GUIDE TO REVIEWING A MANUSCRIPT

The First Read-Through: Overall Impression

Try to bear in mind the following question:

- Is the main question addressed relevant and interesting?
- How original is the topic?
- Is the paper well-written to read?
- Are the conclusions consistent with the evidence and arguments presented?
- Do they have a future direction based on main results?
- If the paper includes tables or figures, what do they add to the paper?

The Second Read-Through: Section by Section Instruction

Here, we provide an example of the peer review with each section's checklist. We hope our guidance is helpful in your constructive review.

You would need to keep in mind the following essential checklists of each section:

<table>
<thead>
<tr>
<th>Checklist-Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Condensation of the paper's content in a few words</td>
</tr>
<tr>
<td>2. Capture the reader's attention</td>
</tr>
<tr>
<td>3. Originality of the paper from other papers of the same subject area</td>
</tr>
<tr>
<td>4. Identification as a randomized trial, if applicable</td>
</tr>
</tbody>
</table>

Title

Pathologic findings at risk-reducing salpingo-oophorectomy in germline BRCA mutation
carriers with breast cancer: significance of bilateral RRSO at the optimal age in germline BRCA mutation carriers

Reviewer's comment: You need to make the title short and clear.

Checklist-Abstract
1. Structured summary of objective, methods, results and conclusion
2. Clear purpose of the study
3. Identical results and conclusions with those of main manuscript

Abstract

Objective: Most BRCA1/2 carriers do not undergo risk-reducing salpingo-oophorectomy (RRSO) by the recommended age of 40.

Methods: We retrospectively reviewed breast cancer patients identified as BRCA mutation carriers who underwent RRSO at OO Hospital from 2010 to 2014. From 2013, both fallopian tubes of all cases were examined according to the SEE/FIM protocol and underwent immunohistochemically (IHC) staining.

Results: RRSO was performed in 63 patients, 27 in 2010–2012 and 36 in 2013–2014. The median age at RRSO was 46.5 years (32–73 years). Occult invasive cancer was detected in 8 patients, of ovarian origin in 5 and of tubal origin in 3. All occult invasive cancer cases with metastasis were detected in patients older than 40 years. Of the 36 patients from the 2013–2014 cohort, 7 showed p53 overexpression, 1 showed Ki-67 overexpression, 2 showed serous tubal intraepithelial carcinoma, and 3 showed occult cancer. The detection rate of premalignant lesions or cancer was 36.1% (13/36). In the analysis according to age, premalignant lesions were more common in BRCA 1 mutation carriers younger than 40 years old (66.7% vs 20.0%). In BRCA 2 mutation carriers, premalignant lesions were only detected.

Reviewer's comment: You should clearly describe the purpose of this study in object part of abstract.

Reviewer's comment: Please, move this sentence to the methods section.
in those older than 40 years of age, indicating the possible faster occurrence of premalignant lesions in BRCA1 mutation carriers.

**Conclusions:** Many patients still tend to delay RRSO until after they are 40 years old. Our findings support the significance of RRSO before the age of 40 in germline BRCA mutation carriers.

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**Checklist-Introduction**

1. Originality of the research aims by demonstrating the need for investigations
2. Highlights gaps in current understanding or conflicts in current knowledge
3. Adequate citation of appropriate references with avoiding a lengthy review
4. Clear purpose in a last paragraph of this section

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**Introduction**

High-grade serous ovarian cancer (HGSOC) is the most common histologic subtype of epithelial ovarian cancer, accounting for almost 70% of cases, and >75% of HGSOC patients are diagnosed at an advanced stage. Up to 20% of patients with HGSOC have a BRCA 1 or 2 germline mutation [1]. BRCA1 germline mutation carriers have a 40%–60% risk of developing HGSOC in their lifetime, whereas the risk is 11%–27% for BRCA2 germline mutation carriers. These carriers are thus offered risk-reducing salpingo-oophorectomy (RRSO) by age 40 by gynecologic oncologists according to National Comprehensive Cancer Network (NCCN) guidelines. However, most BRCA1/2 carriers do not undergo RRSO by this age [2].

Since the discovery that tubal intraepithelial lesions are the premalignant lesion of HGSOC in women with a BRCA mutation, the significance of RRSO until the guideline-
recommended age and comprehensive histopathologic examination of the fallopian tubes obtained has increased [3]. However, there are limited data on the optimal age for RRSO based on the prevalence of premalignant lesions or occult cancer at RRSO. Here, we analyzed the median age of RRSO in BRCA mutation carriers with a history of breast cancer and estimated the prevalence of tubal intraepithelial lesions and occult invasive cancer in RRSO specimens to identify the optimal age to undergo RRSO.

**Checklist-Methods**

1. Trial design with IRB approval
2. Observance of standard guidelines (e.g. the CONSORT for randomized trials)
3. Eligibility criteria for participants
4. Study duration and locations where the data were collected
5. Repeatable methods with sufficient details
6. Completely defined pre-specified primary and secondary outcome measures
7. Sample size determination
8. All statistical methods used in the study

**Materials and Methods**

We retrospectively reviewed patients with breast cancer who were identified as BRCA mutation carriers and underwent RRSO at OO Hospital from 2010 to 2014. Medical records were reviewed for parity, family history, BRCA mutation status, history of tamoxifen use, and age at RRSO. From 2013, both fallopian tubes of all cases were examined according to the SEE/FIM protocol and underwent immunohistochemical staining for p53 and Ki67.

**Reviewer's comment:**

How many in total do you had in your study population?

Pathology slides from 2010 to 2012 were merely reviewed to identify occult cancer. Thus, we could not review the data on tubal intraepithelial lesions because they were not examined
according to the SEE/FIM protocol in the fallopian tube, especially at the fimbriated end. All slides were reviewed by an expert gynecological pathologist. We obtained institutional review board approval for these studies. 

The relationships between variable characteristics and the detection rate of precursor lesions or occult cancer were assessed by univariate analysis using chi-square and Fisher’s exact tests and by multivariate analysis using logistic regression analysis to identify independent risk factors for precursor lesions or occult cancer. To analyze the relationship between patient age and the detection rate of precursor lesions or occult cancer at RRSO, patient age was dichotomized as ≤40 and >40 years. Descriptive statistics and data analysis were performed using SPSS ver.20.0 (SPSS Inc., Chicago, IL).

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**Checklist-Results**

1. Consistency in manuscript, figure, table and abstract
2. Description of all results for all subjects mentioned in method section
3. Simple presentation of the statistical analysis results with exact value
4. Adequate tables and figures use

**Results**

From 2010 to 2014, 63 patients with breast cancer who were identified as BRCA mutation carriers underwent RRSO at our institution, 27 patients from 2010 to 2012 and 36 patients from 2013 to 2014. The characteristics of the 63 patients are summarized in Table 1. Of the 63 patients, 38 were BRCA1 mutation carriers and 25 were BRCA2 mutation carriers. The median age at RRSO was 46.5 years (32–73 years) and only one patient was nulliparous at the time of RRSO. Regarding tamoxifen use, 14 patients were current users, 10 were former users, and 39 had never used tamoxifen. There was a family history of ovarian or tubal or peritoneal cancer in 13 patients and 33 patients had a family history of breast cancer. In
statistical analysis, tamoxifen use (p=0.736) and family history (p=1.000) were not associated with the detection rate of precursor lesions or occult cancer at RRSO. RRSO was performed before the age of 40 in 13 of the 63 patients (20.6%). The frequency of precursor lesions (p53 overexpression, Ki67 overexpression, serous tubal intraepithelial carcinoma [STIC]) and occult cancer in the 36 patients treated from 2013 to 2014 is indicated in Table 2. Of these 36 patients, 7 showed p53 overexpression, 1 showed Ki-67 overexpression, 2 showed STIC, and 3 showed occult cancer according to the SEE-FIM protocol. The primary site of all precursor lesions was the distal tube. The detection rate of premalignant lesions or cancer was 36.1% (13/36).

The relationship between patient age and the detection rate of precursor lesions or occult cancer at RRSO in 2013 to 2014 is indicated in Table 3. There was no statistically significant relationship. However, in the BRCA1 mutation group, patients who underwent RRSO before 40 years of age were more likely to have precursor lesions. In the BRCA2 mutation group, precursor lesions were only detected in patients who were older than 40 when they underwent RRSO. In addition, all three patients who were found to have occult cancer were older than 40 when they underwent RRSO.

In the pathology slides from 2010 to 2012, the presence of occult cancer was only assessed because the fallopian tube was not examined according to the SEE/FIM protocol. Occult cancer was detected in 5 of these 27 patients, 4 of the 15 BRCA1 mutation carriers (26.7%) and 1 of the 12 BRCA2 mutation carriers (8.3%). Of the 5 patients with occult cancer, 1 was younger than 40 at detection (14.3% of the 7 patients younger than 40) and 4 were older than 40 (20.0% of the 20 patients older than 40). Eight occult invasive cancers were identified from 2010 to 2014, five of ovarian origin and three of tubal origin (Table 4). The pathologic type of the identified cancer was papillary serous carcinoma in 6 patients.
mucinous adenocarcinoma in 1 patient, and endometrioid adenocarcinoma in 1 patient; 1 of the 13 cancer cases (7.7%) was detected in a patient younger than 40 and 7 of the 50 cancer cases (14.0%) were detected in patients older than 40.

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**Checklist-Discussion**

1. Focusing on the main results
2. Presentation of expected reasons for the main results
3. Adequate comparison with similar articles
4. Avoiding the repeat of the introduction/results contents
5. Adequate description of the study’s strengths or limitations
6. Suggestion of the future direction

**Discussion**

BRCA 1 and 2 are tumor suppressor genes that play a role in chromosomal damage repair. Mutations in these genes are associated with increased risk of breast and ovarian cancer. Since the discovery that tubal intraepithelial lesions are the premalignant lesion of HGSOC in women with a BRCA mutation, the significance of the optimal age for RRSO in BRCA 1/2 mutation carriers has increased [4,5]. NCCN guidelines suggest RRSO before the age of 40 in these BRCA carriers. However, patients still tend to delay RRSO until after 40 years of age due to worries about the negative hormonal influence of bilateral oophorectomy. The dramatic and rapid decline in estrogen and androgen levels after RRSO may negatively influence patient quality of life and health [6,7]. Short-term hormone replacement treatment seems to improve quality of life and, moreover, does not seem to have an adverse effect on oncologic outcomes in BRCA 1/2 mutation carriers without breast cancer history [8]. In BRCA 1/2 mutation carriers with breast cancer history, greater prudence is required regarding the timing of RRSO due to the impossibility of hormone replacement treatment.

In our current study, the median age at RRSO was 46.5 years (32–73 years), which was...
higher than the recommended age of the NCCN guidelines. Only 13 patients (20.6%) underwent bilateral RRSO before 40 years of age. This result is similar to that obtained by Garcia et al. [2], who evaluated the adherence to the NCCN guidelines of RRSO by age 35–40 and found that only 17% women had undergone RRSO before the age of 40. Comparison of changes in the number of RRSO and the median age at RRSO is indicated in Figure 1. The median age at RRSO was not decreased against steadily increasing number of RRSO. In our current study series, only one woman was nulliparous. This result shows that most women want to undergo RRSO after childbirth, which may be one of the reasons why BRCA carriers delay undergoing RRSO, given that women tend have children at an older age than in previous generations [9]. Statistical analysis of factors such as tamoxifen use and family history revealed no significant relationship with the detection rate of precursor lesions or occult cancer at RRSO in the 2013–2014 cohort.

The frequency of precursor lesions and occult cancer in the 36 patients treated from 2013 to 2014 is listed in Table 2. The primary site of all precursor cases was the distal tube, supporting the value of the SEE-FIM protocol, which sections the fimbriated end at 2–3-mm intervals to expose about a 60% greater surface area [10]. p53 overexpression (p53 signature) is defined as strong nuclear staining in at least six consecutive tubal epithelial cells [5]. Ki-67 is a proliferation marker and its overexpression is defined by Ki-67 staining in >50% of nuclei of p53-overexpressing cells. These characteristics are putative precursors to STIC, which is itself a direct precursor of HGSOC. STIC shows significant atypia, p53 overexpression, and Ki-67 overexpression [11]. In our current study, the detection rate of STIC at RRSO was 5.6% (BRCA 1 mutation carriers, 4.3%; BRCA 2 mutation carriers, 7.7%), which is similar to the results of previous studies reporting STIC detection rates of between 5% and 8% [12,13]. The detection rate of occult cancer was 12.7% (BRCA 1 mutation carriers, 15.8%; BRCA2 mutation carriers, 8.0%) and this is similar to the results of previous studies, which ranged from 4.4% to 17% [14,15,16]. The detection rate of premalignant lesions or cancer at RRSO in the 2013–2014 cohort was 36.1% (13/36). There were no significant differences in the distribution of premalignant lesions and occult cancer between BRCA 1 and BRCA 2
mutation carriers.

In the analysis according to age, premalignant lesions were more common in BRCA 1 mutation carriers younger than 40 years old (66.7% vs 20.0%). In BRCA 2 mutation carriers, premalignant lesions were only detected in those older than 40 years of age, indicating the possible faster occurrence of premalignant lesions in BRCA1 mutation carriers. Occult cancer was detected in three women, all of whom were older than 40 years of age, suggesting that premalignant lesions in women younger than 40 years old were progressing and being detected as occult cancer in women older than 40 years of age. However, no statistically significant relationships were found in this analysis due to the small sample size.

In the analysis of pathology slides obtained in 2010–2012, occult cancer was detected in 5 patients, with a higher detection rate in BRCA 1 mutation carriers than in BRCA 2 mutation carriers (26.7% vs 8.3%). Of the five patients with occult cancer, only one was younger than 40 years old. Eight patients were found to have occult cancer from 2010 to 2014, and only one patient was younger than 40 years old (12.5%). Occult cancer was more commonly detected in women older than 40 years old (7.7% vs 14.0%).

The age at which RRSO provides maximal benefit with minimal adverse hormonal effects is still controversial. The efficacy of RRSO in protecting against ovarian and breast cancer has already been proven [17] and women who undergo RRSO show the same levels of fatigue, fracture incidence, and quality of life as controls [18,19]. However, previous studies showed increased overall and cardiovascular disease mortality in premenopausal women who had undergone bilateral oophorectomy and had not received estrogen replacement therapy [6,20,21]. Moreover, in a study conducted by Michelsen et al. [22], surgical menopause was significantly associated with metabolic syndrome. For this situation, a “two-step prevention strategy” was suggested in which salpingectomy or fimbriectomy with ovarian conservation
would be performed in young women, followed by oophorectomy after menopause [23,24].

Reviewer's comment:
This paragraph could be merged with the first paragraph of discussion into one.

However, this approach remains to be fully investigated.

Reviewer's comment:
What is the unresolved problem with this approach?

Our study had several notable weaknesses. We were unable to collect data on the history of contraceptive use due to the retrospective nature of our analysis. During the 2010 to 2012 period, pathology slide reviews considered occult cancer only because the slides were not assessed according to the SEE/FIM protocol in the fallopian tube. In addition, we were unable to find a statistically significant association between age and the detection of premalignant lesions and occult cancer, probably due to the small sample size of the study. However, we analyzed the differences in the detection rate of premalignant lesions and occult cancer by 40 years of age according to the NCCN guidelines. Additionally, our current analysis only comprised women with breast cancer who were unable to receive estrogen replacement therapy. The optimal timing of RRSO is more crucial for these patients than others who can receive estrogen support. And our study shows the possible faster occurrence of premalignant lesions in BRCA 1 mutation carriers than BRCA 2 mutation carriers.

The continuous increase in BRCA testing is increasing the detection of BRCA mutation carriers, with a consequent more widespread use of RRSO. Nonetheless, women still tend to delay RRSO until after they are 40 years old. However, as shown by our present analysis, occult invasive cancers were more commonly detected in patients older than 40 years old. Our findings support the significance of bilateral RRSO by age 40 in germline BRCA mutation carriers, although there was no significant association found between age and the detection rate of premalignant lesions and occult cancer due to the small sample size. To resolve this matter, a larger and prospective study is required to determine the optimal timing of RRSO for maximal risk reduction with minimal adverse hormonal effects. A prospective study of the usefulness of the two-step prevention strategy using salpingectomy or fimbriectomy is also necessary.

Reviewer's comment:
Please, remove too much homework you could not figure out in your study.
Conflict of interest statement

The authors have nothing to disclose and no conflict of interest to report.

References


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**Checklist-Tables and Figures**

1. Inclusion of sufficient data (e.g. time point to analysis)
2. Support the paper's discussion and conclusions

**Table 1** Characteristics of the 63 patients with breast cancer in this study who were identified as BRCA mutation carriers and underwent RRSO.

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at RRSO, median (range)</td>
<td>46.5 (32–73 years)</td>
</tr>
<tr>
<td>BRCA mutation status</td>
<td></td>
</tr>
<tr>
<td>BRCA 1 mutation</td>
<td>38 (60.3)</td>
</tr>
<tr>
<td>BRCA 2 mutation</td>
<td>25 (39.7)</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>1 (1.6)</td>
</tr>
<tr>
<td>Tamoxifen use</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>14 (22.2)</td>
</tr>
<tr>
<td>Former</td>
<td>10 (15.9)</td>
</tr>
<tr>
<td>Never</td>
<td>39 (61.9)</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
</tr>
<tr>
<td>Ovarian or tubal or peritoneal cancer</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>50 (79.4)</td>
</tr>
<tr>
<td>Yes</td>
<td>13 (20.6)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>30 (47.6)</td>
</tr>
<tr>
<td>Yes</td>
<td>33 (52.4)</td>
</tr>
<tr>
<td>Total</td>
<td>63 (100%)</td>
</tr>
</tbody>
</table>

RRSO = risk-reducing salpingo-oophorectomy.
Table 2 Frequency of precursor lesions and occult cancer in 36 study patients with breast cancer who were identified as BRCA mutation carriers and underwent RRSO in 2013 to 2014.

<table>
<thead>
<tr>
<th></th>
<th>BRCA 1 (N = 23)</th>
<th>BRCA 2 (N = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53 overexpression</td>
<td>4 (17.4%)</td>
<td>3 (23.1%)</td>
</tr>
<tr>
<td>Ki67 overexpression</td>
<td>1 (4.3%)</td>
<td>0</td>
</tr>
<tr>
<td>STIC</td>
<td>1 (4.3%)</td>
<td>1 (7.7%)</td>
</tr>
<tr>
<td>Cancer</td>
<td>2 (8.7%)</td>
<td>1 (7.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>8 (34.8%)</td>
<td>5 (38.5%)</td>
</tr>
</tbody>
</table>

STIC = serous tubal intraepithelial carcinoma

Reviewers’s comment:
p53 overexpression, Ki67 overexpression, and STIC should be realigned as second level category under ‘precursor lesions’ being the first level category.

Reviewers’s comment:
Definition of precursor lesion should be described as footnote of this table.

Table 3 Relationship between patient age and the detection rate of precursor lesion or occult cancer at RRSO in 2013 to 2014.

<table>
<thead>
<tr>
<th></th>
<th>≤40</th>
<th>&gt;40</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>6</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Precursor lesion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA 1</td>
<td>2/3 (66.7%)</td>
<td>4/20 (20.0%)</td>
<td>0.155</td>
</tr>
<tr>
<td>BRCA 2</td>
<td>0/3</td>
<td>4/10 (40.0%)</td>
<td>0.497</td>
</tr>
<tr>
<td>BRCA 1/2</td>
<td>2/6 (33.3%)</td>
<td>8/30 (26.7%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Occult cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA 1</td>
<td>0/3</td>
<td>2/20 (10.0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>BRCA 2</td>
<td>0/3</td>
<td>1/10 (10.0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>BRCA 1/2</td>
<td>0/6</td>
<td>3/30 (10.0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Total</td>
<td>2/3 (66.7%)</td>
<td>6/20 (30.0%)</td>
<td>0.269</td>
</tr>
<tr>
<td>BRCA 1</td>
<td>0/3</td>
<td>5/10 (50.0%)</td>
<td>0.231</td>
</tr>
<tr>
<td>BRCA 1/2</td>
<td>2/6 (33.3%)</td>
<td>11/30 (36.7%)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Reviewers’s comment:
P value of 1.000 should be presented as p>0.999.
Table 4 Characteristics of the study patients with cancers detected at RRSO.

<table>
<thead>
<tr>
<th>#</th>
<th>Age at RRSO</th>
<th>Location</th>
<th>Histologic Type</th>
<th>Stage</th>
<th>Cytology</th>
<th>BRCA 1/2 status</th>
<th>BRCA germline mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>Ov</td>
<td>MAC, WD</td>
<td>IA</td>
<td>Negative</td>
<td>1</td>
<td>5199G&gt;T</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>Ov</td>
<td>EAC, PD</td>
<td>IC2</td>
<td>Negative</td>
<td>1</td>
<td>5312+1G&gt;C</td>
</tr>
<tr>
<td>3</td>
<td>67</td>
<td>Ov</td>
<td>PSC, HG</td>
<td>IIB</td>
<td>Positive</td>
<td>1</td>
<td>1835del A</td>
</tr>
<tr>
<td>4</td>
<td>41</td>
<td>FT</td>
<td>PSC, HG</td>
<td>IIIC</td>
<td>Positive</td>
<td>1</td>
<td>509C&gt;A</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>FT</td>
<td>PSC, HG</td>
<td>IIB</td>
<td>Negative</td>
<td>1</td>
<td>1418delC</td>
</tr>
<tr>
<td>6</td>
<td>52</td>
<td>FT</td>
<td>PSC, HG</td>
<td>IIIC</td>
<td>None a</td>
<td>1</td>
<td>2478 del G</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>Ov</td>
<td>PSC, HG</td>
<td>IIB</td>
<td>None</td>
<td>2</td>
<td>1627A&gt;T</td>
</tr>
<tr>
<td>8</td>
<td>63</td>
<td>Ov</td>
<td>PSC, HG</td>
<td>IVB</td>
<td>Positive</td>
<td>2</td>
<td>5820_5821delCA</td>
</tr>
</tbody>
</table>

RRSO = risk-reducing salpingo-oophorectomy; Ov = ovary; PSC = papillary serous carcinoma; HG = high-grade; MAC = mucinous adenocarcinoma; WD = well differentiated; EAC = endometrioid adenocarcinoma; PD = poorly differentiated; FT = fallopian tube.

a Cytology was not performed.

Fig. 1 Comparison of changes in the number of RRSO and the median age at RRSO