BRCA1 mutation carrier presented in 2001 with metastases in axillary lymph nodes, bone and liver, 3.5 years after BRRM. Histological examination of an axillary lymph node showed adenocarcinoma, being ER/PR-positive and Her2Neu-negative, consistent with breast cancer. Neither preoperative workup in 1998, consisting of clinical breast examination and mammography carried out 3 months before BRRM (no MRI), nor re-examination of pathology slides and other remnant frozen tissue material showed a primary BC. This patient died of BC in 2006.

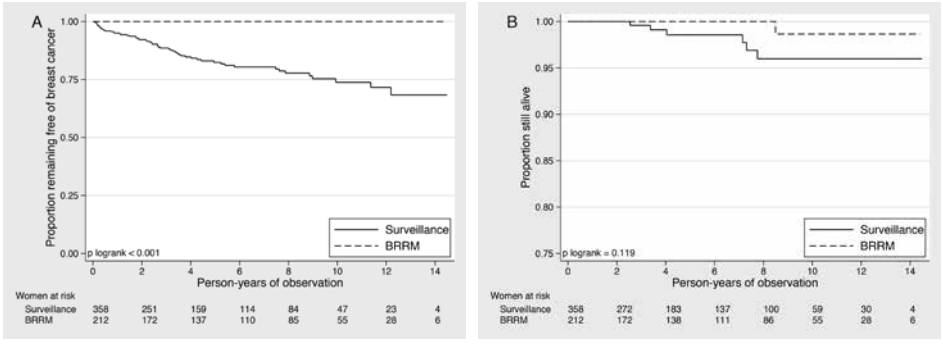

Figure 1. Kaplan-Meier estimates for time to onset of breast cancer (A) or death by all causes (B). BRRM, bilateral risk-reducing mastectomy.

Table 1. Characteristics of the study population

	Surveillance		BRRM		
	N	(%)	*N*	(%)	*P* value
	358	(63)	212	(37)	
Mutation status					
BRCA1	249	(70)	156	(74)	.305
BRCA2	109	(30)	56	(26)	
Year of birth					
<1940	7	(2)	0	(0)	.054
1940–1949	27	(8)	14	(7)	
1950–1959	73	(20)	34	(16)	
1960–1969	96	(27)	79	(37)	
1970–1979	102	(28)	62	(29)	
>1980	53	(15)	23	(11)	
Median (range)	1968	(1933–1993)	1967	(1941–1989)	.774
Age at DNA diagnosis (years)					
< 30	92	(26)	57	(26)	< .001
30–39	115	(32)	104	(49)	
40–49	79	(22)	38	(18)	
50–59	55	(15)	12	(6)	
>60	17	(5)	1	(1)	
Median, years (range)	36	(18–75)	33	(18–64)	< .001
Median year of DNA diagnosis (range)	2006	(1994–2011)	2001	(1994–2011)	< .001
Median age at BRRM, years (range)	-	-	35	(20–65)	
RRSO	137	(28)	114	(54)	< .001
Median age at RRSO, years (range)	47	(33–71)	40	(32–57)	< .001
Breast cancer (BC)	57	(16)	0[a]	(0)	< .001
Median age at BC, years (range)	43	(23–65)	-	-	
Ovarian cancer	6	(2)	5	(2)	.567
Other tumours[b]	8	(2)	5	(2)	.924
Metastatic BC	4	(1)	1	(1)	.424
Death	6[c]	(2)	1	(1)	.207

Abbreviations: BRRM, bilateral risk-reducing mastectomy; RRSO, risk-reducing salpingo-oophorectomy.
[a] One woman presented with metastatic breast cancer 3.5 years after BRRM, no primary breast cancer found.
[b] Skin tumours (except melanoma) excluded.
[c] Two patients died of extra-ovarian peritoneal cancer; all other deaths were due to breast cancer.

Table 2. Efficacy of bilateral risk-reducing mastectomy on breast cancer incidence and mortality

	BC incidence			Mortality					
	PYO	BC cases	Incidence rate[a]	PYO	Deaths (due to BC)	All-cause mortality rate[a]	HR (95% CI)[b]	Breast cancer specific mortality rate[a]	HR (95% CI)[b]
Surveillance	2037	57	28	2253	6 (4)	2.7	Ref.	1.8	Ref.
BRRM	1379	0	0	1384	1 (1)	0.7	0.20 (0.02-1.68)	0.7	0.29 (0.03-2.61)

Abbreviations: BC, breast cancer; PYO, person-years of observation; HR (95% CI), hazard ratio (95% confidence interval); BRRM, bilateral risk-reducing mastectomy.
[a] Per 1000 PYO.
[b] Univariate analysis; adding potential confounding variables to the model did not change the HR with >10%.

All-cause and breast cancer specific mortality

All-cause mortality rates (per 1000 PYO) were 0.7 for the BRRM group and 2.7 for the surveillance group (Table 2), yielding a HR of 0.20 (95% CI 0.02-1.68). Ten-year overall survival was 99% for the BRRM and 96% for the surveillance group (Figure 1B). In the surveillance group two women with previous RRSO died of extra-ovarian peritoneal cancer (diagnosed at age 50 and 61, respectively, no history of BC). The BC-specific mortality rates (per 1000 PYO) were 1.8 for the surveillance group and 0.7 for the BRRM group, resulting in a HR of 0.29 (95% CI 0.03-2.61) (Table 2). Of note, adding potential confounding variables to the model did not change the HR with more than 10%.

Discussion

This prospective cohort study in healthy BRCA1/2 mutation carriers showed that after BRRM the BC incidence rate was substantially reduced, compared with regular surveillance. Further, all cause mortality and BC-specific mortality rates were reduced, although significant survival benefits could not be claimed yet. Moreover, regular surveillance detected BC at mainly favourable stages.

To the best of our knowledge, this is the largest single institution, prospective cohort study on the efficacy of BRRM versus regular surveillance in healthy BRCA1/2 mutation carriers so far. Our data are in line with the results of other published retrospective studies[6-10] and illustrate that BRRM substantially reduces the BC risk. We did not observe any BC after BRRM in 212 mutation carriers after a median follow-up of 6.3 years, while in the studies of Rebbeck et al.[8] and Skytte et al.[10], two and three BC cases were observed after BRRM in 102 and 96 women, respectively.

With the exception of the recent study by Skytte et al.[10], the prospective character of most previous studies is debatable. Especially, strong cancer-induced testing bias is introduced by including patients undergoing genetic testing after BC diagnosis into the surveillance group. Clearly, for ethical reasons it is not possible to perform a randomized clinical trial in this setting. Still, by limiting our study cohort to those women being cancer-free and having both breasts and ovaries in situ at the moment of DNA testing, and by starting to count PYO from the date of individual DNA diagnosis for all women, in our opinion we have approached the most unbiased possible prospective model to study the efficacy of BRRM on BC risk-reduction in healthy BRCA1/2 mutation carriers.

However, the ultimate goal of risk-reducing mastectomy is to improve survival, eventually being the reason for a healthy woman to decide for this drastic intervention. So far, survival after BRRM is only studied in mathematical simulation models describing an improved survival for women who opt for risk-reducing surgery[12-16], although Kurian et al.[14,16] estimated that screening by means of mammography and MRI plus RRSO results in almost

Table 3. Tumour and treatment characteristics of patients with breast cancer in the surveillance group

		All tumours (*n*=57)		BRCA1 (*n*=49)		BRCA2 (*n*=8)	
		N	(%)	*N*	(%)	*N*	(%)
Year of diagnosis	1999-2003	13	(23)	11	(22)	2	(25)
	2004-2007	13	(23)	11	(22)	2	(25)
	2008-2011	31	(54)	27	(56)	4	(50)
TN classification[a]	Tis	6	(10)	4	(8)	2	(25)
	T1aN0	9	(16)	8	(16)	1	(12)
	T1bN0	14	(25)	11	(23)	3	(38)
	T1cN0	14	(25)	12	(25)	2	(25)
	T2N0	6	(10)	6	(12)	0	(0)
	T1N1	6	(10)	6	(12)	0	(0)
	T2N1	2	(4)	2	(4)	0	(0)
ER status[b]	Negative	32	(63)	31	(69)	1	(17)
	Positive	16	(31)	12	(27)	4	(66)
	Unknown	3	(6)	2	(4)	1	(17)
PR status[b]	Negative	36	(71)	34	(76)	2	(33)
	Positive	12	(23)	9	(20)	3	(50)
	Unknown	3	(6)	2	(4)	1	(17)
Her2Neu status[b]	Negative	43	(84)	39	(86)	4	(67)
	Positive	3	(6)	3	(7)	0	(0)
	Unknown	5	(10)	3	(7)	2	(33)
Triple negative[b]	Yes	30	(59)	29	(64)	1	(17)
Presentation	Interval[c]	5	(9)	5	(10)	0	(0)
	Screen-detected	46	(81)	40	(82)	6	(75)
	RRM	6	(10)	4	(8)	2	(25)
Synchronous bilateral BC	Yes	2	(3)	2	(4)	0	(0)
Adjuvant systemic therapy	Chemo	21	(37)	21	(43)	0	(0)
	Endocrine	2	(4)	2	(4)	0	(0)
	Chemo and Endocrine	10	(17)	8	(16)	2	(25)
	None	21	(37)	15	(31)	6	(75)
	Unknown	3	(5)	3	(6)	0	(0)

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; BC, breast cancer.
[a] Classification according to UICC International Union Against Cancer, 6th edition, 2002.
[b] Only for invasive BC.
[c] Clinical symptoms between two screening rounds.

similar survival when compared with BRRM combined with RRSO, suggesting that intensive MRI-based surveillance might be a reasonable alternative to BRRM. Arguments to support the latter are (1) MRI detects BC at an early stage in BRCA1/2 mutation carriers[17-20], (2) in a prospective MRI-detected series of BRCA1/2-associated patients the 5-year cumulative overall survival was 93% (95% CI 79% to 98%)[21], (3) broad implementation of (neo)adjuvant systemic therapy in recent years results in improved BC survival.[22]

The (non-significant) mortality rate reduction after BRRM found in our study corresponds with an absolute survival benefit of 3% according to the Kaplan-Meier estimates, which is comparable to the 3%–5% decrement in survival described by Kurian et al. when MRI-based screening was performed instead of BRRM.[14,16] Our prospective data add to the modelling study of Kurian, since they better reflect real practice, with only 38% of the women undergoing RRSO in the surveillance group of our study (versus 100% in Kurian's model), during surveillance BC mostly detected at a favourable stage (DCIS/T1N0, while Kurian incorporated BC characteristics at symptomatic detection in the model), and 59% of the BCs in the surveillance group of our study being triple-negative (data not incorporated in Kurian's model).

Our data concerning early detection are in concordance with the mentioned literature.[17-20] In the surveillance group 76% of the BCs were detected at favourable stages (DCIS or T1N0). Nevertheless, four patients (all BRCA1 carriers) developed metastatic disease and died of BC, despite early detection or applied adjuvant chemotherapy. Notably, these BCs were all diagnosed before 2007, when mammography and MRI were performed simultaneously once a year. From 2007 on, mammography and MRI were performed alternating every 6 months, while MRI-techniques also have been improved. In this series no significant differences in grade, behaviour, size and nodal-status were observed between invasive BCs diagnosed before 2007 (n = 22) and from 2007 and beyond (n = 29), though the latter BCs tended to be somewhat smaller (T1a/b, 62% versus 36%; P = .175) and more often node-negative (N0, 90% versus 77%; P = .228). However, since the mean follow-up after BC diagnosis in the latter group was only 1.7 years (versus 6.6 years for patients with BC diagnosis before 2007), no conclusions regarding a more favourable outcome can be drawn yet.

Noteworthy, the incidence of breast tumours was lower in BRCA2 than in BRCA1 mutation carriers, all BRCA2-associated tumours were detected at favourable stages, and so far, all these BRCA2 patients remained without recurrent disease. These findings may suggest that for BRCA2 mutation carriers, in contrast to BRCA1 mutation carriers, intensive surveillance indeed may lead to similar survival when compared with BRRM.

In our series, one woman experienced metastatic breast cancer 3.5 years after BRRM. As neither before nor at BRRM a primary tumour was diagnosed, we did not count this as a primary BC event. Reanalysing the data by doing so resulted in BC incidence rates (per 1000 PYO) of 1 versus 28 for the BRRM and surveillance groups, respectively (HR 0.03; 95% CI 0.01–0.22).

Despite preoperative (imaging) examination, unexpected malignant findings were found in the mastectomy specimens of six women (2.8%), emphasizing the need for careful examination of the mastectomy specimens. Reassuringly, none of the women with unexpected malignant findings in our study sample has been diagnosed with recurrent disease, after a mean follow-up of 4.8 years since BC diagnosis.

In our study design, the PYO of the women with unexpected malignant findings at BRRM were allocated to the surveillance group, and also the tumours were counted as events

Table 4. Characteristics of unexpected malignant findings at BRRM

Patient	Mutation status	Year of diagnosis	Age at diagnosis	Laterality	TN classification[a]	Differentiation grade	ER/PR status	Triple negative	Preoperative (<3 months)		
									CBE	Mx	MRI
1	BRCA1	2002	36	Left	T1aN0	III	Negative	Y	Nl	Nl	Nl
				Right	T1bN0	III	Positive[b]	N	Nl	Nl	PB[c]
2	BRCA2	2003	49	Right	Tis	II	NA	NA	Nl	Nl	NE
3	BRCA1	2005	34	Right	T1aN1mic	II	Positive	N	PB[d]	-	PB[e]
4	BRCA1	2008	33	Left	T1aN0	III	Negative	Y	Nl	Nl	Nl
5	BRCA1	2009	34	Left	T1aN0	II	Positive	N	-	-	Nl
				Right	T1bN0	III	Negative	Y	-	-	Nl
6	BRCA2	2009	41	Right	Tis	II	NA	NA	-	-	Nl

Abbreviations: BRRM, bilateral risk-reducing mastectomy; ER/PR, estrogen receptor/progesterone receptor; CBE, clinical breast examination; Mx, mammography; MRI, magnetic resonance imaging; Nl, normal; PB, probably benign; NA, not applicable; NE, not evaluable.
[a] Classification according to UICC International Union Against Cancer, 6th edition, 2002.
[b] PR status negative.
[c] Additional investigations normal.
[d] Palpable tumour; additional ultrasound probably normal.
[e] No additional investigation carried out.

Table 5. Features of patients with metastatic breast cancer in the surveillance group

Patient	Mutation status	Year of diagnosis	Age at diagnosis	Age at MBC	Age at death	Presentation[a]	TN classification[b]	Differentiation grade	ER/PR status	Triple negative	Adjuvant systemic therapy
1	BRCA1	1999	23	25	26	Interval	T2N1	III	Negative	Y	AC
2	BRCA1	2004	45	49	49	Screen detected[c]	T1cN0	II	Positive	N	None
3	BRCA1	2005	58	60	60	Screen detected	T2N0	III	Negative	Y	Chemotherapy[d]
4	BRCA1	2006	33	35	35	Interval	T2N0	III	Negative	Y	TAC

Abbreviations: ER/PR, estrogen receptor/progesterone receptor; BC, breast cancer; MBC, metastatic breast cancer; AC, adriamycine, cyclofosfamide; TAC, taxane, adriamycine, cyclofosfamide.
[a] Interval presentation: clinical symptoms between two screening rounds.
[b] Classification according to UICC International Union Against Cancer, 6th edition, 2002.
[c] Tumour found at first screening round.
[d] Type unknown.

in this group. Although these women initially opted for BRRM, and therefore, according to the intention-to-treat principle, assignment to the BRRM group can be argued, in our opinion this is the most appropriate way to handle these unexpected findings, since these events could not be prevented by BRRM anymore. Then again, this may have led to some overestimation of the BC risk in the surveillance group. Reanalysing the data by excluding the women with unexpected malignant findings at BRRM rendered similar results for BC incidence rates and mortality rates (data not shown), thus not altering the conclusions of our analyses.

Despite the strengths of our study, including a prospective design with a large sample size from a single institution, a sufficiently long follow-up period for BC risk estimates, and regular control visits after BRRM, longer follow-up, and larger sample size are still warranted to truly establish that BRRM indeed results in improved survival when compared with intensive surveillance in BRCA mutation carriers. Moreover, larger numbers of especially BRCA2 mutation carriers are needed to specifically investigate whether a more conservative approach concerning risk-reducing mastectomy in BRCA2 versus BRCA1 mutation carriers may be justified.

In summary, we confirmed that BRRM substantially reduces breast cancer occurrence in healthy BRCA1/2 mutation carriers. Further, this is the first prospective observational study suggesting that BRRM when compared with surveillance is associated with improved survival, although longer follow-up in combination with larger sample size are needed to confirm statistical significance. Our data are certainly worthwhile for the clinic as it provides more accurate information on life expectancy for healthy BRCA1/2 mutation carriers facing the difficult choice between breast cancer surveillance and BRRM.

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Chapter 4

Improved overall survival after contralateral risk-reducing mastectomy in BRCA1/2 mutation carriers with a history of unilateral breast cancer: a prospective analysis

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Abstract

Background

Data on survival of BRCA1/2-associated primary breast cancer (PBC) patients who opt for subsequent contralateral risk-reducing mastectomy (CRRM) are scarce and inconsistent. We examined the efficacy of CRRM on overall survival in mutation carriers with a history of PBC.

Patients and Methods

From a Dutch multicentre cohort, we selected 583 BRCA-associated PBC patients, being diagnosed between 1980 and 2011. Over time, 242 patients (42%) underwent CRRM and 341 patients (58%) remained under surveillance. Survival analyses were performed using Cox models, with CRRM as a time-dependent covariate.

Results

The median follow-up after PBC diagnosis was 11.4 years. In the CRRM group, four patients developed contralateral breast cancer (2%), against 64 patients (19%) in the surveillance group ($p<0.001$). The mortality was lower in the CRRM group than in the surveillance group (9.6 and 21.6 per 1000 person-years of observation, respectively; adjusted hazard ratio 0.49, 95% confidence interval 0.29-0.82). Survival benefit was especially seen in young PBC patients (<40 years), in patients having a PBC with differentiation grade 1/2 and/or no triple-negative phenotype, and in patients not treated with adjuvant chemotherapy.

Conclusion

We conclude that CRRM is associated with improved overall survival in BRCA1/2 mutation carriers with a history of PBC. Further research is warranted to develop a model based on age at diagnosis and tumour and treatment characteristics that can predict survival benefit for specific subgroups of patients, aiming at further personalized counselling and improved decision making.

Introduction

Women with a **BR**east **CA**ncer (BRCA)1 or BRCA2 germline mutation and a history of primary breast cancer (PBC) have a significantly elevated risk of developing contralateral breast cancer (CBC). The estimated cumulative lifetime risk of CBC is 20-83%[1-5] for BRCA1/2 mutation carriers, and 4-15% in sporadic PBC patients.[6,7] CBCs usually are diagnosed at a more favourable stage than PBCs,[8] i.e. at smaller size and more often node negative, suggesting no additional adverse effect on survival. Nevertheless, sporadic patients with CBC show worse survival as compared to unilateral breast cancer (BC) patients.[9,10]

From the available risk-reducing measures, the most effective option for CBC risk reduction is contralateral mastectomy. This procedure significantly reduces the risk of CBC by 83-100%, both in the sporadic breast cancer population and in high-risk and BRCA-associated PBC patients.[11-19]

Still, the ultimate goal of contralateral risk-reducing mastectomy (CRRM) is to improve survival. So far, data on survival of PBC patients who opt for subsequent CRRM are inconsistent. Some studies showed improved survival after CRRM,[14,17,19-21] while others did not.[11,13,16,18] Also, previous studies often suffered from small sample sizes,[11,13,18] short follow-up[13,18,20] and heterogeneous study populations consisting of mixtures of sporadic, high-risk and BRCA-associated PBC patients.[16,18] In fact, only three studies exclusively reported on BRCA1/2 mutation carriers.[13,19,21]

More knowledge on the efficacy of CRRM after BRCA-associated PBC is important as this allows for more accurate and tailored genetic and oncologic counselling for respective patients. In view of the limitations of previous studies, we designed a well-powered prospective study to establish the efficacy of CRRM after BRCA-associated PBC on overall survival.

Methods

Study population

In the context of an ongoing nationwide Dutch study on risk assessment and gene-environment interactions (the HEBON study),[22] members of breast and/or ovarian cancer families have been identified through the departments of Clinical Genetics/Family Cancer Clinics at all Dutch academic centres, the Netherlands Cancer Institute, and the Foundation for the detection of Hereditary Tumours. Linkage to the Netherlands Cancer Registry and the Netherlands Pathology Database, as well as medical files were used to retrieve and prospectively update data on the occurrence of any cancer, tumour characteristics, recurrent disease after previous breast and/or ovarian cancer, on previous and ongoing therapy, and on preventive strategies. According to protocols approved by the Medical Ethical Committees of the participating centres, all included women provided written informed consent.

From this national cohort we identified 905 proven BRCA1 or BRCA2 female

mutation carriers with PBC diagnosed during the period 1980-2011. Further eligibility criteria applicable on the date of study inclusion were (a) no history of bilateral BC or ovarian cancer, (b) no evidence of distant disease activity, and (c) at least one unaffected breast in situ. From the selected cohort, we excluded 85 patients with missing data regarding dates of cancer diagnoses, DNA test results, risk-reducing surgeries, or death. In addition, 237 patients who did not match the eligibility criteria were excluded.

Data collection

For the eligible patients, data on type of mutation (i.e. BRCA1 or BRCA2), dates of birth, disclosure of individual DNA test result, primary and contralateral BC diagnoses, and ovarian cancer diagnosis, dates of and findings at CRRM and/or risk-reducing salpingo-oophorectomy (RRSO), and dates of disease recurrence and death were retrieved. We also collected data on PBC and CBC characteristics (type of histology, differentiation grade, estrogen receptor (ER) status, progesterone receptor (PR) status, HER2 status, and stage) and PBC therapy (surgery, radiotherapy, chemotherapy, endocrine therapy).

Outcome definitions

Overall survival was measured in person-years of observation (PYO), and defined as time from date of study inclusion until death from any cause. CBC was defined as invasive BC or ductal carcinoma-in-situ (DCIS) in the contralateral breast, detected at least three months after diagnosis of PBC.

Statistical analysis

We evaluated patient demographics by comparing women who underwent contralateral risk-reducing mastectomy (CRRM group) with women who were under surveillance (surveillance group). Figure 1 depicts the allocation of PYO to both groups. For the current prospective analyses, we defined the date of study inclusion as either the date of PBC diagnosis or the date of individual DNA diagnosis, whichever came last. Left truncation was used to avoid potential survival bias due to inclusion of patients who underwent genetic testing after breast cancer diagnosis, as recommended by Klaren et al.[23] For the women under surveillance, all PYO were allocated to the surveillance group. For women opting for CRRM, PYO prior to surgery were allocated to the surveillance group, while PYO after surgery were allocated to the CRRM group. The observation ended on the date of death, date of last contact or study closing date (i.e. December 31, 2012), whichever came first.

Further, we investigated potential survival bias due to inclusion of the subgroup of patients who developed distant metastases or died shortly after PBC diagnosis. These patients never may have had the opportunity to opt for CRRM, and counting eventual deaths as events in the surveillance group may result in overestimation of mortality in this group, and consequently in overestimation of the survival benefit from CRRM. To avoid this, we performed an additional

analysis including only those patients who were alive and had remained free of distant metastases for at least 2 years after PBC diagnosis. The choice for 2 years was based on the median time period observed between PBC diagnosis and CRRM. Counting PYO for this additional analysis started either at the date of PBC diagnosis plus 2 years or at the date of individual DNA diagnosis, whichever came last (Figure 1). This resulted in exclusion of 17 patients from the analyses: 10 with distant metastases and/or who died (n=4) within 2 years after PBC diagnosis, and seven who had less than 2 years of follow-up after PBC diagnosis (14 without and three with CRRM).

To estimate the efficacy of CRRM on overall survival, we used a Cox model with CRRM as a time-dependent covariate to obtain hazard ratios (HRs) and accompanying 95% confidence intervals (95% CIs), using the surveillance group as the reference group. The following variables were considered as potential confounders: type of mutation, year of birth, age at DNA diagnosis, age at PBC diagnosis, various tumour characteristics (tumour and nodal stage, differentiation grade, hormone receptor status, HER2 status), different treatments administered for PBC, and RRSO (as a time-dependent covariate). We incorporated a variable in the multivariate Cox model if (*i*) there was a significant difference in the median or in the distribution of the variable between the CRRM group and the surveillance group, and (*ii*) the likelihood-ratio test showed that the model including the variable was significantly different from the model without the variable.

Because the Kaplan-Meier method does not accommodate a time-dependent covariate, we used the Simon and Makuch method, which takes into account the change in an individual's covariate status over time, to graph survival curves for the CRRM and the surveillance groups.[24,25]

Finally, we performed stratified Cox analyses to explore the effect of CRRM within different risk groups. Considered strata were: type of mutation (BRCA1 versus BRCA2), age at PBC diagnosis (<40 versus ≥40 years), various tumour characteristics [DCIS/T1N0 (no versus yes); nodal status (N0 versus N+); differentiation grade (1-2 versus 3); ER status (negative versus positive); triple negative phenotype (no versus yes)], and different types of treatment for PBC [mastectomy versus breast-conserving surgery; radiotherapy (no versus yes); adjuvant chemotherapy (no versus yes); adjuvant endocrine therapy (no versus yes)].

All p-values were two-sided, and a significance level α=0.05 was used. Analyses were performed with STATA (version 12.0; StataCorp, CollegeStation, TX, USA).

Results

Study population

Of the 583 BRCA-associated PBC patients, 193 BRCA1 and 49 BRCA2 mutation carriers eventually opted for CRRM (Table 1), at a median age of 41 and 47 years, respectively (data not shown). Median follow-up after PBC diagnosis was similar in the CRRM and the surveillance group (11.4 and 11.3 years), while median PYO was longer for patients who opted for CRRM

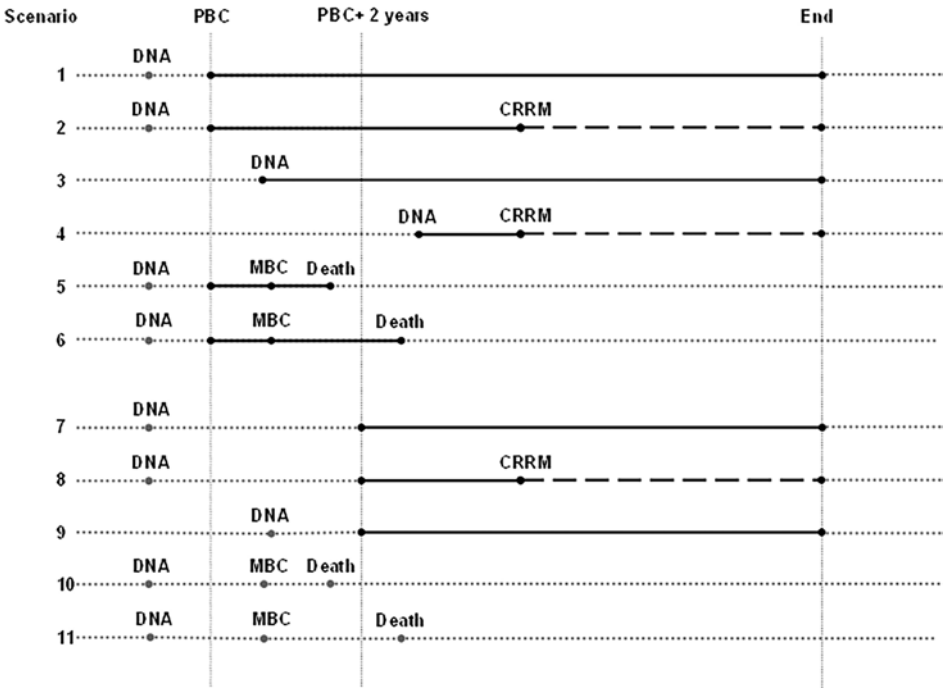

Figure 1

Allocation of person-years of observation to the surveillance group (solid line) and the CRRM group (dashed line) according to different scenarios, as applied to Cox analysis on overall survival.

Abbreviations: PBC, primary breast cancer; CRRM, contralateral risk-reducing mastectomy; MBC, metastatic breast cancer.

Observation started at the date of PBC diagnosis (scenarios 1, 2, 5, 6), unless DNA testing was performed after PBC diagnosis. Survival bias can occur if BRCA1/2 mutation carriers who were tested a long time after PBC diagnosis – thus surviving long enough to undergo DNA testing – are included in the analyses. Including the time between PBC diagnosis and DNA diagnosis would dilute mortality in the surveillance group, thereby underestimating the risk-reduction of CRRM. To control for this, observation for patients with DNA testing after PBC diagnosis started at DNA diagnosis (scenarios 3 and 4).

Counting deaths that occur shortly after PBC diagnosis, or are the result of MBC occurring shortly after PBC diagnosis – thus including patients who are not eligible for CRRM on the basis of the (bad) prognosis of PBC – may lead to an overestimation of numbers of deaths in the surveillance group, and consequently to an overestimation of risk-reduction of CRRM. To investigate this, we performed an additional analysis in which we defined the start of observation as the date of PBC diagnosis plus 2 years (scenarios 7, 8, 9). Patients who deceased or had distant metastases within 2 years after PBC diagnosis were therefore not included in this analysis (scenarios 10 and 11).

(9.6 versus 7.4 years, $p<0.001$). CRRM patients were diagnosed with PBC at younger age (38 versus 42 years, $p<0.001$), and more often opted for RRSO (80% versus 69%, $p=0.002$), the latter also at younger age (43 versus 47 years, $p<0.001$). More of the CRRM patients had been treated with adjuvant chemotherapy for PBC (66% versus 51%, $p=0.001$), while more surveillance patients underwent breast-conserving surgery (52% versus 35%, $p<0.001$) and radiotherapy (67% versus 48%, $p<0.001$). As shown in Table 1, tumour characteristics of the PBCs were similar in both groups.

Contralateral BC

CBC was detected in four patients (2%) after CRRM, and in 64 patients of the surveillance group (19%; Table 1). As shown in Table 2, the majority of the CBCs had a favourable tumour stage, with a Tis/T1 classification in 87%, and node-negative disease in 79% of the patients. Conversely, 73% of the tumours were triple-negative. CBC was diagnosed in 13% of the BRCA1 patients and in 8% of the BRCA2 patients ($p=0.122$; data not shown). Median follow-up after CBC diagnosis was 5.2 years (range 0.1-15.5). Sixteen of the CBC patients (24%) died during follow-up, all in the surveillance group (Table 2).

Overall survival

As shown in Table 1, fewer patients died in the CRRM group (8% versus 19%, $p<0.001$). Accordingly, the mortality was lower in the CRRM group (21.6 versus 9.6 per 1000 PYO); the Cox analysis yielded an HR of 0.49 (95% CI, 0.29-0.82; adjusted for RRSO; Table 3). The additional analysis starting 2 years after PBC diagnosis showed an adjusted HR of 0.55 (95% CI, 0.32-0.95; Table 3). Furthermore, as shown in the survival curves (Figure 2), the 15-year overall survival in the current cohort was better in the CRRM group (86%) than in the surveillance group (74%).

Risk strata

The exploratory analyses (see Figure 3) showed a marked survival benefit after CRRM for mutation carriers of the following subgroups: young PBC patients (<40 years), patients having a PBC with a low differentiation grade (grade 1 or 2) and/or no triple-negative phenotype, and patients not treated with adjuvant chemotherapy.

Discussion

In this prospective Dutch multicentre cohort study in BRCA1/2 mutation carriers with a history of unilateral BC, we observed a significantly improved overall survival after CRRM. In addition, CBC incidence was strongly reduced after CRRM.

Our results are in agreement with recently published data.[19,21] In the British study, describing a cohort of 698 BRCA-associated PBC patients of whom 105 (15%)

Table 1. Characteristics of the study population

	CRRM		Surveillance		
	N	(%)[1]	*N*	(%)[1]	*P* value
	242	(42)	341	(58)	
Follow-up, median yrs (range)					
After PBC	11.4	(1.0-30.6)	11.3	(12.0-32.5)	0.711
In study[2]	9.6	(1.0-17.9)	7.4	(0.2-17.1)	<0.001
Mutation status					
BRCA1	193	(80)	261	(77)	0.365
BRCA2	49	(20)	80	(23)	
Year of birth					
<1940	7	(3)	24	(7)	0.001
1940-1949	35	(14)	75	(22)	
1950-1959	76	(31)	118	(35)	
1960-1969	72	(30)	88	(26)	
1970-1979	43	(18)	31	(9)	
> 1980	9	(4)	5	(1)	
Median (range)	1960	(1929-1982)	1956	(1924-1983)	<0.001
Age at study inclusion[2], median (range)	41	(23-72)	47	(26-80)	<0.001
Age at PBC diagnosis (yrs)					
<40	130	(54)	137	(40)	<0.001
≥40	112	(46)	204	(60)	
Median (range)	38	(23-72)	42	(24-71)	<0.001
Median year (range)	2000	(1982-2011)	2000	(1980-2011)	0.131
DNA diagnosis					
Age, median yrs (range)	40	(22-67)	46	(20-80)	<0.001
Timing					
Before PBC diagnosis	57	(24)	47	(14)	0.003
After PBC diagnosis	185	(76)	294	(86)	
Time after PBC diagnosis, median yrs (range)	1.3	(0.1-16.9)	2.8	(0.1-27.7)	<0.001
PBC tumour characteristics					
T-status[3]					
DCIS	11	(5)	18	(6)	0.712
T1[4]	12	(5)	16	(5)	
T1a/b	41	(18)	47	(15)	
T1c	79	(35)	100	(33)	
T2+	83	(37)	126	(41)	
unknown	16		34		
Positive lymph nodes (n)					
0	145	(63)	199	(64)	0.302
1-3	71	(31)	86	(27)	
≥ 4	13	(6)	28	(9)	
unknown	13		28		
DCIS/T1N0					
No	124	(56)	177	(59)	0.474
Yes	99	(44)	123	(41)	

	CRRM		Surveillance		
	N	(%)[1]	*N*	(%)[1]	*P* value
unknown	19		41		
Differentiation grade[5]					
1	3	(1)	3	(1)	0.871
2	40	(18)	60	(21)	
3	176	(81)	221	(78)	
Unknown	23		57		
Receptor-status					
ER-positive	61	(37)	77	(35)	0.747
PR-positive	49	(31)	60	(29)	0.646
HER2-positive	7	(6)	7	(4)	0.586
Triple negative	77	(49)	107	(52)	0.598
Surgery for PBC					
Breast-conserving therapy	79	(35)	142	(52)	<0.001
Mastectomy	144	(65)	131	(48)	
Unknown	19		68		
Radiotherapy for PBC	106	(48)	207	(67)	<0.001
Systemic therapy for PBC					
Chemotherapy	157	(66)	174	(51)	0.001
Endocrine therapy	46	(19)	57	(17)	0.508
RRSO	193	(80)	234	(69)	0.002
Age, median yrs (range)	43	(32-75)	47	(29-71)	<0.001
Ovarian cancer	4	(2)	13	(4)	0.142
Age, median yrs (range)	48	(41-56)	53	(42-75)	0.234
CRRM					
Age, median yrs (range)	42	(24-74)	-		
Year, median (range)	2004	(1995-2012)	-		
Years after PBC, median (range)	2.0	(0-20.2)			
<6 months after PBC	36	(15)	-		
6 months - 2 yrs after PBC	81	(33)	-		
>2 yrs after PBC	125	(52)	-		
Follow-up after CRRM, median yrs (range)	8.2	(0.3-16.4)	-		
CBC	4[6]	(2)	64	(19)	<0.001
Death[7]	19	(8)	65	(19)	<0.001
Age, median yrs (range)	50	(26-66)	51	(30-85)	0.282

CRRM, contralateral risk-reducing mastectomy; PBC, first primary breast cancer; yrs, years; DCIS, ductal carcinoma in situ; ER, estrogen receptor; PR, progesterone receptor; RRSO, risk-reducing salpingo-oophorectomy; CBC, contralateral breast cancer.
[1] Excluding missing values.
[2] Inclusion date is either date of PBC diagnosis or date of DNA diagnosis, whichever came last.
[3] Classification according to UICC International Union Against Cancer, 6th edition, 2002.
[4] Subgroup classification (i.e. T1a/b/c) not assessed or unknown.
[5] Classification according to Bloom & Richardson.
[6] All incident cases, detected after CRRM.
[7] All causes.

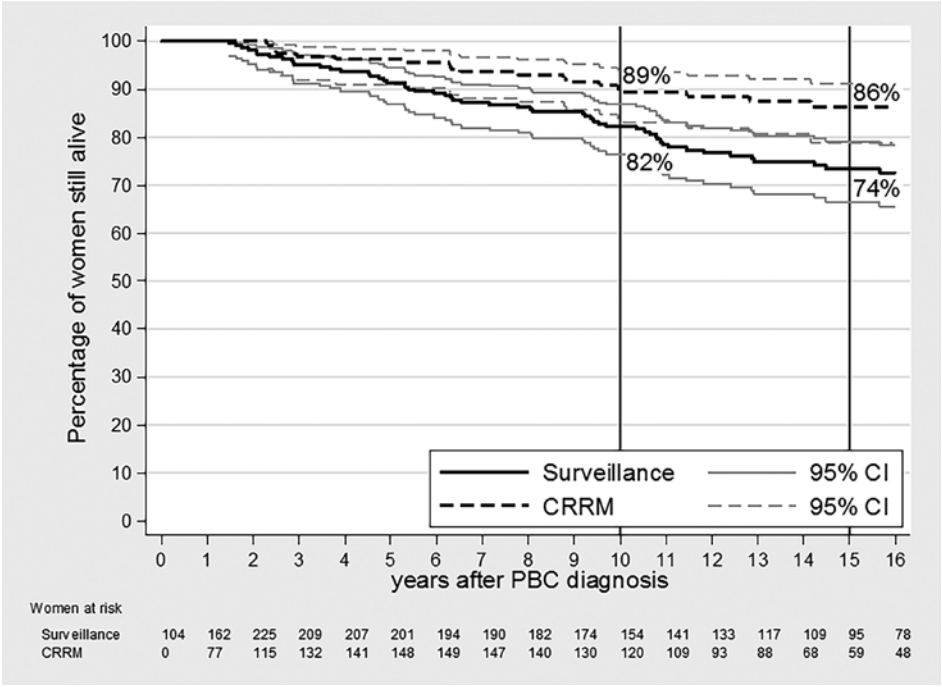

Figure 2

Unadjusted overall survival curves for BRCA1/2-associated breast cancer patients (including patients who deceased or had distant metastases within 2 years after primary breast cancer (PBC) diagnosis) opting for contralateral risk-reducing mastectomy (CRRM) versus not opting for risk-reducing mastectomy (Surveillance), using the Simon and Makuch method – which takes into account the change in an individual's covariate status over time – with years after PBC diagnosis as the time variable.

opted for CRRM (median follow-up period ~9 years), 10-year overall survival was 89% in the CRRM group and 71% in the non-CRRM group. Due to differences in study design, though, it is difficult to directly compare the results of this study with our findings. First, Evans and colleagues compared survival of patients opting for different risk-reducing strategies separately – that is CRRM only, RRSO only and both CRRM and RRSO – with survival of patients not undergoing any risk-reducing surgery. In our analyses, we compared overall survival of patients opting for CRRM with overall survival of patients who did not, and adjusted for RRSO, reflecting clinical practice. Second, to control for survival bias, Evans and colleagues used a matched case-control design (n=105 pairs), while we started the observation period at the date of DNA diagnosis for those patients who underwent genetic testing after PBC diagnosis. Finally, in the current study all women were free of CBC and/or ovarian cancer at the start of the observation, and were followed prospectively.

Table 2. Characteristics of Contralateral Breast Cancer patients and tumours

	N	*(after CRRM)*[1]	(%)[2]
	68	*(4)*	
Mutation status			
BRCA1	58	*(4)*	(85)
BRCA2	10		(15)
Age, median years (range)	48	*(52)*	(32-65)
Years after PBC diagnosis			
< 2	6		(9)
2-5	15		(22)
> 5	47	*(4)*	(69)
Median (range)	7.0	*(7.1)*	(0.6-29.1)
CBC tumour characteristics			
T-status[3]			
DCIS	11		(18)
T1[4]	4		(7)
T1a/b	18		(29)
T1c	20	*(3)*	(33)
T2+	8		(13)
unknown	7	*(1)*	
No. of positive lymph nodes			
0	50	*(2)*	(79)
1-3	11	*(1)*	(18)
≥ 4	2		(3)
unknown	5	*(1)*	
Receptor-status			
ER-positive	13		(27)
PR-positive	8		(17)
Her2-positive	1		(2)
Triple-negative	33	*(3)*	(73)
Follow-up after CBC diagnosis			
Median years (range)	5.2	*(7.6)*	(0.1-15.5)
Death[5]	16	*(0)*	(24)
Age, median years (range)	52		(41-71)
Years after CBC diagnosis, median (range)	5.2		(0.1-15.5)

CRRM, contralateral risk-reducing mastectomy; PBC, primary breast cancer; CBC, contralateral breast cancer; DCIS, ductal carcinoma in situ; ER, estrogen receptor; PR, progesterone receptor.
[1] All incident cases, detected after CRRM.
[2] Excluding missing values.
[3] Classification according to UICC International Union Against Cancer, 6th edition, 2002.
[4] Subgroup classification (i.e. T1a/b/c) not assessed or unknown.
[5] All causes.

Table 3. Efficacy of contralateral risk-reducing mastectomy on overall survival

Analysis[1]	Group	Person years of observation	Deaths	Mortality[2] (95% CI)	HR (95% CI)
(a)	Surveillance	3007	65	21.6 (16.9-27.6)	Ref.
	CRRM	1975	19	9.6 (6.1-15.1)	0.43 (0.26-0.72)[3]
					0.49 (0.29-0.82)[4]
(b)	Surveillance	2673	56	21.0 (16.1-27.2)	Ref.
	CRRM	1837	18	9.8 (6.2-15.5)	0.46 (0.27-0.79)[3]
					0.55 (0.32-0.95)[4]

CRRM, contralateral risk-reducing mastectomy; HR, Hazard ratio; CI, confidence interval.

[1] Analysis (a) is the main analysis with start of observation being either the date of primary breast cancer (PBC) diagnosis or the date of DNA diagnosis, whichever came first. In the additional analysis (b), the observation starts either 2 years after PBC or at the date of DNA diagnosis, whichever came first, to exclude patients who presented with distant meta stases or died within 2 years after PBC diagnosis (*n*=17).

[2] Per 1000 person years of observation.

[3] Univariate analysis.

[4] Multivariate analysis, adjusted for risk-reducing salpingo-oophorectomy. The following variables did not meet the criteria for incorporation in the multivariate Cox model as described in the Methods section, and were therefore not included in the multivariate analysis: type of mutation, year of birth, age at DNA diagnosis, age at PBC diagnosis, T-status, presence of positive lymph nodes, differentiation grade, hormone receptor status, HER2 status and treatments administered for PBC.

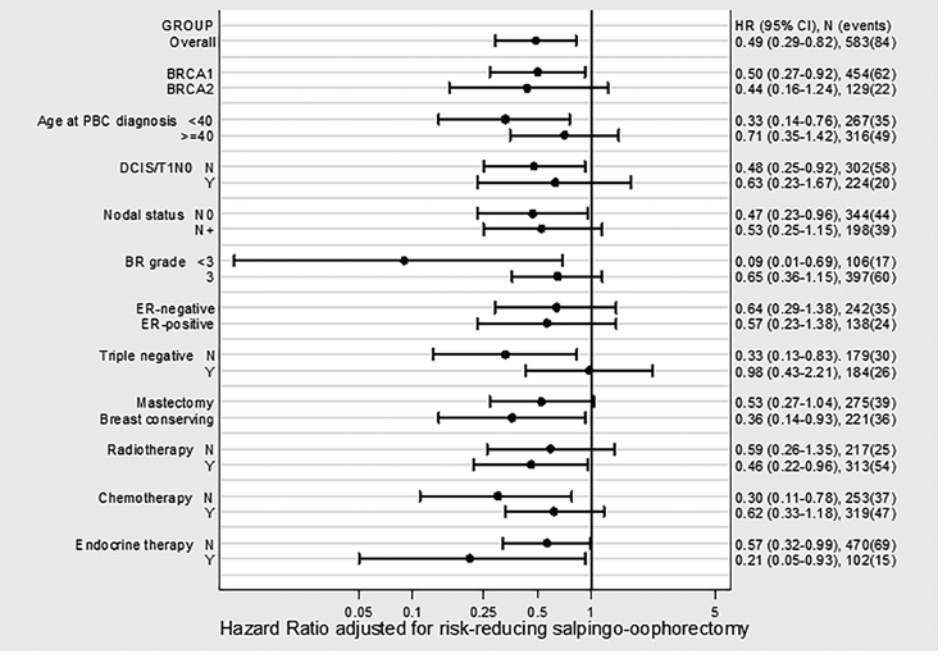

Figure 3

Forest plots of stratified Hazard ratios (HR; solid circles) and 95% confidence intervals (95% CI; whiskers on both sides of the solid circles) for the association between contralateral risk-reducing mastectomy and overall survival for separate risk groups. Hazard ratios were provided by using Cox analyses, with contralateral risk-reducing mastectomy as a time-dependent variable, and adjusted for risk-reducing salpingo-oophorectomy. N (events), number of patients in stratum (number of events in stratum).

The overall survival benefit observed in this study was neither reported in an earlier publication on BRCA-associated PBC patients[13] nor in our own preliminary single-centre data.[26] However, sample sizes were smaller (n=148 and n=375, respectively) and mean follow-up periods shorter (3.5 and 7.4 years, respectively) than in the current study. With a median follow-up of 11.4 years (range 1.0-32.5) after PBC diagnosis and 5.2 years (range 0.1-15.5) after CBC diagnosis, the differences between the present and previously reported results are mainly due to longer follow-up after BC diagnosis. This is also supported by the survival curves (Figure 2), which strongly diverge only after a period of about 10-11 years. Noteworthy, this observation underscores that the benefits of CRRM on survival require a long follow-up period, as has been mentioned before.[27]

In addition, we showed that exclusion of patients who died or had distant metastases within 2 years after PBC diagnosis decreased the mortality in especially the surveillance group. This indicates potential survival bias in the main analyses, since patients in the CRRM group survived long enough to undergo CRRM, while some patients in the surveillance group did not. Because of the incorporation of the adjustments as described, we regard our study design as a strong alternative for a randomized trial – which is clearly not feasible in this setting – for the estimation of mortality reduction associated with CRRM in BRCA-associated PBC patients.

Our findings regarding the significant reduction of CBC incidence after CRRM are in line with results reported in other publications, both in BRCA1/2 mutation carriers,[13] and in the sporadic BC population.[11,14,15] The observation that four patients in the mastectomy group (1.7%) still developed CBC is comparable to the 1.6% reported by van Sprundel et al.[13] These data emphasize that careful surgery aiming at removal of all mammary-gland tissue is important, as residual mammary-gland tissue in BRCA1/2 mutation carriers remains at high risk of BC development.

Of note, the risk of developing CBC is not the same for all PBC patients, and may depend on age at PBC diagnosis,[10,28] ER-status,[29] and given adjuvant systemic therapy.[10,30] Reassuringly, CBCs were mostly diagnosed at a more favourable stage than PBCs – that is more DCIS, smaller tumour size and more node-negative disease –, which may be the result of accurate surveillance after PBC. In this view, the observed survival benefit in the CRRM group cannot completely be explained by the prevention of CBC-associated deaths. However, despite a relatively lower CBC stage, survival of sporadic bilateral BC patients has been shown worse than survival of unilateral BC patients.[9,10] Whether the latter also applies for BRCA1/2 mutation carriers is not clear yet.

Greatest survival benefits after CRRM are expected in subgroups of patients at high risk of CBC and low risk of primary BC-specific mortality. This assumption is supported by our exploratory analyses showing that survival benefit after CRRM was especially seen in young PBC patients (<40 years), in patients having a PBC with differentiation grade 1/2 and/or no triple-negative phenotype, and in patients not treated with adjuvant chemotherapy. Further

research with larger samples and more events is warranted to develop a model based on age at diagnosis and tumour and treatment characteristics of the PBC that can predict survival benefit in more specific subgroups of patients.

We found no differences between the CRRM group and the surveillance group regarding PBC characteristics known as prognostic factors, which suggests similar primary BC-specific mortality risks for both groups at study inclusion. Therefore, other "unknown" prognostic factors or individual combinations of these factors, may account for that part of the survival benefit that cannot be explained by the prevention of CBC-associated deaths. Remarkably, patients in the CRRM group were treated more often with adjuvant chemotherapy, probably because of the younger age at PBC diagnosis. Also, a higher incidence of comorbidities in the surveillance group may have led to a lesser use of adjuvant chemotherapy in this group. Notably, neither adding age at PBC diagnosis nor adding treatment with adjuvant chemotherapy to the multivariate Cox model influenced the association of CRRM with overall survival. In addition, local therapy for PBC more often consisted of breast-conserving surgery and radiotherapy in the surveillance group. However, because previous studies showed that survival after breast-conserving therapy or mastectomy was similar, for both BRCA-associated,[31] and sporadic BC patients,[32] we may assume that differences in local therapy for PBC between the surveillance and CRRM group did not influence the current overall survival analyses.

More patients in the CRRM group also underwent RRSO, which is in line with previously reported data indicating that BRCA1/2 mutation carriers choosing for risk-reducing mastectomy often opt for all available risk-reducing surgeries.[33,34] We observed no significant difference in ovarian cancer incidence rates between the groups. Previously, Domchek et al. reported both improved overall survival and improved BC-specific survival after RRSO in BRCA-associated BC patients.[35] In our current analyses, we adjusted for RRSO by adding RRSO as a time-dependent covariate.

The strength of our study concerns the prospective design with limited influences from selection bias and confounding variables. Further, the study included a sufficient sample size and adequate length of follow-up period for mortality risk-reduction estimates. We are aware, though, of some limitations. First, the current study lacks data on BC-specific mortality. However, since the median age at death was 51 years, and only 5% of the deceased patients (4/84) also had ovarian cancer [being 4 out of 65 (6%) of the deceased patients in the surveillance group], it seems likely that BC was the leading cause of death in this study cohort. Therefore, we expect that BC-specific mortality will not significantly differ from overall mortality. A second limitation concerns the small numbers of BRCA2 mutation carriers, so that analyzing the data separately for BRCA1 and BRCA2 mutation carriers was not possible. Finally, the 15-year overall survival rates in this study are possibly not representative for all BRCA-associated BC patients – both identified and unidentified – as the majority of the currently selected patients had to survive at least until their DNA test. Since patients who died without being identified as a BRCA mutation carrier – because they did not survive long

enough to undergo genetic testing – were not eligible for study inclusion, the survival rates in both groups of the current study may have been overestimated.

In summary, we found that CRRM is associated with improved overall survival in BRCA1/2 mutation carriers with a history of unilateral BC. Also, we confirmed that CRRM strongly reduces CBC incidence in BRCA1/2 mutation carriers. In the absence of randomized clinical trials, these prospective analyses add important information for both clinicians and mutation carriers with unilateral BC who face the difficult decision regarding CRRM. In view of our data, it seems reasonable to discuss CRRM with BRCA-associated PBC patients, taking into account specific patient and tumour features. Ideally, one should offer CRRM to PBC patients with a high CBC risk and a low risk of dying from PBC. A prediction model based on age at PBC diagnosis and tumour and treatment characteristics of the PBC, as mentioned before, will facilitate more personalized advice to BRCA-associated unilateral BC patients. This may avoid drastic and sometimes mutilating surgery in patients with unfavourable prognosis.

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Chapter 5

Lower mitotic activity index in BRCA1/2-associated breast cancers detected after risk-reducing salpingo-oophorectomy

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Abstract

Background

Risk-reducing salpingo-oophorectomy (RRSO) is associated with 50% reduction of BRCA1/2-associated breast cancer (BC) risk, possibly through decreased growth activity. In this pilot study, tumour characteristics and growth rates of BRCA1/2-associated primary BCs (PBCs) detected after RRSO were compared with those of PBCs originating without RRSO.

Methods

From a cohort of 271 women with BRCA1/2-associated screen detected BC we selected 20 patients with PBC detected ≥12 months after RRSO (RRSO group). Controls were 36 BRCA1/2 mutation carriers with PBC detected without RRSO (non-RRSO group) matched for age at diagnosis (±2.5 years) and for BRCA1 or BRCA2 mutation. Pathology samples were revised for histological subtype, tumour differentiation grade, mitotic activity index (MAI), estrogen receptor (ER), progesterone receptor (PR) and HER2 status. Tumour growth rates, expressed as tumour volume doubling times (DT), were calculated from revised magnetic resonance and mammographic images.

Results

Median age at PBC diagnosis was 52 years (range 35-67). PBCs after RRSO had lower MAIs (12 versus 22 mitotic counts/2mm, $P = 0.02$), were smaller (11 versus 17 mm, $P = 0.01$), and tend to be PR-positive more often than PBCs without RRSO (38% versus 13%, $P = 0.07$). Differentiation grade, ER and HER2 status were not different. Median DT was 124 days (range 89–193) in the RRSO group and 93 days (range 54–253) in the non-RRSO group ($P = 0.47$).

Conclusion

BC occurring after RRSO in BRCA mutation carriers features a lower MAI, suggesting a less aggressive biological phenotype. When confirmed in larger series, this may have consequences for BC screening protocols after RRSO.

Introduction

BRCA1/2 mutation carriers face increased lifetime risks by the age of 70 years of developing breast cancer (BC; 55–85%), contralateral breast cancer (CBC; 20–60%) and ovarian cancer (18–54% for BRCA1 and 3–23% for BRCA2 mutation carriers).[1-5] In view of the increased ovarian cancer risk, and the unavailability of an adequate screening tool, the majority of BRCA1/2 mutation carriers opt for risk-reducing salpingo-oophorectomy (RRSO), mostly before 50 years of age.[6,7] RRSO significantly reduces the risk of ovarian/fallopian tube cancer by more than 95%,[6,8,9] while it is also associated with a primary BC (PBC) risk-reduction of about 50%, being most pronounced when performed at premenopausal age.[7,10]

BRCA1/2-associated BCs are often diagnosed at young age and are more often poorly differentiated than sporadic BCs (grade 3 in 50–75% versus 35%, respectively).[11,12] The BRCA1 BC phenotype is mainly estrogen receptor (ER) and progesterone receptor (PR) negative, and does not express HER2, resulting in approximately 60% of the BCs being triple negative.[12] The BRCA2 BC phenotype is quite similar to sporadic BCs regarding ER, PR and HER2 status.[12] Furthermore, shorter tumour volume doubling times (DT), expressing faster tumour growth, have been described for both BRCA1- and BRCA2-associated tumours as compared with non-BRCA1/2-associated tumours in patients of similar age.[13] At increasing age, BRCA1/2-associated tumours have longer DTs,[13] a more favourable differentiation grade and are more often ER positive possibly due to changes in ovarian hormone production.[12,14,15] In view of the mentioned observations and the reduced BC risk after RRSO, we hypothesized that PBCs developing after RRSO-induced menopause might show altered characteristics and decreased tumour growth. The latter is also an observation at our institute, although an earlier study on tumour growth did not find a correlation of menopausal status with tumour growth.[13] To our knowledge, no detailed data are available on this topic. The finding of a lowered growth rate of BCs occurring after RRSO might have consequences for BC screening protocols for the subgroup of BRCA1/2 mutation carriers who underwent RRSO.

We performed a pilot study in a matched cohort of BRCA mutation carriers, and compared tumour characteristics and tumour growth rates of PBCs developing after RRSO with PBCs originating without RRSO.

Methods

Patients

Since the start of the Rotterdam Family Cancer Clinic (FCC, approximately 1991), women at increased risk of hereditary breast and/or ovarian cancer are prospectively followed. From this cohort, we identified BRCA1/2 mutation carriers with a PBC detected at least 12 months after RRSO (RRSO group, cases). Patients were matched for age at PBC (±2.5 years) and type of mutation (BRCA1 or BRCA2), with an intended ratio of 1:2, to obligate or proven BRCA1/2

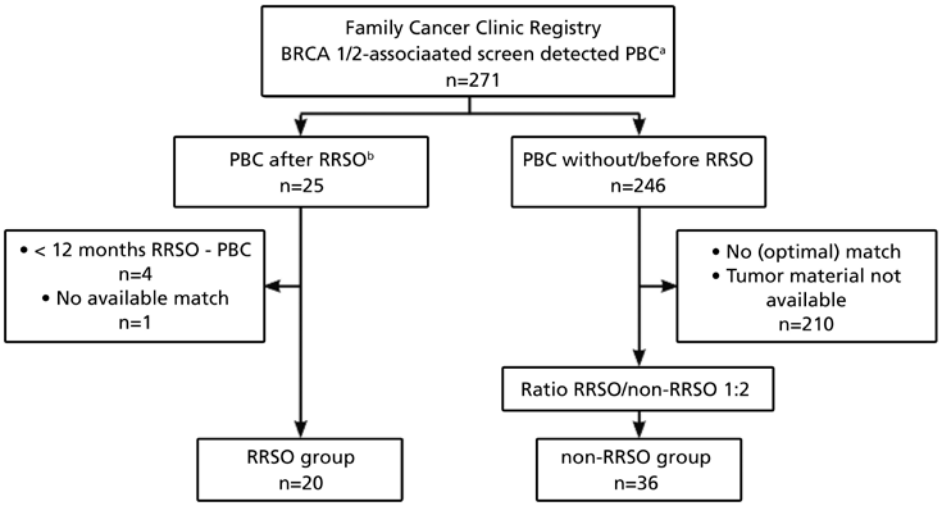

Figure 1. Patient inclusion from the Rotterdam Family Cancer Clinic.
[a]PBC, primary breast cancer; [b]RRSO, risk-reducing salpingo-oophorectomy.

mutation carriers with a PBC developing without RRSO (non-RRSO group, controls) (Figure 1).

Further eligibility criteria included (a) PBC detected at screening or presenting as interval carcinoma between two screening examinations (previous examination within 2 years before diagnosis), and (b) availability of tumour material for pathology revision. Exclusion criteria were risk-reducing mastectomy prior to PBC, neo-adjuvant chemotherapy and/or a history of ovarian cancer. Detailed data on hormonal status and reproductive factors including menarche, number of pregnancies and childbearing, breast feeding, use of oral contraceptives and age at RRSO and/or menopause were collected from medical records.

Written informed consent was obtained according to research protocols approved by the Medical Ethical Committee.

Radiological tumour measurements and growth rate assessment

Radiological images of serial screening examinations (magnetic resonance imaging [MRI] or mammography; previous and at PBC diagnosis) of selected patients were collected. Eligibility criteria for this research question included (a) invasive carcinoma and (b) the availability of at least two well interpretable imaging examinations of the same screening modality (MRI, preferably, or mammography), one made at diagnosis and one within two years prior to PBC diagnosis.

Images were revised by a breast radiologist (IMA Obdeijn) being unaware of RRSO status of the patients, regarding visibility of the lesion and perpendicular tumour diameters. If the tumour was visible on ≥2 comparable, consecutive examinations, the first and the last examination were used for tumour volume calculations. If the tumour was clearly visible

on MRI, three perpendicular tumour diameters were measured. On mammography, three perpendicular diameters were measured if possible, but if only two diameters could be measured, the smaller of the two diameters was used as third diameter. Tumour volumes were calculated by using a formula for obloid spheroids: $V = {}^{4}/_{3}\ \pi * {}^{1}/_{2}{}^{a} * {}^{1}/_{2}{}^{b} * {}^{1}/_{2}{}^{c}$.[13,16] Because of the assumed exponential growth pattern of small tumours,17 an exponential formula was used to calculate tumour volume doubling time: $DT = \frac{\ln 2}{\beta}$ with ß being the slope of the straight line between the logarithms of the tumour volumes versus time.[13,16] If the tumour was only visible on MRI or mammography at diagnosis, the tumour volume of the preceding examination was set corresponding with the assumed lower detection limit of that imaging examination, being 2 mm for MRI, corresponding with a volume of 0.004 cm^3, and 4 mm for mammography, corresponding with a volume of 0.033 cm^3.[13]

Histological tumour characteristics

Pathology slides were revised by a breast pathologist (CHM van Deurzen) unaware of the RRSO status of patients. Items scored concerned: tumour subtype according to the World Health Organization classification, grade according to the modified Bloom & Richardson score (based on tubule formation, nuclear pleomorphism and Mitotic Activity Index [MAI]),[18] and ER, PR and HER2 status. For categorization of MAI, thresholds of the modified Bloom & Richardson grade were used resulting in three categories (low 0–7 mitoses/2 mm^2, moderate 8–12 mitoses/2 mm^2 and high ≥13 mitoses/2 mm^2).[19] For ER and PR, histoscores (H-scores) were calculated as the sum of the percentages of immunoreactive staining of tumour cells, multiplied by ordinal values corresponding to the intensity levels of the staining: H-score (0–300) = % weakly immunoreactive cells x 1 + % moderately immunoreactive cells x 2 + % intensely immunoreactive cells x 3. An H-score of ≥10 was considered positive, since 10% of immunoreactive staining of tumour cells, independent of intensity, is the cut-off point for ER/PR positivity according to Dutch national guidelines.[19] Patients with carcinoma in situ without an invasive component were also included. Data on tumour size and nodal status were obtained from the database and/or pathology reports.

Statistical analysis

Differences between the RRSO and non-RRSO groups were tested by using Chi-square and Fisher's exact tests for categorical variables, and by using Mann-Whitney U tests for continuous variables. The SPSS computer package (version 20.0) was used for statistical analyses.

Results

From a cohort of 271 patients with screen detected BRCA1/2-associated BC retrieved from the FCC database 21 female BRCA1/2 mutation carriers were identified with a PBC detected

at least 12 months after RRSO. One woman with BC after RRSO was excluded from further analysis because no match was available (Figure 1). Of 246 proven or obligate mutation carriers with BC without RRSO, 36 appropriate matches (including two obligate mutation carriers) were found for the non-RRSO group (Figure 1). For four RRSO women only one appropriate match was found.

Patient characteristics and demographics are listed in Table 1. As year of diagnosis was not a matching criterion, median year of PBC diagnosis in the RRSO group was 2009 versus 2001 in the non-RRSO group (P = 0.001). RRSO was performed at a median age of 50 years, and four women (20%) were postmenopausal at RRSO. Nine women (45%) of this group had used hormone replacement therapy (HRT) between RRSO and PBC diagnosis. In the non-RRSO group, 18 women (50%) were postmenopausal at PBC diagnosis, none of them having used HRT. More PBCs were detected by MRI in the RRSO group (14 out of 20, 70% by MRI) than in the non-RRSO-group (8 out of 36, 22% by MRI, P = 0.001) as compared to mammography (Table 1). Both groups were comparable regarding age at PBC (due to matching), parity and other hormonal factors.

Radiological tumour measurements and growth analysis of invasive carcinomas

Tumour volume doubling times (DTs) of invasive BCs, as an expression of tumour growth rate, could be calculated for 12 of 17 tumours (71%) in the RRSO group and for 18 of 34 tumours (53%) in the non-RRSO group (Table 2). In total, 13 tumours (43%) were only visible on the imaging examination at diagnosis (5 on MRI, 8 on mammography), concerning 10 patients of the non-RRSO group. Twelve tumours (40%) were on revision visible on two consecutive examinations, and 5 tumours (17%) were visible on three or more consecutive examinations performed over a time period of 0.5–3.5 years (4 in the RRSO group, 1 in the non-RRSO group). Median DT of the PBCs was 124 days (interquartile range [IQR] 89–193) in the RRSO group and 93 days (IQR 54–253) in the non-RRSO group (P = 0.47) (Figure 2; Table 2).

Histological tumour characteristics

Tumour characteristics are presented in Table 3. The RRSO group comprised three cases of DCIS (two BRCA1, one BRCA2) and 17 invasive PBCs (15 BRCA1, two BRCA2), concerning 15 ductal carcinomas, one lobular and one metaplastic carcinoma. The non-RRSO group comprised two cases of DCIS (one BRCA1, one BRCA2) and 34 invasive ductal carcinomas (30 BRCA1, four BRCA2), including one with metaplastic characteristics. Median tumour size of the invasive PBCs was 10.0 mm (IQR 6.5-16.0) in the RRSO group, versus 17.0 mm (IQR 10.0-25.0) in the non-RRSO group (P = 0.01). The majority of invasive PBCs in both groups was node negative (15/17 in the RRSO group and 25/34 in the non-RRSO group, P = 0.30).

MAI of the PBCs was significantly lower in the RRSO group than in the non-RRSO group, with a median of 12 mitoses/2mm^2 (IQR 1-20) and 22 mitoses/2mm^2 (IQR 14-28.5), respectively (P = 0.02). No differences were found in the amount of tubule formations,

Table 1. Patient characteristics

	RRSO group		Non-RRSO group		*P* value
No. of women (*N*)	20		36		
Mutation status					
BRCA1	17	85%	31	86%	1.0
BRCA2	3	15%	5	14%	
Age PBC (median, IQR), years	52.0	46.0–62.5	50.0	46.0–57.0	0.44
Year PBC diagnosis (median, IQR)	2009	2005–2011	2001	1996–2006	0.001
Screening method that detected PBC	74	(51)	26	(29)	
MRI	14	70%	8	22%	0.001
Mammography	6	30%	28	78%	
Menopausal status at PBC					
Pre-	0	0%	17	49%	
Post-	20	100%	18	51%	
Unknown	0		1		
Age RRSO (median, IQR), years	50.0	41.0–55.0	-		
Menopausal status at RRSO					
Pre-	14	78%	-		
Post-	4	22%	-		
Unknown	2		-		
Months RRSO-PBC (median, IQR)	19.0	14.5–118.0	-		
Hormone replacement therapy (HRT)[a]					
Yes	8	45%	0	0%	0.003
No	12	55%	18	100%	
Age menarche (median, IQR), years	14.0	12.5–15.5	14.0	12.0–14.0	0.93
Oral contraceptive use					
Yes	15	88%	29	97%	0.54
No	2	12%	1	3%	
Unknown	3		6		
Years of oral contraceptive use (median, IQR)	18.0	9.5–22.0	9.0	4.0–18.0	0.42
Parity (mean, standard deviation)	1.9	1.0	1.6	1.1	0.43
Nulliparity					
Yes	3	15%	8	26%	0.49
No	17	85%	23	74%	
Unknown	-		5		
Age at 1st child (median, IQR), years	26.0	21.5–28.0	25.5	24.0–30.0	0.61
Breastfeeding					
Yes	6	40%	17	57%	0.35
No	9	60%	13	43%	
Unknown	5		6		
Months breastfeeding (median, IQR)	4.0	0.5–9.5	6.0	3.0–8.0	0.51

Abbreviations: RRSO, risk-reducing salpingo-oophorectomy; PBC, primary breast cancer; IQR, interquartile range.
[a] Hormone replacement therapy after RRSO (RRSO) group or menopause (Non-RRSO group).

nuclear pleomorphism, overall Bloom & Richardson grade, ER status, or HER2 status. The proportion of PR positive PBCs (PR H-score ≥10) was higher in the RRSO group than in the non-RRSO group (38% versus 13%) without reaching statistical significance (P = 0.07), while median PR H-score was significantly higher in the RRSO than in the non-RRSO group (3 versus 0, *P* = 0.05). As a consequence, the percentage of triple negative PBCs was lower in the RRSO group than in the non-RRSO group (47% versus 68%) without reaching statistical significance (*P* = 0.21).

Table 2. Radiological tumour growth analysis of invasive carcinomas

	RRSO group		Non-RRSO group		*P* value
Invasive carcinomas (*N*)	17		34		
Eligible for growth rate analysis	12	71%	18	53%	
BRCA1	11		17		
MRI screening	9	82%	7	41%	0.05[a]
Mammography screening	2	18%	10	59%	
BRCA2	1		1		
MRI screening	1	100%	0	0%	
Mammography screening	0	0%	1	100%	
Tumour on revision visible on					
1 examination[b]	3	25%	10	56%	0.164[a]
2 examinations	5	42%	7	39%	
≥ 3 examinations	4	33%	1	5%	
Time between two screening examinations (median, IQR), days	344	243–433	397	286–686	0.212[c]
Time between examinations used for tumour growth calculation (median, IQR), days	427	201–586	400	341–686	0.719[c]
Tumour volume doubling time (DT) (median IQR), days	124	89–193	93	54–253	0.472[c]

Abbreviations: RRSO, risk-reducing salpingo-oophorectomy; MRI, magnetic resonance imaging; IQR, interquartile range. All percentages are of invasive carcinomas.

[a] Fisher's Exact Test.

[b] Tumour growth is calculated combined with one baseline examination with no visible tumour (baseline tumour volume 0.004 cm^3 (MRI) or 0.033 cm^3 (mammography).

[c] Mann-Whitney U Test.

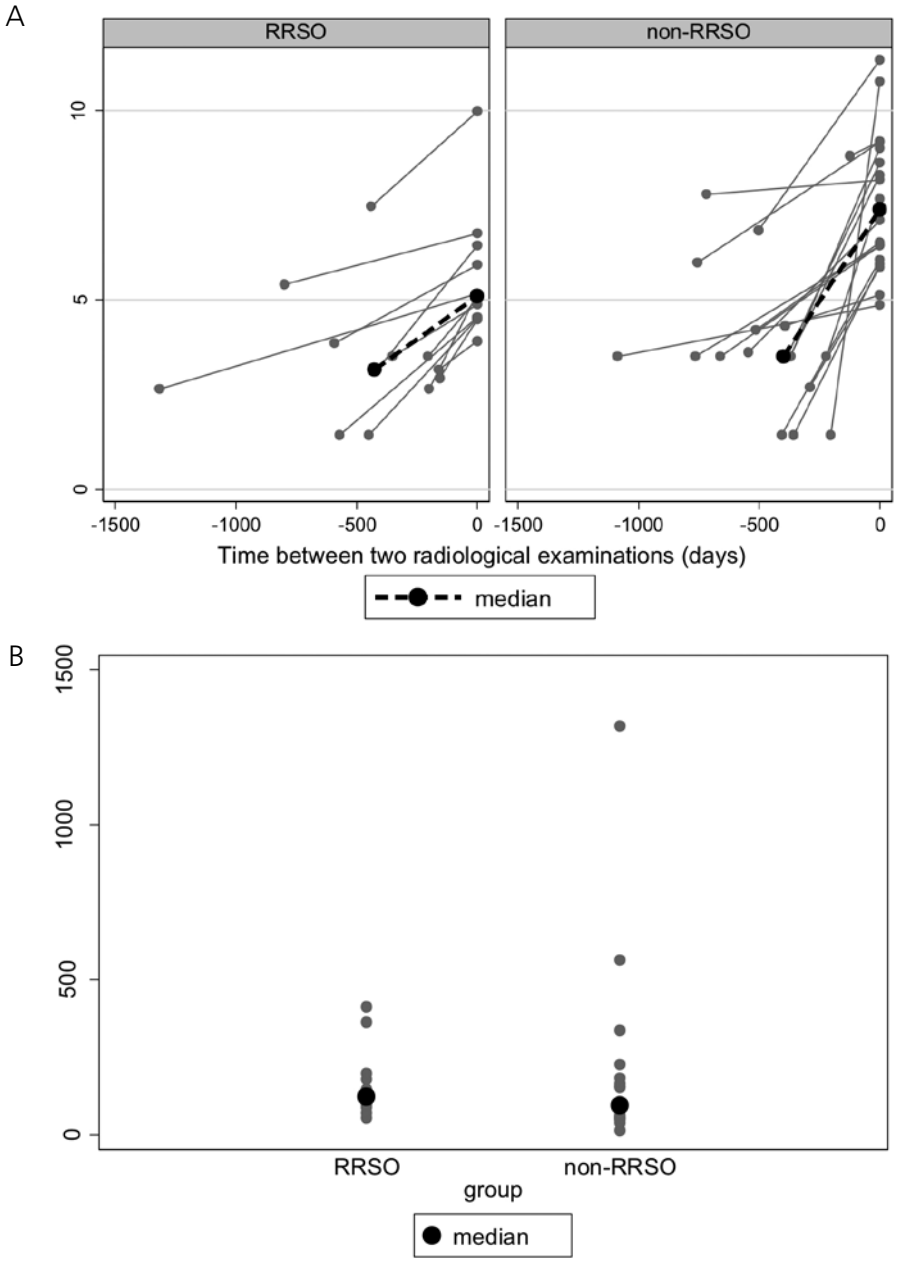

Figure 2. (**A**) Tumour volumes over time and (**B**) tumour volume doubling times (DT s) for primary breast cancers occurring after risk-reducing salpingo-oophorectomy (RRSO) and without RRSO (non-RRSO). ln, natural logarithm; tumour volumes: $V = {}^{4}/_{3}\ \pi * {}^{1}/_{2}{}^{a} * {}^{1}/_{2}{}^{b} * {}^{1}/_{2}{}^{c}$, where a, b and c are perpendicular tumour diameters on MRI or mammography; tumour volume doubling time (DT): DT = (ln2)/ß; ß = slope between natural logarithms of tumour volumes.

Table 3. Histological tumour characteristics

	RRSO group		Non-RRSO group		*P* value
N	20		36		
DCIS	3	15%	2	6%	0.34[a]
Invasive carcinoma	17	85%	34	94%	
T status[b]					
T1a/b	9	53%	10	30%	0.16[c]
T1c	6	35%	13	38%	
T2	2	12%	11	32%	
Size, mm (median, IQR)[b]	11.0	6.0–17.0	17.0	10.0–25.0	0.01[d]
N status[b]					
Negative	15	88%	27	74%	0.29[a]
Positive	2	12%	9	26%	
Tubule formation[b]					
> 75%	0	0%	1	3%	0.74[c]
10–75%	2	12%	3	9%	
< 10%	14	88%	28	88%	
Unknown	1		2		
Nuclear pleomorphism[b]					
Minimal	0	0%	0	0%	
Moderate	6	38%	8	25%	0.50[a]
Extensive	10	62%	24	75%	
Unknown	1		2		
Mitotic count/2 mm^2 (median, IQR)	12	1–20	22	14–29	0.02[d]
Mitotic activity index[b] (mitoses/2 mm^2)					
0–7	6	38%	6	19%	0.008[c]
8–12	3	19%	0	0%	
≥ 13	7	43%	26	81%	
Unknown	1		2		
Bloom and Richardson grade[b]					
1	1	6%	0	0%	0.16[c]
2	6	38%	7	22%	
3	9	56%	25	78%	
Unknown	1		2		
Lymphovascular invasion[b]					
Yes	1	7%	6	19%	0.40[a]
No	14	93%	26	81%	
Unknown	2		2		
ER H-score[b] (median,IQR)	0	0–270	1	0–41	0.63[d]
Positive (H-score ≥ 10)	7	47%	9	29%	0.33[a]
Negative (H-score < 10)	8	53%	22	71%	

	RRSO group		Non-RRSO group		*P* value
Unknown	2		3		
PR H-score[b] (median,IQR)	3	0–150	0	0–1	0.05[d]
Positive (H-score ≥ 10)	6	38%	4	13%	0.07[a]
Negative (H-score < 10)	10	62%	27	87%	
Unknown	1		3		
HER2 status[b]					
Positive	1	6%	0	0%	0.36[a]
Negative	15	94%	28	100%	
Unknown	1		6		
Triple-negative[b,e]					
Yes	7	47%	19	68%	0.21[a]
No	8	53%	9	32%	
Unknown	2		6		

Abbreviations: RRSO, risk-reducing salpingo-oophorectomy; DCIS, ductal carcinoma in situ; IQR, interquartile range; ER, estrogen receptor; H-score, histoscore (0-300) = % weakly immunoreactive cells x 1 + % moderately immunoreactive cells x 2 + % intensely immunoreactive cells x 3; PR, progesterone receptor.
[a] Fisher's Exact Test.
[b] Invasive carcinomas only.
[c] Chi-square test.
[d] Mann-Whitney U Test.
[e] Negative status for ER, PR and HER2.

Discussion

In this pilot study in an age-matched cohort consisting of BRCA1/2-associated BC patients, PBCs occurring after RRSO were featured by significantly lower mitotic counts, a trend for more PR positivity, and (non-significantly) more often ER positivity when compared with PBCs without RRSO. Tumour volume doubling time (DT) was non-significantly longer in the RRSO group. To our knowledge, this is the first report comparing tumour characteristics and growth patterns of PBCs occurring after RRSO to those without RRSO.

The significantly lower mitotic count in PBCs occurring after RRSO when compared with PBCs without RRSO suggests that estrogen depletion induced by RRSO decreases cell proliferation. As the majority of PBCs in this study was ER negative, the mechanism behind this observation remains unclear. Various authors confirm that the development of BRCA1-associated triple negative BCs is susceptible to estrogen depletion or inhibition as achieved by RRSO or tamoxifen.[20-22] It has been hypothesized that the explanation lies in high ER expression of early stages of triple negative BC genesis.[23,24] Estrogens may facilitate BRCA1-mutant cell proliferation and tumour development in premalignant mammary tissue until ER expression extinguishes in later stages, possibly after the loss of transcriptional ER-activation by the second BRCA1 allele.[25] By this mechanism, estrogen depletion by RRSO may inhibit tumour development of triple negative BC in a very early stage. Furthermore, there is some evidence suggesting that in a later stage of tumour development estrogen may induce changes even in ER-negative BCs by affecting the microenvironment of the tumour.[26]

Interestingly, a recent study found that also RRSO performed after natural menopause was associated with BC risk-reduction. The authors suggest that androgens, being produced by the ovaries after menopause, may affect cell proliferation either directly or indirectly through the aromatization to estrogens,[27] and possibly play a role in the risk-reduction of hormone receptor negative breast cancer.

As 85% of the study patients were BRCA1 mutation carriers, our findings are majorly driven by BRCA1. BRCA1-associated BCs are known to have higher mitotic counts than BRCA2-associated and sporadic BCs,[28] possibly because proteins associated with normally functioning BRCA1 genes inhibit cell proliferation.[29,30] Separate analyses of BRCA1 carriers alone revealed comparable results as for the overall group (data not shown). To our knowledge, reduced cell proliferation in BRCA1-associated BCs after RRSO or menopause has not been described so far. Although tubule formation and nuclear pleomorphism, two other components of the Bloom & Richardson grade scoring system, and overall differentiation grade were not significantly different in PBCs after versus without RRSO, there is evidence that MAI is the most important prognostic factor in early stage BCs.[31,32] The finding of lower MAI in PBCs after RRSO therefore suggests a less aggressive biological growth pattern of this subgroup.

Still, 43% of the tumours in the RRSO group had high mitotic counts (≥13 mitoses/2 mm^2). An explanation may be that the time period of 12 months between RRSO and BC diagnosis considered in this study was relatively short, and that some tumours already had developed before RRSO. Of interest, PBCs with a high MAI were detected at a median of 24 months after RRSO, while this was 69 months for tumours with a lower MAI (0-12 mitoses/2 mm^2; data not shown). This supports previous data suggesting that the maximum level of risk-reduction by RRSO is effective more than 12 months post-RRSO, although some risk-reducing effect is already present one year after RRSO.[7]

We observed a trend for more PR positivity in the RRSO group, but without significant difference in ER status (Table 3). Consequently, fewer tumours in the RRSO group (47%) were triple negative, as compared to 68% in the non-RRSO group. The latter percentage is in accordance with data from the literature for BRCA1-associated BC,[12] and mirrors the fact that the majority of our patients were BRCA1 mutation carriers. Earlier studies found that the proportion of ER and PR positive tumours in BRCA1-associated BC increases with increasing age at diagnosis, but is still lower than the percentage of ER-positivity in sporadic tumours irrespective of age.[12,14,15] In these studies however, menopausal status and history of RRSO were not taken into account. As patients in the current study were matched for age, the increased expression of PR in PBCs in the RRSO group, in our opinion, suggests transcriptional activation by ER and therefore can be a sign of increasing ER-functionality.[33-35] Therefore, in a larger series we expect not only increase of PR positivity, but also of ER positivity in PBCs after RRSO. To our knowledge, only one study reported on BC characteristics after RRSO,[36] but due to a different study design and patient cohort, the outcomes of both studies are not comparable.

Tumour size at surgery as reported in pathology reports was significantly smaller in

the RRSO group than in the non-RRSO group. This is most likely a reflection of the differences in screening regimens between the two groups. In the RRSO group all women knew their BRCA mutation status prior to RRSO and consequently were screened by means of annual MRI and mammography, according to Dutch guidelines. The non-RRSO group was more heterogeneous with respect to radiological screening, since 19 of the 36 women had not been genetically tested until PBC diagnosis. First, time intervals between screening examinations were longer in the non-RRSO group. Second, due to our matching criteria and evolving approaches over time regarding RRSO, year of diagnosis ranged from 1999–2012 in the RRSO group and from 1987–2011 in the non-RRSO group with consequently varying quality of radiological screening examinations. These differences between the two groups probably resulted in earlier detection and in smaller tumour sizes at diagnosis in the RRSO group (Table 2). In smaller tumours, mitotic counts may be lower, as has been reported for screen-detected sporadic BCs.[37] Therefore, the reduced mitotic activity we found in BCs developing after RRSO may partly have been a consequence of the smaller tumour size in this group.

Median tumour volume DT was longer in the RRSO group than in the non-RRSO group, but this difference was not statistically significant. As pointed out before, in the RRSO group women were more often screened with MRI, resulting in more precise tumour volume assessment. Because of the better imaging quality of MRI over mammography and of digital mammography in recent years as compared to previous analogue mammography, tumours in the RRSO-group may have been longer visible in retrospect, resulting in lower DTs, suggesting slower growth. Further, in both groups large (interquartile) ranges for DTs were found (Figure 2), suggesting that the formula used for DT was imprecise. Possibly the assumptions of presumed obloid tumour shape and the exponential growth are a too simplified approach of real tumour volume and growth. In combination with small groups, this might be the reason no statistical significant DT difference was found.

Unfortunately, due to the small numbers it was not possible to take menopausal status and HRT use into account regarding differences in histological characteristics and tumour volume DT. Of the non-RRSO group, 51% was naturally postmenopausal at PBC diagnosis and growth in these tumours may already have been restrained due to declined or absent production of ovarian hormones. Moreover, 45% of women in the RRSO group used HRT before PBC. Based on our hypothesis of tumour growth stimulation by estrogens, we expect that the differences between the groups in MAI, ER, and PR status and DT will increase when comparing HRT-naïve patients in the RRSO group with premenopausal PBC patients in the non-RRSO group.

Strikingly, in five patients the tumour was visible on three or more screening examinations over a time period of 0.5–3.5 years before BC diagnosis. All women were BRCA1 mutation carriers and screened by MRI, while four of them had undergone RRSO. In all five cases the lesion was noticed earlier, but classified as "probably benign", while additional ultrasonography showed no signs of malignancy. Our observations support the

fact that radiologists must be aware that in BRCA1 mutation carriers, and especially after RRSO, BC can present during screening as small benign looking lesions.

An important strength of our pilot study concerns the matched design, chosen to adjust for age at PBC and type of mutation (BRCA1 or BRCA2). Furthermore, pathology samples were revised by a breast pathologist, and all imaging examinations were revised by a breast radiologist, both unaware of RRSO status and therefore not biased regarding results. However, we are aware of some relevant limitations. First, only 20 patients were eligible for the RRSO group due to the relatively low number of PBCs detected after RRSO. Women who consult our cancer centre nowadays are encouraged to undergo RRSO as of 40 years of age, and some women already have suffered from BC by that time. Strict inclusion criteria and the matched design restricted further enlargement of the non-RRSO group. While some women in the RRSO group were relatively old at the time of PBC diagnosis (> 60 years), only few BRCA mutation carriers were identified with a first BC occurring at older age without prior RRSO. Groups were too small to perform multivariable analysis to correct for other variables possibly of influence on tumour biology, such as HRT use, menopausal status and tumour size at detection. Second, the group consisted mostly of BRCA1 mutation carriers, as this is most frequently seen in the Netherlands. The number of BRCA2 mutation carriers was too small to perform a subgroup analysis.

In conclusion, the lower MAI and the increased proportion of PR positive BRCA1/2-associated BCs developing after RRSO suggest a less aggressive biological phenotype compared with PBCs occurring without RRSO. This was not confirmed by significantly longer DTs, probably due to small numbers. Our findings in BRCA1/2-associated PBCs occurring after RRSO are the first of this kind, but confirmation is warranted in larger sample sizes, since these findings may have consequences for less intensive breast cancer screening protocols after RRSO in mutation carriers, with possibly less outpatient clinic visits, less distress for the patient, and lower costs.

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Chapter 6

Breast cancer risk after salpingo-oophorectomy in healthy BRCA1/2 mutation carriers: revisiting the evidence for risk-reduction

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Submitted

Abstract

Background

Previous studies have reported a breast cancer (BC) risk-reduction of approximately 50% after risk-reducing salpingo-oophorectomy (RRSO) in BRCA1/2 mutation carriers, but may have been subject to several types of bias. Purpose of this nationwide cohort study was to assess potential bias in the estimated BC risk-reduction after RRSO.

Methods

We selected BRCA1/2 mutation carriers from an ongoing nationwide cohort study on HEreditary Breast and Ovarian cancer in the Netherlands (HEBON). First, we replicated the analytical methods as previously applied in four major studies on BC risk after RRSO. Secondly, we analyzed the data in a revised design in order to further minimize bias. The most important differences between our approach and those of previous studies were the requirement of no history of cancer at the date of DNA diagnosis and the inclusion of person-time preceding RRSO.

Results

For replication of the analytical methods used in four previous studies, we used the data of 551 to 934 BRCA1/2 mutation carriers with a median FU of 2.7 to 4.6 years (versus 426 to 3305 participants and a median FU of 2.3 to 4.7 in the published studies). We found similar protective effects of RRSO on BC risk as published in the previous studies, with hazard ratios (HRs) of 0.36, 0.49, and 0.62 versus published HRs of 0.36, 0.54, and 0.53, and an odds ratio (OR) of 0.61 versus a published OR of 0.46. For the second analysis with start of FU at the date of DNA testing, we selected 822 BRCA1/2 mutation carriers. After a median FU period of 3.2 years, the adjusted Cox analysis with RRSO as a time-dependent variable yielded an HR of 1.07 (95% CI, 0.66-1.73).

Conclusions

In previous studies, BC risk-reduction after RRSO in BRCA1/2 mutation carriers may have been overestimated due to bias. With the current design, a protective effect does not seem to occur.

Introduction

Women with a BRCA1 or BRCA2 mutation have substantially higher risks of developing primary and contralateral breast cancer (BC) and ovarian cancer than women from the general population.[1-5] Options to reduce these increased cancer risks include risk-reducing mastectomy (RRM) and/or risk-reducing salpingo-oophorectomy (RRSO). The uptake of the latter intervention is high (up to 75%) among BRCA1/2 mutation carriers,[6-11] especially since gynaecological screening does not contribute to early ovarian cancer detection.[12-14] Apart from reduction of ovarian cancer risk,15 RRSO has also been associated with a BC risk-reduction, estimated to be 46% to 64%.[16-22]

Accurate knowledge on the efficacy and side effects of risk-reducing interventions may help female BRCA1 and BRCA2 mutation carriers who consider these strategies. However, as health effects of RRSO will not be investigated in a randomized clinical trial, evaluations on the efficacy of this strategy are confined to observational studies. Consequently, risk estimates are more subject to potential biases. Being aware of this, the investigators of previous studies on BC risk after RRSO have taken several precautions to control for one or more types of bias, resulting in a variety of study designs and analyses.[16-22] Since in all studies RRSO was consistently associated with a BC risk-reduction of approximately 50% this observation has been widely communicated in counselling practice. Also, the estimated BC risk-reduction after RRSO has been included in a prediction model regarding BC risks in BRCA1/2 mutation carriers.[23]

In this paper, we revisit the association between RRSO and BC risk in BRCA1/2 mutation carriers, focusing on the impact of different analytical methods and potential types of bias. Additionally, we propose a revised analytical approach for observational studies in BRCA1/2 mutation carriers, in order to minimize bias as much as possible. Finally, we apply this approach in a Dutch cohort of healthy BRCA1/2 mutation carriers.

Theoretical background

Several methodological issues related to observational studies on the efficacy of risk-reducing surgery in female BRCA1/2 mutation carriers have previously been discussed by Klaren et al. and by Wacholder et al.[24,25] Since then four major studies addressing BC risk after RRSO in BRCA1/2 mutation carriers have been published, each using different designs and analytical methods (Table 1). In this section, we will discuss several types of selection bias and the possible consequences for the observed risk estimates in these four studies. Selection bias may occur if the association between the exposure (RRSO) and the event of interest (BC) differs for the participating subjects and for the eligible subjects, including those who do not participate.[26]

Cancer-induced testing bias

In studies on BC risk after RRSO, the main inclusion criterion for participants is carrying a

proven BRCA1 or BRCA2 mutation. Nowadays, women usually undergo DNA testing before RRSO, to avoid unnecessary surgery and removal of healthy ovaries and fallopian tubes. The comparison group without RRSO, however, includes women who proceeded to DNA testing because of a history of BC. Furthermore, the comparison group does not include the potential mutation carriers who remain unidentified as they are not affected with cancer and do not opt for risk-reducing surgery, and therefore do not consider genetic testing. The differential selection of identified mutation carriers in the RRSO group (with DNA test result before surgery) and the non-RRSO group (including women who proceed for DNA testing after BC diagnosis) is called *"cancer-induced testing bias"*, and the over-selection of BC cases in the non-RRSO group may result in an overestimation of BC risk-reduction after RRSO.[24]

Cancer-induced testing bias may have affected the estimates in three of the four discussed studies as patients who underwent DNA testing after BC or ovarian cancer diagnosis – so-called prevalent cases – were included in these studies (Table 1).[18,20,22] Cancer-induced testing bias can be avoided by starting the observation period at the date of the individual DNA test result. This has been applied by Kauff et al.[21], but only for the non-RRSO group. Women undergoing RRSO were followed from the date of RRSO.

Immortal person-time bias

Immortal person-time refers to the follow-up period that participants must have survived event-free to become eligible for the exposure. In cohort studies on BC risk after RRSO, BRCA1/2 mutation carriers are by definition BC-free before RRSO. *"Immortal person-time bias"* may occur if the person-time before surgery is not allocated to the non-RRSO group, thus resulting in less observation time in this group.[27,28] Consequently, BC incidence rates are spuriously increased in the non-RRSO group, leading to an overestimation of BC risk-reduction after RRSO. Immortal person-time bias might have played a role in both reviewed unmatched cohort studies[21,22] but not in the other two reviewed studies due to the matched designs.[18,20] In an unmatched study, this type of bias can be avoided by allocating the observation time between the date of DNA test result and the date of RRSO to the non-RRSO group.[24,27]

Informative censoring

A high familial background risk, for instance when several first degree female family members are diagnosed with BC at young ages, may be the motivation for opting for RRM.[29,30] When a censoring event (e.g. RRM) depends on the (presumed) risk for the study-endpoint (i.e. incidence of BC), censoring is called informative.[31] *"Informative censoring"* can lead to biased results if women with a high familial BC risk more often opt for RRM at younger age and will be censored before their RRSO. The remaining participants who will undergo RRSO, will be those with a lower familial BC risk. Consequently, BC risk-reduction may be incorrectly attributed to the RRSO, while it is in fact the result of censoring for RRM. Although informative censoring can hardly be avoided in cohort studies investigating BC risk after RRSO in BRCA1/2 mutation carriers, so far,

Table 1. Designs and results from the four discussed studies from literature

Author, year	Eisen, 2005[18]	Domchek, 2006[20]	Kauff, 2008[21]	Domchek, 2010[22]
Study design	Case-control	Matched cohort	Unmatched cohort	Unmatched cohort
FU	NA	Prospective	Prospective	Prospective
Start FU RRSO/ non-RRSO	NA	RRSO/Date RRSO of matched surgery subject	RRSO/Date of DNA diagnosis (age≥30)	RRSO/Date of entry into research program[1]
Machtiging	• Year of birth • Country of residence • Type of mutation (BRCA1/BRCA2)	• Age at RRSO	NA	NA
Eligibility criteria	• No history of OC • Both breast in situ • Control had at least no BC diagnosis until age at BC of matched case	• Cancer-free at enrolment2 • Cancer-free before RRSO • Matched controls cancer-free at time of participant's RRSO • Both breasts in situ • No cancer diagnosis within 6 months after enrolment[2]	• At least one ovary in situ at time of DNA testing • No history of bilateral BC or gynaecological cancer before DNA testing • No evidence of metastatic BC at time of DNA testing	• No prior OC diagnosis at time of study entry • Both ovaries and breasts in situ at time of study entry • No cancer diagnosis within 6 months of observation
RRM	Excluded	Excluded	Censored	Censored
Study endpoint	First BC	First BC	First BC or CBC	First BC
Control for bias	• Matching	• Cancer-free at enrolment[2] • Matching	• Start FU at DNA testing (exclusion of prevalent cases)	• Cancer-free at study entry
Potential bias	• Cancer-induced testing bias • Exclusion of participants ever undergoing RRM	• Cancer-induced testing bias • Exclusion of BC-free person-time before RRM	• Immortal person-time bias • Inclusion of patients with a personal history of BC at start FU[3]	• Cancer-induced testing bias • Immortal person-time bias
N RRSO/non-RRSO	166/3139	155/271	303/294	336/1034
FU RRSO/non-RRSO	NA	3.1/2.1[4]	2.5/2.1[5]	4.2/4.9[4]
Age at start FU RRSO/non-RRSO	NA	45(8.5)/43(10.0)[4]	46(32-79)/39(30-88)[5]	44(21-79)/36(18-90)[4]
Year of birth RRSO/ non-RRSO	NR	1955/1957[4]	NR	NR
% DNA testing after cancer diagnosis	NR	NR	0%	NR
Risk-reduction (95% CI)	OR 0.46 (0.32-0.65)	HR 0.36 (0.20-0.67)[6,7]	HR 0.53 (0.29-0.96)[8]	HR 0.54 (0.37-0.79)[6,9]

Abbreviations: RRSO, risk-reducing salpingo-oophorectomy; FU, follow-up; NA, not applicable; OC, ovarian cancer; BC, breast cancer; RRM, risk-reducing mastectomy; N, number; CBC, contralateral breast cancer; NR, not recorded; CI, confidence interval; OR, odds ratio; HR, hazard ratio.

[1] Study entry between 1974 and 2008
[2] Moment of enrolment not specified
[3] RRSO group, 47%; non-RRSO group, 37%
[4] Mean years (standard deviation)
[5] Median years (range)
[6] A robust variance-covariance estimation method was used to correct for non-independence of observations in women from the same family or centres
[7] Adjusted for type of mutation (BRCA1/BRCA2) and centre
[8] Adjusted for age at start of follow-up, parity, previous BC, prior hormone-replacement therapy, type of mutation (BRCA1/BRCA2)
[9] Adjusted for year of birth and stratified by centre

none of the previous studies addressed the potential bias that may have been introduced by this type of censoring. Still, after RRM women are clearly not at risk for BC anymore. Therefore, the two unmatched cohort studies censored for RRM[21,22], while the two matched studies completely excluded women who ever underwent RRM.[18,20] The latter alternative may be less preferable since in this way the BC-free person-years between the start of observation and the RRM are excluded from the analyses, which again may disturb risk-reduction estimates.

Confounding

Estimates of BC risk-reduction after RRSO can be confounded when the study endpoint does not distinguish between a first and a contralateral BC. While baseline risks for first and contralateral BC – estimated to be 45%-88% for first BC[1,4,32-35] and 52%-87% for contralateral BC[32,34,36,37] – may be similar, adjuvant systemic therapy after BC is known to reduce the contralateral BC risk.[37-39] If more women with a history of BC opt for RRSO, the BC risk in the surgery group may be reduced due to adjuvant systemic therapy, and the BC risk-reduction due to RRSO may be overestimated. In fact, in the study by Kauff et al. the percentage of participants with a history of BC was higher in the RRSO group (47%) than in the non-RRSO group (37%).[21] To control for confounding by previous systemic therapy, analyses on BC risk-reduction after RRSO should be restricted to healthy BRCA mutation carriers, as was done in the matched studies by Eisen et al. and Domchek et al. (Table 1).[18,20] Another option is to perform analyses separately for BRCA mutation carriers with and without a history of BC, as was done by Domchek et al. (Table 1).[22]

Proposed design and method of analysis

To minimize bias as much as possible in observational studies investigating the effect of RRSO on BRCA1/2-associated BC risk, we propose the following design and analyses. The observation period should start at the date of DNA test result, and at that date participants should be at risk for a first BC and be eligible for RRSO. Therefore, women with BC or ovarian cancer before DNA testing are ineligible, and participants should have both breasts and both ovaries in situ at the date of DNA test result. Further, person-time before surgery should be taken into account. The proposed design is illustrated in Figure 1.

Theory in practice

First, we replicated the analyses of the four discussed studies within a Dutch cohort, to examine if this cohort was comparable to the cohorts used in the previous studies. Second, we estimated the effect of RRSO on BC risk in the Dutch cohort using the proposed design and analyses.

Subjects and Methods

Study population

In the context of an ongoing nationwide study on hereditary breast and ovarian cancer in

the Netherlands (the HEBON study)[40], members of breast and/or ovarian cancer families tested for a BRCA mutation are being identified through the departments of Clinical Genetics/Family Cancer Clinics at all Dutch academic centres, the Netherlands Cancer Institute, and the Foundation for the detection of Hereditary Tumours (STOET). Data on patient and tumour characteristics and on preventive strategies were retrospectively as well as prospectively retrieved and updated through medical files and questionnaires, and

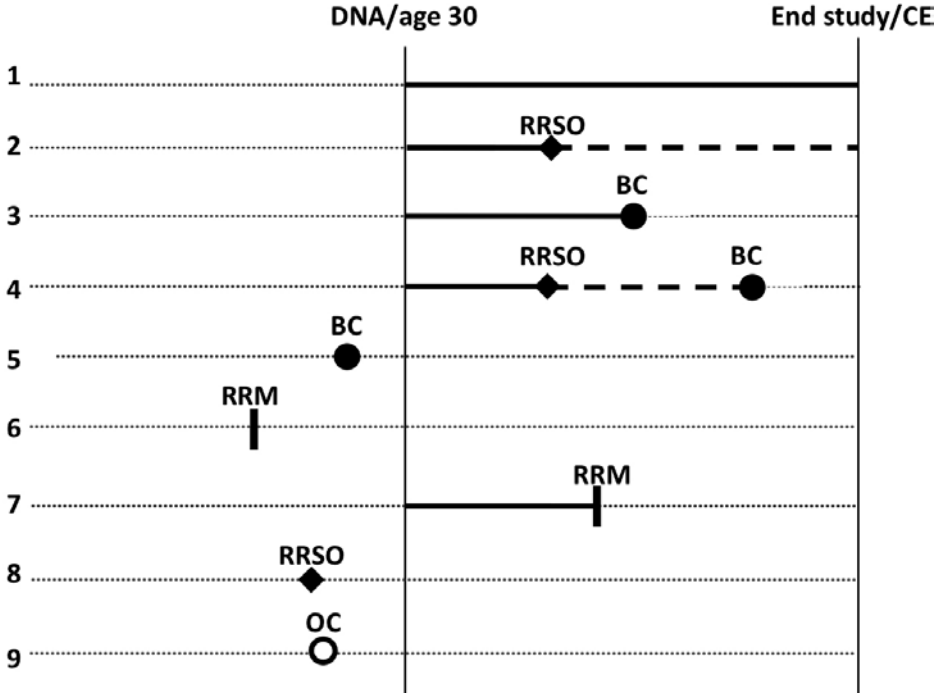

Figure 1

Design of the proposed analytical method and allocation of person-years of observation

Abbreviations: DNA, date of DNA test result; CE, censoring event; RRSO, risk-reducing salpingo-oophorectomy; BC, first breast cancer; RRM, risk-reducing mastectomy; OC, ovarian cancer.

Observation started at the age at DNA test result, or age 30, whichever came last (scenarios 1, 2, 3, 4, 7). For women not opting for RRSO, we allocated all person-years of observation (PYO) to the non-RRSO group (solid lines; scenarios 1, 3, 7). For women undergoing RRSO, we allocated PYO before surgery to the non-RRSO group, and PYO after surgery to the RRSO group (dashed lines; scenarios 2 and 4). The observation ended on the age at BC diagnosis, age at RRM, age at diagnosis of another tumour (including ovarian cancer), age at last contact, age at death or age at study closing date (i.e. June 30, 2013), whichever came first. To avoid selection bias, subjects diagnosed with breast cancer or ovarian cancer before DNA testing (scenarios 5 and 9) or undergoing risk-reducing surgery before DNA testing (scenarios 6 and 8) are not eligible for study inclusion.

through linkages to the Netherlands Cancer Registry and the Dutch Pathology Database. All participants provided written informed consent. The HEBON study was approved by the medical ethics committees of all participating centres.

For replication of the analyses in the four reviewed studies, we selected female BRCA1 and BRCA2 mutation carriers from the national cohort according to the eligibility criteria and designs as indicated in Table 1. For our proposed design, we used the following eligibility criteria: (a) no history of cancer at the date of DNA test result, (b) both breasts and both ovaries in situ at the date of DNA test result, and (c) no cancer diagnosis within the first 6 months of study observation.

Data collection

We retrieved data on type of mutation (i.e. BRCA1 or BRCA2), parity, date of birth, date of DNA test result, date of breast and/or ovarian cancer diagnosis, date of and findings at RRM and/or RRSO, and date of death.

Statistical analyses

For replication of the analyses of the four previous studies within the HEBON cohort, we estimated the effect of RRSO on BC incidence according to the statistical methods described in the respective papers (Table 1). Briefly, for replication of the case-control study, participants were matched on year of birth and type of mutation. The odds ratio (OR) and 95% confidence interval (CI) for BC risk associated with RRSO was calculated using conditional logistic regression. For the cohort studies, we used multivariate Cox proportional-hazard models to obtain hazard ratios (HRs) with 95% CIs. In the replicated matched cohort study, we adjusted for type of BRCA mutation and centre. In the replicated unmatched cohort study described by Kauff et al., we adjusted for age at start of observation, previous BC, any prior hormone-replacement therapy, and type of BRCA mutation. In the replicated unmatched cohort study described by Domchek et al., we adjusted for year of birth. Additionally, for replication of the analyses of both Domchek studies, we used a robust variance-covariance estimation method to correct for non-independence of observations among participants from the same family and centres.

For the proposed analysis, we evaluated person characteristics by comparing women who underwent RRSO (RRSO group) with women who did not (non-RRSO group). We started the observation period at the age of DNA test result or the age of 30 (since the youngest age at RRSO in this cohort was 31), whichever came last. We allocated all person-years of observation (PYO) before surgery as well as a latency period of three months after RRSO to the non-RRSO group. Thereafter, PYO were allocated to the RRSO group (Figure 1). The observation ended at the age at first BC diagnosis, age at RRM, age at diagnosis of another cancer (including ovarian cancer), age at last contact, age at death or age at study closing date (i.e. June 30, 2013), whichever came first. Of note,

BC cases diagnosed during the latency period were counted as events in the non-RRSO group. To estimate the association between RRSO and BC risk, we used a Cox model with RRSO as time-dependent variable to obtain HRs and accompanying 95% CIs, using the non-RRSO group as the reference group. We used a robust variance-covariance estimation method to correct for non-independence of observations in women from the same family. The following variables were considered as potential confounders: year of birth, type of mutation, centre, and parity (yes/no). We incorporated a variable in the multivariate Cox model if 1) there was a significant difference in the median or in the distribution of the respective variable between the RRSO and the non-RRSO group, and 2) the likelihood-ratio test showed that the model including the respective variable was significantly different from the model without the variable. To graph the cumulative BC risk curves for the RRSO group and the non-RRSO group, we used the Simon and Makuch method – which takes into account the change in an individual's covariate status over time – with chronological age as the time variable.[41,42] Additionally, we performed stratified Cox analyses to explore the effect of RRSO for BRCA1 and BRCA2 mutation carriers separately. Further, we performed sensitivity analyses to estimate the effect of RRSO on BC risk in different settings. First, to investigate the effect of excluding the BC-free time before RRM, we estimated BC risk-reduction after RRSO for participants who never underwent RRM. Second, we explored the effect of RRSO on BC risk when the immortal person-time, i.e. the time before RRSO, was excluded from the analysis.

All *p*-values were two-sided, and a significance level α=0.05 was used. Analyses were performed with STATA (version 12.0; StataCorp, CollegeStation, TX, USA).

Results

Replication of four previously published analyses

We used the data from 551 to 934 mutation carriers – according to the respective eligibility criteria –with a median follow-up of 2.7 to 4.6 years (data not shown). As compared with the original studies (Table 1), the numbers for the RRSO and non-RRSO groups separately, depicted in Table 2, were lower for the replicated case-control study[18] and for the non-RRSO group of the replicated unmatched cohort study by Domchek et al.[22], but comparable or higher for the other study groups. Periods of follow-up in the replicated analyses (Table 2) were comparable with or longer than those from the original studies (Table 1). We replicated the approximately 50% BC risk-reduction after RRSO, varying from 38% to 64% (Table 2).

Table 2. Results of the replicated analyses in the HEBON cohort according to eligibility criteria and designs of the four discussed published studies

Author, year	Eisen, 2005[18]	Domchek, 2006[20]	Kauff, 2008[21]	Domchek, 2010[22]
Study design	Case-control	Matched cohort	Prospective cohort	Prospective cohort
N RRSO/non-RRSO	56/849	208/343	333/364[1]	342/592
FU RRSO/non-RRSO[2]	NA	5.3/1.9	4.8/2.8	4.8/4.6
Age at start FU RRSO/non-RRSO[2]	NA	44(32-71)/41(29-74)	46(29-71)/32(30-81)	46(29-71)/30(20-81)
Year of birth RRSO/ non-RRSO[2]	1955/1964	1960/1962	1958/1970	1958/1972
% DNA testing after cancer diagnosis RRSO/ non-RRSO	8%/42%	5%/27%	0%/0%	1%/8%
Risk-reduction (95% CI)	OR 0.61 (0.35-1.08)	HR 0.36 (0.25-0.53)[3]	HR 0.62 (0.39-0.99)[4]	HR 0.49 (0.33-0.71)[3,5]

Abbreviations: RRM, risk-reducing mastectomy; N, number; RRSO, risk-reducing salpingo-oophorectomy; FU, follow-up; NA, not applicable; CI, confidence interval; OR, odds ratio; HR, hazard ratio.

[1] Only participants without a history of breast cancer at start of FU

[2] Median years (range)

[3] A robust variance-covariance estimation method was used to correct for non-independence of observations in women from the same family or centres

[4] Adjusted for age at start of follow-up and type of mutation (BRCA1/BRCA2)

[5] Adjusted for year of birth

Proposed design and analytical method

Patient characteristics

Of the 822 eligible women, 246 BRCA1 and 100 BRCA2 mutation carriers opted for RRSO, at a median age of 45 years (Table 3). The mean observation period was longer among participants ever undergoing RRSO than among those not undergoing RRSO (6.8 versus 3.1 years, $p<0.001$), and started at older age (median 44 versus 33 years, $p<0.01$). In the non-RRSO group, more women were censored because of RRM (51% versus 23%, $p<0.001$), at younger age than in the RRSO group (median 35 versus 45 years, $p<0.001$). Numbers of death were similar in both groups (2%), but in the non-RRSO group women died at younger age (median 44 versus 57 years, $p=0.004$).

Breast cancer incidence

We observed no difference in BC incidence between the RRSO and the non-RRSO group (12% and 10%; Table 3). Likewise, the BC incidence rate after RRSO was not different from that in the non-RRSO group (25.6 versus 21.5 per 1000 PYO; Figure 2), yielding an HR of 1.09 (95% CI, 0.67-1.77; Figure 2). Furthermore, the cumulative BC risk curves for both groups were not different (Figure 2).

Additional analyses

We observed no significant BC risk-reduction after RRSO in the gene-stratified analyses; the HR

was 1.21 (95% CI, 0.72-2.06) for BRCA1 mutation carriers and 0.54 (95% CI, 0.17-1.66) for BRCA2 mutation carriers (data not shown). The sensitivity analyses in subgroups of participants revealed no BC risk-reduction after RRSO; when participants ever undergoing RRM were excluded the HR was 1.10 (95% CI 0.66-1.84), and when the PYO between dates of DNA disclosure and RRSO were left out the HR was 0.78 (95% CI 0.51-1.19) (Data not shown).

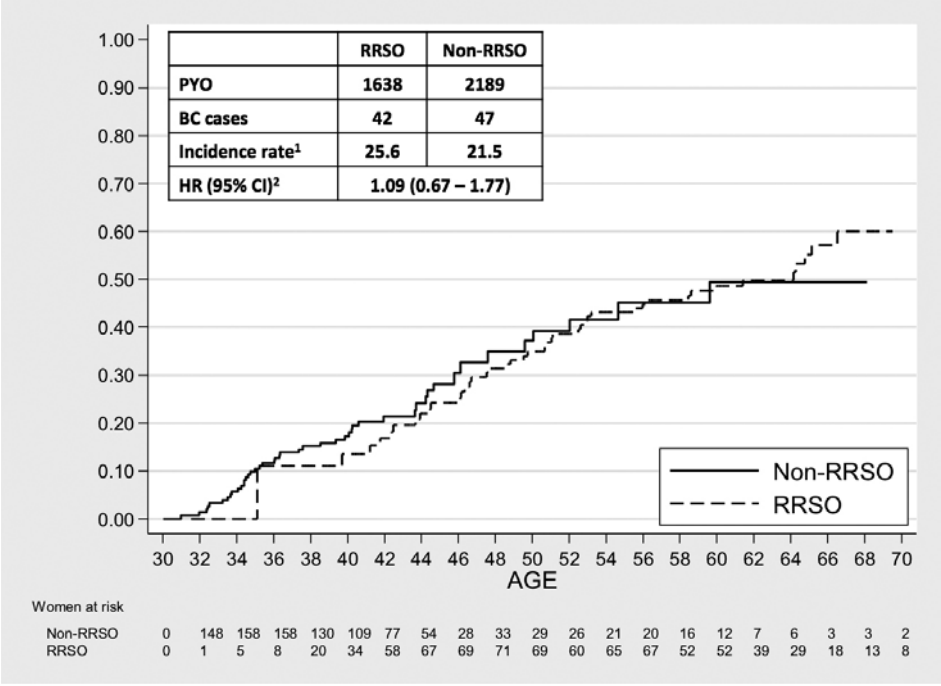

Figure 2. Cumulative breast cancer risk curves for BRCA1/2 mutation carriers opting for risk-reducing salping-oophorectomy (RRSO) versus not opting for risk-reducing salpingo-oophorectomy (non-RRSO), using the Simon and Makuch method – which takes into account the change in an individual's covariate status over time – with chronological age as the time variable.

Discussion

With our proposed method of analysis – designed to overcome bias as much as possible – we found no evidence for BC risk-reduction after RRSO in healthy BRCA1/2 mutation carriers, while replication of previously described designs and analyses yielded a similar approximately 50% BC risk-reduction as estimated before. These findings support our idea that in previous studies the consistent finding of a reduced BC risk after RRSO may at least partly result from bias.

Table 3. Characteristics of the study population for the proposed design and analysis

	RRSO		Non-RRSO		
	N	%	N	%	*P* value
	346	42	476	58	
Observation period, mean years (range)[1]	6.8	0.5-17.4	3.1	0.1-15.9	< .001
Age at start of observation (years)					
30-39	110	32	376	79	< .001
40-49	147	42	75	16	
50-59	78	23	21	4	
≥60	11	3	4	1	
Median (range)	44	30-66	33	30-66	< .001
Median year at start of observation (range)	2003	1994-2011	2003	1994-2013	.466
Mutation status					
BRCA1	246	71	343	72	.814
BRCA2	100	29	133	28	
Median age at DNA testing (range)	44	24-66	33	18-66	< .001
Median year at DNA testing (range)	2003	1994-2011	2002	1994-2013	.136
Year of birth					
1940-1949	61	17	17	4	< .001
1950-1959	117	34	68	14	
1960-1969	134	39	181	38	
1970-1979	34	10	177	37	
≥ 1980	0	0	33	7	
Median (range)	1959	1940-1976	1968	1940-1983	< .001
Parity[2]					
Nulliparous	23	12	59	20	0.046
Parous	162	88	241	80	
Unknown	176		161		
RRSO					
Median age, years (range)	45	31-67	-	-	
Median year (range)	2005	1994-2013	-	-	
Mean observation time, years (range)					
before RRSO	1.9	0.1-11.7	-	-	
after RRSO	4.9	0.3-16.2	-	-	
Censoring events					
Ovarian cancer	5[3]	1	9	2	0.787
Median age, years (range)	56	45-57	39	32-58	0.038
Median year (range)	2003	2000-2013	2003	1998-2010	.499
RRM	79	23	242	51	< .001
Age at RRM, years					
30-39	12	14	182	74	< .001
40-49	48	57	54	22	
50-59	23	28	7	3	
≥60	1	1	2	1	

	RRSO		Non-RRSO		
	N	%	N	%	*P* value
Median (range)	45	33-65	35	30-65	< .001
Median year (range)	2004	1996-2012	2001	1994-2013	.015
Death[4]	8	2	9	2	.805
Median age, years (range)	57	45-68	44	34-60	.004
Median year (range)	2010	2007-2011	2006	2002-2012	.022
History of BC and/or OC					
No	5	62	1	12	.034
BC	3	38	4	44	
OC	0	0	4	44	
BC and OC	0	0	0	0	
Event of interest					
BC	42	12	47	10	.308
Age at BC diagnosis (yrs)					
≤35	0	0	17	36	< .001
35-50	23	55	26	55	
≥50	19	45	4	9	
Median (range)	50	35-66	37	30-71	< .001
Median year (range)	2008	1998-2012	2006	1998-2012	.299
Mean observation time, years (range)[1]	5.5	0.6-14.1	3.9	0.5-12.7	.093
Mean time after RRSO, years (range)	4.2	0.4-13.7	-	-	

Abbreviations: RRSO, risk-reducing salpingo-oophorectomy; RRM, risk-reducing mastectomy; BC, first breast cancer; SD, standard deviation.

[1] Start of observation is either age at DNA diagnosis, or 30th birthday, whichever came last

[2] As reported at the moment of signing the informed consent

[3] Extra-ovarian peritoneal cancer diagnosed after RRSO

[4] All causes.

Estimates of previous studies on BC risk-reduction after RRSO were reproducible in the Dutch cohort when we applied the previously used eligibility criteria and analytical methods of the respective case-control, matched cohort, and unmatched cohort designs. This demonstrates that our cohort is comparable to the previous cohorts. The most important differences between our currently proposed design and the previous designs concern the exclusion of prevalent BC cases and the allocation of the immortal person-time to the non-RRSO group. Exclusion of patients affected with BC before genetic testing results in a lower BC incidence in especially the non-RRSO group, and subsequently leads to attenuated BC risk-reduction estimates after RRSO. Further, allocating the immortal person-time to the non-RRSO group again decreased the BC incidence rate in this group. With our proposed design and analytical method, we have overcome two major types of bias, i.e. cancer-induced testing bias and immortal person-time bias.

Still, some differences between the RRSO group and the non-RRSO group may have influenced the estimates in our proposed analyses. First, the observation period was shorter among participants not undergoing RRSO than among those ever undergoing RRSO (3.1

versus 6.8 mean years). An explanation is that in the non-RRSO group more participants were censored because of RRM than in the RRSO group (51% versus 23%). By considering RRM as a censoring event and including observation time before this intervention into the analyses, the mean observation period in especially the non-RRSO group was drastically decreased, since the mean time period between start of observation and moment of RRM was only 1.4 years in this group (data not shown). The mean observation period for women without RRM in the non-RRSO group was 4.8 years (data not shown). Noticeably, also in the four discussed studies the reported observation period in the non-RRSO groups was short, varying from 2.1 to 4.9 years, while we obtained 1.9 to 4.6 years in the replicated analyses.

In the non-RRSO group, 79% of the women who were censored because of RRM were under the age of 40 at start of observation (data not shown). Those women may not have completed child-bearing yet and/or had not reached the age at which RRSO is advised in the Netherlands (being 35-40 years for BRCA1 and 40-45 years for BRCA2 mutation carriers), and therefore may have opted for RRM before RRSO. This indicates that informative censoring might play a role in this cohort. When participants who ever underwent RRM were excluded from the analyses we found a similar association between RRSO and BC risk as for the total group. This shows that our initial concern regarding disturbed risk-reduction estimates when the BC-free person-years before RRM are excluded from the analyses was unfounded for the current cohort. Still, informative censoring bias cannot be ruled out.

Second, participants opting for RRSO were born in earlier years, and were older at start of observation. The date of DNA test result – and thus the date of start of observation – may be associated with the desire of BRCA1/2 mutation carriers to undergo risk-reducing surgery in the near future. This is supported by the fact that in our cohort participants not undergoing RRSO underwent DNA testing at the median age of 33 years and RRM at the median age of 35 years, while in the RRSO group the median age was 44 years at DNA testing and 45 years at RRSO and at RRM. We adjusted our analyses for differences in age by using chronological age as the time variable. Additionally, including year of birth into the Cox model did not influence the association of RRSO with BC risk. Noteworthy, differences in median ages at start of observation were also reported in the previous cohort studies, being consequently younger for the non-RRSO groups.[20-22]

The cumulative breast cancer risk curves suggest a slight protective effect of RRSO on the BC risk when performed at premenopausal age. Larger numbers of mutation carriers opting for RRSO at premenopausal age are warranted to confirm this, although we do not expect the potential risk-reducing effect to be as high as the previously estimated 50%.

Altered estrogen receptor expression in mammary gland cells is suggested to play a significant role during tumour genesis of BC.[43,44] Given the fact that BRCA2-associated BCs mainly are ER-positive, while the majority of BRCA1-associated BCs is ER-negative[45], a BC risk-reducing effect of RRSO may be expected in BRCA2 mutation carriers rather than in BRCA1 mutation carriers. Unfortunately, in the current cohort the numbers of BRCA2 mutation

carriers, and especially the numbers of events in that specific group, were too small to perform conclusive gene-stratified analyses using the proposed design and analytical method.

In summary, we have shown that the finding of a reduced BC risk after RRSO for BRCA1/2 mutation carriers in previously published studies may at least partly have resulted from bias. Using a simple, more valid method of analysis, we found no evidence for first BC risk-reduction after RRSO in BRCA1/2 mutation carriers. We suggest that counselors, clinicians and researchers should consider the potential impact of bias in previous and future observational studies on this topic. It would be of great interest to examine the risk estimates in the previous study cohorts when using our proposed design and analytical method, in order to validate our findings. Further research with longer follow-up and larger numbers of especially BRCA2 mutation carriers is warranted to explore differential effects on BC risks after RRSO for BRCA1 and BRCA2 mutation carriers. For the present, caution is advised in the message regarding BC risk-reduction after RRSO, at least for BRCA1 mutation carriers.

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Chapter 7

General discussion

Since the discovery in the mid-nineties of the 20th century of the two tumour suppressor genes, BRCA (**BR**east **CA**ncer) 1 and 2,[1,2] options for genetic counselling and testing for mutations in these genes became available for members from families with clustering of breast and/or ovarian cancer. Research over the last two decades regarding the risks, phenotypes and outcome of BRCA1-associated and BRCA2-associated breast and ovarian cancer has provided a lot of information, revealing a highly increased risk of developing a first breast cancer (BC) and a subsequent contralateral BC, already at young age. From age 40, also the risk of ovarian/fallopian tube cancer is highly increased. Therefore, the knowledge of being a BRCA mutation carrier comes with difficult decisions regarding strategies to reduce the risk of developing cancer and subsequent cancer-related death. Possible options include regular surveillance – including magnetic resonance imaging (MRI) – as of young age, and risk-reducing surgeries consisting of prophylactic removal of healthy breasts and/or ovaries and fallopian tubes. Especially since cancer risks are already high at young age,[3,4] choices on cancer risk management play an important role for a long lifetime period. The studies described in this thesis provide additional information on the efficacy of the different strategies, which may be used by clinical geneticists and treating physicians in the counselling process, and thus will ease the decision-making process for female BRCA1/2 mutation carriers.

In this chapter, after recapitulation of the main research findings, additional aspects of studies on risk-reducing strategies in BRCA1/2 mutation carriers are discussed. The chapter ends with clinical implications and several recommendations for future research.

Breast cancer incidence and survival after risk-reducing mastectomy in BRCA1/2 mutation carriers

We confirmed that bilateral and contralateral risk-reducing mastectomy strongly reduces the occurrence of a first and contralateral breast cancer, respectively (Chapter 3 and 4). In addition, we found a trend for improved overall survival and breast cancer-specific survival after bilateral RRM in healthy BRCA1/2 mutation carriers (Chapter 3). In BRCA1/2 mutation carriers with a history of unilateral BC, we observed a significantly improved overall survival after contralateral risk-reducing mastectomy (RRM) when compared with regular surveillance (Chapter 4).

Although the improved survival after bilateral RRM in healthy BRCA1/2 mutation carriers is not significant yet, our findings provide the only available clinical data on survival after bilateral RRM in healthy BRCA1/2 mutation carriers so far, and are in line with the information obtained from mathematical models with simulated cohorts describing an improved survival for women who opt for risk-reducing surgery.[5-9] To establish that bilateral RRM not only prevents the development of BC, but in the end also leads to better survival than intensive surveillance, further research in larger nationwide cohorts and especially with longer periods of follow-up is warranted.

For BRCA1/2 mutation carriers with a history of unilateral BC, counselling may

be more complicated. Although we observed a significant overall survival benefit after contralateral RRM, one should keep in mind that the contralateral BC risk and the prognosis after unilateral BC are not similar for all BRCA1/2-associated BC patients. After all, the risk of developing contralateral BC depends on age at first BC diagnosis,[10] hormone-receptor status, and especially ER-status,[11] and administered adjuvant systemic therapy.[12,13] Possibly, also menopausal status as well as lifestyle factors may play a role. Therefore, contralateral RRM may be redundant for BRCA-associated BC patients with a relatively low contralateral BC risk, i.e. those with a first BC diagnosed at older age or receiving adjuvant hormonal therapy – after diagnosis of ER-positive BC – and/or chemotherapy. We expect the greatest survival benefits after contralateral RRM in subgroups of patients at high risk of contralateral BC and low risk of mortality due to the first BC. When data from larger samples become available, thus providing conclusive data for specific subgroups, the development of a model that can predict survival benefit in more detailed subgroups will be feasible. This will improve the counselling process for BRCA1/2 mutation carriers with a history of unilateral BC, since such a prediction model will facilitate a more personalized advice. Moreover, drastic contralateral RRM may be avoided in patients with an unfavourable prognosis after first BC.

The effect of risk-reducing salpingo-oophorectomy on first breast cancer

Regarding the effect of risk-reducing salpingo-oophorectomy (RRSO) on a first BC, we addressed two aspects in this thesis. First, we observed that breast cancers which developed at least one year after RRSO-induced estrogen depletion showed significantly lower mitotic activity index (MAI) – especially due to significant lower mitotic counts – than breast cancers occurring without previous RRSO (Chapter 5). Additionally, we found a trend for longer tumour volume doubling time in the RRSO group, although the difference with the non-RRSO group was not significant. Our findings suggest a less aggressive biological phenotype after RRSO, possibly also involving slower tumour growth. When confirmed in larger series, this may have consequences for BC screening protocols after RRSO.

Second, we addressed potential types of bias that may have influenced previous estimates from literature on BC risk-reduction after RRSO (Chapter 6). Since gynaecologic screening has limited value in early detection of ovarian/fallopian tube cancer, and ovarian cancer (OC) detection at advanced stages shows an unfavourable prognosis, the only option to prevent ovarian/fallopian tube cancer and OC-related death is to undergo RRSO. When discussing RRSO, counsellors often inform BRCA1/2 mutation carriers that RRSO is also associated with a BC risk-reduction of approximately 50%, based on the data of previous studies. However, as a randomised clinical trial clearly is neither ethical nor feasible in this setting, investigators are confined to observational studies, thus challenging at least two major types of bias, i.e. cancer-induced testing bias and immortal person-time bias. Unfortunately both types of bias may lead to an overestimation of BC risk-reduction after RRSO in BRCA1/2 mutation carriers, as discussed in Chapter 6. We proposed a revised approach of the analysis

in order to overcome these types of bias, resulting in a more valid estimate for the effect of RRSO on first BC risk. Using this method of analysis we found no evidence of first BC risk-reduction after RRSO. The latter finding justifies restraint regarding the message of a 50% BC risk-reduction – above OC risk-reduction – after RRSO.

Regular surveillance in BRCA1/2 mutation carriers

Regular surveillance – including MRI – detects BC at early stages, including ductal carcinoma in situ (DCIS), thus resulting in a more favourable prognosis. Moreover, screening by means of mammography and MRI plus risk-reducing salpingo-oophorectomy (RRSO) may result in almost similar survival as bilateral RRM with RRSO, as estimated by Kurian and colleagues in their mathematical simulation model.[8,9] This suggests that intensive MRI-based surveillance might be a reasonable alternative to the drastic and irreversible bilateral mastectomy procedure. Arguments to support the latter are (1) BC surveillance – including MRI – detects BC at an early stage in BRCA1/2 mutation carriers,[14-18] (2) in a prospective MRI-detected series of BRCA1/2-associated patients the overall survival at six years was 93%,[19] and (3) broad implementation of neo-adjuvant and adjuvant systemic therapy in recent years results in improved BC survival.[20] In this respect, it is important to obtain more data on the efficacy of the current intensive surveillance programs for BRCA1/2 mutation carriers – including MRI – regarding both early BC detection and BC-specific mortality. Since the follow-up time after the introduction of MRI for BC screening is only approximately 10 years so far, conclusive data on mortality are still lacking. Further, in some centres in the Netherlands, breast imaging in BRCA1/2 mutation carriers is carried out biannually with mammography and MRI in an alternating schedule since 2007. It is not clear yet whether this adapted surveillance scheme will further improve stage at BC detection and subsequent prognosis after screen-detected BC. Additionally, the introduction of digital mammography as well as more sophisticated MRI devices, in combination with radiologists becoming more and more experienced in MRI interpretation, may improve early BC detection even more.

BRCA1 versus BRCA2: different entities

BRCA1-associated and BRCA2-associated breast tumours show different characteristics. BRCA1-associated breast tumours usually are poorly differentiated (grade 3), infiltrating ductal carcinomas which are characterised by a so-called triple-negative phenotype, i.e. not expressing receptors for estrogen (ER) and progesterone (PR) nor the human epidermal growth factor receptor 2 (HER2). In comparison, among BRCA2 mutation carriers medium graded (grade 2) tumours, lobular carcinomas or ductal carcinomas with lobular features and carcinomas in situ are more prevalent. Also, BRCA2-associated BCs usually stain positive for ER and PR.[21-23]

In this respect, it may be worthwhile to explore differences between BRCA1 and BRCA2 mutation carriers more thoroughly. We observed lower first BC incidence rates, more

favourable BC characteristics (i.e. more DCIS, smaller tumour size and more node-negative disease), and lower recurrence rates for BRCA2 mutation carriers than for BRCA1 mutation carriers (Chapter 3). This suggests that for BRCA2 mutation carriers – in contrast to BRCA1 mutation carriers – intensive surveillance may lead to similar survival as bilateral RRM and thus that intensive surveillance may be a reasonable alternative for bilateral RRM. Additionally, we observed lower contralateral BC incidence rates for BRCA2 mutation carriers (Chapter 4). With all BRCA2-associated contralateral BCs being DCIS or ≤ 2 cm in size and 80% of them having a negative nodal status (T1N0), and 14% of the BRCA1-associated contralateral BCs being > 2 cm and 67% having a negative nodal status, also contralateral BCs were detected at a more favourable stage for BRCA2 mutation carriers.

Further, we observed a non-significant association between RRSO and first BC risk-reduction among BRCA2 mutation carriers and no BC risk-reduction after RRSO in BRCA1 mutation carriers (Chapter 6). This supports the previous suggestion by other investigators that altered estrogen expression may play a significant role during tumour genesis,[24,25] and that the estrogen deprivation induced by RRSO results in a decreased incidence of especially ER-positive tumours. In our pilot study comparing tumour characteristics of first BCs preceded by RRSO or not (Chapter 5), however, we observed a trend for more progesterone receptor (PR)-positive tumours and (non-significantly) more ER-positive tumours in women who previously underwent RRSO than in women who developed BC without previous RRSO. This suggests that also hormone receptor-negative BCs are susceptible to estrogen depletion.[26-28] We speculate that the explanation might lie in high ER expression at early stages of tumour genesis of eventually hormone receptor-negative BCs. Estrogen may facilitate cell proliferation and tumour development in premalignant mammary tissue until ER expression extinguishes at later stages of tumour development.[29,30] By this mechanism, RRSO-induced estrogen depletion might inhibit tumour development of hormone receptor-negative BC at a very early stage. Another previously proposed explanation is based on the assumption that all breast cancer stem cells are ER-negative, while the surrounding cells are ER-positive.[22,31] These surrounding cells might respond to estrogen, which has shown to have anti-apoptotic effects,[32] and subsequently send pro-survival signals to the ER-negative stem cells.[33] This mechanism may be inhibited after RRSO-induced estrogen depletion.

Potential of bias in studies involving BRCA1/2 mutation carriers

Evaluation of the efficacy of risk-reducing surgery in BRCA1/2 mutation carriers is confined to observational studies. Consequently, estimates may be influenced by several types of potential selection bias. According to Rothman, selection biases are distortions that result from procedures used to select participants and from factors that influence study participation.[34] For example, BRCA1/2-associated cancer cases may be over-selected because patients underwent DNA testing only after cancer diagnosis. Also, healthy women may be over-selected in the risk-reducing surgery groups when unaffected women elect for DNA

testing and subsequent RRM and/or RRSO because their sisters were diagnosed with BC and/or OC. Due to this differential patient selection, with more patients in the non-surgery group and more healthy women in the surgery group, the intervention may appear protective, while there is in fact no protection at all. If the intervention does have a protective effect, this will be exaggerated.

In Chapter 6 we discussed three major types of bias – i.e. cancer-induced testing bias, immortal person-time bias, and bias due to informative censoring –, and we showed that the consistent finding of reduced BC risk after RRSO in previous studies may at least partly have resulted from bias. We proposed a revised method of analysis, in order to minimize bias as much as possible (Chapter 6). In this section we will discuss two other types of bias that may hamper valid cancer risk estimations and estimates of the effects of risk-reducing surgeries in BRCA1/2 mutation carriers.

Familial testing bias

The BRCA1/2 mutation carriers selected for the studies in this thesis came from BRCA1/2 families which were identified through Clinical Genetic Centers. Members of these families sought advice based on their family history and/or personal history of BC and/or OC. It is possible that in the past especially families with the strongest clustering of BC and/or OC were identified as BRCA1/2 families. Possibly, families with – presumed – lower risks, for example small families or families with the majority of offspring being male, less likely may have presented themselves in the past. Therefore, risk and risk-reducing estimates may be overestimated in family-based studies, and may not be directly generalizable to the real – though partly unrecognized – population of BRCA1/2 families. Previously, family-based studies showed higher penetrance estimates at age 70 for BRCA1/2-associated BC (68%-85% for BRCA1 mutation carriers and 75%-84% for BRCA2 mutation carriers)[35-37] than population-based studies (46%-69% for BRCA1 and 43%-74% for BRCA2 mutation carriers).[3,38,39] Likewise, BRCA1/2-associated OC penetrance estimates were higher in family-based studies (60%-63% for BRCA1 and 27%-30% for BRCA2 mutation carriers)[35-37] than in population-based studies (39%-46% for BRCA1 mutation carriers and 11%-22% for BRCA2 mutation carriers).[3,38,39]

Fortunately, after identification of large families with a strong family clustering of BC and/or OC shortly after genetic testing became available in 1995, nowadays also more lower-risk families are being tested, due to declined required levels of personal and/or family history to qualify for genetic testing. In this respect it is important to spend effort and money in expanding and updating existing BRCA1/2 databases, and re-estimate risks, at institutional, national and international levels. The latter may be facilitated by large consortia such as the International BRCA1/BRCA2 Carriers Cohort Study (IBCCS) and the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA).

Confounding by indication

It is conceivable that the choice for risk-reducing surgery is associated with the presence of more cancer cases in the family, and that the prevalence of one cancer type in the family may influence the choice for a particular type of risk-reducing surgery. The risk of developing BC and/or OC is not the same among all BRCA1/2 families. While in one family BC may be more prevalent, OC may occur more often in another family. A mutation on the central region of the BRCA1 gene has been associated with a higher risk of OC and a lower risk of BC.[40] Consequently, women from a family with a mutation in the central region of the gene may be more likely to undergo RRSO. Due to this overrepresentation of the number of mutation carriers from such families in the surgery group, the baseline risk for OC may be higher and the baseline BC risk may be lower in this group. Subsequently, this may lead to an underestimation of OC risk-reduction and to an overestimation of the BC risk-reduction after RRSO.

This phenomenon is called 'confounding by indication' and in practice it is difficult to properly take into account this type of bias, due to the wide range of variations in BRCA1/2 mutations. One statistical feature to adjust for clustering of participants from the same family is the use of a robust variance-covariance estimator method as we performed in the analyses on the effect of RRSO on first BC risk in Chapter 6.

Risk-reducing salpingo-oophorectomy and non-cancer related health effects

The protective effect of RRSO on the development of OC and subsequent OC-related mortality has been convincingly demonstrated. Still, relevant adverse health effects may accompany RRSO, especially when performed at premenopausal age, which is usually the case for BRCA mutation carriers where the median age at RRSO is [44.41-43] Early menopause after RRSO leads to loss of fertility, potential problems with sexual functioning, and may increase both the risk of osteoporosis and the risk of cardiovascular disease on the long term.[44,45] In general, early menopause is associated with increased all-cause mortality.[46,47] Further research is warranted to investigate to what extent these health effects apply also to BRCA1/2 mutation carriers and whether the non-cancer related effects affect quality of life. In view of increasing evidence that ovarian cancer may arise from the fallopian tube,[48-52] and the adverse consequences of early menopause as mentioned, several studies on the efficacy and safety of prophylactic salpingectomy followed by delayed oophorectomy as an alternative for early RRSO in BRCA mutation carriers are currently ongoing.[53-55]

Clinical implications

The study findings described in this thesis yield important information for clinical genetic counsellors, oncologists and other physicians and nurses involved in the care of BRCA mutation carriers, and especially for BRCA1/2 mutation carriers who consider risk-reducing surgery. Moreover, the findings can contribute to the optimization of the counselling process for

BRCA1/2 mutation carriers. Although with some caution, counsellors can inform healthy BRCA1 mutation carriers that bilateral RRM results in survival benefit, by preventing the development of BC. For healthy BRCA2 mutation carriers, early BC detection by intensive surveillance may lead to similar survival as BC prevention through bilateral RRM. Further, it seems reasonable to discuss the option of contralateral RRM with BRCA1/2 mutation carriers who have a favourable prognosis after unilateral BC diagnosis. Last, towards BRCA1/2 mutation carriers who opt for RRSO in order to prevent ovarian/fallopian tube cancer, we recommend restraint regarding the message of a subsequent 50% BC risk-reduction after RRSO.

Recommendations for future studies

Additional prospective studies with larger sample sizes and longer observation periods are warranted to confirm survival benefit after both bilateral and contralateral RRM. For the current study on the efficacy of bilateral RRM, we selected healthy BRCA1/2 mutation carriers from the Rotterdam Family Cancer Clinic registry database. This cohort can be extended with BRCA1/2 mutation carriers from a national cohort, existing in the context of an ongoing nationwide study in the Netherlands on risk assessment and gene-environment interactions (the HEBON study).[56] To examine the efficacy of contralateral RRM on survival, we identified BRCA1/2 mutation carriers with a history of unilateral BC from the national HEBON cohort. Therefore, for extension of the latter patient selection international collaboration is required.

Our findings clearly indicate that BRCA1 mutation carriers and BRCA2 mutation carriers should be analysed separately, both in healthy women and in BC patients. Larger sample sizes are necessary in order to investigate whether bilateral RRM results in survival benefit for healthy BRCA1 mutation carriers, and whether regular surveillance yields similar survival as bilateral RRM in healthy BRCA2 mutation carriers. Further, separate analyses in larger samples are needed to confirm that RRSO results in BC risk-reduction in BRCA2 mutation carriers, and has no effect on BC risk in BRCA1 mutation carriers.

Results from larger samples with longer observation periods will enable the development of a prediction model based on type of BRCA mutation, and patient, tumour and treatment characteristics. Such a model will facilitate more personalized advice to BRCA-associated BC patients regarding the difficult choice between regular surveillance and contralateral RRM.

Findings from our pilot study on first BCs developing after RRSO suggest a less aggressive biological phenotype of a first breast tumour in this setting. However, numbers in this study were very small. The results should be confirmed in larger series.

Our study on the risk of first BC after RRSO hopefully will open the discussion on the potential of bias in observational studies in BRCA1/2 mutation carriers. We are very interested in the validation of the estimates of our revised method of analysis in the patient cohorts used in previously published studies reporting on BC risk-reduction after RRSO.

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Chapter 8

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PhD portfolio

Summary

Women with a mutation in one of the two **BR**east **CA**ncer genes (BRCA1 and BRCA2) have highly increased risks of developing breast cancer and/or ovarian cancer, usually already at young ages. In order to prevent early cancer-related death, BRCA1/2 mutation carriers can opt for regular surveillance aiming at early cancer detection with more favourable prognosis. However, surveillance will neither prevent the development of breast cancer, nor guarantee detection before spreading to for example the lymph nodes has occurred. Additionally, gynaecological screening does not add to the early detection of ovarian cancer, and is thus not effective in the reduction of ovarian cancer-specific mortality. Therefore, part of the BRCA1/2 mutation carriers opts for risk-reducing mastectomy and/or risk-reducing salpingo-oophorectomy, aiming at cancer prevention. The latter involves difficult decisions, especially for unaffected women considering prophylactic removal of their healthy breasts and/or ovaries and fallopian tubes. Risk-reducing mastectomy may have large consequences on body image, self-esteem, and sexuality for women, while removal of the ovaries and fallopian tubes by means of risk-reducing salpingo-oophorectomy at young age results in early menopause with specific problems, such as infertility, and increased risks of osteoporosis and cardiovascular disease later in life. In order to adequately inform and support BRCA1/2 mutation carriers who face the difficult choice between surveillance and risk-reducing surgery, we need accurate information on the efficacy of risk-reducing strategies regarding cancer prevention and life expectancy. The aims of this thesis were to obtain additional information (1) on breast cancer risk-reduction and survival after risk-reducing mastectomy in both healthy BRCA1/2 mutation carriers and BRCA1/2-associated breast cancer patients and (2) on the risk of first breast cancer and tumour characteristics after risk-reducing salpingo-oophorectomy.

Experiences with risk-reducing-mastectomy at the Rotterdam Family Cancer Clinic

At the Rotterdam Family Cancer Clinic we have long-term experience with large numbers of women undergoing bilateral or contralateral risk-reducing mastectomy. Previously, investigators from our institute reported on the occurrence of breast cancer after risk-reducing mastectomy in healthy BRCA1/2 mutation carriers, on complications after risk-reducing mastectomy with breast reconstructions, and on psychological aspects of risk-reducing mastectomy in combination with breast reconstruction. In **Chapter 2**, we confirmed in an updated and extended series (n=358) with a median follow-up of 4.5 years, that the risk of developing breast cancer after bilateral or contralateral risk-reducing mastectomy is very low (<0.5%) in both unaffected and affected BRCA1/2 mutation carriers and women from a hereditary breast and ovarian cancer family without a proven BRCA mutation. Further, almost half of the women opting for risk-reducing mastectomy reported complications, mainly related to cosmetic outcome and capsular formation, and almost always leading to

additional surgery.

Additionally, we observed unexpected (pre)malignant changes in the mastectomy specimens of 3% of the women undergoing risk-reducing mastectomy, thus emphasising the necessity of preoperative imaging and careful histological examination afterwards. The number of unexpected malignant findings might be decreased, though, since the implementation of institutional guidelines in 2000 concerning preoperative breast examination, and especially since the introduction of magnetic resonance imaging (MRI), being more sensitive in detecting tumours in high-risk women than mammography.

Bilateral risk-reducing mastectomy in healthy BRCA1/2 mutation carriers

In **Chapter 3**, we investigated whether bilateral risk-reducing mastectomy – aiming at prevention of first BC – also results in improved survival when compared with intensive surveillance in healthy BRCA1/2 mutation carriers. Especially since the addition of magnetic resonance imaging (MRI) to the surveillance program for BRCA1/2 mutation carriers, and the current availability of modern adjuvant and neo-adjuvant treatment strategies, it can be hypothesized that intensive surveillance – aiming at early breast cancer detection with better prognosis – may lead to similar survival when compared to bilateral risk-reducing mastectomy.

In a cohort study with a prospective design and a large sample of healthy BRCA1/2 mutation carriers (n=570), we found substantially reduced first breast cancer incidence rates after bilateral risk-reducing mastectomy, when compared with first breast cancer incidence rates during intensive surveillance. In the risk-reducing mastectomy group, we observed no breast cancer cases, while 57 women in the surveillance group (16%) were diagnosed with breast cancer. Additionally, all-cause mortality and breast cancer-specific mortality were reduced after bilateral risk-reducing mastectomy, although significant survival benefits could not be claimed yet.

Further, we observed a lower breast cancer incidence rate in BRCA2 mutation carriers (7%) than in BRCA1 mutation carriers (20%). In addition, all BRCA2-associated tumours had favourable stages at diagnosis (i.e. ductal carcinoma *in situ* (DCIS) or invasive tumours smaller than 2 cm and without metastases in the lymph nodes (T1N0)), while 28% of the BRCA1-associated breast tumours were detected at less favourable stages. Moreover, all BRCA2 mutation carriers were still alive at the end of the observation period. Although numbers of BRCA2 mutation carriers were small, our findings suggest that opting for intensive surveillance and opting for bilateral risk-reducing mastectomy may lead to similar survival in BRCA2 mutation carriers.

Contralateral risk-reducing mastectomy in BRCA1/2 mutation carriers with a history of unilateral breast cancer

In **Chapter 4**, we prospectively examined the efficacy of contralateral risk-reducing mastectomy on overall survival. For this study we selected 583 BRCA1/2 mutation carriers with a history of unilateral breast cancer from the **H**ereditary **B**reast and **O**varian Cancer

study group in the **N**etherlands (HEBON). We confirmed that contralateral risk-reducing mastectomy strongly reduces contralateral breast cancer incidence. In the contralateral risk-reducing mastectomy group, four patients (2%) developed contralateral breast cancer, against 64 patients (19%) in the surveillance group. In addition, we observed a significantly improved overall survival after contralateral risk-reducing mastectomy. Fifteen-year overall survival was 86% in the contralateral risk-reducing mastectomy group and 74% in the surveillance group. Further, exploratory analyses showed that survival benefit may especially be achieved in young breast cancer patients (<40 years), in patients having a first breast cancer with differentiation grade 1-2 and/or no triple-negative phenotype, and in patients not treated with adjuvant chemotherapy.

Characteristics of first breast cancer after risk-reducing salpingo-oophorectomy

Risk-reducing salpingo-oophorectomy successfully prevents the development of ovarian cancer and fallopian tube cancer in BRCA1/2 mutation carriers, the primary objective of this intervention. Additionally, previous studies also reported a 50% breast cancer risk-reduction after risk-reducing salpingo-oophorectomy. The underlying mechanisms of the latter phenomenon are not exactly clear yet, although the deprivation of estrogen is assumed to play a role. In addition, no detailed data are available on characteristics and tumour growth of breast tumours developing after risk-reducing salpingo-oophorectomy. In **Chapter 5**, we described the results of a pilot study in a matched cohort of BRCA1/2 mutation carriers, in which we compared tumour characteristics and tumour growth rates of first breast cancers developing with (n=20) and without previous risk-reducing salpingo-oophorectomy (n=36). We observed that breast tumours occurring after risk-reducing salpingo-oophorectomy showed significantly lower mitotic activity index (MAI) – and more specifically significantly lower mitotic counts (12 versus 22 mitotic counts/2 mm)– than breast tumours not preceded by risk-reducing salpingo-oophorectomy. Additionally, tumour volume doubling time was (non-significantly) longer in the risk-reducing salpingo-oophorectomy group. Our findings suggest a less aggressive biological breast tumour phenotype after risk-reducing salpingo-oophorectomy.

Risk of first breast cancer after risk-reducing salpingo-oophorectomy

Studies on the efficacy of risk-reducing strategies in BRCA1/2 mutation carriers are confined to observational studies, and therefore previous estimates on breast cancer risk-reduction after risk-reducing salpingo-oophorectomy may be subject to potential biases. In **Chapter 6**, we explored the influence of different methodological procedures on estimates of the association between risk-reducing salpingo-oophorectomy and first breast cancer risk. We reviewed four previous studies reporting a breast cancer risk-reduction of approximately 50%, using four different designs and methods of analyses, and discussed the potential biases associated with these analyses. Next, we applied these analyses in a large cohort of BRCA1/2 mutation carriers selected from the HEBON database, and showed a similar breast

cancer risk-reduction of approximately 50%.

In addition, we proposed a revised approach to the analyses in order to minimize bias as much as possible in observational studies in BRCA1/2 mutation carriers. In contrast with the previous analyses, we excluded prevalent cases – i.e. women with breast cancer and/or ovarian cancer diagnosis before DNA testing – in this proposed method of analysis. In addition, we included the breast cancer-free observation time before risk-reducing salpingo-oophorectomy and risk-reducing mastectomy thus better reflecting reality. With our proposed method of analysis, we found no evidence for breast cancer risk-reduction after risk-reducing salpingo-oophorectomy. This strengthens our idea that bias may indeed have played a role in previous estimates. Further, we observed a trend for breast cancer risk-reduction among BRCA2 mutation carriers, but not in BRCA1 mutation carriers.

General discussion

In **Chapter 7** the results described in this thesis are discussed and conclusions are drawn. Further, clinical implications and recommendations for future studies are described. Concerning the aims of this thesis we first concluded that risk-reducing mastectomy significantly reduces the development of breast cancer, both in healthy BRCA1/2 mutation carriers and in BRCA1/2 mutation carriers with a history of unilateral breast cancer. Maybe even more important is the conclusion that the prevention of breast cancer eventually results in improved survival. Although survival benefit after bilateral risk-reducing mastectomy needs to be confirmed, we observed a significantly improved overall survival in BRCA1/2 mutation carriers with a history of unilateral breast cancer who opted for contralateral risk-reducing mastectomy. Since survival benefit has shown not to apply to all BRCA1/2-associated breast cancer patients, we recommend the development of a prediction model – based on characteristics of the first tumour, age at diagnosis and type of mutation – which may facilitate more personalized advice regarding the option of contralateral risk-reducing mastectomy for BRCA1/2 mutation carriers with a history of unilateral breast cancer.

Another important conclusion of this thesis is that the breast cancer risk-reduction after risk-reducing salpingo-oophorectomy is overestimated in previously published studies, due to various types of bias. Although bias can probably not completely be ruled out in observational studies in BRCA1/2 mutation carriers, the influence of some important types of bias is minimized in our proposed method of analysis. With this method of analysis, we found no evidence of breast cancer risk-reduction after risk-reducing salpingo-oophorectomy in BRCA1/2 mutation carriers. We are very interested in the validation of the estimates of our method of analysis in the patient cohorts used in previously published studies reporting on breast cancer risk-reduction after risk-reducing salpingo-oophorectomy. Hopefully, the results of our study will open the discussion on the potential of bias in observational studies in BRCA1/2 mutation carriers. For the present, we recommend cautiousness about the message regarding breast cancer risk-reduction after risk-reducing salpingo-oophorectomy.

Finally, incidences and characteristics of BRCA1- and BRCA2-associated breast tumours have shown to be different. Therefore risk estimates for breast cancer occurrence and survival may be different for BRCA1 and BRCA2 mutation carriers. We observed lower incidence rates of both first and contralateral breast cancer cases for BRCA2 mutation carriers than for BRCA1 mutation carriers. Moreover, BRCA2-associated breast tumours were detected at more favourable stages. Although numbers of BRCA2 mutation carriers were too small to draw firm conclusions, our findings suggest that for BRCA2 mutation carriers, regular surveillance may lead to similar survival as risk-reducing mastectomy. Therefore, for BRCA2 mutation carriers, regular surveillance may be a reasonable alternative for this drastic and irreversible intervention. Further, although on average we found no evidence of breast cancer risk-reduction after risk-reducing salpingo-oophorectomy, for BRCA2 mutation carriers, risk-reducing salpingo-oophorectomy was (non-significantly) associated with a reduction of first breast cancer risk while this was not observed for BRCA1 mutation carriers. Confirmation of our findings in larger study populations – especially larger numbers of BRCA2 mutation carriers are needed to enable separate analyses for BRC1 and BRCA2 mutation carriers – will facilitate better tailored counselling regarding risk-reducing strategies, taking also into account the type of BRCA mutation.

Samenvatting

Vrouwen die draagster zijn van een kiembaanmutatie in één van de twee borstkanker genen (**BR**east **CA**ncer) BRCA1 en BRCA2 hebben een sterk verhoogd risico op het krijgen van borst- en/of eierstokkanker, vaak al op jonge leeftijd. BRCA1/2 mutatiedraagsters wordt geadviseerd om zich regelmatig te laten controleren, d.w.z. elke half jaar klinisch borstonderzoek en jaarlijks beeldvorming door 'magnetic resonance imaging' (MRI) en mammografie. Deze periodieke controle heeft als doel borstkanker in een zo vroeg mogelijk stadium te ontdekken en daarmee de kans op overlijden ten gevolge van borstkanker te verminderen. Regelmatige controle kan echter het ontstaan van borstkanker niet voorkomen, en geeft ook niet altijd de garantie dat kanker wordt ontdekt voordat uitzaaiingen naar bijvoorbeeld de lymfeklieren zijn ontstaan. Verder is gebleken dat gynaecologische controle niet leidt tot een vroege opsporing van eierstokkanker, en dus niet effectief is in het verlagen van eierstokkanker-specifieke mortaliteit. Een deel van de mutatiedraagsters kiest daarom voor risicoreducerende mastectomie (preventieve verwijdering van borstklierweefsel) en/of risicoreducerende salpingo-ovariëctomie (preventieve verwijdering van eierstokken en eileiders). Dit zijn ingrijpende beslissingen, zeker voor gezonde vrouwen die uit voorzorg de verwijdering van gezonde borsten en/of eierstokken en eileiders overwegen. Risicoreducerende mastectomie kan grote consequenties hebben voor het lichaamsbeeld, de zelfwaardering en de seksualiteit van vrouwen. Risicoreducerende salpingo-ovariëctomie op jonge leeftijd resulteert in een vroege menopauze met specifieke problemen, zoals onvruchtbaarheid en een verhoogd risico op botontkalking en hart- en vaatziekten op latere leeftijd. BRCA1/2 mutatiedraagsters die voor de moeilijke keus tussen regelmatige controle en risicoreducerende chirurgie staan, hebben baat bij nauwkeurige informatie over de effectiviteit van beide strategieën ten aanzien van het voorkómen van borstkanker en het overlijden aan deze ziekte. Het doel van dit proefschrift is meer inzicht te verkrijgen over (1) de effectiviteit van risicoreducerende mastectomie ten aanzien van het risico op borstkanker en de overleving in zowel gezonde BRCA1/2 mutatiedraagsters als in BRCA1/2 mutatiedraagsters met een voorgeschiedenis van borstkanker, en (2) het risico op en de tumorkarakteristieken van een eerste borstkanker na risicoreducerende salpingo-ovariëctomie.

Risicoreducerende mastectomie in het Erasmus Medisch Centrum te Rotterdam

Bij de Polikliniek Erfelijke Tumoren van het Erasmus Medisch Centrum te Rotterdam wordt een groot aantal vrouwen die bilaterale of contralaterale risicoreducerende mastectomie hebben ondergaan al vele jaren gevolgd. In het verleden hebben onderzoekers van ons instituut gerapporteerd over het vóórkomen van borstkanker na risicoreducerende mastectomie bij gezonde BRCA1/2 mutatiedraagsters, over complicaties na risicoreducerende mastectomie in combinatie met een borstreconstructie, en over psychologische aspecten van risicoreducerende mastectomie in combinatie met een borstreconstructie. In **Hoofdstuk 2**

van dit proefschrift wordt in een grotere, bijgewerkte serie (n=358) met een mediane follow-up van 4,5 jaar bevestigd dat het risico op het ontwikkelen van borstkanker na risicoreducerende mastectomie erg laag is (minder dan 5%) voor zowel gezonde als aangedane BRCA1/2 mutatiedraagsters, en voor vrouwen uit families met een verhoogd risico op borst- en/of eierstokkanker zonder bewezen BRCA mutatie. Verder bleek bijna de helft van de vrouwen die risicoreducerende mastectomie hadden ondergaan complicaties te krijgen, voornamelijk gerelateerd aan cosmetiek en kapselvorming. Deze complicaties leidden bijna altijd tot noodzakelijke aanvullende operaties.

Bij 3% van de vrouwen werden onverwachte (pre)maligne veranderingen in de mastectomiepreparaten gevonden. Dit benadrukt de noodzaak van preoperatieve beeldvorming en zorgvuldig histologisch onderzoek achteraf. Waarschijnlijk is het aantal onverwachte maligne bevindingen wel verminderd na de invoering van institutionele richtlijnen in het jaar 2000 ten aanzien van preoperatief borstonderzoek. In de richtlijnen wordt beeldvormend onderzoek maximaal 3 maanden vóór de ingreep geadviseerd, bij voorkeur door middel van MRI, aangezien dit screeningsinstrument gevoeliger is in het ontdekken van tumoren in jonge, hoog-risico vrouwen dan mammografie.

Bilaterale risicoreducerende mastectomie bij gezonde BRCA1/2 mutatiedraagsters

Door de toevoeging van MRI aan het screeningsprogramma voor BRCA1/2 mutatiedraagsters en de huidige beschikbaarheid van moderne (neo)adjuvante systemische therapieën, is gesuggereerd dat regelmatige controle – gericht op de vroege opsporing van borstkanker, met een gunstige prognose – leidt tot vergelijkbare overleving als bilaterale risicoreducerende mastectomie. In **Hoofdstuk 3** hebben we onderzocht of voor gezonde BRCA1/2 mutatiedraagsters bilaterale risicoreducerende mastectomie – bedoeld om borstkanker te voorkómen – toch resulteert in betere overleving in vergelijking met regelmatige controle.

In een prospectieve cohort studie met 570 BRCA1/2 mutatiedraagsters vonden we een aanzienlijke verlaging van de borstkankerincidentie na bilaterale risicoreducerende mastectomie, in vergelijking met de borstkankerincidentie bij vrouwen die onder regelmatige controle waren; in de mastectomiegroep werd geen borstkanker gevonden, terwijl 57 vrouwen in de controlegroep (16%) werden gediagnosticeerd met borstkanker. Bovendien waren zowel de algemene als de borstkanker-specifieke sterftecijfers lager na risicoreducerende mastectomie, hoewel de overlevingswinst nog niet significant was in deze studie.

Daarnaast vonden we een lagere borstkankerincidentie in BRCA2 mutatiedraagsters (7%) dan in BRCA1 mutatiedraagsters (20%). Bovendien werden alle BRCA2-geassocieerde tumoren in een gunstig stadium gevonden (tumoren kleiner dan 2 cm zonder uitzaaiingen in de lymfeklieren), terwijl 28% van de BRCA1-geassocieerde borsttumoren werden gedetecteerd in een minder gunstig stadium. Alle BRCA2 mutatiedraagsters waren nog in leven aan het eind van de observatieperiode. Hoewel het aantal BRCA2 mutatiedraagsters klein is in deze studie, suggereren onze bevindingen dat intensieve controle en bilaterale risicoreducerende

mastectomie tot vergelijkbare overleving leiden voor BRCA2 mutatiedraagsters.

Contralaterale risicoreducerende mastectomie in BRCA1/2 mutatiedraagsters met een voorgeschiedenis van eenzijdig borstkanker

In **Hoofdstuk 4** hebben we onderzocht of het voorkómen van contralateraal borstkanker door contralaterale risicoreducerende mastectomie ook leidt tot verbeterde overlevingskansen. Voor deze prospectieve studie selecteerden we 583 BRCA1/2 mutatiedraagsters met een voorgeschiedenis van eenzijdig borstkanker uit het bestand van de landelijke werkgroep **HE**reditair **B**orst en **O**variumkanker **N**ederland (HEBON). We konden bevestigen dat de contralaterale borstkankerincidentie sterk is verlaagd na contralaterale mastectomie; in de contralaterale mastectomiegroep ontwikkelden 4 patiënten (2%) contralateraal borstkanker, tegen 64 patiënten (19%) in de controlegroep. Bovendien vonden we een significant verbeterde overleving na contralaterale risicoreducerende mastectomie. De 15-jaars overleving was 86% in de contralaterale mastectomiegroep en 74% in de controlegroep. Aanvullende analyses toonden aan dat vooral jonge borstkankerpatiënten (40 jaar of jonger ten tijde van de diagnose), patiënten van wie de eerste borstkanker een gunstige differentiatiegraad vertoont en/of ten minste een receptor voor oestrogeen, progesteron of de 'human epidermal growth factor 2' (HER2) draagt, en patiënten die niet zijn behandeld met adjuvante chemotherapie profijt lijken te hebben van contralaterale risicoreducerende mastectomie.

Karakteristieken van borsttumoren gediagnosticeerd na risicoreducerende salpingo-ovariëctomie

Door risicoreducerende salpingo-ovariëctomie wordt de ontwikkeling van eierstokkanker en kanker in de eileiders met succes voorkomen, wat het primaire doel van deze interventie is. Eerdere studies hebben bovendien gerapporteerd dat na risicoreducerende salpingo-ovariëctomie het risico op borstkanker met 50% daalt. Het onderliggende mechanisme hiervoor is tot op heden niet duidelijk, hoewel verondersteld wordt dat het verlies van oestrogenen een rol kan spelen. Het is tot nu toe niet bekend of risicoreducerende salpingo-ovariëctomie invloed heeft op de karakteristieken en de groei van borsttumoren die zich na de ingreep manifesteren. In **Hoofdstuk 5** worden de resultaten beschreven van een pilot studie waarin de tumorkarakteristieken en de groeisnelheid zijn vergeleken van BRCA1/2-geassocieerde borstkankers die werden ontdekt met (n=20) en zonder voorafgaande risicoreducerende salpingo-ovariëctomie (n=36). Uit deze studie blijkt dat borsttumoren die zich manifesteren na risicoreducerende salpingo-ovariëctomie een significant lagere mitotische activiteit vertonen dan borsttumoren die niet zijn voorafgegaan door risicoreducerende salpingo-ovariëctomie. Vooral het aantal mitosen bleek significant lager, namelijk 12 mitosen/mm in de ovariëctomiegroep en 22 mitosen/mm^2 in de controlegroep. Verder bleek de verdubbelingstijd van het tumor volume (niet-significant) langer in de ovariëctomiegroep. Onze bevindingen suggereren dat na risicoreducerende salpingo-ovariëctomie borsttumoren

een minder agressief biologisch fenotype hebben.

Het risico op een eerste borstkanker na risicoreducerende salpingo-ovariëctomie

Studies die de effectiviteit van risicoreducerende strategieën in BRCA1/2 mutatiedraagsters onderzoeken kunnen op ethische gronden niet in een klinisch gerandomiseerd studieverband worden uitgevoerd, en zijn daarom gebonden aan een observationeel ontwerp. Hierdoor kunnen bepaalde vormen van selectiebias (een systemische fout in de selectie van de individuen die participeren in een studie) invloed hebben gehad op berekeningen in eerdere studies betreffende de vermindering van het borstkankerrisico na risicoreducerende salpingo-ovariëctomie. In **Hoofdstuk 6** bestudeerden we verschillende methodologische aspecten die van invloed kunnen zijn geweest op de berekening van de associatie tussen risicoreducerende salpingo-ovariëctomie en het optreden van een eerste borstkanker. We bespraken vier recente studies uit de literatuur die allemaal een vermindering van het borstkankerrisico van ongeveer 50% rapporteerden. De betreffende onderzoekers hebben verschillende methodes gebruikt om de data te analyseren, en in dit hoofdstuk bediscussieerden we mogelijke vormen van bias die geassocieerd kunnen zijn met deze analyses. Daarna hebben we dezelfde analysemethodes toegepast op onze eigen data, en ook wij vonden een vermindering van het borstkankerrisico van ongeveer 50%, waarmee is aangetoond dat ons cohort niet wezenlijk verschilt van de in eerdere studies gebruikte cohorten. Voor deze analyses selecteerden we BRCA1/2 mutatiedraagsters uit het landelijk HEBON bestand.

Vervolgens hebben we een voorstel gedaan voor een aantal aanpassingen in de analyses om bias nog meer te beperken in observationele studies in BRCA1/2 mutatiedraagsters. Eén van de belangrijkste verschillen met de eerdere analyses is dat bij deze aangepaste analysemethode vrouwen met een borst- en/of eierstokkankerdiagnose vóór de DNA test worden uitgesloten van de studie. Een ander belangrijk verschil is dat de borstkanker-vrije observatietijd die voorafgaat aan een risicoreducerende ingreep wel wordt meegenomen in de studie. Met deze aanpassingen in de analyse vonden we geen bewijs voor vermindering van het borstkankerrisico na risicoreducerende salpingo-ovariëctomie. Dit versterkt ons idee dat bias inderdaad een rol heeft gespeeld in de risicoschattingen uit het verleden. Overigens vonden we wel een trend voor vermindering van het borstkankerrisico bij BRCA2 – maar niet bij BRCA1 – mutatiedraagsters.

Algemene discussie

In **Hoofdstuk 7** worden de resultaten uit de voorafgaande hoofdstukken in een breder perspectief geplaatst en worden conclusies getrokken. Verder worden de klinische implicaties en aanbevelingen voor toekomstige studies beschreven. Voor wat betreft de doelstellingen van dit proefschrift concludeerden we in de eerste plaats dat het risico op borstkanker zowel in gezonde BRCA1/2 mutatiedraagsters als in BRCA1/2 mutatiedraagsters met een voorgeschiedenis van eenzijdig borstkanker significant is verminderd ten gevolge

van risicoreducerende mastectomie. Misschien nog wel belangrijker is de conclusie dat het voorkómen van borstkanker uiteindelijk resulteert in een verbeterde overleving. Hoewel de overlevingswinst voor gezonde mutatiedraagsters nog definitief bevestigd moet worden, vonden we voor BRCA1/2-geassocieerde borstkankerpatiënten die contralaterale risicoreducerende mastectomie ondergingen wel een significant verbeterde algemene overleving. Verder bleek dat deze overlevingswinst waarschijnlijk niet voor alle BRCA1/2-geassocieerde borstkankerpatiënten geldt. Het verdient daarom aanbeveling om contralaterale risicoreducerende mastectomie alleen te adviseren voor die patiënten met een laag risico op het overlijden aan de eerste borstkanker en een hoog risico op het krijgen van contralateraal borstkanker. Een in de toekomst te ontwikkelen predictie-model – gebaseerd op tumorkenmerken van de eerste borstkanker, leeftijd bij diagnose en type mutatie – kan helpen bij een meer persoonsgericht advies ten aanzien van contralaterale risicoreducerende mastectomie voor BRCA1/2 mutatiedraagsters met een voorgeschiedenis van borstkanker.

Een andere belangrijke conclusie in dit proefschrift is dat de in eerdere studies gevonden vermindering van het borstkankerrisico van ongeveer 50% na risicoreducerende salpingo-ovariëctomie mogelijk is overschat als gevolg van verschillende vormen van bias. Hoewel bias waarschijnlijk nooit helemaal is uit te sluiten in observationele studies in BRCA1/2 mutatiedraagsters, is in de door ons voorgestelde methode van analyseren de invloed van een aantal belangrijke vormen van bias geminimaliseerd. Wanneer we deze methode van analyseren toepasten, vonden we geen bewijs voor vermindering van het borstkankerrisico na risicoreducerende salpingo-ovariëctomie in BRCA1/2 mutatiedraagsters. Het zou bijzonder interessant zijn om te weten welke risicoschattingen andere onderzoekers vinden wanneer zij de door ons voorgestelde methode van analyseren zouden toepassen. Hopelijk vormen de resultaten van onze studie een opening tot discussie rondom bias in observationele studies in BRCA1/2 mutatiedraagsters. Vooralsnog lijkt het raadzaam om de boodschap dat risicoreducerende salpingo-ovariëctomie, naast de sterke vermindering van het risico op eierstokkanker en kanker in de eileiders, ook leidt tot een vermindering van het risico op borstkanker met terughoudendheid te brengen.

Tot slot is gebleken dat er verschillen zijn ten aanzien van de incidentie en tumorkarakteristieken van BRCA1- en BRCA2-geassocieerde borsttumoren, met mogelijke implicaties voor het klinisch beleid. Wij vonden voor BRCA2 mutatiedraagsters een lagere incidentie van zowel de eerste als de contralaterale borsttumoren dan voor BRCA1 mutatiedraagsters. Bovendien werden BRCA2-geassocieerde borsttumoren in een gunstiger stadium gevonden. Hoewel het aantal BRCA2 mutatiedraagsters in onze studies te klein is om significante verschillen aan te tonen, suggereren onze bevindingen dat voor BRCA2 mutatiedraagsters regelmatige controle mogelijk tot eenzelfde overleving zou kunnen leiden als risicoreducerende mastectomie. Regelmatige controle zou voor BRCA2 mutatiedraagsters daarom een redelijk alternatief kunnen zijn voor deze ingrijpende en onomkeerbare ingreep. Verder bleek dat voor BRCA2 mutatiedraagsters, hoewel we overall geen vermindering van

het borstkankerrisico na risicoreducerende salpingo-ovariëctomie vonden, risicoreducerende salpingo-ovariëctomie (niet-significant) was geassocieerd met een vermindering van het risico op een eerste borstkanker, terwijl we dit niet zagen voor BRCA1 mutatiedraagsters. Wanneer onze bevindingen worden bevestigd in grotere studiepopulaties, in het bijzonder met grotere aantallen BRCA2 mutatiedraagsters, zodat BRCA1 en BRCA2 mutatiedraagsters apart geanalyseerd kunnen worden, kunnen adviezen met betrekking tot risicoreducerende strategieën gerichter worden gegeven waarbij ook het type mutatie in ogenschouw kan worden genomen.

Dankwoord

Het is volbracht. Er is een boekje en mijn naam staat erop! Wie mij een kleine 20 jaar geleden had voorspeld dat dit moment toch nog zou komen had ik voor gek verklaard. Maar wat ben ik er blij mee! Een proefschrift komt echter nooit tot stand zonder de hulp van velen. Graag wil ik iedereen die op wat voor manier dan ook een bijdrage heeft geleverd van harte bedanken. Met veel plezier wil ik een aantal mensen bij naam noemen.

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onze beschikking hebben en die heel veel data voor de studies beschreven in dit proefschrift heeft gegenereerd. Maar er is meer. Jan, ik herinner me nog goed dat je tijdens mijn sollicitatiegesprek vroeg of mijn ambities niet verder reikten dan de functie van datamanager. Ik meende dat ik na de hectiek van het ICT-wereldje, in combinatie met een gezin met toen nog jonge kinderen, mijn ei voorlopig wel kwijt zou kunnen in deze functie. Jan, je had gelijk. Cecile, ik denk dat jij eerder dan ik in de gaten had dat Jan misschien wel gelijk had. Je gaf me alle ruimte om mijn werkzaamheden zelf in te vullen, binnen de grenzen van de functie-omschrijving maar zeker ook daarbuiten. Daarnaast bood je me, samen met Caroline, de mogelijkheid om mijn eerste artikel over dit onderwerp te schrijven. Bedankt Jan en Cecile!

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Dr. M. Rookus, beste Matti, je enthousiasme voor epidemiologisch onderzoek werkt aanstekelijk. Bedankt voor al je input voor m.n. het laatste artikel, onze samenwerking heeft een mooi stuk opgeleverd. Nu maar hopen dat de reviewers dat ook vinden!

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Curriculum vitae

Annette Heemskerk-Gerritsen werd geboren op 18 april 1965 te Maarheeze. Zij behaalde haar VWO diploma in 1984 aan de Scholengemeenschap Werenfridus te Hoorn en startte na veel twijfels met de studie Nederlandse Taal- en Letterkunde aan de Universiteit van Amsterdam. Tijdens het propedeusejaar bleek deze opleiding toch niet aan haar verwachtingen te voldoen, waarna zij in 1985 begon met de studie Medisch Biologie aan de Vrije Universiteit van Amsterdam. Na het behalen van haar doctoraal examen in 1990, werkte zij een jaar als research analist in het Jan van Breemen Instituut te Amsterdam. Van 1992 tot 1996 deed zij promotie-onderzoek bij de afdeling Longziekten van het Leids Universitair Medisch Centrum, wat echter niet resulteerde in de totstandkoming van een proefschrift. Na een jaar gewerkt te hebben als trial assistent bij de firma Bicom Medical B.V., besloot zij tot een carrière-switch en maakte de overstap naar de ICT. Van 1998 tot 2004 was zij werkzaam als ontwerper, ontwikkelaar en consultant bij PinkRoccade, Capelle a/d IJssel, en bij Bergler ICT, Breda. De medisch-wetenschappelijke onderzoekswereld bleef echter trekken, en in 2004 begon zij als datamanager bij de Werkgroep Erfelijke Tumoren binnen de afdeling Interne Oncologie van het Erasmus MC Kankerinstituut te Rotterdam, een functie waarin zij haar biomedische achtergrond en haar kennis en ervaring op het gebied van databases perfect kon combineren. In 2009 begon zij aan haar promotie-traject bij dezelfde werkgroep, met als afsluiting dit proefschrift. In 2010 startte zij met de Master of Science opleiding Clinical Epidemiology aan het Netherlands Institute for Health Sciences te Rotterdam, en behaalde het diploma in 2012. Momenteel houdt zij zich bezig met het schrijven van een aantal vervolgprojecten en het aanvragen van de benodigde subsidies, met als doel haar onderzoek bij vrouwen met een verhoogd risico op het krijgen van borst- en/of ovariumkanker als gevolg van een BRCA1/2 mutatie als post-doc te vervolgen.

List of publications

Heemskerk-Gerritsen BAM, Rookus MA, Aalfs CM, Aussems MGEM, Collée JM, Jansen L, Kets C, Keymeulen KBMI, Koppert LB, Meijers-Heijboer HEJ, Mooij TM, Tollenaar RAEM, Vasen HFA, HEBON, Hooning MJ, Seynaeve C. Improved overall survival after contralateral risk-reducing mastectomy in BRCA1/2 mutation carriers with a history of unilateral breast cancer: a prospective analysis. *International Journal of Cancer:* DOI: 10.1002/ijc.29032 [Epub ahead of print], 2014

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Heemskerk-Gerritsen BAM, Menke-Pluijmers MBE, Jager A, Tilanus-Linthorst MMA, Koppert LB, Obdeijn IMA, van Deurzen CHM, Collée JM, Seynaeve C, Hooning MJ. Substantial breast cancer risk reduction and potential survival benefit after bilateral mastectomy when compared with surveillance in healthy BRCA1 and BRCA2 mutation carriers: a prospective analysis. *Annals of Oncology* **24**:2029-2035, 2013

Vencken PMLH, Kriege M, Hooning M, Menke-Pluymers MB, **Heemskerk-Gerritsen BAM**, van Doorn LC, Collée JM, Jager A, van Montfort C, Burger CW, Seynaeve C. The risk of primary and contralateral breast cancer after ovarian cancer in BRCA1/BRCA2 mutation carriers. *Cancer* **119**:955-962, 2013

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Heemskerk-Gerritsen BAM, Dijkman JH, ten Have-Opbroek AA. Stereological methods: a new approach in the assessment of pulmonary emphysema. *Microscopy Research and Technique* **34**:556-562, 1996.

PhD Portfolio

Summary of PhD training and teaching

Name PhD student: BAM Heemskerk-Gerritsen — PhD period: 1-10-2009 – 1-3-2014
Erasmus MC Department: Medical Oncology — Promotor: Prof. dr. J Verweij
Research School: NIHES — Supervisors: Dr. C Seynaeve, Dr. MJ Hooning

PhD training	**Year**	**Workload (ECTS)**
General courses		
Methodologie van patiëntgebonden onderzoek en voorbereiding van subsidieaanvragen	2005	0.5
Oncologisch spectrum	2008	1.4
BROK 'Basiscursus Regelgeving Klinisch Onderzoek'	2010	0.3
English Biomedical Writing and Communication	2013	4
Specific courses		
NIHES, Master of Science programme Clinical Epidemiology		
Erasmus Summer Programme		
- Principles of research in medicine	2010	0.7
- Clinical decision analysis	2010	0.7
- Methods of public health research	2010	0.7
- Clinical trials	2010	0.7
- Pharmaco-epidemiology	2010	0.7
- Markers and prognostic factors	2010	0.7
- Conceptual foundation of epidemiologic study design	2011	0.7
- The practice of epidemiologic analysis	2011	0.7
Core Curriculum		
- Study design	2010	4.3
- Methodologic topics in epidemiologic research	2010	1.4
- Biostatistical methods I: Basic principles	2011	5.7
- Biostatistical methods II: Popular regression models	2011	4.3
- Clinical epidemiology	2011	5.7
- Research proposal	2012	2.5
Advanced short courses		
- Course for the quantitative researcher	2010	1.4
- Missing values in clinical research	2011	0.7
- Prognosis research	2011	0.9
- Principles of epidemiologic data-analysis	2011	0.7

- Repeated measurements in clinical studies	2012	1.4
- Bayesian statistics	2012	1.1
- Cancer epidemiology	2012	1.4
Skill courses		
- English language	2010	1.4
- Introduction to medical writing	2010	1.1
- Working with SPSS for windows	2010	0.5

Oral presentations

Annual HEBON Workshop	2008	1
Scientific Meeting Department of Medical Oncology, Erasmus MC	2010	1
Annual HEBON Workshop	2011	1
35th Symposium Werkgroep Epidemiologisch Onderzoek Nederland	2011	1
Annual HEBON Workshop	2012	1
49th Annual Meeting American Society of Clinical Oncology	2013	1
Scientific Meeting Department of Medical Oncology, Erasmus MC	2013	1
Joint Meeting UK/Dutch Clinical Genetics Societies & Cancer Genetics Groups	2014	1
9th European Breast Cancer Conference	2014	1

Poster discussion presentations

31st San Antonio Breast Cancer symposium	2008	1
7th European Breast Cancer Conference	2010	1
8th European Breast Cancer Conference	2012	1

Poster presentations

5th European Breast Cancer Conference	2006	1
Joint Meeting of the UK Cancer Genetics Group and Dutch Cancer Society with the 10th International meeting on the Psychosocial Aspects of Genetic Testing	2007	1
17th Annual Meeting European Cancer Organization	2013	1

International conferences

5th European Breast Cancer Conference (Nice, France)	2006	1
Joint Meeting of the UK Cancer Genetics Group and Dutch Cancer Society with the 10th International meeting on the Psychosocial Aspects of Genetic Testing (Manchester, UK)	2007	1
31st San Antonio Breast Cancer symposium (San Antonio, Tx, USA)	2008	1
7th European Breast Cancer Conference (Barcelona, Spain)	2010	1
34th Symposium Werkgroep Epidemiologisch Onderzoek Nederland (Nijmegen, the Netherlands)	2010	1

35th Symposium Werkgroep Epidemiologisch Onderzoek Nederland (IJmuiden, the Netherlands)	2011	1
8th European Breast Cancer Conference (Vienna, Austria)	2012	1
49th Annual Meeting American Society of Clinical Oncology (Chicago, Ill, USA)	2013	1
17th Annual Meeting European Cancer Organization (Amsterdam, the Netherlands)	2013	1
Joint Meeting UK/Dutch Clinical Genetics Societies & Cancer Genetics Groups (Leiden, the Netherlands)	2014	1
9th European Breast Cancer Conference (Glasgow, UK)	2014	1

Seminars and workshops

Annual HEBON Workshop 2008	2008	1
Annual HEBON Workshop 2009	2009	1
Annual HEBON Workshop 2011	2011	1
Annual HEBON Workshop 2012	2012	1
Annual HEBON Congress Day 2013	2013	1