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Breast Cancer Risk After Salpingo-Oophorectomy in Healthy BRCA1/2 Mutation Carriers: Revisiting the Evidence for Risk Reduction

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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. The 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. Cox proportional hazards models were used to calculate hazard ratios and conditional logistic regression to calculate odds ratios. Secondly, we analyzed the data in a revised design in order to further minimize bias using an extended Cox model with RRSO as a time-dependent variable to calculate the hazard ratio. 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: Applying the four previously described analytical methods and the data of 551 to 934 BRCA1/2 mutation carriers with a median follow-up of 2.7 to 4.6 years, the odds ratio was 0.61 (95% confidence interval [CI] = 0.35 to 1.08), and the hazard ratios were 0.36 (95% CI = 0.25 to 0.53), 0.62 (95% CI = 0.39 to 0.99), and 0.49 (95% CI = 0.33 to 0.71), being similar to earlier findings. For the revised analysis, we included 822 BRCA1/2 mutation carriers. After a median follow-up period of 3.2 years, we obtained a hazard ratio of 1.09 (95% CI = 0.67 to 1.77).

Conclusion: In previous studies, BC risk reduction after RRSO in BRCA1/2 mutation carriers may have been overestimated because of bias. Using a design that maximally eliminated bias, we found no evidence for a protective effect.

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Women with a BRCA1 or BRCA2 mutation have substantially higher risks of developing both 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 because 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 subject to more 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). Because in all studies RRSO was consistently associated with a BC risk reduction of approximately 50%, this observation has been widely communicated in counseling practice. Also, the estimated BC risk reduction after RRSO has been included in a prediction model regarding BC risks in female BRCA1/2 mutation carriers (23).

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). Three major types of selection bias—ie, cancer-induced testing bias, immortal person-time bias, and informative censoring—may have had consequences for the observed risk estimates in these four studies. For a detailed description of these types of selection bias and the possible consequences, please see the Supplementary Materials (available online), in which a theoretical background is presented.

In this article, 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.

Methods

First, we replicated the analyses of four previous studies, performed by Eisen et al. (18), Kauff et al. (21), and Domchek et al. (20,22), within a Dutch cohort, to examine if this cohort was comparable with the cohorts used in the previous studies. Second, we estimated the effect of RRSO on BC risk in the Dutch cohort using the following design and analyses in order to minimize bias as much as possible. For the proposed design, the observation period starts 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.

Study Population

In the context of an ongoing nationwide study on hereditary breast and ovarian cancer in the Netherlands (the Hereditary Breast and Ovarian Cancer in the Netherlands [HEBON] study) (26), 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 centers, the Netherlands Cancer Institute, and the Foundation for the Detection of Hereditary Tumours. Data on patient and tumor characteristics and on preventive strategies were retrospectively as well as prospectively retrieved and updated through medical files and questionnaires, and 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 centers.

For replication of the analyses in the four reviewed studies, we selected female BRCA1 and BRCA2 mutation carriers from the HEBON cohort according to the eligibility criteria and designs as indicated in Table 1. For our proposed design, we used the following eligibility criteria to select female BRCA1/2 mutation carriers from the HEBON cohort: 1) no history of cancer at the date of DNA test result, 2) both breasts and both ovaries in situ at the date of DNA test result, and 3) no cancer diagnosis within the first six months of study observation.

Data Collection

We retrieved data on type of mutation (ie, 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 by Eisen et al. (18), 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, using the non-RRSO group as the reference group. For the cohort studies by Kauff et al. (21) and by Domchek et al. (20,22), we used multivariate Cox proportional-hazard models to obtain hazard ratios (HRs) with 95% CIs. In the replicated matched cohort study (20), we adjusted for type of BRCA mutation and center. In the replicated unmatched cohort study described by Kauff et al. (21), 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. (22), we adjusted for year of birth. Additionally, for replication of the analyses of both Domchek studies (20,22), we used a robust variance-covariance estimation method to correct for nonindependence of observations among participants from the same family and centers.

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

Eligibility criteria/study design	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 y)	RRSO/date of entry into research program*
Matching	Year of birth Country of residence Type of mutation (BRCA1/BRCA2)	Age at RRSO	NA	NA
Eligibility criteria	No history of OC Both breasts in situ Control patient had at least no BC diagnosis until age at BC of matched case patient	Cancer-free at enrolment† Cancer-free before RRSO Matched control patients cancer-free at time of participant's RRSO Both breasts in situ No cancer diagnosis within six months after enrolment†	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 six 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† 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‡	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§	2.5/2.1‖	4.2/4.9§
Age at start FU RRSO/non-RRSO	NA	45(8.5)/43(10.0)§	46(32–79)/39(30–88)§	44(21–79)/36(18–90)§
Year of birth RRSO/non-RRSO, y	NR	1955/1957§	NR	NR
% DNA testing after cancer diagnosis	NR	NR	0%	NR
Risk reduction (95% CI)	OR = 0.46 (0.32 to 0.65)	HR = 0.36 (0.20 to 0.67)¶,#	HR = 0.53 (0.29 to 0.96)**	HR = 0.54 (0.37 to 0.79)¶,††

* Study entry between 1974 and 2008. BC = breast cancer; CBC = contralateral breast cancer; CI = confidence interval; FU = follow-up; HR = hazard ratio; N = number; NA = not applicable; NR = not recorded; OC = ovarian cancer; OR = odds ratio; RRM = risk-reducing mastectomy; RRSO = risk-reducing salpingo-oophorectomy.
† Moment of enrolment not specified.
‡ RRSO group, 47%; non-RRSO group, 37%.
§ Mean years (standard deviation).
‖ Median years (range).
¶ A robust variance-covariance estimation method was used to correct for nonindependence of observations in women from the same family or centers.
# Adjusted for type of mutation (BRCA1/BRCA2) and center.
** Adjusted for age at start of follow-up, parity, previous BC, prior hormone replacement therapy, type of mutation (BRCA1/BRCA2).
†† Adjusted for year of birth and stratified by center.

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 years (because the youngest age at RRSO in this cohort was 31 years), 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 (ie, 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 hazard ratios and accompanying 95% confidence intervals, using the non-RRSO group as the reference group. We used a robust variance-covariance estimation method to correct for nonindependence of observations in women from the same family. The following variables were considered as potential confounders: year of birth, type of mutation, center, and parity (yes/no). We incorporated a variable in the multivariate Cox model if: 1) there was a statistically significant difference in the

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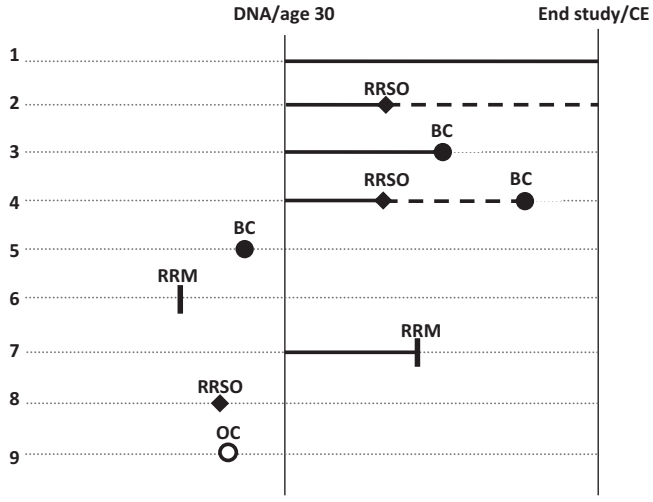

Figure 1. Design of the proposed analytical method and allocation of person-years of observation. Observation started at the age at DNA test result, or age 30 years, whichever came last (scenarios 1, 2, 3, 4, 7). For women not opting for risk-reducing salpingo-oophorectomy (RRSO), we allocated all person-years of observation (PYO) to the non-RRSO group (**solid lines**, scenarios 1, 3, 7). For women undergoing RRSO, we allocated PYO before surgery as well as a latency period of three months after RRSO to the non-RRSO group, and PYO thereafter to the RRSO group (**dashed lines**, scenarios 2 and 4). The observation ended on the age at first breast cancer diagnosis, age at risk-reducing mastectomy, age at diagnosis of another tumor (including ovarian cancer), age at last contact, age at death or age at study closing date (ie, 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. BC = first breast cancer; CE = censoring event; DNA = date of DNA test result; OC = ovarian cancer; RRM = risk-reducing mastectomy; RRSO = risk-reducing salpingo-oophorectomy.

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 (27,28). 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, ie, the time before RRSO, was excluded from the analysis. Third, we examined the effect on BC risk when RRSO was performed under the age of 51 years, being the mean age of postmenopausal status in the Netherlands, and at the age of 51 years and above.

All P values were two-sided, and a statistical significance level $\alpha = .05$ was used. Analyses were performed with STATA (Version 12.0, StataCorp, College Station, TX).

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%. The respective hazard ratios were 0.36 (95% CI = 0.25 to 0.53), 0.62 (95% CI = 0.39 to 0.99), and 0.49 (95% CI = 0.33 to 0.71), and the odds ratio was 0.61 (95% CI = 0.35 to 1.08) (Table 2).

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 vs 3.1 years, $P < .001$), and started at older age (median 44 vs 33 years, $P < .001$). In the non-RRSO group, more women were censored because of RRM (50.8% vs 22.8%, $P < .001$) at younger age than in the RRSO group (median 35 vs 45 years, $P < .001$). Numbers of death were similar in both groups (2.3% in the RRSO group and 1.9% in the non-RRSO group), but in the non-RRSO group women died at younger age (median 44 vs 57 years, $P = .004$).

Breast Cancer Incidence

We observed no difference in BC incidence between the RRSO and the non-RRSO group (12.1% and 9.9%, $P = .31$) (Table 3) after a median follow-up period of 3.2 years. Likewise, the BC incidence rate after RRSO was not different from that in the non-RRSO group (25.6 vs 21.5 per 1000 PYO) (Figure 2), yielding a hazard ratio of 1.09 (95% CI = 0.67 to 1.77) (Figure 2). Furthermore, the cumulative BC risk curves for both groups were not different (Figure 2).

Additional Analyses

We observed no statistically significant BC risk reduction after RRSO in the gene-stratified analyses; the hazard ratio was 1.21

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

Eligibility criteria/study design	Eisen, 2005 (18)	Domchek, 2006 (20)	Kauff, 2008 (21)	Domchek, 2010 (22)
Study design	Case-control	Matched cohort	Unmatched cohort	Unmatched cohort
N RRSO/non-RRSO	56/849	208/343	333/364*	342/592
FU RRSO/non-RRSO†	NA	5.3/1.9	4.8/2.8	4.8/4.6
Age at start FU RRSO/ non-RRSO†	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†	1955/1964	1960/1962	1958/1970	1958/1972
% DNA testing after cancer diagnosis RRSO/ non-RRSO	7.6/42.3	4.5/27.3	0	0.91/8.3
Risk reduction (95% CI)	OR = 0.61 (0.35 to 1.08)‡	HR = 0.36 (0.25 to 0.53)§	HR = 0.62 (0.39 to 0.99)‖	HR = 0.49 (0.33 to 0.71)§,¶

* Only participants without a history of breast cancer at start of follow-up. CI = confidence interval; FU = follow-up; HEBON = Hereditary Breast and Ovarian Cancer Research Group Netherlands; HR = hazard ratio; N = number; NA = not appicable; OR = odds ratio; RRM = risk-reducing mastectomy; RRSO = risk-reducing salpingo-oophorectomy.
† Median years (range).
‡ The non-RRSO group was used as the reference group.
§ A robust variance-covariance estimation method was used to correct for nonindependence of observations in women from the same family or centers.
‖ Adjusted for age at start of follow-up and type of mutation (BRCA1/BRCA2).
¶ Adjusted for year of birth.

(95% CI = 0.72 to 2.06) for BRCA1 mutation carriers and 0.54 (95% CI = 0.17 to 1.66) for BRCA2 mutation carriers (Table 4). The sensitivity analyses in subgroups of participants revealed no BC risk reduction after RRSO; when participants ever undergoing RRM were excluded the hazard ratio was 1.10 (95% CI = 0.66 to 1.84), and when the PYO between dates of DNA disclosure and RRSO were left out the hazard ratio was 0.78 (95% CI = 0.51 to 1.19) (Table 4). Further, we obtained a hazard ratio of 1.11 (95% CI = 0.65 to 1.90) when RRSO was performed under the age of 51 years and a hazard ratio of 1.78 (95% CI = 0.52 to 6.15) when RRSO was performed at age 51 years and above (data not shown).

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.

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 with 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, ie, 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 vs 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 (50.8% vs 22.8%). 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, because 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, 79.8% of the women who were censored because of RRM were under the age of 40 years 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

Table 3. Characteristics of the study population selected from the HEBON cohort used for the proposed design and analysis

Variable	RRSO No. (%)	Non-RRSO No. (%)	*P*
Total	346 (42.1)	476 (57.9)	
Observation period, mean (range), y*	6.8 (0.5–17.4)	3.1 (0.1–15.9)	<.001¶
Age at start of observation, y			
30–39	110 (31.8)	376 (79.0)	<.001#
40–49	147 (42.5)	75 (15.8)	
50–59	78 (22.5)	21 (4.4)	
≥60	11 (3.2)	4 (0.8)	
Median (range)	44 (30–66)	33 (30–66)	<.001¶
Median year at start of observation (range)	2003 (1994–2011)	2003 (1994–2013)	.47¶
Mutation status			
BRCA1	246 (71.1)	343 (72.1)	.81**
BRCA2	100 (28.9)	133 (27.9)	
Median age at DNA testing (range), y	44 (24–66)	33 (18–66)	<.001¶
Median year at DNA testing (range)	2003 (1994–2011)	2002 (1994–2013)	.14¶
Year of birth			
1940–1949	61 (17.6)	17 (3.6)	<.001#
1950–1959	117 (33.8)	68 (14.3)	
1960–1969	134 (38.7)	181 (38.0)	
1970–1979	34 (9.9)	177 (37.2)	
≥ 1980	0 (0)	33 (6.9)	
Median (range)	1959 (1940–1976)	1968 (1940–1983)	<.001¶
Parity†			
Nulliparous	23 (12.4)	59 (19.7)	.05**
Parous	162 (87.6)	241 (80.3)	
Unknown	176	161	
RRSO			
Median age (range), y	45 (31–67)	-	
Median year (range)	2005 (1994–2013)	-	
Mean observation time (range), y			
before RRSO	1.9 (0.1–11.7)	-	
after RRSO	4.9 (0.3–16.2)	-	
Censoring events			
Ovarian cancer	5‡ (1.5)	9 (1.9)	.79**
Median age, y (range)	56 (45–57)	39 (32–58)	.04¶
Median year (range)	2003 (2000–2013)	2003 (1998–2010)	.50¶
RRM	79 (22.8)	242 (50.8)	<.001**
Age at RRM, y			
30–39	12 (14.3)	182 (74.3)	<.001#
40–49	48 (57.1)	54 (22.0)	
50–59	23 (27.4)	7 (2.9)	
≥60	1 (1.2)	2 (0.8)	
Median (range), y	45 (33–65)	35 (30–65)	<.001¶
Median year (range)	2004 (1996–2012)	2001 (1994–2013)	.02¶
Death§	8 (2.3)	9 (1.9)	.81**
Median age (range), y	57 (45–68)	44 (34–60)	.004¶
Median year (range)	2010 (2007–2011)	2006 (2002–2012)	.02¶
History of BC and/or OC			
No	5 (62.5)	1 (11.2)	.03#
BC	3 (37.5)	4 (44.4)	
OC	0 (0)	4 (44.4)	
BC and OC	0 (0)	0 (0)	
Event of interest			
BC	42 (12.1)	47‖ (9.9)	.31**
Age at BC diagnosis, y			
≤35	0 (0)	17 (36.2)	<.001#
35–50	23 (54.8)	26 (55.3)	
≥50	19 (45.2)	4 (8.5)	
Median (range), y	50 (35–66)	37 (30–71)	<.001¶
Median year (range)	2008 (1998–2012)	2006 (1998–2012)	.30¶

Table 3. *Continued*

Variable	RRSO No. (%)	Non-RRSO No. (%)	*P*
Mean observation time (range), y*	5.5 (0.6–14.1)	3.9 (0.5–12.7)	.09¶
Mean time after RRSO (range), y	4.2 (0.4–13.7)	-	

* Start of observation is either age at DNA diagnosis or 30th birthday, whichever came last. BC = first breast cancer; HEBON = Hereditary Breast and Ovarian Cancer Research Group Netherlands; RRM = risk-reducing mastectomy; RRSO = risk-reducing salpingo-oophorectomy.
† As reported at the moment of signing the informed consent.
‡ Extra-ovarian peritoneal cancer diagnosed after RRSO.
§ All causes.
‖ Two BCs were diagnosed within the latency period of three months after RRSO.
¶ Two-sided Wilcoxon rank-sum (Mann-Whitney) test.
# Two-sided Pearson Chi-square test.
** Two-sided Fisher's exact test.

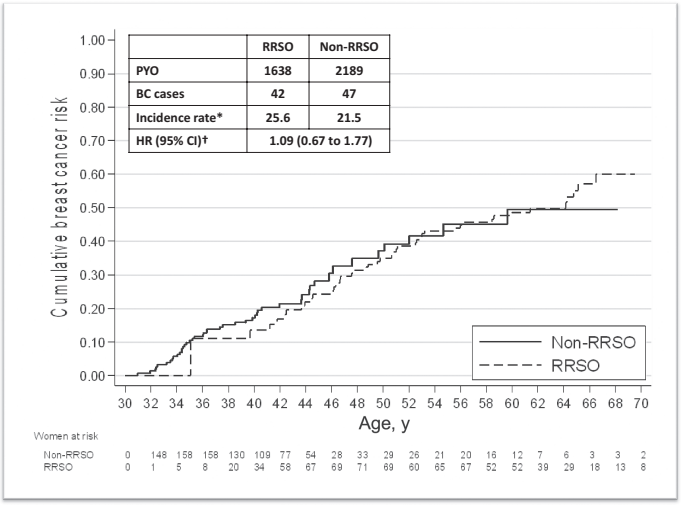

Figure 2. Cumulative breast cancer risk curves for BRCA1/2 mutation carriers selected from the Hereditary Breast and Ovarian Cancer Research Group Netherlands cohort opting for risk-reducing salpingo-oophorectomy (RRSO) vs not opting for risk-reducing salpingo-oophorectomy (non-RRSO). The Simon and Makuch method was used, with chronological age as the time variable. This method takes into account the change in an individual's covariate status over time. *per 1000 person-years of observation. †Univariate analysis. Variables that were considered as potential confounders—ie, type of mutation, year of birth, and center—did not meet the criteria for incorporation in a multivariable Cox model as described in the Methods section and were therefore not included in the analysis. Eventually, parity was not considered as a potential confounder because of the large proportion (41.0%) of missing data on this variable. A robust variance-covariance estimation method was used to correct for nonindependence of observations in women from the same family. BC = breast cancer; CI = confidence interval; HR = hazard ratio; PYO = person-years of observation.

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. Notably, 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 slightly 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 an important role during tumor genesis of BC (29,30). Given the fact that BRCA2-associated BCs mainly are ER positive, while the majority of BRCA1-associated BCs are ER negative (31), 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

Table 4. Additional analyses

Type of analysis	PYO with/without RRSO	BC cases with/without RRSO	Incidence rate* with/without RRSO	HR (95% CI)†
Stratified analyses				
BRCA1	1238/1609	36/39	29.1/24.2	1.21 (0.72 to 2.06)
BRCA2	400/580	6/8	14.9/13.8	0.54 (0.17 to 1.66)
Sensitivity analyses‡				
I	1521/1754	40/40	26.3/22.8	1.10 (0.66 to 1.84)
II	1638/1463	47/42	25.6/32.1	0.78 (0.51 to 1.19)

* Per 1000 person-years of observation. BC = first breast cancer; CI = confidence interval; HR = hazard ratio; PYO = person-years of observation; RRSO = risk-reducing salpingo-oophorectomy.

† Univariate analysis. A robust variance-covariance estimation method was used to correct for nonindependence of observations in women from the same family.

‡ Sensitivity analyses concerning:

I, exclusion of participants ever undergoing risk-reducing mastectomy;

II, no allocation of PYO between date of DNA test result and date of RRSO.

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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