Pre-menopausal bilateral salpingo-oophorectomy and breast cancer risk for carriers of *BRCA1* and *BRCA2* pathogenic variants: A pooled cohort analysis

Whether risk-reducing bilateral salpingo-oophorectomy (rrBSO) reduces breast cancer (BC) risk for *BRCA1* and *BRCA2* mutation carriers is uncertain[1]. All studies of rrBSO and BC risk are observational in nature, and subject to various forms of bias and confounding, thus limiting conclusions that can be drawn about causation. Early studies generally supported a statistically significant protective association for rrBSO on BC risk[2-15]. However, these historical studies were hampered by the presence of several important biases, including immortal person-time bias, confounding by indication, informative censoring, and confounding by other risk factors, which may have led to over-estimation of any protective benefit (Table 1)[16-19].

Contemporary studies, specifically designed to reduce some of these biases, have yielded contradictory results[19-27]. Several of these studies had overlapping samples. It may be most relevant to focus on studies of pre-menopausal rrBSO, given that any protective association between rrBSO and BC risk would only be biologically plausible for pre-menopausal rrBSO, because post-menopausal rrBSO does not alter circulating levels of female hormones. Of the five studies[20,23-25,26] that assessed the association between pre-menopausal rrBSO and BC risk in *BRCA1* mutation carriers (using the average age of menopause in the general

population, 50 or 51 years, as a surrogate for actual menopausal status), only Stjepanovic et al[25] showed clear evidence of a protective association. The other four studies reported HRs between 0.84 and 1.55, and confidence intervals including 1. Conversely, all four studies[20,23-25] of rrBSO in pre-menopausal *BRCA2* mutation carriers reported point estimates <1 (HR 0.17-0.77) however, apart from Kotsopoulos et al. 2017[20], the confidence interval included 1 in the other three studies. Of note, Kotsopoulos et al. 2017 only included BCs diagnosed before age 50, which differs from the design of the other studies. Despite the wide confidence intervals, given that the point estimates for pre-menopausal rrBSO for *BRCA2* were consistently <1, it is plausible that a clear protective association was not demonstrated due to underpowered individual analyses.

Not surprisingly, there is no consensus in guidelines regarding whether rrBSO should be offered to *BRCA1* and *BRCA2* mutation carriers specifically to reduce their BC risk. The U.S. National Comprehensive Cancer Network (NCCN) states that pre-menopausal oophorectomy likely reduces the risk of developing BC but the magnitude is uncertain and may be gene-specific; no overt guidance is provided regarding whether to undergo rrBSO specifically for BC risk reduction[28]. The U.K. National Institute for Health and Care Excellence (NICE) recommend a discussion of rrBSO to reduce BC risk after completion of childbearing[,29]. In contrast, the European Society of Medical Oncology (ESMO)[30] and the Australian Government guidelines (eviQ)[31] do not recommend rrBSO specifically for BC risk reduction.

Conversely, the guidance regarding rrBSO for reduction of tubo-ovarian cancer risk is clear; major guidelines recommend rrBSO between age 35-40 years for *BRCA1* pathogenic variant carriers and between age 40-45 for *BRCA2* pathogenic

variant carriers. If rrBSO does reduce breast cancer risk, biologically it would be expected to do so only if women were pre-menopausal and still had a reasonable period of expected ongoing ovarian function (eg > 2 years). It is interesting that some studies have suggested that BRCA1 pathogenic variant carriers may lose ovarian function earlier than non-carriers[32,33]. This could support a hypothesis that rrBSO is unlikely to reduce breast cancer risk substantially unless it is done at a relatively early pre-menopausal age. Conversely, early loss of ovarian function has not been clearly shown in *BRCA2* pathogenic variant carriers, so it is possible that rrBSO may be a more efficacious intervention for *BRCA2*. If rrBSO done before age 40 were shown to reduce breast cancer risk for *BRCA2* pathogenic variant carriers, that would be a practice-changing finding, because it would support discussion of rrBSO with *BRCA2* pathogenic variant carriers younger than 40 years.

Thus, currently there is no clear and consistent evidence for a role of pre-menopausal rrBSO in reducing BC risk in *BRCA1* or *BRCA2* mutation carriers, and no clarity in international guidelines. Randomised trials of premenopausal rrBSO versus no rrBSO are not considered feasible, because women are unlikely to accept such a randomisation. Thus, we propose an analysis of pooled individual data from published cohorts, using an optimised analytical design to minimise bias and confounding.

HYPOTHESIS

1. Pre-menopausal rrBSO is associated with reduced risk of breast cancer for BRCA2, but not BRCA1, pathogenic mutation carriers.

2. Pre-menopausal rrBSO before age 40 years is associated with greater reduced risk of breast cancer than pre-menopausal rrBSO after age 40 years.

AIM

1. To estimate the effect of pre-menopausal rrBSO on breast cancer risk for (i) BRCA1 mutation carriers and (ii) BRCA2 mutation carriers.
2. To test whether any effect of rrBSO is stronger when carried out at younger ages.

ELIGIBILITY CRITERIA

Any cohort participants that meet the following criteria will be accepted for this study.

- Carrier of pathogenic or likely pathogenic variant (class 4 or 5) in BRCA1 or BRCA2
- born after 1920
- aged at least 18 years at cohort entry
- no personal history of cancer (except cervix carcinoma in situ or non-melanoma skin cancer) at cohort entry
- no personal history of risk-reducing bilateral mastectomy at cohort entry
- follow-up information available (for at least invasive BC, DCIS and death)

STATISTICAL METHODS

Cox regression models will be used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for BC (invasive disease or ductal carcinoma in situ) associated with pre-menopausal BSO, with age as the timescale, entry being at the latter of cohort enrolment and positive genetic test, and censoring at the earlier of

bilateral mastectomy, death, diagnosis of another cancer or last follow-up. Separate analyses will undertaken for *BRCA1* and *BRCA2* mutation carriers; women with mutations in both will be analysed with *BRCA1* mutation carriers. The non-independence of data from members of the same family will be accounted for by clustering on family. Analyses will be stratified on study with equal coefficients across strata but baseline hazard distinct for each stratum, and adjusted for the following other pre-defined potential confounders:

- year of birth (continuous)
- body mass index (continuous)
- number of first- degree relatives with BC (continuous)
- number of second-degree relatives with BC (continuous)
- number of first- degree relatives with ovarian cancer (continuous)
- number of second-degree relatives with ovarian cancer (continuous)
- age at menarche (continuous)
- *parity (ever/never, plus continuous)
- *age at first birth (continuous)
- *hormonal contraceptive (HC) use (cumulative duration of use, continuous)
- *cumulative duration of breastfeeding (continuous)
- *menopausal status (binary)
- *HRT use (binary)
- Interaction term between BMI and menopausal status?

We will assess confounding of associations with premenopausal BSO by each of these factors by adding them, one-by-one, to the univariable model and retaining

those for which the estimated HR for premenopausal BSO changes by more than 10%.

Age at menopause will be defined using self-reported data, but where self-reported age is unavailable or unreliable (for example, women who had a hysterectomy but ovaries were not removed, had a hormonal IUD in or were on the OCP) the menopause age will be assumed to be 50 years.

RRBSO will be modelled as a time-varying exposure, as will potential confounders marked above with an asterisk.

Sensitivity analyses:

- Assess incident cancers by follow-up time and consider excluding up to the first six months of follow-up if there appears to be a peak in incidence
- Exclude women with missing information on age or reason for menopause at baseline
- Assume rrBSO occurred at questionnaire at which rrBSO was reported retrospectively
- Exclude women with prevalent rrBSO at baseline (or more than 5 years prior to baseline)
- Exclude women who had used cHRT at baseline, and censor at commencement cHRT use during follow-up.

Table 1: Possible Sources of Bias in Studies of rrBSO and Breast Cancer Risk

TYPE OF BIAS	DEFINITION AND EXAMPLE	IMPACT	MITIGATION STRATEGY
Confounding by indication	May be introduced if women who choose rrBSO have a different BC risk to those who do not have rrBSO. For example, within *BRCA1* and *BRCA2* there are areas, of each gene which, when mutated, increase TOC risk and decrease BC risk compared with mutations in other regions. Carriers with an inherently higher risk of TOC and lower risk of BC may be more likely to choose rrBSO because they have a stronger family history of TOC [18].	The potential benefit of rrBSO on BC risk may be overestimated as women opting to undergo rrBSO may do so because of a strong FHx of TOC and may have been at comparatively lower risk of developing BC [18].	Adjust for FHx of ovarian cancer
Survival bias from competing risk of TOC	This bias is closely related to confounding by indication and describes the observation that women, who are at inherently higher risk of TOC than BC, who do not undergo rrBSO, may contribute fewer person-years at risk during follow-up if they die from TOC before censoring for another reason. If these women are over-represented in the control group, the bias introduced by indication and survival is accentuated [18].	Overestimation of the protective association between rrBSO and BC risk, further amplifying confounding by indication [18].	Adjust for FHx of ovarian cancer
Informative censoring	When a censoring event, for example, rrBM, depends on the study endpoint (BC risk) then the censoring becomes "informative." Carriers with higher familial BC risk may	The potential benefit of rrBSO on BC risk may be overestimated due to an excess of	Adjust for FHx of breast cancer

	be more likely to undergo early rrBM, before rrBSO, compared to carriers with lower familial BC risk. The censoring event, rrBM, is considered “informative” because the group of women who undergo rrBM were more likely to develop BC than women who proceed to either rrBSO or other risk-reducing options [18,19].	lower-risk women in the rrBSO group.	
Cancer-induced testing bias	Cancer-induced testing bias explains the observation that diagnosis of BC often prompts genetic testing. Women who are then found to carry a *BRCA1* or *BRCA2* mutation may be recommended to undergo rrBSO for TOC risk reduction. Thus, an analysis of BC incidence before and after rrBSO may be enriched for BC events in the non-rrBSO period [18,19].	May lead to overestimation of the association between BC risk and rrBSO.	Exclusion of women with a personal history of BC prior to genetic testing. Starting the observation period at the time of genetic testing, if after enrolment [18,19].
Immortal person-time bias	1. Related to the follow-up period that participants survived BC-free before rrBSO. This bias is introduced if the person-time before rrBSO is not allocated to the non-rrBSO group [19]. 2. Related to the inclusion of women who had rrBSO prior to recruitment. If rrBSO is related to reduced breast cancer risk and longer survival, women who didn’t	1. Results in misallocation of observation time away from the non-rrBSO group and consequently, an increase in BC events per person-year in this group, biasing towards a protective association between rrBSO and BC [19]. 2. Results in bias towards the null	1. Consider rrBSO as a time-varying exposure[19]. 2. Sensitivity analysis excluding women who had rrBSO prior

	have rrBSO (and developed breast cancer - or died - before recruitment) will be under-represented in the eligible cohort.		to baseline (or had rrBSO more than 5 years prior to baseline).
Confounding by other risk factors	Confounding by other risk factors for BC also needs to be taken into account when assessing the efficacy of rrBSO [18]. e.g., parity - parous women may be more likely to undergo rrBSO compared with nulliparous women. If parous carriers are also at lower risk of BC, the association between rrBSO and reduced BC risk may appear spuriously stronger.	May lead to over- or underestimation of the association between rrBSO and BC risk depending on risk factor.	Adjustment for confounders
Missing data	Due to the nature of observational studies, it is not always possible to collect data points of interest on all patients [17].	Depending on the volume of missing data and its relationship to the main study outcomes, missing data may affect the integrity of the results [17].	Apply multiple imputation [34,35].
Other	Age at rrBSO – if the association between rrBSO and reduced BC risk only occurs for women who have early pre-menopausal rrBSO and not for those who have peri or post-menopausal rrBSO (which is biologically plausible) then including women with peri and post-menopausal rrBSO in the analysis will tend to weaken the association seen between rrBSO and reduced BC risk.	Any association between rrBSO and reduced BC risk may be underestimated or missed	Analyses stratified by age at rrBSO.

	cHRT – women who undergo pre-menopausal rrBSO may be more likely to receive subsequent cHRT than women who do not have rrBSO. If cHRT increases BC risk in carriers, any association between rrBSO and reduced BC risk may be spuriously weaker	Any association between rrBSO and reduced BC risk may be underestimated or missed	Adjustment for use of cHRT Sensitivity analysis restricted to women who had not used cHRT at baseline and censoring at commencement cHRT use during follow-up.

BC=breast cancer; TOC = tubo-ovarian cancer; rrBSO = risk-reducing bilateral salpingo-oophorectomy; rrBM = risk-reducing bilateral mastectomy; FHx = family history; cHRT = combined hormone replacement therapy

SIGNIFICANCE

This analysis of pooled cohort data will provide more definitive evidence than is currently available of the association between rrBSO and breast cancer risk in BRCA1 and BRCA2 pathogenic variant carriers. Depending on the results, the findings may alter clinical guidelines around timing of rrBSO.

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