Gynecologic Oncology 153 (2019) 326–334

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Retrospective study of a 16 year cohort of *BRCA1* and *BRCA2* carriers presenting for RRSO: Prevalence of invasive and in-situ carcinoma, with follow-up

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HIGHLIGHTS

- The majority of high grade serous carcinoma in our cohort were of tubal origin.
- High grade serous carcinoma was more common in *BRCA1/2* carriers undergoing RRSO after the recommended age.
- Isolated STIC can give rise to peritoneal serous carcinoma >7 years post-RRSO.

ARTICLE INFO

Article history:
Received 8 November 2018
Received in revised form 28 February 2019
Accepted 2 March 2019
Available online 18 March 2019

Keywords:
BRCA1 gene
BRCA2 gene
Salpingo-oophorectomy
Serous cystadenocarcinoma
STIC

ABSTRACT

Objectives. Carriers of *BRCA1* and *BRCA2* mutations are at increased risk of high grade serous carcinoma and are therefore offered risk-reducing salpingo-oophorectomy (RRSO) by 40–45 years. Most of these carcinomas are believed to arise in the fallopian tube from serous tubal intraepithelial carcinoma (STIC). We conducted a retrospective study on the prevalence of high grade serous carcinoma and STIC in *BRCA1/2* carriers presenting for RRSO, and their follow-up.

Methods. Consecutive *BRCA1/2* carriers presenting for an RRSO at Erasmus MC (2000–2016) were studied. SEE-FIM pathology protocol was followed from 2010 onwards. For the cases with carcinoma and/or STIC, the histology was reviewed and immunohistochemistry (p53 & MIB-1) was performed. Next Generation Targeted Sequencing (NGTS) for *TP53* mutation was used to establish clonality in 2 cases.

Results. Of the 527 included patients, 68% were *BRCA1*, 31.6% were *BRCA2*, and 0.4% carried both mutations. The prevalence of high grade serous carcinoma was 2.3% (12/527); 59% of these were of tubal origin. High grade serous carcinoma was more common in patients operated on after the recommended age ($p = 0.03$). Isolated STIC was present in 0.8% (4/527). Two *BRCA1* carriers with isolated STIC at RRSO developed peritoneal serous carcinoma >7 years later. Identical *TP53* mutations in the peritoneal serous carcinoma and the preceding STIC established their clonal origin.

Conclusions. High grade serous carcinoma is more common in *BRCA1/2* carriers presenting for RRSO after the recommended age, and is more often of tubal origin. Longer follow up of patients with STIC at RRSO should be considered.

1. Introduction

Carriers of germline mutation of breast cancer susceptibility genes types 1 and 2 (*BRCA1/2*) are at a high risk of developing high grade serous carcinoma, which is known to have a dismal overall survival rate [1]. The lifetime risk of high grade serous carcinoma for the general population is <2% [2], but for *BRCA1* carriers the risk is up to 40%, and for *BRCA2* up to 25% [2,3]. Therefore, a risk reducing salpingo-oophorectomy (RRSO) is recommended to *BRCA1* carriers between 35 and 40 years, and to *BRCA2* carriers between 40 and 45 years [3]. Risk-reducing salpingo-oophorectomy effectively lowers ovarian cancer risk and ovarian cancer-specific mortality [3,4].

Growing scientific evidence, gleaned primarily from histological examination of the RRSO specimens of *BRCA1/2* carriers, has established

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https://doi.org/10.1016/j.ygyno.2019.03.003
0090-8258/

the distal fallopian tube as the most important site of origin of high grade serous carcinoma [5–8]. Serous tubal intraepithelial carcinoma (STIC) is the widely accepted precursor lesion, which bears morphological resemblance and identical *TP53* mutation patterns to high grade serous carcinoma, and often exists in a continuum with an invasive tubal carcinoma [7–9]. This new hypothesis for the pathogenesis of high grade serous carcinoma raises the possibility of a two-stage surgery for *BRCA1/2* carriers: a premenopausal risk-reducing salpingectomy (RRS), with a delayed oophorectomy (DO). This alternative risk-reducing surgery is currently being evaluated in some clinical trials [10–14]. These ongoing trials underline the importance of furthering our understanding and knowledge of the biology of in situ and invasive carcinoma, and the site of origin of these carcinomas in high risk women.

We carried out a retrospective study on a 16 year cohort of *BRCA1/2* carriers presenting for an RRSO at our institution, to measure the prevalence of high grade serous carcinoma and/or in-situ lesions (STIC), and to investigate the primary site (fallopian tube vs ovary) of these carcinomas. We also describe here 2 interesting cases of *BRCA1* carriers with isolated STIC at RRSO, who developed peritoneal serous carcinoma >7 years later.

2. Materials and methods

Details of all women who presented for a risk reducing salpingo-oophorectomy (RRSO) between 2000 and 2016 in the Department of Gynecologic Oncology, Erasmus MC Cancer Institute, were retrieved from the electronic patient records. Only women with proven *BRCA1* and/or *BRCA2* mutation were included. For all the included cases, a pre-operative measurement of the CA-125 level and transvaginal ultrasound were performed [15]. Risk reducing salpingo-oophorectomy was performed laparoscopically; both ovaries, fallopian tubes, and mesosalpinx were removed. From 2007, as per National Comprehensive Cancer Network (NCCN) guidelines [3], peritoneal washing was collected for cytology prior to the removal of tubes and ovaries. This study was approved by the Medical Research Ethics Committee of Erasmus MC (MEC code 2015-037) [16].

2.1. Data collection

The following information was collected: age of the patients, menopausal status at RRSO, history of breast carcinoma, pre-operative CA-125 levels, findings on the pre-operative transvaginal ultrasound, reports of the peritoneal wash cytology, and follow-up information, particularly regarding the development of malignancies. For the cases with a high grade serous carcinoma, the stage as per The International Federation of Gynecology and Obstetrics (FIGO) classification, details of any additional surgical procedures, and chemotherapy administered were recorded. All the data were anonymized.

2.2. Histopathology

For the handling of RRSO specimens, the Sectioning and Extensively Examining the Fimbriated End (SEE-FIM) protocol was followed at least from 2010 [17]. The macroscopy protocols for our pathology laboratory prior to 2010 are not available, and therefore the exact date of the introduction of SEE-FIM, and the exact procedure for handling of RRSO specimens before 2010 cannot now be established. In our laboratory the tissue is cut as close to 2 mm as possible at the time of grossing. Serial sections are not cut on the slides unless there is a suspicion of a high grade serous carcinoma/STIC on the first section, and this has always been the approach.

The histology of those cases with a high grade serous carcinoma and/or STIC at the time of RRSO was reviewed by two pathologists (PEG & SDG); PEG is an experienced gyneco-pathologist. The primary site of these carcinomas was allocated based on the consensus statement published by Singh et al. [18]. For cases allocated as ovarian origin high grade serous carcinoma, and for isolated STIC, additional sections of the tubes were prepared for the study. In all of these cases the tube had been totally enclosed. Histological diagnosis of STIC was based on the presence of atypical tubal epithelial cells exhibiting nuclear enlargement and pleomorphism, prominent nucleoli, abnormal chromatin, mitotic figures, apoptotic bodies, loss of cilia, with epithelial stratification, and loss of polarity.

2.3. Immunohistochemistry

Immunohistochemistry with p53 and MIB-1 was conducted for the cases with a high grade serous carcinoma and/or STIC. Sections of 4 μ were prepared from the formalin fixed paraffin embedded (FFPE) tissue. p53 (BP53-11, Ready to use, Ventana) and MIB-1 (clone 30-9, Ready to use, Ventana) staining was carried out on Benchmark Ultra Immunostainer (Roche), according to the manufacturer's instructions. Diffuse intense staining with p53 was noted as 'mutation pattern', and complete absence of staining was noted as 'null-pattern'. Mutation or null-pattern p53 staining with a MIB-1 labeling index ≥10% were considered confirmatory for the diagnosis of STIC.

2.4. Molecular analysis

A *TP53* mutation analysis by Next Generation Targeted Sequencing (NGTS) was conducted for the patients who had isolated STIC at RRSO, and developed peritoneal serous carcinoma on follow-up, to investigate the clonal relationship.

In order to extract DNA for NGTS, FFPE lesional tissue and normal tissue were manually micro-dissected and tissue fragments were subjected to proteinase K digestion for 16 h at 56 °C in the presence of 5% Chelex 100 resin (BioRad Laboratories, Hercules, CA). The extracted DNA was used without further purification after inactivation of proteinase K and the removal of cell debris and the Chelex resin. DNA concentration was measured with Qubit 2.0 fluorometer (Thermo Fisher Scientific Inc., Wilmington, DE), as described by the manufacturer [19].

Next Generation Targeted Sequencing (NGTS) of *TP53* was performed by semiconductor sequencing with the Ion Torrent Personal Genome Machine following the suppliers' materials and protocols (Life Technologies, Carlsbad, CA). The library and template were prepared consecutively with the AmpliSeq Library Kit 2.0-384 LV (ThermoFisher Scientific Inc.) and the Ion Personal Genome Machine Template OT2 200 kit (ThermoFisher Scientific Inc.). Templates were sequenced using the Ion Personal Genome Machine Sequencing 200 Kit v2 (ThermoFisher Scientific Inc.) on an Ion 318v2 chip (ThermoFisher Scientific Inc.). Sequence information was analyzed with Variant Caller v3.6 (Life Technologies), and variants were annotated in a local Galaxy pipeline using ANNOVAR version 2014-11-12 (Wang Genomics Lab, University of Southern California, Los Angeles, CA, http://annovar.openbioinformatics.org/en/latest, last accessed February 2019) [19].

2.5. Statistical analysis

Data were analyzed using IBM SPSS Statistics 21 (SPSS, Chicago, IL, USA). Descriptive statistic was used for patient characteristics. Student's *t*-test was used for continuous data, and Chi-square test or Fischer's exact test for binary data, depending on the sample size to derive the *p*-value. A p-value <0.05 was considered significant.

3. Results

During the 16 year study period, 612 patients presented for a risk reducing salpingo-oophorectomy (RRSO) at Erasmus MC. Eighty three of these patients were excluded because no *BRCA* mutation was proven. Two patients were excluded because they underwent only salpingectomy. Eight of the included patients underwent additional salpingectomy

between 2000 and 2016 after a previous oophorectomy between 1995 and 2005. Two patients underwent a unilateral salpingo-oophorectomy; one had a history of hysterectomy with removal of one adnexa, and the other had a prior unilateral salpingectomy due to ectopic pregnancy.

In total, 527 patients were included. Sixty eight percent (358/527) of these patients carried a *BRCA1* mutation, 31.6% (167/527) carried a *BRCA2* mutation, and 0.4% (2/527) carried both *BRCA1* and *BRCA2* mutations. Median age at RRSO of all the included patients was 47 years (range 32–78 years). The menopausal status was available for 504 patients, of which 63.1% (318/504) were pre-menopausal, and 36.9% (186/504) were post-menopausal. Thirty eight percent (202/527) of all included patients had a previous breast carcinoma. Thirty nine percent (141/358) of *BRCA1* carriers had a previous breast carcinoma, and for *BRCA2* carriers, this was 37% (61/167). The patient characteristics are elaborated in Table 1.

For *BRCA1* carriers, 32% (115/358) underwent RRSO by the recommended age (≤40 years). For *BRCA2* carriers, 47% (78/167) were operated on by the recommended age (≤45 years). The average age of those operated on after the recommended age was 50.6 years (range: 41–78 years, median: 50.1 years) for *BRCA1* carriers, and 56.7 years (range: 46–71 years, median: 55.5 years) for *BRCA2* carriers.

The prevalence of high grade serous carcinoma in the whole cohort was 2.3% (12/527); in *BRCA1* carriers this was 3% (11/358) and in *BRCA2* carriers 0.6% (1/167). The difference in the prevalence of high grade serous carcinoma between *BRCA1* and *BRCA2* carriers was not statistically significant ($p = 0.12$).

The median age of the *BRCA1/2* carriers with high grade serous carcinoma was 52 years (range: 38–65 years). Of the *BRCA1* carriers with a high grade serous carcinoma, 91% (10/11) underwent RRSO after the recommended age (>40 years). The 1 *BRCA2* carrier with a high grade serous carcinoma had also undergone RRSO after the recommended age (>45 years). The rates of high grade serous carcinoma at RRSO in both *BRCA1* and *BRCA2* carriers were significantly higher for women operated on after the recommended age ($p = 0.03$). The exact ages of the *BRCA1/2* carriers with a high grade serous carcinoma are provided in Table 2.1, and the prevalence of lesions in *BRCA1* carriers by age decade in Supplementary Table 1.

Out of the 12 patients with a high grade serous carcinoma, the pre-operative CA-125 level was elevated in 3 patients, and in 2 of them a mass suspicious for malignancy was identified on the pre-operative ultrasound [Table 2.1]. The third patient with an elevated pre-operative CA-125 was morbidly obese at the time of her pre-operative ultrasound. Although she had lost weight in order to be fit for RRSO, the ultrasound was not repeated. This may be the reason why her ovarian mass was not detected prior to RRSO. None of the other 9 patients with a carcinoma had any abnormalities in their CA-125 level or on the pre-operative ultrasound. Thus, the prevalence of an unsuspected high grade serous carcinoma in our cohort was 1.7% (9/527).

Table 1
Patient characteristics of all included *BRCA1/2* carriers who underwent RRSO.

Type of mutation ($n = 527$)	Number of cases (Percentage)
— *BRCA1*	358 (67.9)
— *BRCA2*	167 (31.6)
— Both	2 (0.4)
Menopausal status at RRSO ($n = 504$)	Number of cases (Percentage)
— Premenopausal	318 (63.1)
— Postmenopausal	186 (36.9)
Previous breast carcinoma (n = 527)	Number of cases (Percentage)
— Present	202 (38.3)
Follow up ($n = 525$)	Median follow up interval – 56.9 months
Outcome on follow up	Number of cases (Percentage)
— Deceased	19 (3.6)
— Breast carcinoma	55 (10.5)
— Peritoneal serous carcinoma	2 (0.4)
— Other malignancies	25 (4.7)
— No evidence of disease	424 (80.8)

The peritoneal wash cytology of the patients with a high grade serous carcinoma was positive for malignant cells in 33% (4/12), suspicious for malignant cells in 8% (1/12), and was negative in 59% (7/12) of cases [Table 2.2].

The tumor size of the high grade serous carcinoma ranged from 0.5 to 90 mm. Following the consensus statement [18], the primary site was allocated to the fallopian tube in 59% (7/12), and to the ovary in 33% (4/12). In one patient with high grade serous carcinoma in the ovary, the primary site of the tumor could not be ascertained with certainty, as the fallopian tubes were not completely sampled. In 86% (6/7) of the high grade serous carcinoma allocated as tubal-origin, an ipsilateral co-existing serous tubal intra-epithelial carcinoma (STIC) was identified [Table 2.2]. On immunohistochemistry with p53, 75% (9/12) of the high grade serous carcinoma showed a mutation pattern, and 25% (3/12) showed a null-pattern. All the cases with high grade serous carcinoma had MIB-1 labelling index >10%.

Twenty five percent (3/12) of the high grade serous carcinoma were FIGO Stage IA, and 33% (4/12) were FIGO Stage IIIC. The rest (42%) comprised a single case each of FIGO Stages IC, IIB, IIC, IIIA, and IIIB. These patients were treated according to the Dutch National Guidelines [15]; cytoreductive surgery was carried out for the 4 patients with a Stage IIIC carcinoma, and for the single patient with a Stage IIIB carcinoma. All the 12 patients with carcinoma received post-operative chemotherapy, as detailed in Table 2.2.

In total, 10 STIC lesions were detected, of which 6 co-existed with a high grade serous carcinoma, and 4 were isolated STIC lesions. Thus, the prevalence of all STIC lesions was 1.9% (10/527), and that of isolated STIC 0.8% (4/527). Three (75%) of the 4 isolated STIC were found in *BRCA1* carriers and 1 (25%) was found in a *BRCA2* carrier.

The median age of the patients with an isolated STIC was 54 years (range: 46–71 years). All the women with an isolated STIC had undergone RRSO after the recommended ages (median age: 54 years). The difference in the median age of patients with an isolated STIC and patients with a high grade serous carcinoma was not statistically significant ($p = 0.37$).

For the patients with an isolated STIC, no abnormalities were detected in the pre-operative CA-125 levels or on the pre-operative ultrasound. The peritoneal wash cytology reports were available for 2 of these patients, and both of them were negative for malignant cells. The details of the cases with an isolated STIC are shown in Table 3.

All the cases of STIC showed characteristic histological appearances, with compatible p53 and MIB-1 immunohistochemistry. Three out of the four isolated STIC showed mutation pattern, and 1 showed null-pattern on p53 immunohistochemistry.

In accordance with the Dutch National Guidelines [15], none of the patients with an isolated STIC underwent any additional surgery, staging procedure, or received post-operative chemotherapy.

In addition to the lesions described above, other findings were: a serous borderline tumor, an adult type granulosa cell tumor, a Brenner tumor, an adenomatoid tumor, a Leydig cell tumor and in one case a focus of metastatic breast carcinoma.

Follow-up information was available for 525 patients; the median follow-up duration was 56.9 months. Eleven percent (55/525) developed breast carcinoma, 5% (25/525) developed other malignancies, 4% (19/525) died, and 0.4% (2/525) developed peritoneal serous carcinoma. Out of the 19 patients in our cohort who expired on follow-up, 1 patient died from complications related to high grade serous carcinoma in the ovary, 11 patients died from complications related to breast carcinoma, and the other 7 patients died from causes unrelated to breast carcinoma or high grade serous carcinoma.

From our cohort, two patients developed peritoneal serous carcinoma on follow-up. Both of these patients were *BRCA1* carriers, and had isolated STIC at RRSO. The peritoneal wash cytology reports were not available for both patients, as this was not carried out routinely at the time of their RRSO. For both the patients, the fallopian tubes were

Table 2.1
Cases with high grade serous carcinoma at RRSO – Patient characteristics.

Patient	Age at RRSO (yrs.)	*BRCA* status	Previous breast carcinoma	CT received for previous breast carcinoma	Menopausal status	CA-125	Pre-operative ultrasound
1	51	*BRCA1*	absent	–	pre-menopausal	not elevated	NAD
2	57	*BRCA1*	present	yes	unknown	elevated	NAD
3	43	*BRCA1*	present	yes	pre-menopausal	not elevated	NAD
4	61	*BRCA1*	absent	–	pre-menopausal	not elevated	NAD
5	50	*BRCA1*	absent	–	pre-menopausal	not elevated	NAD
6	65	*BRCA1*	present	yes	post-menopausal	not elevated	NAD
7	53	*BRCA1*	present	yes	pre-menopausal	not elevated	NAD
8	57	*BRCA1*	absent	–	unknown	not elevated	NAD
9	38	*BRCA1*	absent	–	pre-menopausal	not elevated	NAD
10	42	*BRCA1*	present	yes	pre-menopausal	not elevated	NAD
11	56	*BRCA2*	absent	–	post-menopausal	elevated	mass suspicious for malignancy
12	46	*BRCA1*	absent	–	pre-menopausal	elevated	mass suspicious for malignancy

RRSO: Risk-reducing salpingo-oophorectomy, yrs.: years, BRCA: breast cancer susceptibility gene, CT: chemotherapy, NAD: no abnormality detected.

totally enclosed at the time of pathology handling. There was no invasive carcinoma in additional sections prepared for the study.

Patient 1, aged 46 years at RRSO, developed peritoneal serous carcinoma 118 months later. She was pre-menopausal at the time of RRSO, and did not have a previous breast carcinoma. The pre-operative CA-125 level was not elevated, and no abnormalities could be detected on the pre-operative ultrasound. On immunohistochemistry with p53, both the preceding STIC and the peritoneal serous carcinoma showed 'mutation pattern'. On NGTS, the same somatic missense mutation in *TP53* exon 8 (c.817C > T;p.R273C) was detected in both lesions, which is in accordance with the observed p53 'mutation pattern'.

Patient 2 was aged 53 years at RRSO, and developed peritoneal serous carcinoma after 80 months. She was also pre-menopausal at RRSO. This patient had a breast carcinoma 13 years prior to the RRSO, which was treated with surgical excision and chemotherapy. Her pre-operative CA-125 levels and the ultrasound findings were within normal limits. Immunohistochemistry with p53 showed a 'null-pattern' in both the STIC and the peritoneal serous carcinoma. Identical somatic frameshift mutation in *TP53* exon 4 (c.249_250delGG) was detected in both lesions on NGTS, and is in accordance with the observed p53 'null-pattern'.

For both of these patients, based on the identical p53 mutations detected in the STIC and peritoneal serous carcinoma, a clonal relationship between these lesions was established. The treatment for both the patients comprised 3 cycles of neoadjuvant chemotherapy, followed by interval cytoreductive surgery, and 3 cycles of post-operative chemotherapy. Both the patients were alive at the time of submission of the manuscript. The histology and immunohistochemistry of the lesions from patient 2 are illustrated in Fig. 1, and the corresponding NGTS results are illustrated in Fig. 2 (A and B).

4. Discussion

The risk of high grade serous carcinoma is significantly higher in carriers of *BRCA1/2* germline mutations, compared to the general population. This risk becomes even more substantial after the age of 40 years for *BRCA1* carriers, and after 45 years for *BRCA2* carriers [2]. Risk reducing salpingo-oophorectomy is therefore routinely offered to *BRCA1/2* carriers by 40–45 years, which is known to reduce all-cause mortality, breast cancer specific mortality, and ovarian cancer specific mortality for these women [2,3]. Over the past decade, the distal fallopian tube has come to be established as the source of these high grade serous carcinomas, with serous tubal intraepithelial carcinoma (STIC) being

Table 2.2
Cases with high grade serous carcinoma at RRSO – Pathology findings and follow-up.

Patient	Age at RRSO (yrs.)	PWC	Primary site	Tumor size (mm)	STIC	FIGO stage	Treatment	Follow-up	
								Outcome	Follow-up duration (months)
1	51	negative	FT	Paratubal: 3	Present	IC	None	NED - 2017	161.4
2	57	negative	FT	RO: 60, RFT: 5	Absent	IIIC	6× carbo/pacli and debulking	NED – 2016	83.8
3	43	positive	FT	RFT: <1, RO: 0.6	Present	IIIA	9× carbo/pacli	Colon carcinoma (pT3N2) – 2015	147.3
4	61	negative	FT	FT: 2	Present	IIB	6× carbo/pacli	NED – 2016	107.7
5	50	positive	FT	FT: 18, BO: 5	Present	IIIB	9× carbo/pacli	Lost in follow-up	45.7
6	65	negative	FT	FT: 1	Present	IA	None	NED – 2016	72.8
7	53	negative	FT	FT: 2	Present	IA	None	NED – 2016	65.7
8	57	positive	Ovary	LO: 18 RO: superficial	Absent	IIIC	6× carbo/pacli	Breast carcinoma - 2017	98.1
9	38	positive	Ovary	BO: 0.5	Absent	IIC	6× carbo/pacli	NED – 2016	82.0
10	42	negative	Ovary	RO: 0.6	Absent	IA	None	Breast carcinoma - 2011 NED – till 2015	54.3
11	56	suspicious for malignant cells	Ovary	RO: 90	Absent	IIIC	6× carbo/pacli	Lost in follow-up	1.3
12	46	negative	Unclear* (assigned to ovaries)	RO: 70, LO: <1	Absent	IIIC	9× carbo/pacli	Deceased at 50 yrs. of age	57.4

*Tubes not fully sampled.
PWC: peritoneal wash cytology, FT: fallopian tube, RFT: right fallopian tube, LFT: left fallopian tube, RO: right ovary, LO: left ovary, BO: both ovaries, FIGO: The International Federation of Gynecology and Obstetrics, NED: No evidence of disease.

Table 3
Cases with isolated STIC at RRSO.

Patient	Age at RRSO (yrs.)	BRCA status	Previous breast carcinoma	CT received for breast carcinoma	Menopausal status	CA-125 level	Pre-operative ultrasound	PWC	Follow-up	
									Outcome	Duration (months)
1	46	*BRCA1*	absent	–	pre-menopausal	not elevated	NAD	NA	Peritoneal serous carcinoma – 2015	135.7
2	53	*BRCA1*	present	yes	pre-menopausal	not elevated	NAD	NA	Peritoneal serous carcinoma – 2012	118.1
3	55	*BRCA1*	absent	–	post-menopausal	not elevated	NAD	negative	NED – till 2015	6.0
4	71	*BRCA2*	absent	–	post-menopausal	not elevated	NAD	negative	NED – till 2014	2.1

yrs.: years, BRCA: Breast carcinoma susceptibility gene, CT: chemotherapy, PWC: peritoneal wash cytology, NA: not available, NED: no evidence of disease.

identified as the precursor lesion. For further development of risk reduction strategies for high-risk women, information on in-situ and invasive carcinoma from retrospective RRSO cohorts can be invaluable. We studied a single institutional 16 year cohort of *BRCA1/2* carriers presenting for RRSO, and we present here the prevalence of high grade serous carcinoma and STIC, and the follow-up.

Our cohort comprised 527 patients, of which 67.9% were *BRCA1* mutation carriers, 31.6% were *BRCA2* mutation carriers, and 0.4% were carriers of both mutations. The proportion of *BRCA1* carriers in our study was higher compared to 2 recent studies from the Netherlands, which had 56% and 44% *BRCA1* carriers [20,21]. We noticed that only 32% of *BRCA1*, and 47% of *BRCA2* carriers underwent RRSO by the NCCN recommended ages [3]; this was partly due to the later detection of the carrier status in these women. Similar findings have been reported by Lee et al. and Garcia et al., where 21% and 17% of women in their respective cohorts underwent RRSO by the recommended ages [22,23].

The prevalence of high grade serous carcinoma in our entire cohort was 2.3% (12/527), and the rates reported in the literature vary between 0.9 and 17% [4,24–28]. Our findings reflect that of Powell et al., who reported a carcinoma prevalence of 2.7%, pooling together 3030 RRSOs from 13 studies [29]. High grade serous carcinoma was detected in 3% of the *BRCA1* carriers, and in 0.6% of the *BRCA2* carriers in our cohort. Detection of an invasive carcinoma at RRSO is known to be more common for *BRCA1* than for *BRCA2* carriers [29]. The reported rates of malignancy in *BRCA1* and *BRCA2* carriers vary between 2.1 and 11%, and 0–6% respectively [4,24–29].

The median age of patients (*BRCA1* and *BRCA2*) with a high grade serous carcinoma was 52 years in our study. In a single institutional study on 345 RRSO conducted by Connor et al., the mean age of patients with a neoplasia was 54.4 years [24]. For both *BRCA1* and *BRCA2* carriers, a higher rate of high grade serous carcinoma was noted in the women undergoing RRSO after the recommended ages ($p = 0.03$). This is line with the findings from Finch et al., who reported an almost two-fold increase in the rates of high grade serous carcinoma in *BRCA1/2* women who underwent RRSO after 40 years of age [26]. Fifty percent (6/12) of the high grade serous carcinoma from our cohort were FIGO Stage III, and

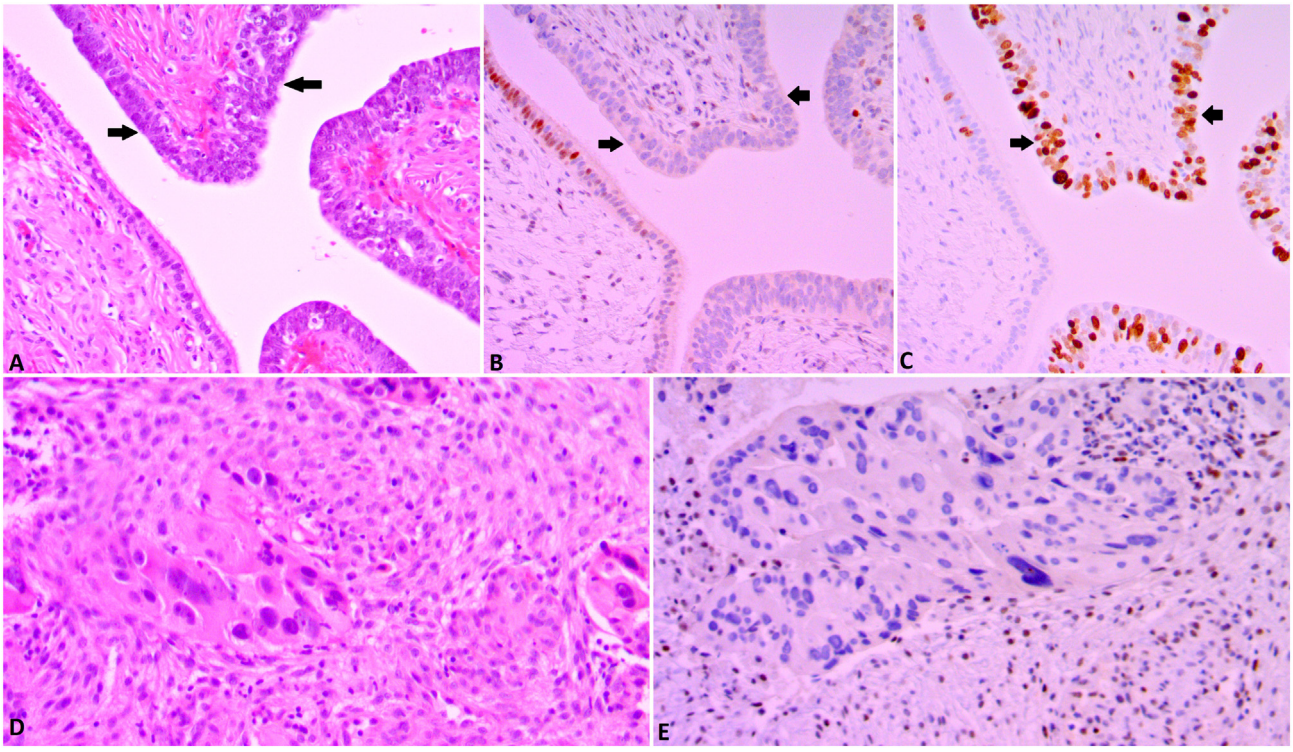
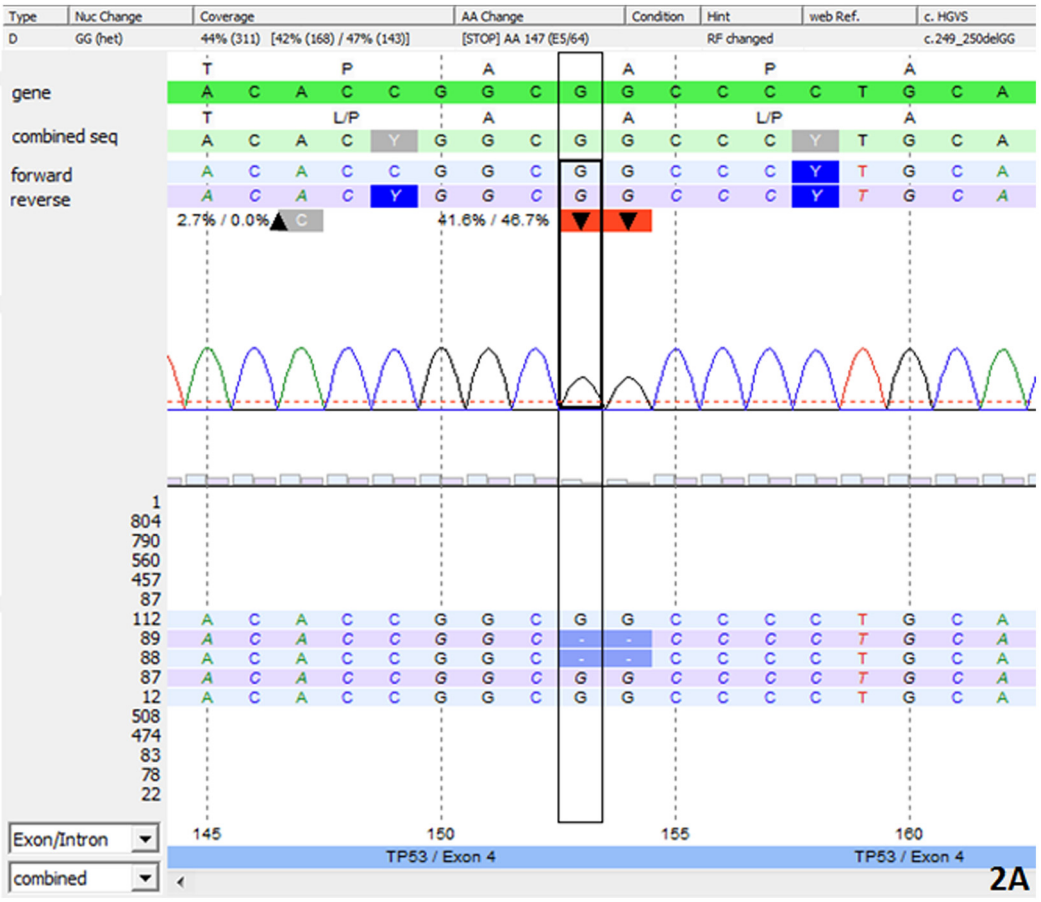


Fig. 1. Histology and immunohistochemistry of the serous intraepithelial carcinoma (STIC) present at RRSO (A-C), and the peritoneal serous carcinoma (D,E). A. Characteristic histological appearance of the STIC (indicated by arrows) can be appreciated on a Hematoxylin Eosin (H-E) stained section. Atypical tubal epithelial cells exhibit nuclear pleomorphism, abnormal chromatin, mitotic figures, loss of cilia, and epithelial stratification. B. Immunohistochemistry with p53 shows a null-pattern (indicated by arrows). C. MIB-1 labelling index is >10% (indicated by arrows). D. Histological appearance of peritoneal serous carcinoma, H-E stained section. E. Similar to the preceding STIC lesion, the peritoneal serous carcinoma also shows null-pattern on p53 immunohistochemistry.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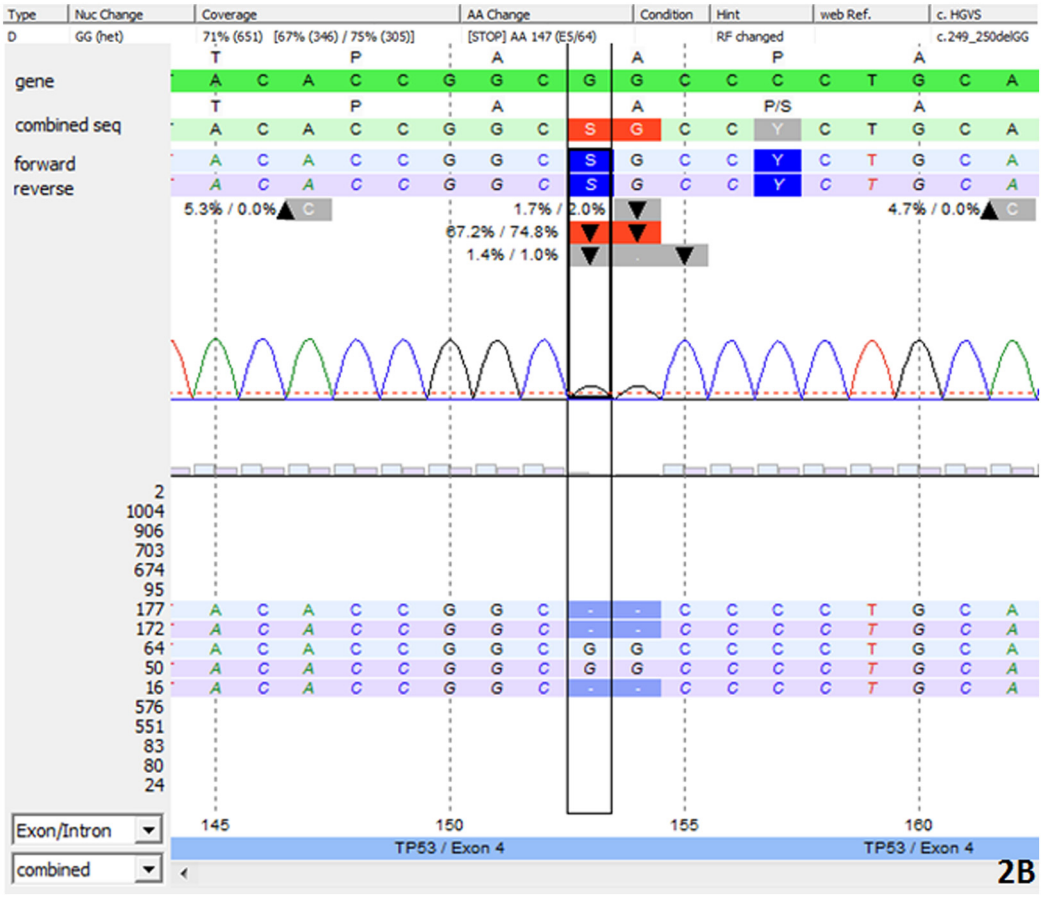
AA Change
Condition
Hint
web Ref.
c. HGVS
D
GG (het)
44% (311) [42% (168) / 47% (143)]
[STOP] AA 147 (E5/64)
RF changed
c.249_250delGG
gene
combined seq
forward
reverse
2.7% / 0.0%
41.6% / 46.7%
Exon/Intron
combined
145
150
155
160
TP53 / Exon 4
TP53 / Exon 4
2A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GG (het)
71% (651) [67% (346) / 75% (305)]
[STOP] AA 147 (E5/64)
RF changed
c.249_250delGG
gene
combined seq
forward
reverse
5.3% / 0.0%
1.7% / 2.0%
67.2% / 74.8%
1.4% / 1.0%
4.7% / 0.0%
Exon/Intron
combined
145
150
155
160
TP53 / Exon 4
TP53 / Exon 4
2B

all of them were present in women who underwent RRSO after 40 years. This further emphasizes the importance of undergoing RRSO by the recommended age.

Nine out of the 12 high grade serous carcinoma from our study were unsuspected malignancies, where no abnormalities were found in the pre-operative CA-125 levels or on pre-operative ultrasound. Thus, the prevalence of unsuspected high grade serous carcinoma in our cohort was 1.7% (9/527). This is lower than the rates reported by Finch et al. (4.4%), Manchanda et al. (5.1%), Connor et al. (5.4%), Mingels et al. (7.1%), and Powell et al. (7.9%) [25,26,29–31]. All of the women with an unsuspected carcinoma from our cohort were *BRCA1* carriers. This mirrors the findings of Zakhour et al., who reported *BRCA1* mutation status to be a predictor of occult neoplasia at RRSO [32].

The primary site of high grade serous carcinoma was allocated to the fallopian tube in 59% (7/12) of our cases, following the recently published consensus statement [18]. Of these, 86% (6/7) had an accompanying STIC, supporting an origin from this precursor lesion. In the single case of tubal origin high grade serous carcinoma without a co-existing STIC lesion, it is probable that a STIC lesion was subsumed in invasive carcinoma, or that a small STIC lesion was missed. A potential shortcoming of our study is the possible lack of uniformity in the pathology handling of the RRSO specimens, as the laboratory protocols prior to 2010 are not available.

In view of the fact that up to 70% of occult/invasive carcinoma at RRSO can be of tubal origin [29], and to circumvent the adverse effects of surgical menopause, an earlier (pre-menopausal) salpingectomy with a delayed (post-menopausal) oophorectomy is currently being evaluated in clinical trials, as an alternative to RRSO. The ongoing trials include the MD Anderson study, Radical Fimbriectomy study, TUBA study, Stand up to Cancer: WISP study, and the PROTECTOR study [10–14]. In our cohort, 33% (4/12) of the high grade serous carcinoma were allocated as ovarian origin. However, in view of the limitation of this study regarding pathology handling of RRSO specimens, we cannot draw any firm conclusion regarding the potential benefit from an earlier salpingectomy. As also stated by Gaba et al., we believe that till further data is available from the ongoing trials, an earlier salpingectomy with a delayed oophorectomy should be only offered within a controlled research environment [33].

The prevalence of isolated STIC in our study was 0.8% (4/527). Patrono et al. combined the data from 15 studies with 3850 patients, and reported the rate of isolated STIC to be around 2% (range 0.4–11%) [34]. Three of the four isolated STICs in our study were discovered in *BRCA1* carriers. Van der Hoeven et al., combining the data from 15 studies with 2863 patients, reported that 64% of women with isolated STIC at RRSO were *BRCA1* carriers, and 36% *BRCA2* carriers [21]. This observation was also corroborated by Powell et al., who reported that an isolated STIC lesion can be three times more common in *BRCA1* carriers than in *BRCA2* carriers [29]. For *BRCA1/2* carriers with an isolated STIC, the peritoneal wash cytology may be positive for malignant cells in up to 14% of cases [29]. Reports of peritoneal wash cytology were available for 2 of our patients with an isolated STIC, and they were both negative.

A particularly interesting finding of our study was the development of peritoneal serous carcinoma in 2 *BRCA1* carriers, who had isolated STIC at RRSO. These women were aged 46 years and 53 years at RSSO, and developed peritoneal serous carcinoma after 118 months and 80 months respectively. In the retrospective RRSO cohort of Zakhour et al. comprising 257 *BRCA1/2* carriers, 2 *BRCA1* carriers with isolated STIC developed peritoneal serous carcinoma 42 months and 32 months respectively after RRSO [32]. Peritoneal carcinomatosis in patients with isolated STIC at RRSO may occur in 0–22% of cases, and is more common in *BRCA1* carriers [20]. The reported median interval to peritoneal carcinomatosis is 54.5 months (Range 11–292 months) [20]. The novel finding of our study was the demonstration of a clonal relationship between the STIC and the peritoneal serous carcinoma, based on the detection of identical *TP53* mutations by NGTS.

The possible implication of our findings is that STIC lesions possess a metastatic potential, and may be the precursor lesion of peritoneal serous carcinoma. One of our patients (patient 2, aged 53 years at RRSO) had a breast carcinoma 13 years prior to the RRSO, and had received chemotherapy for the same. There is a theoretical possibility that a focus of occult tubal invasive carcinoma existed in the patient, which receded due to the chemotherapy, leaving behind a small STIC lesion, and that occult carcinoma was the source of metastasis. However, in view of the fact that the patient received chemotherapy for the breast carcinoma 13 years prior to the RRSO, such an association seems unlikely. Interestingly, Chay et al. reported the development of a peritoneal serous carcinoma in a *BRCA1* carrier, with isolated 'low grade' STIC lesion at RRSO, after an interval of 18 months [35]. This patient also had a breast carcinoma 7 years prior to the RRSO, and had received chemotherapy for the same [35]. Due to the lack of molecular testing on these samples, the authors could not establish a clonal relationship between the tubal precursor and the peritoneal carcinoma.

The exact significance of the interplay of tubes and ovaries in high grade serous carcinoma pathogenesis still remains unknown. A recent study proposes that genotoxic insults to the fallopian tubes lead to the development of tubal precursor lesions (early serous proliferations and STIC), the cells of which may escape and emerge later in the pelvis; a mechanism described as 'precursor escape' [36]. This hypothesis is supported by the findings of Soong et al., who established lineage identity between early serous proliferations in the distal fallopian tube and metastatic high grade serous carcinoma on the basis of shared site-specific *TP53* mutations [37]. Intidhar Labidy-Galy et al. also demonstrated identical somatic *TP53* mutations in STIC, high grade serous carcinoma of ovarian or of tubal origin (both sporadic and familial), and peritoneal serous carcinoma through whole-exome sequencing and copy number analysis [38]. A recent case report also establishes the link between concurrently occurring retroperitoneal high grade serous carcinoma and STIC, on the basis of identical somatic mutations of *TP53* (c.536A > G, p.179H > R), and *BRCA2* (c.6385G > T, p.2129E > X) [39].

An alternate hypothesis is that the normal tubal epithelium ingrains itself into the peritoneum prior to RRSO, and undergoes malignant transformation over the course of several years. Harmsen et al. noted the presence of remnants of fallopian tube fimbriae in peritoneal biopsies taken from patients subsequent to their RRSO [20]. It is not improbable that a STIC develops in these fimbrial tissue implants and eventually transforms into a high grade serous carcinoma.

The optimal management strategy of patients with isolated STIC at RRSO also needs to be determined. Currently in The Netherlands, patients with an isolated STIC do not undergo staging procedures, or receive additional treatment [15]. Van der Hoeven et al. in their systematic review reported a lower risk of recurrence in patients who were staged or administered chemotherapy after the diagnosis of isolated STIC at RRSO [21]. Although isolated STIC lesions with positive cytology have been reported, there has been no report of an isolated STIC with a positive finding at staging [29]. Powell et al. therefore opined that for isolated STICs with positive cytology, a formal staging procedure is unlikely to have yield, and is unnecessary as long as invasive carcinoma has been thoroughly ruled out histologically [29]. Gaba et al. recommend a full staging procedure for patients with an isolated STIC with positive cytology, or abnormal staging CT-scan [33].

In order to assess the relevance of routine staging or adjuvant treatment for patients with isolated STIC, higher quality evidence, and pooling of data will be necessary, as even in a comparatively large cohort such as ours the number of malignancies is limited. Detailed histopathological examination of RRSO specimens for detection of STIC is

Fig. 2. Next Generation Targeted Sequencing results proving the clonal relationship between the STIC present at RRSO and the peritoneal serous carcinoma. Both the STIC (A) and the peritoneal serous carcinoma (B) show an identical frameshift mutation (c.249_250delGG) in the *TP53* gene on chromosome 17.

crucial if the biology of these lesions is to be properly understood. Longer follow-up should be considered for women found to have STIC at RRSO, in view of the development of peritoneal serous carcinoma after long lag periods. We hope that the data that we present here can be useful for gynecologic counseling of *BRCA1/2* carriers, and will facilitate the development of future risk reducing strategies.

5. Conclusion

The high grade serous carcinoma in our cohort was more commonly of tubal origin, and was more frequent in *BRCA1/2* carriers undergoing RRSO after the recommended age. Prospective research on the natural history of STIC, including detailed molecular analysis must be carried out to determine the metastatic potential of STIC, and whether patients with an isolated STIC merit staging. Longer follow up of patients with STIC at RRSO should be considered, as STIC can give rise to peritoneal serous carcinoma after long lag periods.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2019.03.003.

Conflict of interest statement

The authors have no conflicts of interest, or competing interests. Winand N. M. Dinjens reports personal fees from Roche and Bristol-Myers Squibb, outside the submitted work. No external funding was received for this study.

Author contributions

Fleur Blok: Designed the study, collected the data, searched the literature, did the statistical analysis and wrote the manuscript.

Shatavisha Dasgupta: Searched the literature, did the histological analysis, arranged the figures, co-wrote and edited the manuscript.

Winand N. M. Dinjens: Supervised the molecular analyses, provided the NGTS data, and revised the manuscript critically for important intellectual content.

E.M. Roes: Revised the manuscript critically for important intellectual content.

Heleen J. van Beekhuizen: Designed the study, provided scientific input, and revised the manuscript critically for important intellectual content.

Patricia C. Ewing-Graham: Co-wrote the manuscript, did the histological analysis, provided scientific input, and revised the manuscript critically for important intellectual content.

All the authors gave their approval for the version to be published and agree to be accountable for all of the aspects of the study.

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