Pathology findings and clinical outcomes after risk reduction salpingo-oophorectomy in BRCA mutation carriers: A multicenter Spanish study.

Running title: Pathology findings after prophylactic surgery in BRCA mutation carriers.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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ABSTRACT

Objective: To determine the incidence of serous tubal intraepithelial carcinoma (STIC) in BRCA mutation carriers after risk-reduction salpingo-oophorectomy (RRSO) in referral Spanish hospitals; as well as to describe clinical and oncological outcomes after RRSO

Material and methods: Patients with documented BRCA mutation who had undergone a RRSO were evaluated in this retrospective multicenter observational study. Patients were also included when fallopian tubes were analyzed following the protocol for Sectioning and Extensively Examining the FIMbria (SEE-FIM). Surgeries were performed between June 2010 and April 2017 at eight Spanish hospitals.

Results: A total of 359 patients met the inclusion criteria. STIC was diagnosed in 3 (0.8%) patients; one patient underwent surgical staging due to positive peritoneal washing. The pathology analysis indicated absence of disease. None of the three patients received adjuvant chemotherapy. Fallopian tube and ovarian carcinoma was diagnosed in 5 (1.4%) and 1 (0.3%), respectively. At a median (range) follow-up time of 29 (3 to 92) months, 5 patients had a newly diagnosed breast cancer. Other types of cancer were diagnosed during the follow-up time and included: serous primary peritoneal carcinoma (n=1), serous endometrial carcinoma (n=1), colon (n=1), pancreas (n=1), jaw (n=1), and lymphoma (n=1). Seven patients died due to different types of cancer: breast (n=4), pancreas (n=1), jaw (n=1), and colon (n=1).

Conclusion: The incidence of STIC after RRSO in BRCA mutation carriers is low (0.8% in this study), and it presents an excellent oncological outcome. These patients, however, run the risk to develop other types of cancer during follow-up and should be properly advised after the prophylactic surgery. This study adds more evidence,

contributing to build more robust recommendations regarding clinical management of STIC in the future.

KEYWORDS: BRCA, risk reduction salpingo-oophorectomy, serous tubal intraepithelial carcinoma, ovarian cancer, primary peritoneal carcinoma.

INTRODUCTION

According with recent findings, serous tubal intraepithelial carcinoma (STIC) is the earliest morphologically recognizable lesion of genital high-grade serous carcinoma. (Kurman 2013) In addition, several studies have shown that the distal part of the fallopian tube represents the site of origin of these types of tumors. (Morrison 2015, Li 2014)

The prevalence of isolated STIC is variable in the studies published in the literature. A great body of evidence has described the pathologic and molecular characteristics, however, very few studies report the clinical and oncological data of STIC in Breast Cancer susceptibility gene (BRCA) mutation carriers. A recent review, including 15 studies with 3850 patients, observed that the incidence of STICs after risk-reduction salpingo-oophorectomy (RRSO) in BRCA mutation carriers ranged from 0.4% to 11%. (Patrono 2017) However, based on the limited evidence, some controversial issues still remain unresolved. (Patrono 2015) These include the role of peritoneal washing at the time of RRSO, the role of surgical staging and adjuvant chemotherapy in patients with STICs, as well as the best surveillance strategy in this group of high risk patients. Thus, increasing the evidence with more reported cases, would contribute to build stronger recommendations in the near future. (Zakhour 2016) Moreover, no data has been reported in the Spanish population to date. Therefore, the objective of this study was to determine the incidence of STIC in BRCA mutation carriers after RRSO in referral Spanish Hospitals; as well as to describe clinical and oncological outcomes after RRSO.

PATIENTS & METHODS

The institutional review board of each center approved this retrospective multicenter observational study. Patients with documented BRCA mutation who had undergone an RRSO were evaluated. Patients were also included when fallopian tubes were analyzed following the protocol for Sectioning and Extensively Examining the FIMbria (SEE-FIM), described by Madeiros et al in 2006. (Madeiros 2006) In addition, women were asymptomatic, with normal level of CA-125 (defined as less than 35 IU/mL), as well as with normal ovaries at preoperative pelvic ultrasound. Prophylactic surgeries were performed between June 2010 and April 2017 at eight Spanish hospitals: Instituto Valenciano de Oncología (IVO), Valencia (n=149); Hospital Universitario H Vall d`Hebron, Barcelona (n=135); Hospital Univeritario 12 de Octubre, Madrid (n=21); Hospital Universitario Príncipe de Asturias de Alcalá de Henares, Madrid (n=17); Hospital Universitario Reina Sofía, Córdoba (n=15); Hospital Universitario Quirón, Madrid (n=7); Hospital Universitario de Getafe, Madrid (n=5); and Complejo Hospitalario Universitario Insular Materno-Infantil de Canarias (CHUIMI), en Gran Canaria (n=4).

Surgical procedure

Specialized gynecologists performed salpingo-oophorectomies by laparoscopy following the NCCN guidelines. (NCCN) Unilateral or bilateral adnexectomy was performed according with the surgical history of each patient. Pelvic washing was done in all cases at the beginning of the procedure, and tubes were removed at the uterine insertion. Concomitant hysterectomy was performed according with the patients´ preference and findings at the preoperative pelvic ultrasound.

Variables

Tubular intraepithelial serous carcinoma (STIC) was defined using a combination of morphologic evaluations to distinguish it from p53 signatures, STIL, and invasive carcinoma. (Mehrad 2010) Immunohistochemistry was performed only when nuclear atypia was present, and a diagnosis of STIC was considered based on the review of sections stained by hematoxylin and eosin. Morphologic considerations included the following: nuclear/cytoplasmic ratio, nuclear pleomorphism, epithelial stratification with loss of polarity, irregular epithelial thickness, and exfoliation of cells into the tubal lumen. Immunohistochemical stains included p53 and Mib-1. Elevated Mib-1 (>15% nuclear cell staining) and abnormal p53 staining (null phenotype or >60% nuclear cell staining) were used as supportive evidence of the diagnosis. Specialized pathologists in each institution analyzed the specimen and the fallopian tubes following the SEE-FIM protocol as previously mentioned.

Epithelial ovarian cancer or primary peritoneal carcinoma (PPC) was staged following the current classification by the International Federation of Gynaecology and Obstetrics (FIGO). (Prat 2014)

Follow-Up

Although a protocol of follow-up was not homogeneous across all Institutions, patients were generally followed-up with pelvic ultrasound and CA-125 every 6 months. Disease status was recorded at the most recent follow-up visit.

Statistical analysis

Kolmogorov-Smirnov with Lilliefors correction was used to evaluate the normal distribution of the data of the collected variables. Whereas frequencies and proportions were used as summary statistics for categorical variables, mean (standard deviation) or

medians and interquartile (IQ) range were used for the continuous. Statistical analysis was performed using the IBM SPSS version 20.0 program.

RESULTS

A total of 359 patients were identified in the institutional databases of the participating referrals hospitals across Spain. Table 1 outlines the patients´ baseline characteristics. Mean (±SD) age was 49,3 (9,0) years. Of note, 146 (40%) women had less than 45 years, and 160 (44.6%) underwent a surgical menopause. Mean (±SD) preoperative CA 125 level was 13,4 (8,6) IU/mL, with a mean (±SD) BMI of 26,1 (5,7) kg/m^2. A total of 218 patients (60.7 %) carried germline BRCA1 mutations, 136 patients (37.8 %) BRCA2 mutations, and 5 patients (1.5 %) carried both BRCA 1 & 2 mutations. Breast cancer was previously diagnosed in 225 (62.6%) of patients at a median (range) interval time from diagnosis to RRSO of 43 (6 to 345) months. Almost all patients underwent bilateral salpingo-oophorectomy, n= 341 (95%). Unilateral salpingo-oophorectomy and concomitant hysterectomy was performed in 7 (2%) and 11 (3%), respectively. Other types of cancer diagnosed before the prophylactic surgery included: colorectal cancer (n= 5), esophagus cancer (n=2), skin cancer (n=1), melanoma (n=1), gastric cancer (n=1), lung cancer (n=1), tongue cancer (n=1), and pancreatic cancer (n=1). (Table 1 & Figure 1)

Table 2 describes the oncological outcomes of all patients with benign findings at RRSO. At a median (range) follow-up time of 29 (3 to 92) months, 5 patients had a newly diagnosed breast cancer, three of them at the time of a prophylactic mastectomy. A total of 14 out of 223 women previously diagnosed of breast cancer, experienced a relapse of the disease. Other patients, were diagnosed with different types of cancer during follow-up time and included: serous primary peritoneal carcinoma (n=1), serous

endometrial carcinoma (n=1), colon adenocarcinoma (n=1), pancreatic adenocarcinoma (n=1), squamous cell carcinoma of the jaw (n=1), lymphoma (n=1). Interval time from the RRSO to the diagnosis of the new cancer is described in Table 2 and in Figure 1. The patient diagnosed with a PPC, had breast cancer in 2003, and she was BRCA 1 mutation carrier. She underwent the RRSO at 57 years of age, in 2014, with normal pathology findings, and she developed PPC 24 months after the prophylactic surgery. She underwent primary debulking surgery with complete tumor resection. Her final FIGO stage was IIIC and she underwent six cycles of I.V. carboplatin and paclitaxel. At her last follow-up, she is alive without relapse of disease. Seven patients died due to different types of cancers: breast (n=4), pancreas (n=1), jaw (n=1), and colon (n=1). (Figure 1)

Patients with abnormal pathology findings are described in Table 3. STIC was diagnosed in 3 (0.8%) patients, while fallopian tube and ovarian carcinoma was diagnosed in 5 (1.4%) and 1 (0.3%), respectively. One additional patient, who underwent concomitant hysterectomy, was diagnosed of well-differentiated endometrioid endometrial carcinoma. Only one patient with STIC underwent comprehensive surgical staging due to positive cytology. After careful evaluation, the specimen was free of disease and she did not received adjuvant chemotherapy. She is now free of disease 11 months after the RRSO. The five patients with fallopian tube cancer, all had concomitant STIC at histopathology analysis, and they underwent full surgical staging. The two patients with final FIGO stage IA did not receive adjuvant chemotherapy, while the remaining three patients with FIGO stage IIIA1, IC1, IIIA2 received adjuvant carboplatin and paclitaxel intravenously (n=2) and intraperitoneally (n=1). The patient with FIGO stage IIIA1 experienced a peritoneal relapse 34 months

after finishing the adjuvant chemotherapy. She underwent a secondary debulking surgery and she is currently under treatment with adjuvant chemotherapy. One woman, with normal fallopian tubes, had serous epithelial ovarian cancer. She underwent surgical debulking with a final FIGO stage IIIC, and she completed the treatment with carboplatin and paclitaxel intravenously. Lastly, a patient who underwent a concomitant hysterectomy at the time of RRSO was diagnosed with a well-differentiated endometrioid endometrial carcinoma FIGO stage IA.

DISCUSSION

The present study observed a 0.8% incidence of serous tubal intraepithelial carcinoma in asymptomatic BRCA mutation carriers after a risk-reduction salpingo-oophorectomy when samples were analyzed following the SEE-FIM protocol. Serous fallopian tube carcinoma where, however, diagnosed in 1.4% of the included patients. At a median (range) time of follow-up of 29 (3 to 92) months after the RRSO, 6 (1.7%) patients had a newly diagnosed cancer and, one of them, was a primary peritoneal carcinoma. All of these patients had benign findings at RRSO.

The incidence of STIC showed in the present study is in accordance with a recent review of the literature, which retrieved 15 articles with 3850 BRCA mutation carriers who underwent RRSO. The study observed that the incidence ranged from 0.4% to 11%. (Patrono 2017) This broad range might be partially explained due to the lack of comprehensive sectioning of the fallopian tubes following the SEE-FIM protocol, insufficient information regarding preoperative work-up, different inclusion criteria, as well as the small cohort size across the published studies. (Zakhour 2016)

In the present study, RRSO also allowed to diagnose unsuspected invasive carcinoma at fallopian tubes (n=5), and at the ovary (n=1) in asymptomatic BRCA

mutation carriers with normal findings at pelvic ultrasound and normal level of CA 125. In this sense, other authors were also able to observe the same findings. Powel *et al*. compared 15 cases of invasive carcinomas with 17 STICs in BRCA mutation carriers after RRSO. The authors noted that 26 out of 32 patients had BRCA1 mutated genes, and that invasive carcinoma was diagnosed at a younger age in comparison with STIC. Seven out of 15 patients (47%) with invasive carcinoma recurred at a median time of 32.5 months; while three died of the disease. (Powel 2013) Other reports observed the same results and, in agreement with our study, were also able to diagnose endometrioid endometrial cancer in patients with concomitant hysterectomy at the time of RRSO. (Zakhour 2016)

Risk-reduction salpingo-oophorectomy was associated with surgical menopause in 160 (44.6%) patients in the present case series. In fact, 146 (40.5%) women were younger than 45 years old. Large studies have demonstrated that an anticipated menopause is associated with a higher incidence of cardiovascular and neurologic adverse events, osteoporosis, as well as with deterioration in the quality of life. (King 2011) Thus, hormone replacement therapy (HRT) emerged as a possible topic of discussion with young BRCA mutation carriers after RRSO. Even though it has been demonstrated that RRSO is associated with 56% risk reduction of breast cancer in BRCA mutation carriers [HR: 0.44 (95% CI, 0.26 to 0.76)] (Domchek 2010), concerns still persist regarding the potential increased risk of breast cancer after HRT. In this sense, however, prospective cohort studies failed to demonstrate an increased risk of breast cancer with HRT in women after RRSO. (Rebbeck 2005, Eisen 2008) Nevertheless, available data is still scarce due to the limited level of evidence with small cohort of patients, as well as different HRT scheme and duration of treatment.

(Chlebowski 2008) Therefore, a careful individualization of patients with an appropriate counseling, taking into consideration the pre-HTR risk of death, as well as other co-morbidities, is mandatory in these patients.

A total of 225 (62.6%) women were diagnosed with breast cancer before RRSO in this study, 13 (3.6%) additional women had colon, gastric, esophagus, lung, tongue, pancreatic cancer or melanoma before prophylactic surgery on the ovaries. After the RRSO, moreover, 11 (3%) patients were diagnosed with breast cancer, colon cancer, pancreatic cancer, jaw cancer, lymphoma as well as serous primary peritoneal carcinoma, and serous endometrial carcinoma. As it has been described, BRCA mutation carriers are at an increased risk to develop other types of cancer rather than breast and ovarian malignancies. (Thompson 2002, Brose 2002)In this regard, large population studies have shown that BRCA mutations carries have a higher risk of pancreatic cancer (RR=2.26, 95% confidence interval [CI]=1.26 to 4.06, p=.004) and cancer of the uterine body and cervix (uterine body RR=2.65, 95% CI=1.69 to 4.16, p<.001; cervix RR=3.72, 95% CI=2.26 to 6.10, p<.001). Overall, the risk for cancer at sites other than the breast or ovary was increased more than twice in women (RR=2.30, 95% CI=1.93 to 2.75, p=.001). (Thompson 2002)

Clinical management of STIC is, currently, a matter of debate. This study described 3 cases of STIC. The patient with positive peritoneal washing, was the only who underwent surgical staging including hysterectomy, omentectomy and pelvic/para-aortic lymphadenectomy. The analyzed specimens revealed no disease and she did not receive adjuvant chemotherapy. Nowadays, there is no standard clinical management recommendation for patients with isolated STIC in BRCA mutation carriers after RRSO. Whether patient with isolated STIC should undergo a comprehensive surgical

staging procedure is not clear.(Patrono 2017) Based on the available literature, no evidence of disease in the uterus, omentum or lymph nodes was observed, among the 13 patients who underwent surgical staging after isolated STIC was found.(Patrono 2017) This is in agreement with the case of STIC who underwent surgical staging in the present study.

Whereas negative peritoneal washing are reassuring, the real significance of positive peritoneal washing is unknown. Moreover, whether positive or atypical washings are associated with a higher risk of PPC is undetermined. To date, no patients with positive peritoneal washing have developed PCC during the follow-up. (Block 2016) There is insufficient evidence in the literature to support adjuvant chemotherapy after isolated STIC with or without positive peritoneal washing aimed to decrease the rates of PPC. Patients who developed PPC were diagnosed at an average median time from the RRSO of 42.5 months (range; 18-72). (Patrono 2017) Even though none of these patients received adjuvant chemotherapy, the time interval seems to be too long to hypothesize that adjuvant chemotherapy might have a potential role in these patients.

One study demonstrated that women with STIC had a higher risk of developing pelvic serous carcinoma than those women with benign pathology findings at the time of RRSO. (Zakhour 2016) A recent review of the literature showed that 6 out of 80 cases (7.5%) of STIC during RRSO in BRCA mutation carriers had subsequent serous PPC at a median (range) follow-up time of 42.5 months (18 to 72 months). (Patrono 2017) There is, however, a current controversy whether a PPC diagnosed after RRSO is interpreted as a relapse of the STIC or as a new primary pelvic serous carcinoma in these BRCA mutation carriers. Based on the evidence available to date, high-grade serous tubal, ovarian, and PPC currently trend to be classified as pelvic serous

carcinomas. (Reitsma 2013, Jazaeri 2011) However, longer follow-up, as well as better understanding on carcinogenesis in this setting of tumors will probably clarify the natural history of these tumors. Even though we did not report any cases of PPC in the three cases with STIC, probably due to the short follow-up time (median time: 29 months), we reported two cases of serous carcinoma during the surveillance period after RRSO with benign pathology findings. One woman was diagnosed with PPC while the other patient had serous endometrial cancer. In this regard, Powell *et al.* reported the rate of PPC following benign RRSO with comprehensive pathologic sectioning at 1% in a study of 101 patients with a median follow-up of 50 months. (Powell 2011) Zakhour *et al*. also observed PPC or pelvic serous carcinoma in 3 out of 257 BRCA mutation carriers after benign RRSO. (Zakhour 2016) Although serous pelvic or peritoneal cancer can be diagnosed during follow-up in BRCA mutation carriers after benign pathology findings at RRSO, the incidence seems to be low. Therefore, whether surveillance (either clinical, imaging or tumor markers) is useful or not in these women, needs to be clarified in the future. For this purposes, a multi-investigator collaborative study was designed to identify and accrue early stage pelvic-ovarian cancers in women, specifically early malignancies in the distal fallopian tube, or tubal intraepithelial carcinomas (STIC). The purpose of this effort is to accumulate, in a relatively short time, a large series of cases that, through pathologic evaluation and follow-up, will yield a clearer understanding of the frequency and outcome of this early phase of pelvic cancer. More information is available in the website. (BWH)

Even though the present study has strengths, which include the first study in Spanish population, as well as the detailed description of oncological history before and after RRSO, our results have limitations. First, the retrospective nature of the study

might have led to variance in interpretation of the data collected from multiple centers, as well as with unidentified patients. Second, even though the study included referral centers in Spain with specialized pathologists, a central pathology review was not performed. Third, the median follow-up time might be short. Lastly, even though the patients' characteristics are homogeneous, the sample size is still small to reach strong results and conclusions should be, therefore, be interpreted with caution.

CONCLUSION

In agreement with the literature previously published, the incidence of serous tubal intraepithelial carcinoma after risk-reduction salpingo-oophorectomy in BRCA mutation carriers is low (0.8% in this study) with an excellent oncological outcome. These patients, however, have also risk to develop other types of cancer during follow-up and should be properly advised after the prophylactic surgery. This study adds more evidence, contributing to build more robust recommendations regarding clinical management of STIC in the future.

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FIGURE LEGENDS

Figure 1: Oncological outcomes of patients with non-breast cancer diagnosed before risk-reduction salpingo-oophorectomy (RRSO) (Patient # 1 – 13), as well as those patients with newly diagnosed cancer after RRSO (Patient # 14 – 23).

REFERENCES