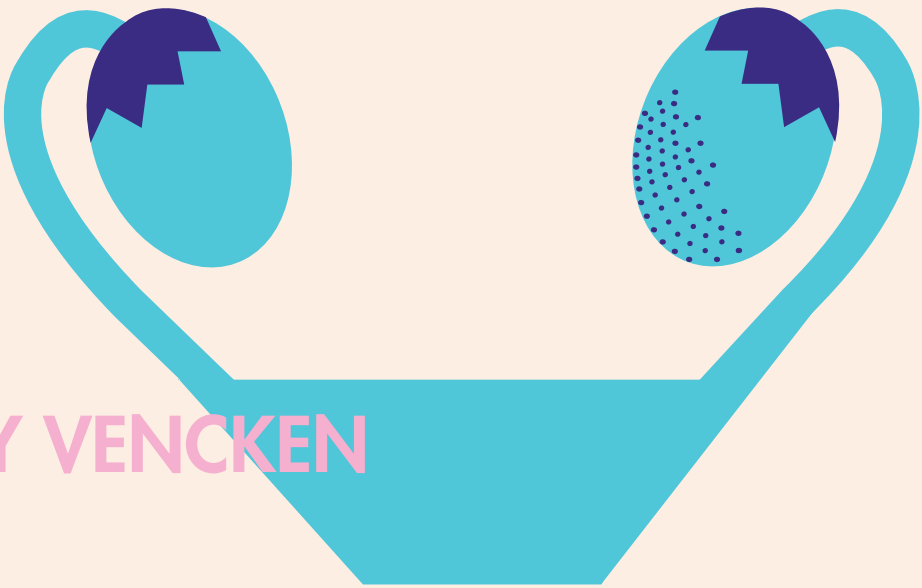
BRCA1- AND *BRCA2*-ASSOCIATED OVARIAN CANCER: DIFFERENT DISEASES?



PEGGY VENCKEN

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Thesis, Erasmus University Rotterdam, The Netherlands

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BRCA1- AND *BRCA2*-ASSOCIATED OVARIAN CANCER: DIFFERENT DISEASES?

BRCA1- en *BRCA2*-geassocieerd ovariumcarcinoom:

verschillende entiteiten?

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GENERAL INTRODUCTION

1.1. Ovarian cancer: epidemiology, treatment and survival

Ovarian cancer will develop in approximately 1.4% of the Dutch women accounting for approximately 1250 new patients yearly in the Netherlands, which is comparable with the incidence in other Western world countries. The disease mainly develops in women of 40 years of age and older, with the highest incidence around 60 years of age.[1-3] Despite this relatively low incidence, ovarian cancer is the leading cause of death from gynaecological malignancies in the Netherlands, with about 1000 deaths annually.[1,4-6]

Ninety per cent of all ovarian cancers are of epithelial origin, and therefore can exhibit features of the different epithelia originating from the Mullerian ducts i.e. the epithelium lining the fallopian tubes, cervix, uterus, and part of the vagina. In this way, epithelial ovarian cancers (EOC) can be subdivided into serous, mucinous, endometrioid, clear cell, undifferentiated carcinomas and Brenner tumors.[2,7] Most ovarian cancers are of serous histology (65-70%). Traditionally, differentiation grade has been classified in grade 1, 2 and 3, according to the Silverberg criteria.[8] More recently another classification system for ovarian cancer has been introduced, subdividing into low-grade (Type I) and high-grade (Type II) tumors.[9,10] Low-grade tumors have frequent mutations in the KRAS, BRAF, ERBB2, CTNNB1, PTEN, PIK3CA, ARID1A, and PPP2R1A genes, lack TP53 mutations and have a better outcome than high-grade tumors.[10] Low-grade carcinomas exhibit low-grade nuclei with infrequent mitotic figures. This entity comprises low-grade serous, low-grade endometrioid,[11] clear cell and mucinous carcinomas and Brenner tumors.

High-grade carcinomas have high-grade nuclei and numerous mitotic figures, feature a high growth rate, are characterized by TP53 mutations and lack mutations of KRAS, BRAF, or ERBB2 and have molecular alterations that disturb expression of *BRCA* either by a mutation in the gene or by promoter methylation.[10] High-grade carcinomas are assumed to originate de novo and comprise high-grade serous, high-grade endometrioid, mixed mesodermal tumors (carcinosarcomas) and undifferentiated carcinomas.[10]

More recently, it was suggested that the origin of low-grade and high-grade ovarian cancer may be intraepithelial carcinoma located in the fallopian tube, (also known as serous tubal intraepithelial carcinomas (STIC) or tubal

intraepithelial carcinomas (TIC)), rather than the ovarian surface epithelium as previously believed.[10,12]

To determine extensiveness of the disease, ovarian cancer is classified into different stages according to the International Federation of Gynaecology and Obstetrics (FIGO) staging system.[6,7] Due to the non-specific symptoms at the beginning of the disease, the majority (70%) of the patients with ovarian cancer is diagnosed with advanced stage disease (FIGO stage IIb and higher; see table 1).[13]

The prognosis of ovarian cancer is mainly determined by the histology, grade, stage and age at diagnosis. Residual disease after debulking surgery is also an important prognostic factor. Five year survival of high stage (stage IIb-IV) epithelial ovarian cancer is only 5-60%, whereas women with low stage disease (FIGO I-IIa) have a 5 years survival of 75-100%.[1,6]

Standard treatment of ovarian cancer consists of surgery in combination with chemotherapy for women with stage IIb and higher. Surgery aims to remove all tumor or as much tumor deposits as possible, because over time it became clear that complete resection of all macroscopic disease, whether performed as primary treatment or after neoadjuvant chemotherapy is an important prognostic factor for survival.[14-17] A higher rate of optimal cytoreduction is observed in patients treated by gynaecologic oncologists and when surgery is performed in high volume institutions.[16,18,19] Furthermore, surgical staging is essential for low-volume disease, when the disease seems to be restricted to the ovaries, since performing a staging procedure results in approximately 30% of the ovarian cancer patients with a presumed stage I or II to upstaging of the disease.[20] Depending on the disease presentation, women with advanced stage (stage IIb and higher) epithelial ovarian cancer are either treated with debulking surgery, followed by chemotherapy or with neoadjuvant chemotherapy (=chemotherapy given before surgery), followed by interval debulking surgery, and postoperative chemotherapy. The survival after both treatment strategies is comparable, but in case of bulky disease postoperative mortality and morbidty is lower in the neoadjuvant chemotherapy group, probably by reducing the tumor load and consequently increasing the surgical resectability.[14]

The standard chemotherapeutic regimen nowadays consists of carboplatin in combination with paclitaxel. During the past decades, the chemotherapeutic treatment of ovarian cancer has considerably changed. The first frequently used chemotherapy agents for ovarian cancer were non-platinum-based combinations, such as HexaCAF (hexamethylmelamine, cyclophosphamide, methotrexate, 5-fluorouracil). In 1979 the cisplatin-based CHAP-5-regimen (cyclophosphamide, hexamethylmelamine, adriamycine, cisplatin) was introduced for ovarian cancer since this resulted in a more favourable progression free survival (PFS) and overall survival (OS), compared with the previously used non-platinum based chemotherapy combination.[21,22] Later, the combination of cisplatin/cyclophosphamide became standard, because it was associated with less toxicity and shorter hospital admission time.

Table 1: FIGO stages of ovarian cancer (2006)

Stage I	Tumor limited to the ovaries	
	Stage Ia	Tumor in one ovarium, capsule intact, no ascites
	Stage Ib	Tumor in both ovaries; capsule intact, no ascites
	Stage Ic	Stage Ia or Ib with tumor on surface ovarium, capsule rupture or positive ascites
Stage II	Ovarian tumor with pelvic extension	
	Stage IIa	Extension and/or metastases to the uterus and/ or fallopian tubes
	Stage IIb	Extension to other pelvic tissues
	Stage IIc	Stage IIa or IIb with ascites, capsule rupture or positive ascites
Stage III	Tumor involving the upper abdomen or lymph nodes	
	Stage IIIa	Microscopic disease outside the pelvis with negative lymph nodes
	Stage IIIb	Tumor outside the pelvis ≤2 cm and lymph nodes negative
	Stage IIIc	Tumor outside the pelvis>2 cm or nodal involvement
Stage IV	Distant organ metastasis, including pleural space or hepatic or splenic parenchyma	

In 2000 the European Organisation for Research and Treatment of Cancer (EORTC)/GOG study showed that the addition of paclitaxel besides cisplatin resulted in an increased median PFS from 12 to 16 months (P<0.001) and an increased median OS from 25 to 35 months (P=0.001).[23] In further studies cisplatin was replaced by carboplatin, and it was shown that the combination of carboplatin/paclitaxel was as effective as cisplatin/paclitaxel, but associated with less side effects. Consequently, carboplatin/paclitaxel (with six 3-weekly cycles) became the new standard, with platinum being the main component. Treatment with gemcitabine, liposomal doxorubicine or topotecan did not

improve survival compared with carboplatin/paclitaxel.[22] Recently, van der Burg et al published the data of a dose dense regimen consisting of six weekly administrations of paclitaxel/carboplatin followed by six 3-weekly cycles, resulting in high response rates in both platinum-resistant and platinum-sensitive patients.[24]

Over the last years, studies have been performed investigating the value of vascular endothelial growth factor (VEGF) inhibitors (i.e. bevacizumab) added to platinum-based chemotherapy for recurrent ovarian cancer. While the combination has resulted in an increased PFS, OS was not improved. In view of the costs and the toxicity of bevacizumab the combination is not yet accepted as standard first line therapy for ovarian cancer.[25] Potentially, bevacizumab may be of greater value for particular subgroups of patients, such as high-grade serous cancers, but this remains to be studied. Another interesting class of new agents under investigation are the poly ADP-ribose polymerase (PARP) inhibitors, which will be further discussed in paragraph 1.6.

1.2. *BRCA* (BReast CAncer) genes and *BRCA* mutations

The *BRCA1* and *BRCA2* genes have been identified in 1994 and 1995, respectively through genome wide linkage studies using large pedigrees of families with clustering of multiple women, affected by breast cancer at a relatively young age. Later, it became clear that women, carrying a mutation in one of these genes not only conferred an increased risk of breast cancer, but also of ovarian cancer. The *BRCA1* gene is localized on chromosome 17q and encodes a protein of 1863 amino acids; *BRCA2* is a 13q-linked gene encoding a 3418 amino acid protein.[26-28]

BRCA genes, which operate as tumor suppressor genes, preserve chromosomal stability, and influence transcription and cell-cycle control.[29] Both *BRCA1* and *BRCA2* genes are critical for homologous recombination, the preferred pathway for repairing DNA double strand breaks that either arise spontaneously or are induced by exogenous factors such as chemotherapy or radiation.[30] While the tumor supression function of *BRCA2* is mainly restricted to double-strand DNA break repair by homologous recombination, namely by regulating the RAD51 protein, *BRCA1* plays a versatile role in tumor suppression through its ability to participate in DNA damage response, checkpoint control, mitotic spindle

assembly, centrosome duplication and sister chromatid decatenation.[31,32]
Worldwide more than 2000 different mutations in the *BRCA1* and *BRCA2* genes have been identified (listed in an international database), which are scattered throughout the genes.
It is known that the frequency of the various mutations differ between countries and even regionally. Mutations in the *BRCA1* gene are more common than *BRCA2* mutations.
The highest recorded rates of ovarian cancer have been reported among Israeli Jews born in Europe or North America. The majority of these women are of Ashkenazi origin and in this population three *BRCA* founder mutations have been identified, two in *BRCA1* and one in *BRCA2*. The founder mutations in *BRCA1* involve the deletion of an adenine and guanine c.68_69delAG, p.Glu23fs (*BRCA1*, exon2) and the insertion of a cytosine c.5266dupC, p Gln1756fs (*BRCA1*, exon 20), whereas the mutation in *BRCA2* involves the deletion of a thymine (c.5946delT, p.Ser1982fs (*BRCA2*, exon 11-H).[33]
One out of 43 individuals of Ashkenazi Jewish heritage test positive for one of the three "founder" mutations, which is at least ten times higher than the frequency of mutations in the non-Ashkenazi population.[33,34] Founder mutations in *BRCA1* or *BRCA2*, conferring higher mutation carrier rates in a particular population, have also been described in other geographic regions, such as Iceland, Greenland, Sweden[32] and the Netherlands.[35]
Overall, it is assumed that approximately 10-20% of all ovarian cancer cases are due to a genetic predisposition, with a higher frequency in women with high grade serous carcinomas.[29] So far, it is thought that mutations in the *BRCA1* and *BRCA2* genes are responsible for more than 90% of the hereditary ovarian cancer cases, while the other hereditary cases are due to mutations in the mismatch repair genes *MLH1, PMS2, MSH2* and *MSH6* and other yet unidentified genes.[6]

1.3. Risk of breast and/or ovarian cancer in female *BRCA* mutation carriers

The cumulative lifetime risk (CLTR) of a primary breast cancer (PBC) has been estimated to range between 50-80%, accounting for both *BRCA1* and *BRCA2* female mutation carriers.[36-38] *BRCA1*- and *BRCA2*–associated BC is often

diagnosed at a young age. The breast cancer risk becomes relevant from the age of 30 years, with an annual risk of 1-2%, and the highest risk between 40-50 years. The reported mean age at onset of a first breast cancer is about 39-44 years for *BRCA1* mutation carriers and about 43-48 years for *BRCA2* mutation carriers respectively.[39-42] The 10-year risk of a de novo breast cancer in the contralateral breast (CBC) is also increased and has been estimated to be 29-39% in *BRCA1*- and 23-35% in *BRCA2*-associated unilateral BC patients.[36-39,43-46] Risk of CBC is dependent on age at diagnosis of PBC and administered adjuvant systemic therapy (both endocrine, and chemotherapy). Female *BRCA1* mutation carriers have a 18%-54% CLTR of developing EOC, whereas for *BRCA2* mutation carriers the estimated CLTR is lower, ranging between 2.4%-19%.[36,37,43,47,48] The reported median age of developing ovarian cancer is about 51 years in the *BRCA1* group and about 55 years in the *BRCA2* group.[49,50]
In view of the increased BC risks of *BRCA* mutation carriers, it has been questioned whether the risk of developing BC after treatment for EOC may be influenced by the treatment (either surgery or chemotherapy) for EOC. So far, no data are yet available about the BC risk after *BRCA*-associated EOC.

1.4. Screening and risk reducing salpingo-oophorectomy (RRSO) for female *BRCA* mutation carriers

Results from yearly gynaecological screening programs (pelvic examination, transvaginal ultrasound, and Ca 125 analysis), aiming at early detection of EOC for women at high risk for hereditary ovarian cancer have not shown to be effective. EOC in high-risk women, detected during surveillance had a similar FIGO stage distribution, compared with non-screened *BRCA1/2* ovarian cancer patients and non-screened sporadic ovarian cancer patients.[51-53]
Therefore, the most safe and effective option for the prevention of ovarian/fallopian tube cancer in mutation carriers remains risk reducing salpingo-oophorectomy (RRSO) from the age of 35-40 years for *BRCA1* mutation carriers and 40-45 years for *BRCA2* mutation carriers respectively.[51,54] After this procedure the residual risk of peritoneal cancer is estimated to be around 1-2%.[56,57]

In view of the current thoughts on the origin of high-grade serous carcinomas, possibly located in the fallopian tubes, the option of prophylactic bilateral salpingectomy (instead of salpingo-oophorectomy) has been suggested as alternative. The great advantage of this strategy is that the very early menopause by oophorectomy with associated morbidity (a.o. sexuality, postmenopausal symptoms, quality of life) can be postponed till later age.[58] However, there are no study data yet regarding efficacy and safety of this strategy. Kwon et al showed that bilateral salpingectomy with delayed oophorectomy is a cost-effective strategy and may be an acceptable alternative, but they also did not provide data concerning the safety of this procedure.[59]

1.5. *BRCA*-associated breast cancer

Compared to sporadic breast cancer *BRCA1*-associated breast cancer is more often characterised by a triple negative phenotype (negative for estrogen receptor (ER), progesterone receptor (PR), and HER2 expression) in 75% of the cases, and frequently shows a medullary histotype.[55,60-62] In contrast, the characteristics of *BRCA2*-associated BC are comparable to these of sporadic BC, with 75% of the cases being hormone receptor positive without specific histotype. Both *BRCA1*- and *BRCA2*-associated BCs are mainly of poor differentiation grade, and as already said present at young age (median age<50 years)
Studies of the outcomes of women with *BRCA1*- or *BRCA2*- associated breast cancer have yielded conflicting results.[63,64] However, after correcting for differences in tumor characteristics, most studies show a comparable survival in *BRCA1*- and *BRCA2*- associated breast cancer.[63,64]

1.6. *BRCA*-associated epithelial ovarian cancer

BRCA1- and *BRCA2*- associated epithelial ovarian cancers are mostly of high-grade and of serous histology, although other histological subtypes are also described.[50,55] To our knowledge, no literature exists on specific histological differences between *BRCA1*- and *BRCA2*-associated EOC, but data are scarce and pathology review has formally not been performed.
The majority (70%) of the patients with *BRCA*-associated ovarian cancer are diagnosed with advanced stage disease, which is comparable with sporadic ovarian cancer patients.
Early studies have described that the overall survival of *BRCA*-associated EOC patients was improved compared to sporadic EOC patients.[31,51,65-75] As explanation it has been suggested that the longer survival may be caused by a better sensitivity to (platinum-based) chemotherapy,[65,68] but most of the studies reporting on survival in *BRCA1/2*-associated EOC patients lacked and still lack detailed data about chemotherapy.[51,66,68,70,75] The hypothesis on an improved chemosensitivity, however, was the result of data of preclinical work and indicated that patients with germline mutations in either the *BRCA1* or *BRCA2* genes have an impaired ability to repair double-stranded DNA breaks via homologous recombination, possibly explaining the increased sensitivity to platinum-based chemotherapy.[76]
Tan et al were the first to describe a phenomenon, called *BRCA*ness.[65] This phenomenon includes: high response to platinum based chemotherapy, long treatment free interval between relapses and improved overall survival. Considering this phenomenon *BRCA1*- and *BRCA2*-associated ovarian cancer have not been studied as a separate entity and it is insufficiently known whether the outcomes of *BRCA1*- and *BRCA2*-associated EOC differ. From the available literature, it appears that the survival of *BRCA2*-associated EOC may be more favourable, compared to *BRCA1*-associated EOC[42,66,68,75,77] but most studies were too small to show survival differences between *BRCA1* and *BRCA2*-associated EOC patients.
Little attention has previously been given to the role of optimal surgical cytoreduction with respect to the improved overall survival in *BRCA1/2*-associated ovarian cancer patients. In fact, this has only recently been addressed in two studies of small sample size.[50,78] Both studies did not describe an association of *BRCA* mutation status and rate of optimal tumor

debulking at primary surgery, although Boyd et al observed a non-significant trend towards a more favourable optimal debulking in women with *BRCA*-associated ovarian cancer.
In view of the advanced stage at disease presentation most patients, including those with a *BRCA1* or *BRCA2* mutation, will unfortunately get a recurrence, require further chemotherapy and will eventually develop resistance to chemotherapeutic agents. Little is known about recurrent disease in *BRCA*-associated ovarian cancer compared to sporadic ovarian cancer. A small study described that *BRCA1/2*-associated EOC frequently metastasizes to the viscera, whereas sporadic EOC commonly remains confined to the peritoneum.[79] In another small study by Tan et al, including 17 *BRCA*-associated patients with recurrent EOC, the response to chemotherapy after second- and third-line treatment was significantly higher in *BRCA*-associated patients compared with the nonhereditary EOC group[65], which was also confirmed by Alsop et al.[80]

1.7. Aims and outline of the thesis

The aims of this thesis were to further investigate tumor characteristics, disease presentation, the efficacy of different types of therapy and survival in Dutch *BRCA1*- and *BRCA2*-associated and sporadic EOC patients, regarding both primary and recurrent disease. Furthermore, we focussed on potential differences between *BRCA1*- and *BRCA2*-associated EOC. Additionally, the risk of developing a subsequent breast cancer (BC) in *BRCA*-associated EOC patients was another research question, since data hereon for this specific subgroup were not available. To answer our questions we performed three retrospective nationwide multicenter studies and two local studies in the Erasmus MC, Rotterdam.
Tumor characteristics, survival and response to therapy were separately evaluated for *BRCA1*-associated, *BRCA2*-associated and sporadic ovarian cancer patients, since most previous studies did not distinguish between *BRCA1*- and *BRCA2*- associated ovarian cancer. Further, it is insufficiently known if the more favourable survival of *BRCA*-associated compared with sporadic ovarian cancer is the result of a better response to treatment (chemotherapy, surgery and the treatment of recurrent disease). Another issue of interest concerns the risk of developing breast cancer after treatment

for *BRCA*-associated EOC, which has not yet been studied. Data hereon are important for a more optimal counselling of *BRCA*-associated EOC patients regarding breast cancer screening and preventive measurements.
In view of these various uncertainties regarding *BRCA*-associated EOC the following questions were addressed:

1. What is the outcome at the end of primary therapy (including chemotherapy) and what is the progression-free and overall survival after primary therapy in women with a *BRCA1*- or *BRCA2* gene mutation, compared with women with sporadic epithelial ovarian cancer (chapter 2.1)?

2. What are the differences between *BRCA1*- and *BRCA2*-associated epithelial ovarian cancer, regarding sensitivity to first line therapy, survival and tumorcharacteristics (chapter 2.2)?

3. Are there any differences in the residual tumor size and survival of surgical treatment between *BRCA1*-associated, *BRCA2*-associated and sporadic epithelial ovarian cancer patients (chapter 3.1)?

4. What is the breast cancer risk after therapy for *BRCA*-associated ovarian cancer compared to mutation carriers without ovarian cancer (chapter 4.1)?

5. What are the characteristics of recurrent EOC with respect to presentation, treatment and outcome in *BRCA1*-associated, *BRCA2*-associated and sporadic epithelial ovarian cancer patients, respectively (chapter 5.1)?

Finally, chapter 6.1 discusses the relevance of the findings, and the implication of the results of the various studies for clinical practice. Additionally, recommendations for future research are addressed.

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2

CHEMOSENSITIVITY AND SURVIVAL OF *BRCA1*, *BRCA2* AND SPORADIC OVARIAN CANCER

2.1

Chemosensitivity and outcome of *BRCA1*- and *BRCA2*-associated ovarian cancer patients after first line chemotherapy compared with sporadic ovarian cancer patients

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ABSTRACT

Background

Because it is insufficiently clear whether *BRCA*-associated epithelial ovarian cancer (EOC) is more chemosensitive than sporadic EOC, we examined response to chemotherapy, progression-free (PFS) and overall survival (OS) in *BRCA1*- and *BRCA2*-associated versus sporadic EOC-patients.

Methods

Data about patient characteristics, response to and outcome after primary therapy, including chemotherapy, were collected from 99 *BRCA1*, 13 *BRCA2*, and 222 sporadic patients. Analyses were performed using a χ^2 test, and Kaplan-Meier and Cox-regression methods.

Results

Complete response (CR) or no evidence of disease (NED) was observed in 87% of the *BRCA1* patients, progressive disease (PD) in 2%, being 71% and 15% respectively, in sporadic EOC patients (p=0.002). In *BRCA2* patients 92% had CR/NED, and none PD (p=0.27). Median PFS in *BRCA1*, *BRCA2* and sporadic patients was 2.1 (95%CI 1.9-2.5) years (p=0.006), 5.6 (95%CI 0.0-11.5) (p=0.008), and 1.3 (95%CI 1.1-1.5) years, respectively. Median OS in the three groups was 5.9 (95% CI 4.7-7.0) (p<0.001), >10 (p=0.008), and 2.9 (95% CI 2.2-3.5) years, respectively. A trend for a longer PFS and OS in *BRCA2* compared to *BRCA*1 patients was observed.

Conclusion

Compared to sporadic EOC patients, both *BRCA1*- and *BRCA2*-associated patients have improved outcomes after primary therapy, including chemotherapy.

INTRODUCTION

Despite advances in cytoreductive surgery and the use of the most effective chemotherapy (currently consisting of carboplatin/paclitaxel) epithelial ovarian cancer (EOC) is still the leading cause of death from gynaecological malignancies in the Western world [1,2]. The strongest known risk factor for ovarian cancer is a family history of breast/ovarian cancer; mutations in either the *BRCA1* or *BRCA2* gene are estimated to account for 10% of the EOC cases [3-7].

Female carriers with a *BRCA1* mutation have a 18%-54% cumulative lifetime risk (CLTR) of developing EOC, whereas for *BRCA2* mutation carriers the CLTR is lower, ranging between 2.4%-19% [3, 8-11].

The few studies published so far found that *BRCA*-associated EOC patients have a longer survival compared to sporadic EOC patients [3, 12-17]. It has been suggested that this longer survival is caused by a better response to (platinum-based) chemotherapy. In the study of Chetrit et al, it was indeed observed that the longer survival was mainly seen in the patient group treated with first-line chemotherapy [12].

In vitro testing showed that *BRCA1* and *BRCA2* deficient cells are associated with a higher proliferation rate, chromosomal instability and a deficiency to repair double strand DNA breaks by homologous recombination [18-20]. The latter biological mechanism may be responsible for an increased chemosensitivity, which might result in a longer PFS and OS, compared to sporadic patients. This mechanism may be especially valid for platinum (analogues) since this type of drugs act at the DNA level by formation of cross-links, leading to double strand DNA breaks and replication arrest [21,22].

Data about response to platinum-based therapy in *BRCA1/2* EOC patients are scarce. Most of the studies reporting a longer survival in *BRCA1/2*-associated EOC patients lack detailed data about chemotherapy [12,14,15,23,24]. So far, only one small study by Tan et al (22 *BRCA* positive patients) has investigated the response to platinum-containing chemotherapy in *BRCA1/2*-associated EOC patients [3]. They found that *BRCA* mutation carriers had a better response to platinum-based chemotherapy.

Whether the outcomes of *BRCA1*- and *BRCA2*-associated EOC differ is insufficiently known. From the available literature, it appears that the survival of *BRCA2*-associated EOC may be more favourable, compared to *BRCA1*-linked EOC [12,14,15,23,25].

In this analysis, we evaluated the response to, as well as the progression-free (PFS) and overall survival (OS) after primary therapy, including first line chemotherapy in 99 *BRCA1*- and 13 *BRCA2*-associated EOC patients, respectively, in comparison with 222 matched sporadic EOC patients.

PATIENTS AND METHODS

From the database of the Rotterdam Family Cancer Clinic of the Erasmus University MC-Daniel den Hoed Cancer Center, we identified all EOC patients, belonging to a proven *BRCA1* or *BRCA2* mutation family, diagnosed between January 1st, 1980 and January 1st, 2009. Follow up information was collected until June 1st 2009. The *BRCA1/2* mutation-carriers were matched (1:2) with sporadic EOC patients for age at (±5 years) and period of diagnosis (±5 years). Sporadic patients were selected from the cancer registry of the institution or the Comprehensive Cancer Center Rotterdam. Sporadic EOC patients with a family history of breast and/or ovarian cancer, defined as two relatives (first or second degree) with breast cancer, one relative (first or second degree) with breast cancer diagnosed before the age of 55, and/or one relative (first or second degree) with ovarian cancer, irrespective of age, were excluded. Inclusion criteria were: data availability about patient and tumor characteristics, first line chemotherapy administered as part of primary treatment and adequate follow-up data. Excluded were patients with a borderline ovarian tumor, suspicion of primary or recurrent breast cancer, or another malignancy before the development of ovarian cancer. For all the *BRCA*-positive patients DNA testing was performed at the Clinical Genetics Department of the Erasmus MC Rotterdam. Methods of DNA testing have previously been described.[22]
We identified 113 *BRCA1*- and 16 *BRCA2*-associated EOC patients diagnosed between 1980 and 2009. Ten patients were excluded because of incomplete follow-up. In total 105 *BRCA1*- and 14 *BRCA2*-associated

patients were matched with 238 sporadic EOC patients. Twenty patients, consisting of 5 *BRCA1* (5%), 1 *BRCA2* (7.7%), and 14 sporadic (6.3%) patients were not treated with chemotherapy as part of primary treatment and were excluded from further analyses. Sixteen of these patients had a FIGO stage I, being the reason for not administering chemotherapy, two patients refused chemotherapy and two patients died before chemotherapy could be given. In addition, three patients not being evaluable for response to chemotherapy as they only received 1 chemotherapy cycle were also excluded. Ultimately 99 *BRCA1*-, 13 *BRCA2*-associated and 222 sporadic ovarian cancer patients were included in the analyses.

For all eligible patients, information concerning patient and tumor characteristics, surgical procedure and residual tumor size, type and duration of chemotherapy, response to treatment, PFS and OS was retrospectively retrieved from medical files. Missing information was assembled as much as possible by treating physicians in regional community hospitals. Response was evaluated after end of first line chemotherapy treatment. As most patients were treated before the introduction of the RECIST criteria (2000), the WHO-criteria were used to evaluate response to chemotherapy [27,28]. The response to chemotherapy could not be determined in four patients, mostly concerning patients who were treated in the early 80's. We decided not to exclude these patients, because we did have information about PFS and OS.

The study was approved by the medical ethics committee of the Erasmus MC, Rotterdam, the Netherlands.

STATISTICAL ANALYSIS

Differences in patient- and tumor-characteristics between *BRCA1/2*-, *BRCA1*- and *BRCA2*-associated patients, respectively, and sporadic ovarian cancer patients were tested with the Pearson's Chi-square test or the Fisher Exact test (categoric variables) or by the Students' T-test (continuous variables).

Study endpoints were response to first-line chemotherapy, PFS, ovarian cancer specific (OCSS) and OS. The response to chemotherapy in the *BRCA1*, *BRCA2* and sporadic ovarian cancer patient groups, respectively, was evaluated for all the chemotherapeutic regimens together, and separately for the patients

being treated with platinum/taxol, a platinum-based regimen (without taxol), and non-platinum-based chemotherapy. Differences in response to first-line chemotherapy were tested with the Pearson's chi-square test or, in case of small numbers, with the Fisher Exact test.
Both PFS and OS were measured using the Kaplan-Meier survival method. PFS was defined as the time between the start of chemotherapy and the date of progressive disease or first recurrence. OS was defined as the time between the start of chemotherapy and the date of death or last follow up and OCSS as the time between the date of diagnosis and the date of death due to ovarian cancer or last follow up.
Patients were censored in the analyses for PFS, OS and OCSS by date of last visit at the clinic or end date of this study (1 June 2009). In the analyses for PFS and OCSS patients were also censored by date of death due to other reasons than ovarian cancer. Differences in PFS and OS between the three patient groups were examined in a multivariate Cox proportional hazard regression model, adjusting for possible confounders including FIGO stage, tumor grade and morphology, CA125 at diagnosis, residual tumor after debulking surgery (<1cm or >1cm), type of chemotherapy, history of and adjuvant chemotherapy for breast cancer. All analysis were performed with SPSS (version 15.0). A two-sided p-value less than 0.05 was considered as statistically significant.

RESULTS

Patient and tumor characteristics

Table 1 depicts the patient and tumor characteristics. Mean age at diagnosis was 52 years (range 31.4-74.0). As expected, significantly more *BRCA1* (29%) and *BRCA2* (54%) associated patients had a history of breast cancer in contrast with the sporadic group (4%). The median disease-free interval between breast and ovarian cancer was 8.1 years (range 0.02-30.1) in the *BRCA1*-, 3.2 (range 1.5-9.7) in the *BRCA2*-, and 9.3 years (range 0.7-17.7) in the sporadic group respectively.
Regarding tumor characteristics, no significant differences in tumor grade, FIGO stage, histology and CA 125 values were observed between the hereditary groups and the sporadic group. In the three patient groups,

tumors were predominantly poorly differentiated, and of serous histology. As a result of the selection criteria, the majority of the patients had an unfavourable FIGO stage, namely stage III/IV in 78% of *BRCA1*, 69% of *BRCA2* and 79% of the sporadic cases, respectively. However, none of the *BRCA2*-associated patients had a FIGO stage IV. In the majority of patients, the CA 125 level at diagnosis was higher than 500 kU/L. More *BRCA1* (64%, p=0.006) and *BRCA2* (85%, p=0.009) patients had a tumor residue < 1cm after cytoreductive surgery compared to sporadic patients (47%).

Response to chemotherapy

No significant differences were observed in the type of administered chemotherapy between the three groups (table 2). *BRCA1*-associated patients obtained a CR/NED after first line chemotherapy in 87% (N=83) of the cases, compared to in 71% (N=158) of the sporadic patients (p=0.002).

Progressive disease was observed in only two *BRCA1* patients (2%), compared to in 34 (15%) sporadic patients. In the *BRCA2* group, the response to chemotherapy was also more favourable than in the sporadic group, and none of the *BRCA2* patients had progressive disease. This, however, was not statistically significant due to the low number of *BRCA2* patients (N=13). None of the *BRCA1* and *BRCA2* patients treated with a platinum-based regimen had PD, in contrast with 15% of the sporadic patients (p<0.001). Remarkably, the two *BRCA1* patients, having PD after first-line chemotherapy did receive a non-platinum based regimen, while both sporadic patients receiving a non-platinum-based regimen obtained a CR/NED (table 3).
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Progression-free survival and overall survival

PFS was significantly longer in the *BRCA1* and *BRCA2* group, compared to the sporadic group, namely 2.1, 5.6 and 1.3 years, respectively (table 4). There was a trend for a longer PFS in *BRCA2*-, compared to *BRCA1* patients (p=0.05). While the PFS at 2 years was higher in both hereditary groups, compared to the sporadic group, the 5-years PFS rate remained high in *BRCA2*-associated patients (54%), and relatively decreased to 28% in *BRCA1*-linked patients (figure Ia and table 4).

Table 1: Patient and tumor characteristics in *BRCA1*-associated, *BRCA2*-associated and sporadic ovarian cancer patients, respectively

	BRCA1			***BRCA2***			**Sporadic**	
	N	**%**	**P**	**N**	**%**	**P**	**N**	**%**
Total	99			13			222	
Age at diagnosis								
Median	51.4		0.63	53.2		0.37	52.1	
Range	32.6-72.8			41.8-68.3			31.4-74.0	
Mean	52.1			54.9			52.6	
Year of diagnosis								
1980-1989	19	19	0.54	2	15	0,81	52	23
1990-1999	49	50		8	62		112	51
2000-2008	31	31		3	23		58	26
Breast cancer before ovarian cancer								
Yes	29	29	<0.001	7	54	<0.001	9	4
No	70	71		6	46		213	96
Tumor grade								
1 (=well differentiated)	6	8	0.17	2	17	0.82	17	8
2 (=moderately differentiated)	27	33		4	33		92	43
3 (=poorly differentiated)	48	59		6	50		103	49
Unknown	18			1			10	
FIGO stage								
I	7	8	0.98	3	23	0.15	28	13
II	13	14		1	8		19	9
III	59	63		9	69		126	58
IV	14	15		0	0		45	21
Unknown	1			0	-		4	-

Table 1: Continued

	BRCA1			*BRCA2*			Sporadic	
	N	%	P	N	%	P	N	%
Histology								
Serous	67	70	0.37	8	63	0.35	141	64
Mucinous	4	4		2	15		19	9
Endometroid	13	14		0	0		24	11
Clear cell	2	2		0	0		12	5
Undifferentiated	10	10		3	23		24	11
Unknown	3	-		0	0		2	-
Ca 125 (kU/L) before treatment								
≤35	10	15	0.59	1	11	0.49	10	7
35-500	25	37		3	33		71	48
>500	33	48		5	56		68	65
Unknown	31	-		4	-		73	-
Tumor size after primary surgery								
No operation	1	-	0.006	0		0.009	0	
<1cm	56	64		11	85		98	47
>1cm	31	36		2	15		111	53
Unknown	11	1		0			13	
Tumor size after interval debulking								
No interval debulking	74	78	0.45	10	83	1.00	159	73
<1cm	18	19		2	17		45	21
>1cm	3	3		0	0		14	6
Unknown	4			1			4	
Tumor size after either primary surgery and/or interval debulking								
No operation	1		0.06	0	-	0.20	0	-
<1 cm	66	78		12	100		138	67
>1cm	19	22		0	0		69	33
Unknown	13			1	-		15	-

Table 2: Type of and response to first-line chemotherapy in *BRCA1/2*-, *BRCA1*-, and *BRCA2*-associated and sporadic EOC

	BRCA1/2			***BRCA1***			***BRCA2***			**Sporadic**	
	N	**%**	**p**	**N**	**%**	**P**	**N**	**%**	**P**	**N**	**%**
Total	112			99			13			222	
Type of first line chemotherapy											
Platinum/paclitaxel	51	45	0.25	47	47	0.13	4	31	0.62	90	40
Platinum without paclitaxel	58	52		49	50		9	69		130	59
Non-platinum regimen	3	3		3	3		0	0		2	1
Response to first line chemotherapy											
NED/CR	95	88	<0.001	83	87	0.002	12	92	0.27	158	71
PR/ Stable	11	10		10	11		1	8		30	14
PD	2	2		2	2		0	0		34	15
Unknown	4	-		4	-						

NED/CR= no evidence of disease/ complete response, PR=partial response, PD=progressive disease

Table 3: Response after end of first line chemotherapy in *BRCA1*-associated, *BRCA2*-associated and sporadic ovarian cancer patients for different chemotherapy regimens

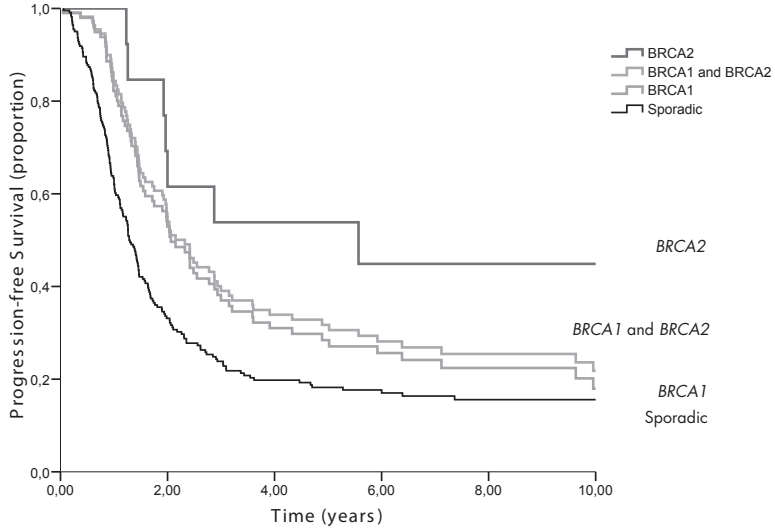
	Response	*BRCA1*			*BRCA2*			Sporadic	
		N	%	P	N	%	P	N	%
Platinum with Paclitaxel	NED/CR	43	94	0.01	4	100	1,00	66	73
	PR/SD	3	6		0	0		14	16
	PD	0	0		0			10	11
	Unknown	1	-		0				
Platinum without Paclitaxel	NED/CR	40	87	0.007	8	89	0.42	90	69
	PR/SD	6	13		1	11		16	12
	PD	0	0		0	0		24	19
	Unknown	3	-		0	-		0	-
Non-platinum based Chemotherapy	NED/CR	0		0.20	0			2	100
	PR/SD	1	33		0			0	
	PD	2	67		0			0	
	Unknown				0				

NED/CR= no evidence of disease/ complete response, PR=partial response, PD=progressive disease

Table 4: Progression-free- (PFS), overall- (OS) and ovarian cancer-specific survival (OCSS) in *BRCA1*-associated, *BRCA2*-associated and sporadic ovarian cancer patients.

	BRCA1/2		*BRCA1*		*BRCA2*		Sporadic	
	N	%	N	%	N	%	N	%
PFS								
Median in years (95% CI)	2.3	(1.9-2.8)	2.1	(1.7-2.5)	5.6	(0.0-11.5)	1.3	(1.1-1.5)
At 2 years	56	54	48	53	8	62	68	33
At 5 years	28	32	21	28	7	54	39	20
At 10 years	12	22	8	18	4	45	12	16
P-value	P=0.001		P=0.006		P=0.008			
OS								
Median in years (95% CI)	6.3	(5.3-7.3)	5.9	(4.7-7.0)	>10 yr	Not calculable	2.9	(2.2-3.5)
2 years	88	85	76	84	12	92	132	62
5 years	54	63	44	60	10	85	58	34
10 years	22	35	18	32	4	53	16	18
P-value	P<0.001		P<0.001		P=0.002			
OCSS								
Median in years (95% CI)	6.8	(4.9-8.8)	6.5	(5.0-7.9)	>10 yr		3.2	(2.6-3.7)
2 years	87	86	75	85	12	92	132	64
5 years	52	67	42	64	10	85	57	37
10 years	21	42	17	39	4	60	16	24
P-value	P<0.001		P=0.002		P=0.008			

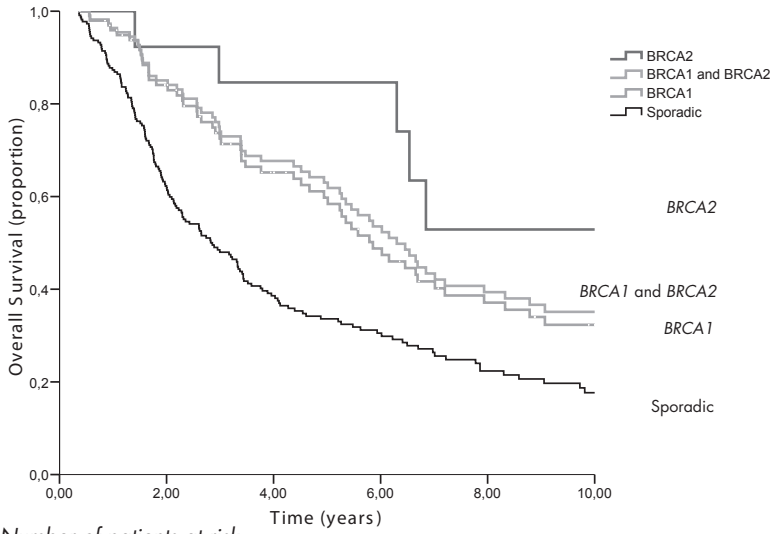
Figure Ia: PFS in *BRCA1*, *BRCA2*, *BRCA1/2* and sporadic patients



Number of patients at risk

BRCA2	8	*7*	4
BRCA1/2	56	28	12
BRCA1	48	21	8
Sporadic	68	39	12

Figure Ib: OS in *BRCA1*, *BRCA2*, *BRCA1/2* and sporadic patients



Number of patients at risk

BRCA2	12	10	4
BRCA1/2	88	54	22
BRCA1	76	44	18
Sporadic	132	58	16

Compared to patients with sporadic EOC, the hazard ratio (HR, *BRCA* versus sporadic patients) by univariate analysis for the risk of progression was 0.66 (95% CI: 0.5-0.87) for the *BRCA1* group, and 0.35 (95% CI 0.17-0.76) for the *BRCA2* group (table 5). In a multivariate analysis, after adjusting for FIGO stage and residual tumor after debulkingsurgery, *BRCA* carriership remained significantly associated with improved PFS (*BRCA1*: HR 0.67 (0.50-0.89), *BRCA2*: HR 0.45 (0.21-0.97), Table 5). Other tested variables (see methods) were not found to be confounders in the analysis, and therefore not included in the multivariate model.
Compared to the sporadic cohort, the OS in the the *BRCA1*- and *BRCA2*-associated patients was significantly longer as well (figure Ib and table 4). The observed median OS was 5.9 years in the *BRCA1*-, and more than ten years in the *BRCA2* group, in comparison with 2.9 years in the sporadic group. Also, both the 2- and 5-years OS rates were significantly higher in the *BRCA* groups, being 84% and 60% in the *BRCA1* group, 92% and 85% in the *BRCA2* patients, in contrast with 64% and 37% in the sporadic group. In accordance with the pattern seen for PFS, relatively more *BRCA2*-associated patients remain alive at 5 as well as at 10 years in comparison with *BRCA1*-associated patients (p=0.06). The hazard ratio (HR, *BRCA* versus sporadic groups) by univariate analysis for the risk of death was 0.56 (95% CI 0.41-0.76) for the *BRCA1*-, and 0.29 (95% CI 0.12-0.71) for the *BRCA2* group. This remained statistically significant after correction for FIGO stage and residual tumor after cytoreductive surgery (Table 5).

Since more *BRCA* patients had a history of breast cancer before the EOC diagnosis, the ovarian cancer specific survival (OCSS) was also of interest (table 4). Although the high proportion of patients with a history of breast cancer before the EOC diagnosis in the *BRCA1* and *BRCA2* cohorts, respectively, only two patients (2%) in the *BRCA1* group and two sporadic patients (1%) died of breast cancer. This resulted in a significantly better OCSS in the *BRCA1* (median 6.5 years, p=0.002) and *BRCA2* groups (median >10 years, p=0.008), respectively, compared to the sporadic group (median 3.2 years).

Table 5: Univariate and multivariate analysis of Progression free and Overall Survival in *BRCA1*- and *BRCA2*-associated, respectively, vs sporadic ovarian cancer patients. HR, hazard ratio; CI, confidence interval.

	N	HR and 95% CI Univariate	HR and 95% CI Multivariate
PROGRESSION FREE SURVIVAL			
Patient group			
Sporadic	222	1	1
BRCA1	99	0.66 (0.5-0.87)	0.67 (0.50-0.89)
BRCA2	13	0.35 (0.17-0.76)	0.45 (0.21-0.97)
FIGO			
I and IIa	43	1	
IIb/c and III	222	3.47 (2.08-5.54)	3.14 (1.91-5.14)
IV	59	6.1 (3.53-10.55)	4.81 (2.74-8,42)
Unknown	10	4.27 (1.9-9.59)	3.18 (1.37-7.36)
Residual tumor after interval debulking and primary surgery			
<1cm	216	1	1
>1cm	88	2.34 (1.77-3.09)	1.71 (1.28-2.28)
Unknown	30	2.33 (1.55-3.49)	2.28 (1.49-3.49)
OVERALL SURVIVAL			
Patient group			
Sporadic	222	1	1
BRCA1	99	0.56 (0.41-0.76)	0.54 (0.39-0.74)
BRCA2	13	0.29 (0.12-0.71)	0.38 (0.16-0.94)
FIGO			
I and IIa	43	1	1
IIB/C and III	222	2.3 (1.4-3.7)	2.14 (1.32-3.49)
IV	59	4.5 (2.6-7.7)	3.54 (2.03-6.16)
Unknown	10	3.0 (1.3-7.3)	1.87 (0.76-4.60)
Residual tumor after interval debulking and primary surgery			
<1cm	216	1	1
>1cm	88	2.21 (1.64-2.97)	1.63 (1.20-2.23)
Unknown	32	2.55 (1.65-3.93)	2.85 (1.80-4.50)

DISCUSSION

To our knowledge, the current report is the first study exploring the response to first line chemotherapy in *BRCA1*- and *BRCA2*-associated EOC patients separately, compared to sporadic EOC patients. We found that *BRCA1*- as well as *BRCA2*-associated patients with EOC have a better response after first-line chemotherapy. In fact, none of the *BRCA*-associated patients were refractory to first-line platinum-based chemotherapy, contrary to sporadic patients. The PFS and OS were significantly longer as well in both hereditary groups, in comparison with sporadic EOC patients and a trend for a longer PFS and OS in *BRCA2* compared to *BRCA1*-associated ovarian cancer patients was observed.

The response to first-line chemotherapy was also studied by Tan et al in 22 *BRCA1/2*-associated compared to sporadic EOC patients.[3] In this smaller study, a significantly higher CR rate (81.8% vs 43.2%, p=0.004) and longer median OS (8.4 vs 2.9 years, p<0.002) were found for the *BRCA*-associated patients, being in accordance with our data. Because only 22 *BRCA1/2*-linked patients were included, a separate evaluation of the outcome in *BRCA1* and *BRCA2* patients was not performed. In our study 92 % of the *BRCA2* patients obtained CR/NED and none had progressive disease compared to 71% and 15%, respectively, in the sporadic patients.

Compared to the sporadic patients, we found a significantly longer PFS for both the *BRCA1*- and *BRCA2*-associated patients (p=0.006, and p=0.008, respectively). In our opinion this is potentially a reflection of the improved chemosensitivity to platinum-based chemotherapy of the hereditary patients and this is in accordance with previously reported data. Boyd et al also reported a significantly improved PFS for *BRCA* mutation carriers after first line chemotherapy [15]. This study, however, only included Ashkenazy-Jewish patients having one of the founder mutations in *BRCA1/2* which is not representative for the Dutch population of *BRCA* patients. In the study of Tan et al an improved PFS in *BRCA1/2* positive patients was described as well, although not statistically significant (18 vs 12 months, p=0.115), which is probably due to the small number of patients [3].

Although the improved response to chemotherapy might be an important reason for the longer PFS, other factors might also play a role. In our *BRCA2*

cohort, 2 patients (15%) had a mucinous carcinoma, compared to 4 (4%) *BRCA1* and 19 sporadic patients (9%). It is known that mucinous EOC has a better prognosis, compared to serous carcinoma [29]. However, restricting our analyses to serous carcinomas only, 92% of the *BRCA1* group (p=0.003), 88% of the *BRCA2* group (p=0.84) and 72% of the sporadic group obtained a complete response/NED after chemotherapy. Also, median PFS remained significantly longer in the hereditary groups, being 2.4 years (p<0.001) in the *BRCA1*, and 2.9 years (p=0.03) in the *BRCA2* group, versus 1.3 years in the sporadic group (data not shown). Other variables possibly playing a role in the better outcome as observed in our *BRCA2* group might be: no FIGO stage IV, no residual tumor >1cm after surgery, and no *BRCA2* patient with a clear cell or endometroid histology. Further research in a greater *BRCA2*-associated cohort is certainly warranted.

The OS in both the *BRCA1*- and *BRCA2*-cohort of our study was significantly better, compared to the sporadic cohort and again most pronounced in the *BRCA2* cohort. These results remained significantly better in favour of the *BRCA* groups if we restricted our analyses to serous carcinomas only (median OS in *BRCA1*: 6.5 years (p=0.001), in *BRCA2*: 6.8 years (p=0.03), and 2.8 years in the sporadic group). This means that our data are in accordance with the results of other studies about OS for *BRCA* associated EOC patients [12,14,15,23,24,30].

The better outcome in *BRCA1* and *BRCA2*-associated EOC patients might partly be caused by the higher percentage of patients with tumor residue <1 cm after primary surgery (table 1). However, in multivariate analyses after correcting for tumor residue, PFS and OS remained significantly better in both *BRCA* groups. In view of the data of Tan et al. [3] who observed a better response to second and third line chemotherapy (without surgery) as well as a prolonged therapy free interval after each chemotherapy line in mutation carriers, our data suggest that chemotherapy contributes to the improved outcome in *BRCA1*- and *BRCA2*-associated, compared to sporadic patients.

Survival or ascertainment bias can occur by preferably selecting long-living patients who were tested a long time after their EOC diagnosis. This can especially happen in patients, diagnosed with EOC before 1995 when

DNA tests for a *BRCA1* or *BRCA2* mutation were not available yet. In order to minimize this type of bias, we also included first-degree family members of mutation carriers affected with ovarian cancer who were not tested themselves, but based on their personal history and the position in the pedigree, these patients were considered as obligate mutation carrier. In addition, we performed a subanalysis after exclusion of 25 *BRCA*-associated patients who underwent genetic testing more than one year after the diagnosis of EOC. All the differences between *BRCA1* and sporadic patients remained significant, while PFS and OS in the *BRCA2* group were not significantly longer anymore, which is probably due to the low number of patients in this group.

A limitation of our study is that genetic testing in the sporadic group was not performed. Although patients with a family history of breast/ovarian cancer were excluded this does not rule out that some of the sporadic EOC patients might be a mutation carrier. However, the differences in outcome parameters between the proven *BRCA1/2* mutation carriers and sporadic patients might then even be bigger as we described.

As the prognosis of early stage EOC is significantly better compared to advanced stage EOC, we also performed a subanalysis excluding patients with FIGO stage I and IIa. In these analyses, PFS and OS remained significantly better for *BRCA1*- and *BRCA2*-associated patients, compared to sporadic patients. Response to chemotherapy remained statistically significantly better for *BRCA1*-associated patients, while the response in the *BRCA2* group was better as well, but not statistically significant.

In conclusion, *BRCA1* and *BRCA2* mutation carriers have a better outcome after primary therapy, including chemotherapy, compared to sporadic EOC patients. It appears that *BRCA1*- and *BRCA2*-associated EOC may very well be different entities. More fundamental research and further comparison of *BRCA1*- and *BRCA2*-associated EOC is urgently needed to specifically define the most effective treatment for the separate patient groups. Confirmation of the present findings may lead to new guidelines for the counselling and treatment of *BRCA1* and *BRCA2* EOC patients.

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2.2

Outcome of *BRCA1*- compared with *BRCA2*-associated ovarian cancer: a nationwide study in the Netherlands

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ABSTRACT

Background

Recent studies suggested an improved overall survival (OS) for *BRCA2*- versus *BRCA1*-associated epithelial ovarian cancer (EOC), whereas the impact of chemotherapy is not yet clear. In a nationwide cohort, we examined results of primary treatment, progression-free survival (PFS), treatment-free interval (TFI) and OS of *BRCA1* versus *BRCA2* patients.

Methods

245 *BRCA1*- and 99 *BRCA2*-associated EOC patients were identified through all Dutch university hospitals. Analyses were performed with the Pearson's chi-square test, Kaplan-Meier and Cox-regression methods.

Results

BRCA1 patients were younger at EOC diagnosis than *BRCA2* patients (51 versus 55 years; $P<0.001$), without differences regarding histology, tumor grade and FIGO stage. Complete response rates after primary treatment, including chemotherapy, did not differ between *BRCA1* (86%) and *BRCA2* patients (90%). *BRCA1* versus *BRCA2* patients had a shorter PFS (median 2.2 versus 3.9 years, respectively; P=0.006), TFI (median 1.7 versus 2.8 years; P=0.009) and OS (median 6.0 versus 9.7 years; P=0.04). Differences could not be explained by age at diagnosis, FIGO-stage or type of treatment.

Conclusions

PFS and OS were significantly longer in *BRCA2*- compared to *BRCA1*-associated EOC patients. While response rates after primary treatment were similarly high in both groups, TFI, as surrogate for chemosensitivity, was significantly longer in *BRCA2* patients.

INTRODUCTION

It is assumed that 8-16% of all epithelial ovarian cancer (EOC) cases is due to a *BRCA1* or *BRCA2* germline mutation.[1-5] For *BRCA1* mutation carriers the cumulative lifetime risk of developing EOC up to the age of 70 is estimated to be 18-54%, and for *BRCA2* mutation carriers 2.4-23%.[6, 7]. Both *BRCA1*- and *BRCA2*-associated ovarian cancers are mostly of serous histology (63-70%) and poorly differentiated (73-88%).[8]

In previous studies, it has been observed that *BRCA1/2*-associated EOC patients have a longer progression-free survival (PFS) and overall survival (OS) compared to women with sporadic EOC.[9-12] It is assumed that this is due to an increased sensitivity to DNA cross-linking agents such as platinum analogues,[10] since it has been shown that *BRCA* deficient cells have an impaired ability to repair DNA by means of homologous recombination. These effects might be intensified by treatment with poly(ADP-ribose) polymerase (PARP) inhibitors).[9, 13, 14] However, the early studies concerning *BRCA1/2*-associated EOC survival mainly included Ashkenazi Jewish patients, did not provide data about chemosensitivity, were of small sample sizes, and survival of *BRCA1* and *BRCA2* mutation carriers was not separately analyzed.[9, 15-17]

More recent data suggested a non significantly longer PFS and OS in *BRCA2*- compared with *BRCA1*-associated EOC patients, while a high response to first-line chemotherapy was observed in both patient groups.[12, 18, 19] A more favorable OS of *BRCA2*- compared to *BRCA1*-associated EOC patients was recently confirmed in two other studies,[20, 21] both not evaluating response to chemotherapy, while Hyman et al. compared small groups of patients including 45% of Ashkenazi Jewish ancestry.[21]

Therefore, in the current analyses, we further explored the clinicopathological characteristics and the results after primary treatment, including chemotherapy, PFS, treatment-free interval (TFI), and OS in a large nationwide cohort of *BRCA1*- versus *BRCA2*-associated EOC patients in the Netherlands.

METHODS

Study population and design

In this retrospective nationwide multicenter study, *BRCA1*- and *BRCA2*-associated EOC patients were identified through the databases of family cancer clinics (FCC), the departments of Clinical Genetics and of Gynecologic Oncology at all eight Dutch university hospitals, the Netherlands Cancer Institute (NKI) and the Netherlands Foundation for the Detection of Hereditary Tumors (STOET). Eligibility criteria were: EOC cases (defined according to the FIGO guidelines [22]), diagnosed between January 1st, 1980 and January 1st, 2011, identified with a deleterious *BRCA1* or *BRCA2* mutation or being a first-degree family member of a proven *BRCA* mutation carrier, adequate data about patient, tumor and treatment characteristics, and without a history of another primary malignancy besides breast cancer (BC). Excluded were patients with a borderline ovarian tumor or suspicion of primary or recurrent BC at time of primary diagnosis of EOC. Both *BRCA1*- and *BRCA2*-associated EOC patients were selected from the Erasmus MC-Daniel den Hoed Cancer Center, University Medical Center Groningen, University Medical Center St Radboud, and the NKI; we exclusively selected consecutive *BRCA2*-associated EOC patients from the Leiden University Medical Center (LUMC), Amsterdam Medical Center (AMC), VU Medical Center Amsterdam (VUMC), University Medical Center Utrecht (UMCU), Maastricht University Medical Center (MUM) and the STOET aiming at expanding the number of *BRCA2* cases. The study was approved by the Institutional Review Board of the Erasmus MC, Rotterdam, and according to the Dutch law, no further institutional Review Board approval was needed. We identified a total number of 245 *BRCA1*- and 99 *BRCA2*-associated EOC patients fulfilling the in- and exclusion criteria.

Data collection

For all eligible patients, data concerning patient- and initial tumor characteristics, primary treatment, results after primary treatment (including response to chemotherapy), PFS, TFI and OS were retrieved from existing institutional databases and medical records. Missing relevant information was assembled by contacting the treating physicians in regional community hospitals.

Outcome measures and data definition

Primary outcome measures were results of primary treatment, including response to first-line chemotherapy, PFS and OS. Secondary outcome measures were TFI and ovarian cancer-specific survival (OCSS). Since the RECIST-criteria were introduced in 2000 and 46% of the included patients were treated before 2000, response to primary treatment including chemotherapy was evaluated at the end of therapy by means of the WHO-criteria and classified as one of the following categories: no evidence of disease (NED) or complete response (CR), partial response (PR) or progressive disease (PD).[23] PFS was calculated as the time period between the date of initial diagnosis and the date of progressive disease or first recurrence. TFI was defined as the time period between end of first-line chemotherapy and the date of progressive disease or first recurrence. Consequently, TFI was not assessed for patients who were not treated with chemotherapy as part of primary treatment. OS was calculated as the time period between the date of diagnosis and the date of death, and OCSS as the time between the date of diagnosis and the date of death due to EOC.

Most patients underwent genetic testing after their EOC diagnosis, which may cause a survival bias. To account for the time elapsed between date of diagnosis and DNA testing, we also calculated time under observation in the survival analyses from date of genetic testing in patients who were tested after EOC (left-truncation). Left-truncated survival analysis is considered to give unbiased effect estimates, if the event time and delayed entry time are independent, given the covariates.[24, 25]

Statistical analysis

Differences in patient-, tumor- and treatment characteristics between *BRCA1*- and *BRCA2*-associated EOC patients were compared with the Pearson's Chi-square test or, in case of small numbers, with the Fisher's Exact test (categorical variables) or by the Student's T-test (continuous variables). Response to chemotherapy was evaluated for all types of chemotherapeutic regimens together, and separately for the patients being treated with the combination platinum/paclitaxel, a platinum-based regimen (without paclitaxel), and non-platinum-based chemotherapy. Differences in response rates between *BRCA1* and *BRCA2* mutation carriers were tested with the Pearson's Chi-square test. Cumulative survival (PFS, TFI, OS and OCSS) was calculated

using the Kaplan-Meier method and survival differences between *BRCA1* and *BRCA2* mutation carriers were tested by using a logrank test. In addition, differences in PFS and OS between the two patient groups were examined in a univariate and multivariate Cox proportional hazard's model to estimate hazard ratios (HR) and 95% confidence intervals (CIs), and to adjust for possible confounders including age at and year of diagnosis, FIGO stage, differentiation grade, histological type, CA125 level at diagnosis, residual tumor after debulking surgery (<1 cm or ≥1 cm) and type of chemotherapy. Patients were censored in the analyses for PFS, OS and OCSS by date of last visit at the clinic or end date of this study (January 1st, 2011). In the analyses for PFS and OCSS, patients were also censored by date of death due to other reasons than EOC. Statistical analyses were performed with SPSS 20.0 for Windows (SPSS, Chicago, IL). A two-sided *P*-value less than 0.05 was considered statistically significant.

RESULTS

Patient and Tumor Characteristics

Patient and tumor characteristics of the 245 *BRCA1*- and 99 *BRCA2*-associated EOC patients are listed in Table 1. EOC was diagnosed at a younger age in *BRCA1* compared with *BRCA2* mutation carriers (median 51.0 versus 55.5 years; *P*<0.001). Overall, 33% of all mutation carriers had a history of BC preceding the EOC diagnosis, which again was diagnosed at a younger age in *BRCA1* compared with *BRCA2* mutation carriers (median 43.3 versus 51.5 years) (P<0.001) (Table 1). Other variables did not significantly differ between the two patient groups. EOC was generally diagnosed at advanced stage (FIGO-stage III or IV; 74%), mainly poorly differentiated (73%), and of serous histology (64%). Median follow-up time was 5.0 years (range 0.1-28.1) and 4.9 years (range 0.3-28.7) in *BRCA1* and *BRCA2* mutation carriers, respectively. In total, 88% of the patients (*BRCA1*, 89%; *BRCA2*, 85%) had complete follow-up until date of death or end date of the study.

89% of the *BRCA1* patients, and 99% of the *BRCA2* patients were proven mutation carriers; while 26 (11%) *BRCA1* and 1 (1%) *BRCA2* patient were not tested themselves but had at least one first degree family member with a proven mutation (with a very high probability of being mutationcarrier as well).

Primary Treatment of EOC

The primary treatment of the majority of patients consisted of both surgery and chemotherapy (94%, Table 2). Residual tumor after primary and/or interval debulking surgery was not significantly different between *BRCA1*- and *BRCA2*-associated EOC patients and was <1 cm in 80% (169/212) and 86% (70/81) of the *BRCA1* and *BRCA2* patients, respectively.

Chemotherapy generally consisted of a platinum-containing regimen, mostly in combination with paclitaxel, except for five *BRCA1* mutation carriers who were treated with non-platinum-based chemotherapy (Table 2). At the end of primary treatment, including chemotherapy, CR/NED was obtained in 86% of the *BRCA1*- versus 90% of the *BRCA2*-associated patients (P=0.36), while progressive disease (PD) during first-line chemotherapy was not observed in any of the *BRCA2* patients, but in five (2%) *BRCA1* patients (table 2).

Additionally, after stratifying for type of chemotherapy (platinum/paclitaxel, platinum-based without paclitaxel, and non-platinum-based), response to primary treatment was not significantly different in any of the subgroups between *BRCA1*- and *BRCA2*-associated EOC patients (Table 3).

PFS, TFI, OS, and OCSS

As shown in Figure 1 and Table 4, the median PFS after primary treatment for EOC was significantly longer in *BRCA2*- compared with *BRCA1*-associated EOC patients, being 3.9 years (95%-CI 2.5-5.3) versus 2.2 years (95%-CI 1.9-2.5), respectively (*P*=0.006). At univariate analysis the HR (*BRCA2* versus *BRCA1*) for risk of progression was 0.65 (95% CI 0.48-0.88), and at multivariate analyses 0.60 (95% CI 0.43-0.83) both in favor of the *BRCA2* group. Also in the left-truncated survival analysis (see methods section) *BRCA2* mutation carriers still had a significantly longer PFS compared with *BRCA1* mutation carriers (HR 0.58; 95%-CI 0.37-0.90; *P*=0.02; data not shown).

Table 1. Patient and tumor characteristics of *BRCA1*- and *BRCA2*-associated EOC patients

	BRCA1		*BRCA2*		
	N	(%)	N	(%)	P
Total	245	(71)	99	(29)	
Age at diagnosis					
Median in years (range)	51.0	(23.1-72.7)	55.5	(29.8-78.2)	<0.001
Mean in years (SD)	51.4	8.5	55.8	8.8	
Year of diagnosis					
1980-1989	33	(14)	8	(8)	0.08
1990-1999	86	(35)	31	(31)	
2000-2008	126	(51)	60	(61)	
Breast cancer before EOC					
Yes	83	(34)	30	(30)	0.52
No	162	(66)	69	(70)	
Median age at BC diagnosis in years (range)	43.3	(25.9-68.8)	51.5	(32.2-75.8)	<0.001
CA125 (kU/L) at primary diagnosis					
≤35	31	(18)	6	(9)	0.46
35-500	69	(39)	34	(49)	
>500	77	(44)	29	(42)	
Unknown	68	-	30	-	
Histology					
Serous	153	(64)	63	(66)	0.90
Mucinous	7	(3)	5	(5)	
Endometroid	25	(11)	10	(10)	
Clear cell	2	(1)	1	(1)	
Undifferentiated	18	(8)	6	(6)	
Adenocarcinoma NOS	31	(13)	11	(12)	
Other	3	(1)	0	(0)	
Unknown	6	-	3	-	
Tumor grade					
1 (well differentiated)	11	(5)	4	(5)	0.62
2 (moderately differentiated)	48	(22)	18	(20)	
3 (poorly differentiated)	155	(72)	67	(75)	
Unknown	31		10		
FIGO stage					
I	25	(10)	18	(18)	0.16
II	35	(15)	11	(11)	
III	138	(57)	53	(54)	
IV	44	(18)	16	(16)	
Unknown	3	-	1	-	

FIGO, International Federation of Gynecology and Obstetrics

Table 2. Type of primary treatment for EOC

	BRCA1		*BRCA2*		
	N	(%)	N	(%)	P
Total	245	(71)	99	(29)	
Surgery only	12	(5)	5	(5)	0.35
Chemotherapy only	1	(0)	2	(2)	
Surgery and chemotherapy	232	(95)	92	(93)	
Type of Surgery					
Primary surgery	221	(91)	85	(88)	0.43
Interval debulking	23	(9)	12	(12)	
Tumor size after primary surgery and/or interval debulking					
No operation	1	-	2	-	0.19
<1 cm	169	(80)	70	(86)	
>1 cm	43	(20)	11	(14)	
Unknown	32	-	16	-	
Type of first-line chemotherapy					
Platinum/paclitaxel	148	(64)	68	(73)	0.14
Platinum without paclitaxel	79	(34)	25	(27)	
Non-platinum-based	5	(2)	0	(0)	
Unknown	1	-	1	-	

In addition, TFI after first line chemotherapy was significantly longer in *BRCA2* than in *BRCA1* patients (Table 4). A TFI of more than six months (=chemotherapy-sensitive) was observed in 192 *BRCA1* (86%) and in 77 (93%) *BRCA2*-associated EOC patients (P=0.11). In the left-truncated analysis *BRCA2* patients still had a longer TFI than *BRCA1* patients (HR 0.61; 95% CI 0.41-0.92, P=0.02; data not shown).

Further, as shown in Figure 2a and Table 4, the median OS was significantly longer in *BRCA2*- than in *BRCA1*-associated EOC patients, being 9.7 years (95%-CI 5.0-14.3) versus 6.0 years (95%-CI 5.1-6.9), respectively (*P*=0.04). The HR for the risk of death by univariate analysis was 0.70 favoring the *BRCA2* group, and remained significant in the multivariate analysis (HR 0.67; 95%-CI 0.47-0.96) (Table 4). In the left-truncated analysis, however, the OS was not significantly longer anymore for *BRCA2* mutation carriers (HR 0.79; 95%-CI 0.53-1.17; *P*=0.24) (data not shown).

Findings for OCSS and OS were comparable (Figure 2b, Table 4), showing a significantly longer median OCSS in *BRCA2* (10.4 years) compared to *BRCA1* patients (6.3 years, *P*=0.02).

Table 3. Outcome after primary treatment, including chemotherapy, in *BRCA1*- and *BRCA2*-associated EOC patients for different chemotherapy regimens

	BRCA1		*BRCA2*		
	N	**(%)**	**N**	**(%)**	**P**
All treatment regimens					
NED/CR	178	(86)	71	(90)	0.36
PR/ Stable	23	(11)	8	(10)	
PD	5	(2)	0	(0)	
Unknown	26	-	15	-	
Platinum with Paclitaxel					
NED/CR	114	(87)	51	(91)	0.38
PR/ Stable	16	(12)	5	(9)	
PD	1	(1)	0	(0)	
Unknown	17		12		
Platinum without Paclitaxel					
NED/CR	62	(90)	19	(86)	0.95
PR/ Stable	5	(7)	3	(14)	
PD	2	(3)	0	(0)	
Unknown	10	-	3	-	
Non-platinum based chemotherapy					
NED/CR	2	(40)	-	-	
PR/ Stable	1	(20)	-	-	
PD	2	(40)	-	-	

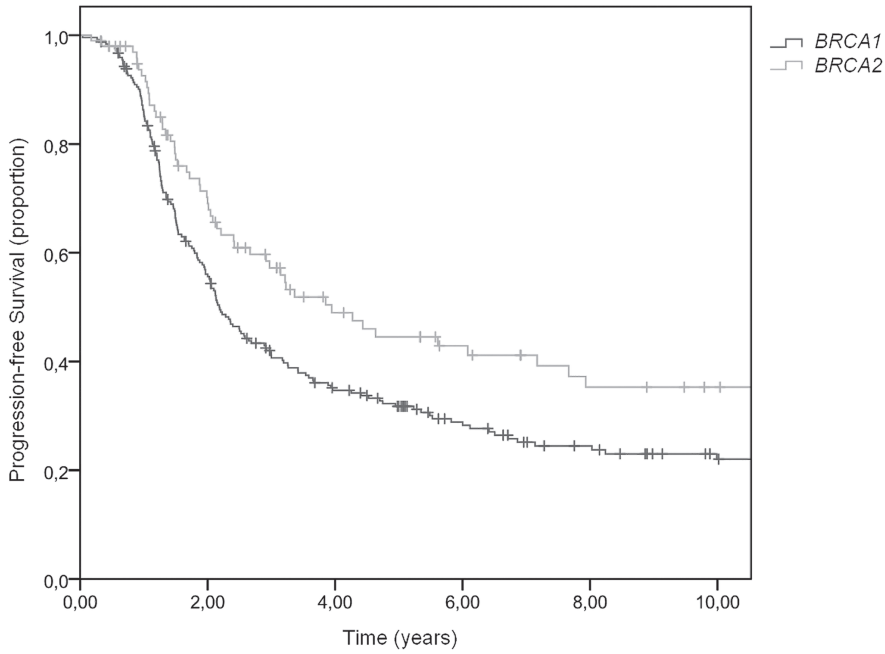
NED/CR, no evidence of disease/complete response; PR, partial response; PD, progressive disease; TFI, treatment free interval

Table 4. Progression-free survival (PFS), treatment free interval (TFI), overall survival (OS) and ovarian cancer-specific survival (OCSS) in *BRCA1*- and *BRCA2*-associated EOC patients

	BRCA1		*BRCA2*		
	N	(%)	N	(%)	P
PFS					
Median in years (95%-CI)	2.2	(1.9-2.5)	3.9	(2.5-5.3)	0.006
Median FIGO III/IV	1.8	(1.5-2.2)	2.2	(1.2-3.2)	0.046
2 years	130	56	60	69	
5 years	62	32	30	45	
10 years	23	22	15	35	
Univariate HR (95%-CI)		1		0.65 (0.48-0.88)	
Multivariate HR (95%-CI)		1		0.60 (0.43-0.83)*	
TFI					
Median in years (95%-CI)	1.7	(1.4-2.0)	2.8	(1.5-4.2)	0.009
Median FIGO III/IV	1.4	(1.0-1.8)	1.7	(0.8-2.3)	0.04
6 months	191	86.3	77	92.9	
2 years	92	43.0	44	57.0	
5 years	44	26.9	22	40.7	
10 years	18	19.9	13	34.5	
Univariate HR (95%-CI)		1		0.66 (0.48-0.90)	
Multivariate HR (95%-CI)		1		0.61 (0.44-0.86)*	
OS					
Median in years (95%-CI)	6.0	(5.1-6.9)	9.7	(5.0-14.3)	0.04
Median FIGO III/IV	5.3	(4.8-5.8)	6.5	(4.0-9.1)	0.05
2 years	198	86	84	93	
5 years	120	62	48	70	
10 years	44	36	21	49	
Univariate: HR (95%-CI)		1		0.70 (0.50-0.99)	
Multivariate HR (95%-CI)		1		0.67 (0.47-0.96)*	
OCSS					
Median in years (95%-CI)	6.3	(5.0-7.6)	11.4	-	0.02
Median FIGO III/IV	5.3	(4.7-6.0)	6.5	(4.0-9.1)	0.04
2 years	198	86	84	93	
5 years	120	63	48	70	
10 years	44	37	21	53	
Univariate HR (95%-CI)		1		0.64 (0.44-0.92)	
Multivariate HR (95%-CI)		1		0.60 (0.41-0.88)*	

HR: Hazard Ratio; * Adjusted for age at EOC diagnosis, year of EOC diagnosis, FIGO stage, differentiation grade, type of histology, CA125 level at diagnosis, residual tumor after debulking surgery (<1 cm or ≥1 cm), and type of chemotherapy.

Figure 1. Progression-free survival in *BRCA1*- (dark line) versus *BRCA2*-associated (light line) EOC patients

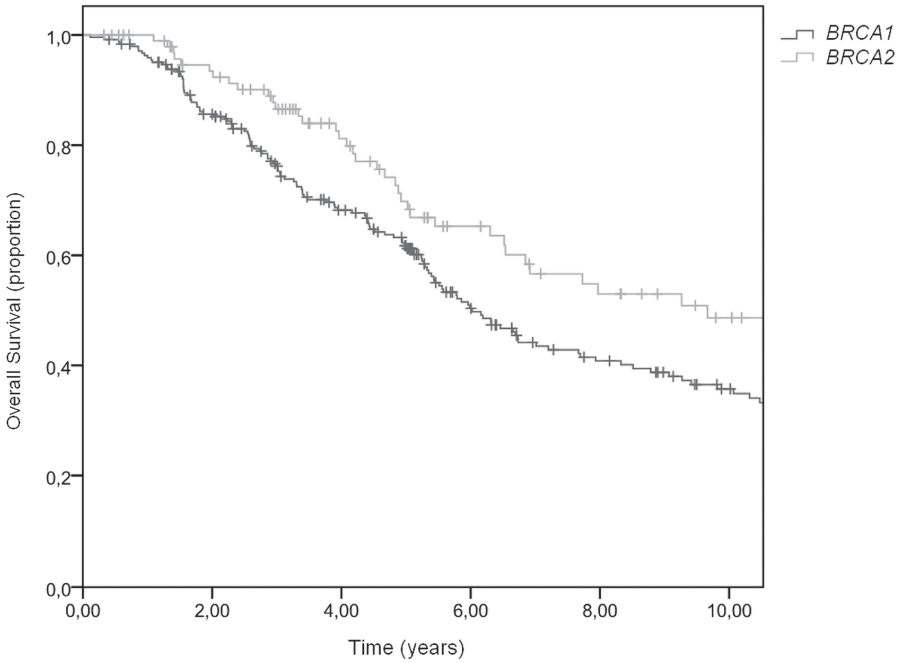


Number of patients at risk

	0 yrs	2 yrs	5 yrs	10 yrs
BRCA1	245	130	62	23
BRCA2	99	60	30	15

logrank *P*=0.006

Figure 2a. Overall survival in *BRCA1*- (dark line) versus *BRCA2*-associated (light line) EOC patients

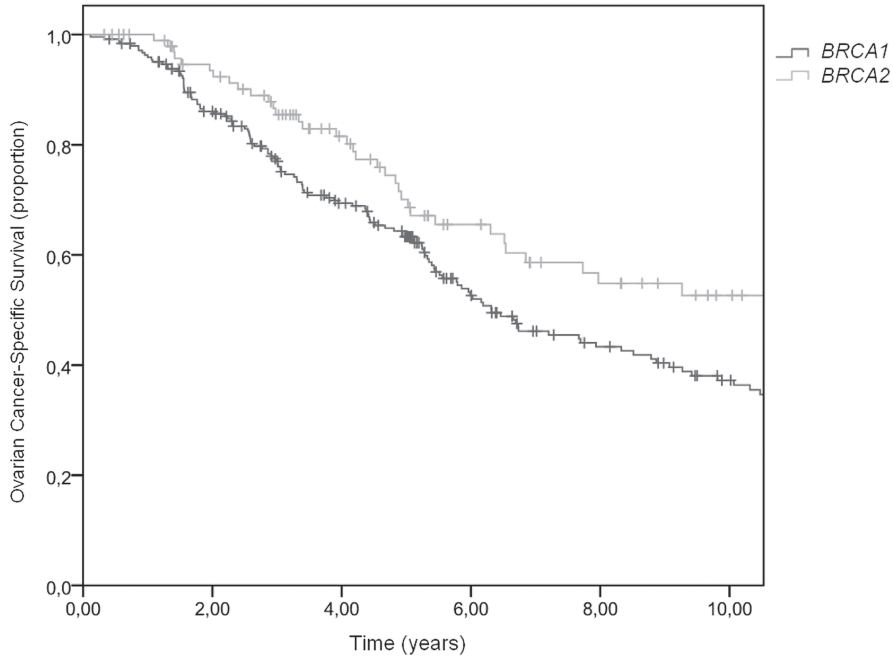


Number of patients at risk

	0 yrs	2 yrs	5 yrs	10 yrs
BRCA1	245	198	120	44
BRCA2	99	84	48	21

logrank *P*=0.04

Figure 2b. Ovarian cancer specific survival in *BRCA1*- (dark line) versus *BRCA2*-associated (light line) EOC patients



Number of patients at risk

	0 yrs	2 yrs	5 yrs	10 yrs
BRCA1	245	198	120	44
BRCA2	99	84	48	21

logrank *P*=0.02

DISCUSSION

To our knowledge, this is the largest reported series of *BRCA*-associated EOC patients comparing both results of primary treatment, including chemotherapy, and survival between *BRCA1*- and *BRCA2*-patients. PFS and OS were significantly longer in *BRCA2*- compared with *BRCA1*-associated EOC patients, which could not be explained by differences in age at diagnosis, FIGO stage, differentiation grade, residual tumor after surgery, and type of chemotherapy. Importantly, PD during chemotherapy was not observed in *BRCA2* patients, and TFI, as a surrogate for chemosensitivity, was significantly longer in *BRCA2* than in *BRCA1* patients.

BRCA2 patients had a higher median age at diagnosis compared to *BRCA1* patients (55.5 versus 51.0 years; $P<0.001$), which is consistent with other studies and reflects the same pattern as is seen in *BRCA1* versus *BRCA2* associated BC, indicative of a differential penetrance in *BRCA1* compared to *BRCA2* mutation carriers.[18-20] Further, if older age would be a negative prognostic factor, one would expect a worse outcome for *BRCA2* patients.[26]

In our study cohort primary treatment consisted of both surgery and chemotherapy in 94% of patients enabling to evaluate response to chemotherapy. Complete response/NED was observed in 86% of *BRCA1* and 90% *of BRCA2* patients ($P=0.36$). PD was rarely observed; only five *BRCA1* patients experienced PD and none of the *BRCA2* patients. Of note, treatment-free interval, considered as a surrogate measure for chemosensitivity, was significantly longer in *BRCA2* than in *BRCA1* patients. While patient selection and definitions are not completely similar, our findings are in line with the results of Yang et al. [18], who published data of an observational study including 35 *BRCA1*- and 27 *BRCA2*-associated EOC patients describing a higher chemosensitivity rate in *BRCA2* versus *BRCA1* mutation carriers (100% versus 80%; $P=0.05$), and a longer platinum-free interval (18 versus 12.5 months). However, our *BRCA* series are much larger than their study cohorts, and for the TFI endpoint we did not exclude patients undergoing incomplete debulking surgery, which is known as a negative prognostic factor.[26] Other studies have not provided information regarding chemotherapy for *BRCA1* and *BRCA2* patients separately.

Additionally, our study is the first that observed a significantly improved PFS after primary treatment of EOC in *BRCA2* compared with *BRCA1* patients, being 3.9 years (95%-CI 2.5-5.3) versus 2.2 years (95%-CI 1.9-2.5) respectively (P=0.006). Yang et al. [18] also found an improved PFS for *BRCA2* versus *BRCA1* reflecting a trend for significance (PFS rate at 3 years 44% versus 22%, P=0.05), whereas the study of Alsop et al. [5], observed a similar PFS (19.4 versus 20 months).

Overall survival was also significantly better in *BRCA2*- compared with *BRCA1*-associated EOC patients, with a median of 9.7 years and 6.0 years, respectively (P=0.04). These results are in line with the findings of Bolton et al. [20] who reported a 5-year OS of 44% (95%-CI 40-48) for *BRCA1*- and 52% (95%-CI 46-58) for *BRCA2*-associated EOC patients (P<0.01). The much smaller study of Hyman et al. [27] evaluating 30 *BRCA1*- and 17 *BRCA2*-associated, compared with 143 sporadic EOC patients, observed a non-significantly different OS between *BRCA2*- and *BRCA1*-associated EOC patients, possibly due to small numbers (3 year OS: 100% versus 91%, P=0.06). In the Australian study [5] the median OS was 5.8 years (range: 4.0-7.6) in *BRCA2* compared with 5.2 years (range: 3.7-6.7) in *BRCA1* EOC patients. It is however not mentioned if this difference is significant.

The mechanism behind the observed improved survival of *BRCA2*- compared with *BRCA1*-associated EOC patients remains unclear. It has been postulated that an improved life expectancy for *BRCA2* compared with *BRCA1* mutation carriers might be attributed to the biological characteristics of the tumors and a better response to cancer treatment.[9] Since in our study the results of debulking surgery were not different, as well as the response to chemotherapy was similarly high for both BRCA cohorts, the longer PFS in *BRCA2* patients could not be explained by the response to primary treatment. However, the longer TFI observed in *BRCA2*, compared to *BRCA1* patients, might reflect an improved sensitivity to chemotherapy of *BRCA2* mutation carriers. We postulate that DNA repair after chemotherapy might be less efficient in *BRCA2* compared to *BRCA1* mutation carriers. Therefore, in our opinion, the improved PFS and OS in *BRCA2* mutation carriers might be explained by a longer duration of response to chemotherapy, and could possibly be explained on a molecular biological level or underlying differences in tumor biology.

Functionally, both BRCA1 and BRCA2 proteins were reported to play key roles in DNA damage repair, but appear to have distinct functions. *BRCA1* plays a versatile role in tumor suppression through its ability to participate in DNA damage response, checkpoint control, mitotic spindle assembly, centrosome duplication and sister chromatid decatenation. *BRCA2* has one main function in double-strand break repair by homologous recombination, namely by regulating the RAD51 protein. Also, it was suggested that *BRCA2* mutations are more instable and that the promotor region is less often hypermethylated compared to *BRCA1*.[18] Overall, the mechanisms through which *BRCA1* and *BRCA2* mutations may result in different tumor biology require further research.

Strengths of our study are the consecutive series of *BRCA1*- and *BRCA2*-associated EOC patients, the large sample size of both BRCA cohorts, the availability of detailed clinical information including debulking status and type of chemotherapy for most patients, the long follow-up period, and the evaluation of PFS, TFI, OS, OCSS as well as response to chemotherapy. However, we are aware that some limitations have to be considered as well. We could not exclude survival bias in the *BRCA1* and *BRCA2* cohorts potentially occurring by preferably selecting long-living EOC patients who were referred for genetic testing a long time after the initial EOC diagnosis. To deal with this possible bias we performed left-truncated survival analyses and observed that the longer PFS for *BRCA2* mutation carriers persisted (HR 0.58; 95%-CI 0.37-0.90; P=0.02), although the OS did not reach statistical significance anymore (HR 0.79; 95%-CI 0.53-1.17; P=0.24).

Nonetheless, the finding of a survival benefit for *BRCA2*- compared with *BRCA1*-associated EOC patients is obvious, and in line with other observations, and may have several clinical implications. Primarily, EOC patients with a *BRCA1* or *BRCA2* mutation may be informed more accurately about the expected survival and chemosensitivity. Furthermore, the improved survival of *BRCA2* compared with *BRCA1* mutation carriers may have implications for future clinical trial designs, since a substantial part (16-21%) of the patients with high-grade serous EOC have a *BRCA1/2* mutation.[5] Further, trials examining agents that target homologous recombination should particularly stratify for *BRCA1* versus *BRCA2* mutation status. This is also of

importance for the currently ongoing phase 1 and phase 2 trials concerning PARP inhibitors showing anti-tumor activity especially in EOC patients with *BRCA1*/2 mutations.[28-31] Our results indicate that the specific mutation status should be taken into account in the results analyses. Additionally, our observations and these of others underscore that it is important to perform genetic testing at EOC diagnosis, since this may have implications for counseling and therapy.

In conclusion, we found a significantly longer PFS, TFI and OS in *BRCA2*-compared with *BRCA1*-associated EOC patients. These data are of importance for both counseling and therapy of BRCA-associated EOC patients, and indicate that genetic testing in EOC patients is important. Also, in upcoming studies concerning the efficacy of new chemotherapeutic modalities or PARP inhibitors stratification for *BRCA1* and *BRCA2* mutation status should be incorporated.

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3

SURGICAL TREATMENT OF *BRCA* OVARIAN CANCER

3.1

Outcome of debulking surgery in patients with *BRCA1*-associated, *BRCA2*-associated and sporadic epithelial ovarian cancer patients: a Dutch analysis

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Submitted

ABSTRACT

Objective

To assess the optimal debulking rate after surgery and to compare the impact of optimal debulking on survival in *BRCA1*-associated, *BRCA2*-associated and sporadic epithelial ovarian cancer (EOC) patients.

Methods

Data of 158 *BRCA1* and 68 *BRCA2* EOC patients with FIGO stage IIb and higher and of a reference group of 181 sporadic patients, were retrospectively retrieved from medical files. Residual tumor after definitive surgery, being primary and/or interval debulking, was categorized as optimal or incomplete resection (residual tumor <1cm or ≥ 1cm). Analyses were performed using the Kaplan Meier survival method and Cox proportional hazard analyses.

Results

The optimal debulking rate after primary surgery was higher in *BRCA1* and *BRCA2* than in sporadic patients (46%, 51% and 40%, respectively), but was comparable after definitive surgery in the three groups (68%, 60% and 60%, respectively). Optimal versus incomplete resection was associated with a more favorable progression-free survival (PFS) in sporadic (HR 1.98; 95% CI1.34-2.92) and *BRCA1* (HR 2.01; 95% CI1.07-3.75), but not in *BRCA2* (HR 0.36; 95% CI 0.12-1.08) patients. Optimal versus incomplete resection was associated with a longer overall survival (OS) in sporadic (40 versus 23 months; HR 1.88; 95% CI1.26-2.81), a non-significantly longer OS in *BRCA1* patients (69 versus 63 months, HR 1.46; 95%CI 0.68-3.15), but not with a longer OS in *BRCA2* patients (35 versus 92 months, HR 0.53; 95% CI 0.15-1.93).

Conclusion: The optimal debulking rate after definitive surgery was not different between the three groups. Optimal debulking is a less strong prognostic factor for survival in *BRCA*-associated EOC, especially in *BRCA2* patients.

INTRODUCTION

The standard treatment of epithelial ovarian cancer (EOC) FIGO stage IIb and higher consists of primary cytoreductive surgery, followed by six cycles of platinum-based chemotherapy or three cycles of neoadjuvant chemotherapy followed by interval debulking surgery and subsequently another three cycles of chemotherapy.[1] Overall, survival after both treatment strategies is comparable, but in case of wide spread disease postoperative morbidity and mortality is lower in patients treated with neoadjuvant chemotherapy, probably by reducing the tumor load and consequently increasing the surgical resectability.[1] Optimal debulking surgery (residual tumor<1cm) and even more complete debulking (no macroscopic residual tumor), have been identified as an important prognostic factor of EOC,[1-5] which is more frequently reached in patients operated by gynaecologic oncologists and in higher volume institutions.[4,6,7]

BRCA1 or *BRCA2* germline mutations are assumed to occur in 8-16% of all EOC cases.[8-14] Particular features of *BRCA1/2*-associated EOC include a mainly high grade serous subtype and a relative young age at onset in mutation carriers.[15,16] In addition, the overall survival (OS) of *BRCA1*- and *BRCA2*-associated patients is improved, compared to sporadic patients, whereas recent reports described an improved OS for *BRCA2*-versus *BRCA1*-associated EOC patients.[12, 17-21] To date, the improved OS of *BRCA1*- and *BRCA2*-associated EOC patients is thought to be the result of an increased sensitivity to platinum-based chemotherapy, since *BRCA1/2* deficient cells have an impaired ability to repair DNA damage by means of homologous recombination.[16,17,22-24] However, there is limited knowledge about the possible role of optimal cytoreductive surgery in relation to the improved OS of *BRCA1/2*-associated EOC patients.

To our knowledge, only two small studies investigated the role of surgical cytoreduction in *BRCA1/2*-associated versus sporadic EOC patients previously. [25,26] Hyman et al. observed. in the time period 2001-2010, a significantly higher percentage of optimal surgical resections in 69 *BRCA1/2*, compared with 298 sporadic EOC patients, all having FIGO stage III/IV and undergoing primary debulking surgery only. However this difference was not significant anymore after correcting for age at diagnosis, and not significantly different when considering *BRCA1* and *BRCA2* separately.

Boyd et al. demonstrated a comparable optimal debulking rate in 88 *BRCA1/2*-associated and 101 sporadic EOC patients with FIGO stage III/IV EOC, undergoing primary debulking surgery only. [26] Because of the high percentage of patients of Ashkenazy Jewish ancestry in both studies, it is unknown whether the results of these studies are representative for other *BRCA*-associated EOC populations, including the Dutch population. Furthermore, the study of Boyd et al. did not distinguish between *BRCA1*- and *BRCA2*-associated EOC patients, and both studies did not investigate whether residual tumor was a prognostic factor for survival in both groups. Also, general operative characteristics, like operating time, amount of blood loss and complications were not described, being important factors in view of morbidity and quality of life.[27]

In the current analyses, we explored the differences in residual disease after primary and definitive surgery in sufficiently large groups of *BRCA1/2*-associated and sporadic EOC patients, enabling to distinguish between *BRCA1*- and *BRCA2*-associated EOC patients. We also evaluated whether residual tumor size after definitive surgery (<1cm or ≥1cm) is associated with progression-free survival (PFS) and OS in *BRCA1*, *BRCA2* and sporadic EOC patients, respectively.

METHODS

Study population

BRCA1- and *BRCA2*-associated patients with EOC were selected from a nationwide dataset and sporadic patients from a local dataset, both available from previous studies.[17,21]

The nationwide study included EOC patients, identified with a deleterious *BRCA1* or *BRCA2* mutation or EOC patients being a first-degree family member of proven *BRCA* mutation carriers. *BRCA1* and *BRCA2* patients were recruited from databases of the Erasmus University MC Cancer Institute, University Medical Center Groningen, Radboud University Medical Center and the Netherlands Cancer Institute – Antoni van Leeuwenhoek hospital (NKI-AVL). To expand the *BRCA2* group, *BRCA2*-associated EOC patients were also recruited from databases of the Leiden University Medical Center (LUMC), Amsterdam Medical Center (AMC), VU Medical Center Amsterdam (VUMC), University Medical Center Utrecht (UMCU), Maastricht University

Medical Center (MUMC) and the Netherlands Foundation for the Detection of Hereditary Tumors (STOET).
Sporadic EOC patients were selected from the cancer registry of the Erasmus University MC Cancer Institute or the Comprehensive Cancer Center Rotterdam for a previous study, as has been described in detail earlier.[17] In this previous study, sporadic patients were matched (ratio 1:2) with individual *BRCA1*- and *BRCA2*-associated EOC patients from the Rotterdam family cancer clinic database for age at (±5 years) and year of diagnosis (±5 years).[17]

Inclusion criteria for the current study were: primary EOC stage IIb-IV (defined according to the FIGO guidelines 2009), [28] diagnosed between January 1st 1980 and January 1st 2011, adequate data about patient, tumor and treatment characteristics and follow-up data after diagnosis of EOC, no history of another primary malignancy besides breast cancer (BC), surgical treatment (either primary debulking and/or interval debulking) as part of primary treatment and known residual tumor after definitive surgery. Definitive surgery was defined as the final operation, being primary debulking if this was the only operation, and being interval debulking if this operation followed primary debulking or neoadjuvant chemotherapy. Patients with a borderline ovarian tumor and patients with suspicion of primary or recurrent BC at time of primary diagnosis of EOC were excluded. Additionally, sporadic EOC patients with a strong family history of breast and/or ovarian cancer, defined as two relatives (first or second degree) with breast cancer, one relative (first or second degree) with breast cancer diagnosed before the age of 55 years and/or one relative (first or second degree) with ovarian cancer, irrespective of age, were excluded to reduce the likeliness of including untested *BRCA1/2* mutation carriers.

In total, we identified 275 *BRCA1*, 115 *BRCA2* and 235 sporadic EOC patients from the databases. Excluded were: 38 *BRCA1*, 20 *BRCA2* and 43 sporadic patients, having FIGO stage I/IIa disease, three *BRCA1* and four *BRCA2* patients with no follow-up data, 17 *BRCA1* and four *BRCA2* patients diagnosed with EOC before 1980 or after 2011, 58 *BRCA1*, 18 *BRCA2* and 10 sporadic EOC patients with unknown residual tumor after definitive debulking surgery, and one *BRCA1*, one *BRCA2* and one sporadic patient,

treated with chemotherapy without interval debulking surgery. Eventually we included 158 *BRCA1*, 68 *BRCA2* and 181 sporadic EOC patients in the current analyses.
The study was approved by the Institutional Review Board of the Erasmus University MC Cancer Institute, Rotterdam, and according to the Dutch law, no further institutional Review Board approval was needed.

Data collection

For all eligible selected patients, information concerning patient- and tumor characteristics, surgical procedure (either primary debulking and/or interval debulking), characteristics of surgery including time of surgery, amount of blood loss, complications, length of hospital stay, re-admission(s), and assistance of a gynecological oncologist during surgery, residual tumor (< or ≥ 1 cm) after primary and/or definitive surgery and date of progressive or recurrent disease and date of death was retrospectively retrieved from medical files. The performance status at diagnosis was evaluated according to the World Health Organisation (WHO) criteria.[29]
Operative notes were reviewed by PV, DM or JR (supervised by PV) to accurately determine the volume of residual disease (<1cm or ≥1cm). Information about leaving no macroscopic residual disease behind after surgery (complete debulking) is not available in our study, since this was not commonly documented before 1990.

Outcome measures and data definition

Primary study endpoints were residual disease (optimal resection <1cm, incomplete resection ≥1cm) after either primary and/or definitive surgery, and whether residual tumor after definitive surgery is a prognostic factor regarding PFS, OS and ovarian cancer specific survival (OCSS) in *BRCA1*- and *BRCA2*-associated and sporadic EOC patients respectively. PFS was defined as the time between date of diagnosis and date of progressive disease or first recurrence. OS was defined as the time between the date of diagnosis and of death and OCSS as the time between the date of diagnosis and of death due to ovarian cancer. Censoring events were date of last visit at the clinic or end date of this study (1 June 2011). In the analyses for PFS and OCSS patients were also censored at date of death due to other reasons than ovarian cancer.

Statistical analyses

Differences in patient- and tumor- characteristics between *BRCA1*- and *BRCA2*-associated and sporadic EOC patients, respectively, were tested with the Pearson's Chi-square test or the Fisher Exact test (categoric variables) or by the Kruskal-Wallis test (continuous variables).

Differences in residual disease after debulking surgery were tested by the Pearson's Chi-square test and examined by means of logistic regression, adjusting for possible confounders including age at and period of diagnosis, tumor characteristics, FIGO stage, CA125 level at diagnosis, history of breast cancer, treatment with platinum containing chemotherapy, type of hospital and presence of a gynecologic oncologist during operation.

Absolute differences of both PFS and OS were measured using the Kaplan-Meier survival method, separately for *BRCA1*, *BRCA2* and sporadic EOC patients, and differences between the groups were tested by a Wilcoxon test. Adjusted relative differences in PFS and OS between the three patient groups were examined in a multivariate Cox proportional hazard regression model. In this model we adjusted for possible confounders including age at and period of diagnosis, FIGO stage, tumor grade and morphology, CA125 at diagnosis, type of chemotherapy for EOC, gynaecological oncologist present during surgery and history of and adjuvant chemotherapy for breast cancer.

All analysis were performed with Stata 12 for Windows. A two-sided p-value less than 0.05 was considered as statistically significant.

RESULTS

Patient- and tumor characteristics

The patient characteristics are depicted in Table 1. *BRCA2*-associated patients were significantly older (median age *57* years) at diagnosis, compared with *BRCA1*-associated (median 50 years; P<0.001) and sporadic EOC patients (median 52 years, P=0.008). *BRCA1* and *BRCA2* patients were more often diagnosed between 2000 and 2010, and sporadic EOC patients more often before 2000. Thirty percent of the *BRCA1/2*-associated EOC patients had a history of breast cancer, which was only 5% in the sporadic cohort. Poor differentiation grade was more often seen in *BRCA* mutation carriers (*BRCA1*: *57%*, *BRCA2:* 65%, sporadic 48%, respectively). More

sporadic patients (51%) had WHO status 0, compared with *BRCA2* patients (43%, p=0.03). Other variables did not significantly differ between the three groups, including FIGO stage at diagnosis. Follow-up was complete for 82% of the patients (*BRCA1*, 78%; *BRCA2*, 71%, sporadic, 90%).

Debulking surgery

As depicted in table 2, in total 141 (89%) *BRCA1*-associated, 57 (84%) *BRCA2*-associated and 169 (93%) sporadic EOC patients underwent primary debulking surgery, while 17 *BRCA1* (11%), 11 *BRCA2* (16%) and 12 sporadic patients (7%) were treated with neoadjuvant chemotherapy, followed by interval debulking. Primary debulking was followed by interval debulking in 52 *BRCA1*-associated, 22 *BRCA2*-associated and 55 sporadic EOC patients (Table 2).

Table 1. Patient- and tumor characteristics in *BRCA1* associated, *BRCA2* associated and sporadic epithelial ovarian cancer patients.

	BRCA1		*BRCA2*		Sporadic		P		
	N	%	N	%	N	%	(*BRCA1* vs *BRCA2*)	(*BRCA1* vs sporadic)	(*BRCA2* vs sporadic)
Total	158		68		181				
Age at diagnosis									
Median	50		57		52		<0.001	0.09	0.008
Mean	51		55		52				
Range	32-72		29-69		31-73				
Year of diagnosis									
1980-1989	20	12	5	7	37	21	0.28	<0.001	<0.001
1990-1999	57	37	21	31	93	51			
2000-2010	81	51	42	62	51	28			
Breast cancer before ovarian cancer									
Yes	49	31	19	28	9	5	0.74	<0.001	<0.001
No	102	65	44	65	172	95			
Unknown	7	4	5	7	0	0			
Tumor grade									
1	10	6	1	1	15	8	0.26	0.003	0.004
2	32	20	15	22	73	40			
3	90	57	44	65	86	48			
Unknown	26	16	8	12	7	4			
FIGO stage									
IIb	3	2	4	6	8	4	0.24	0.07	0.85
IIc	15	9	3	4	8	4			
III	109	69	46	68	117	65			
IV	29	18	14	21	46	25			
Unknown	2	1	1	1	2	1			
Histology									
Serous	125	79	55	81	141	78	0.13	0.08	0.17
Mucinous	4	3	3	4	14	8			
Endometrioid	19	12	4	6	16	9			
Clear cell	1	1	0		6	3			
Undifferentiated	2	1	4	6	3	2			
Unknown	7	4	2	3	1	1			

Table 1: Continued

	BRCA1		*BRCA2*		Sporadic		P		
	N	%	N	%	N	%	(*BRCA1* vs *BRCA2*)	(*BRCA1* vs sporadic)	(*BRCA2* vs sporadic)
CA125 (kU/L) before treatment									
≤35	13	8	4	6	7	4	0.46	0.14	0.58
35-500	50	32	25	37	71	39			
≥500	52	33	17	25	59	33			
Unknown	43	27	22	32	44	24			
WHO status before surgery									
0	78	49	29	43	93	51	0.27	0.46	0.03
1	22	14	16	24	25	14			
2	6	4	4	6	3	2			
3	1	1	0	0	0	0			
4	0		0		0				
Unknown	51	32	19	28	60	33			

Table 2: Type of surgical treatment in *BRCA1* associated, *BRCA2* associated and sporadic epithelial ovarian cancer patients

	BRCA1		*BRCA2*		Sporadic	
	N	%	N	%	N	%
Total number of included patients	158		68		181	
Primary debulking	141	89	*57*	84	169	93
Primary debulking only (no interval debulking)	*89*	*56*	*35*	*51*	*114*	*63*
Primary debulking followed by interval debulking	*52*	*33*	*22*	*32*	*55*	*30*
Interval debulking after neoadjuvant chemotherapy	17	11	11	16	12	*7*

Optimal resection after primary debulking surgery was significantly more often achieved in *BRCA1*- (46%) and *BRCA2*-associated (51%) , compared with sporadic EOC patients (40%) (both P<0.001). After correcting for possible confounders, *BRCA1* mutation status was no longer significantly associated with a more favourable residual tumor load (OR 1.47 (95% CI 0.76-2.83), whereas this remained significant for *BRCA2* mutation status (OR 3.02 (95% CI 1.23-7.37) (Table 3).

BRCA2 patients were significantly more often (28%) operated in the presence of a gynecologic oncologist, compared with the *BRCA1* patients (18%, P=0.03). This probably reflects the fact that more *BRCA2*-associated patients were operated in a university hospital, as a result of the selection of especially the *BRCA2* patients (see methods section). Other characteristics of primary debulking, e.g. blood loss, surgery time, complications, hospital stay and re-admissions were equally distributed among the three patient groups. However, unfortunately much information was missing, which made it impossible to calculate reliable p-values.

After definitive surgery an optimal debulking rate (residual disease<1cm) was reached in 68% of the *BRCA1*, 60% of the *BRCA2* and 60% of the sporadic EOC patients respectively, which was not significantly different between the groups (Table 4).

Table 3: Primary debulking of *BRCA1* associated, *BRCA2* associated and sporadic epithelial ovarian cancer patients

	BRCA1		*BRCA2*		Sporadic		*P (#)*		
	N	%	N	%	N	%	(*BRCA1* vs *BRCA2*)	(*BRCA1* vs sporadic)	(*BRCA2 vs sporadic*)
Total	141		57		169				
Residual disease									
<1cm	65	46	29	51	67	40	0.47	<0.001	<0.001
≥1cm	64	45	21	37	102	60			
Unknown	12	9	7	12	0				
Univariate OR (95% CI) *								1.54 (0.97-2.45)	2.10 (1.11-3.99)
Multivariate OR (95% CI) *								1.47 (0.76-2.83)	3.02 (1.23-7.37)
Type of hospital									
University hospital	32	23	15	26	43	25	0.57	0.83	0.78
No university teaching hospital	40	28	17	30	48	28			
No university, no teaching hospital	69	49	22	39	78	46			
Unknown	0		3	5	0				
Gynecologic oncologist present during operation									
Yes	26	18	16	28	42	25	0.03	0.32	0.13
Unkown	34	24	20	35	29	17			
Time (minutes)									
Median	120		138		138				
Range	40-202		40-233		15-411				
Unknown	95		41		95				

Table 3: Continued

	BRCA1		*BRCA2*		Sporadic		*P (#)*		
	N	%	N	%	N	%	*(BRCA1 vs BRCA2)*	*(BRCA1 vs sporadic)*	*(BRCA2 vs sporadic)*
Blood loss (ml)									
Median	800		1000		1000				
Range	50-6800		5-5000		50-6500				
Unknown	81		29		85				
Complication									
Yes	34	24	15	26	60	36	0.95	0.09	0.44
Unknown	35	25	14	25	35	21			
Type of complication									
Lesion intestines	3		2		6				
Lesion bladder	1		0		2				
≥ 1 liter bloodloss	27		15		50				
Infection	1		1		3				
Platzbauch	1		0		1				
Deceased	0		0		0				
Other	4		0		6				
Hospital stay (days)									
Median	10		10		10				
Range	1-41		4-23		1-75				
Unknown	44		25		33				

Table 3: Continued

	BRCA1		*BRCA2*		Sporadic		*P (#)*		
	N	%	N	%	N	%	*(BRCA1 vs BRCA2)*	*(BRCA1 vs sporadic)*	*(BRCA2 vs sporadic)*
Re-admission within three weeks									
Yes	1	1	2	6	8	6			
Unknown	10	10	1	3	6	5			
Hospital stay during re-admission (days)									
Median	5		4		8				
Range	5-5		4-4		3-21				
Gastro-intestinal Surgeon required during debulking									
Yes	19	13	15	26	32	19	0.05	0.51	0.13
Unknown	44	31	14	25	31	18			
Reason gastro-intestinal surgeon required									
Stoma required	3	16	1	7	10	31			
Lesion intestines	4	21	2	14	3	9			
Resection part of intestines	8	42	5	33	13	41			
Resection other organ (eg spleen)	2	11	3	20	4	13			
Adhaesions	1	5	1	7	1	3			
Other	3	16	6	40	8	25			

OR=odds ratio, CI=confidence interval

* Adjusted for age at and period of diagnosis, tumor characteristics, FIGO stage, CA125 at diagnosis, type of hospital,history of breast cancer, treatment with platinumcontaining chemotherapy and gynecologic oncologist present during operation

fields without p-values had to many "unknowns" to calculate a reliable p-value

Survival

As shown in Figure 1 and Table 4, sporadic patients with optimal debulking (residual disease <1 cm) compared with incomplete resection (residual disease ≥1 cm) after definitive surgery had a longer PFS (median: 17 versus 11 months, P=0.01) and a longer OS (median: 40 versus 23 months, P=0.01). At multivariate analyses, observations regarding the association of optimal resection with PFS (Hazard Ratio (HR) 1.98; 95% CI 1.34-2.92) and OS (HR 1.88; 95% CI 1.26-2.81) remained significantly different.

BRCA1 patients with an optimal versus an incomplete resection, after definitive surgery, also had a longer PFS (median: 25 versus 19 months, P=0.01; multivariate HR 2.01; 95% CI 1.07-3.75), and a non-significantly longer OS (median: 69 versus 63 months, P=0.07; multivariate HR 1.46; 95% CI 0.68-3.15).

Remarkably, in the *BRCA2* group, optimal resection versus incomplete resection after definitive surgery was not significantly associated with neither a longer PFS (median: 35 versus 92 months, P=0.11), nor with a longer OS (median: 93 versus 145 months, P=0.64). Also, at multivariate analyses, optimal resection after definitive surgery (< 1 cm) was not predictive for outcome, neither for PFS (HR 0.36; 95% 0.12-1.08), nor for OS (HR 0.53; 95% 0.15-1.93).

Findings for OCSS were comparable with those of OS (data not shown).

Table 4: Residual disease after definitive surgery in relation to PFS and OS

	BRCA1					***BRCA2***	
	<1cm		**≥1cm**			**<1cm**	
	N	**%**	**N**	**%**	**P**	**N**	**%**
Residual tumor size	108	68	50	32	*	41	60
PFS							
Median in months (95% CI)		25		19	**0.01(#)**		35
2 yrs	56	54	22	44		27	68
5 yrs	23	28	5	10		12	34
10 yrs	11	22	0	3		5	26
Univariate HR (95%-CI)		**1**	**1.60 (#) (1.12-2.29)**				**1**
Multivariate HR (95%-CI)		**1**	**2.01 (#) (1.07-3.75)**				**1**
OS							
Median in months (95% CI)		69		63	**0.07 (#)**		93
2 yrs	90	89	38	76		36	90
5 yrs	47	61	23	55		22	70
10 yrs	19	35	5	16		7	41
Univariate HR (95%-CI)		**1**	**1.48 (#) (0.98-2.23)**				**1**
Multivariate HR (95%-CI)		**1**	**1.46 (#) (0.68-3.15)**				**1**

HR: Hazard Ratio, Adjusted for age at diagnosis, period of diagnosis, FIGO stage, tumor grade and morphology, CA125 at diagnosis, type of chemotherapy, history of and adjuvant chemotherapy for breast cancer, gynaecologic oncologist present during operation

fields without p-values had to many "unknowns" to calculate a reliable p-value

* P-value of *BRCA1/BRCA2*: 0.24, *BRCA1*/sporadic: 0.12 and *BRCA2*/sporadic: 0.99
# P-value of/HR for *BRCA1*<1cm versus *BRCA1*>1cm
∩ P-value of/HR for *BRCA2*<1cm versus *BRCA2*>1cm
^ P-value of/HR for Sporadic<1cm versus Sporadic>1cm

BRCA2			Sporadic				
≥1cm			<1cm		≥1cm		
N	%	P	N	%	N	%	P
27	40	*	109	60	72	40	*
	92	**0.11** (∩)		17		11	**0.01** (^)
15	73		41	38	14	19	
8	52		19	19	5	7	
4	43		8	15	2	4	
0.58 (∩) **(0.28-1.16)**				**1**	**1.79** (^) **(1.30-2.45)**		
0.36 (∩) **(0.12-1.08)**				**1**	**1.98** (^) **(1.34-2.92)**		
	145	**0.64** (∩)		40		23	**0.001** (^)
20	95		72	68	35	49	
10	59		36	37	13	19	
5	53		11	21	3	8	
0.83 (∩) **(0.38-1.81)**				**1**	**1.72** (^) **(1.24-2.38)**		
0.53 (∩) **(0.15-1.93)**				**1**	**1.88** (^) **(1.26-2.81)**		

Figure 1: Progression free survival

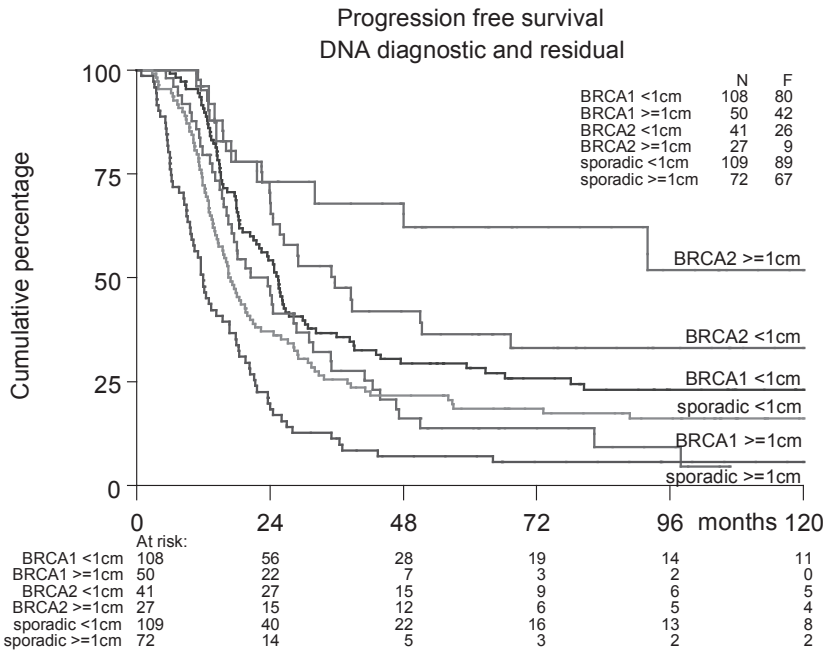
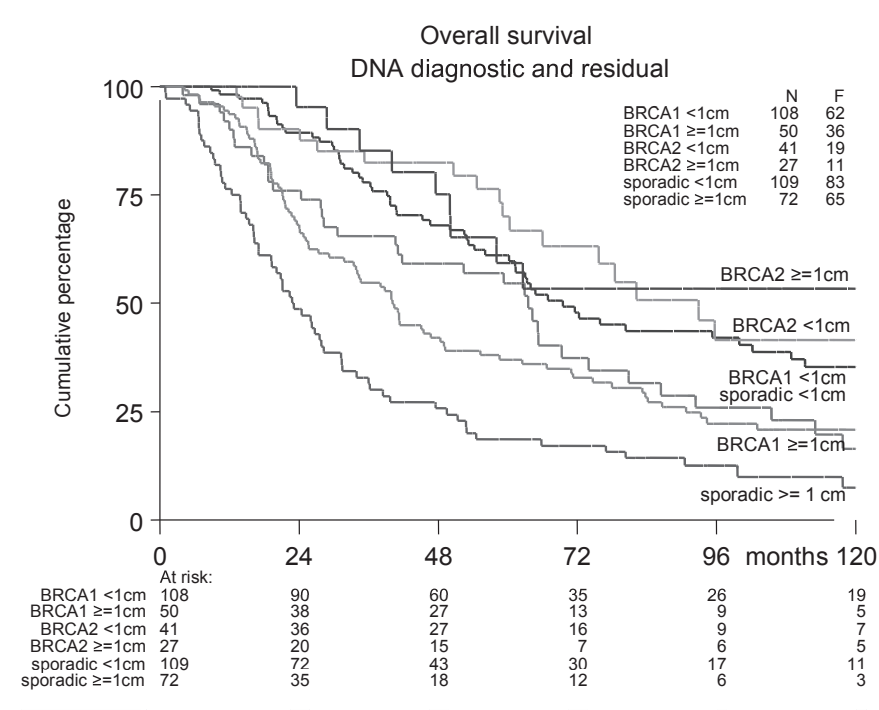


Figure 2: Overall survival



DISCUSSION

In the current study we found that *BRCA1* and *BRCA2* patients more often had an optimal surgical resection after primary surgery compared with sporadic patients, which remained significant after multivariate analyses for *BRCA2* patients, but not for *BRCA1* patients. After definitive surgery no significant differences in optimal debulking rate between the three patient groups were observed anymore. Optimal resection after definitive surgery was associated with a more favorable PFS and OS in sporadic patients, a more favorable and non-significantly longer OS in *BRCA1* patients, whereas in *BRCA2* patients optimal resection was not associated with neither PFS nor OS.

The observation of a higher optimal surgical resection rate after primary debulking surgery in *BRCA2*-associated compared to sporadic EOC patients, remaining in the multivariate analyses, suggests that the more favourable debulking surgery results in the *BRCA2* group could not totally be explained by the contribution of the gynaecologic oncologist during surgery (more frequently present for *BRCA2 (*28%), than for *BRCA1* patients (18%)), since this was one of the tested possible confounders on multivariate analyses. In the studies of Boyd et al. and Hyman et al. no significant differences were observed in residual tumor after primary debulking between *BRCA1/2* and sporadic EOC patients.[25,26] Boyd et al. described a comparable optimal debulking rate in 88 *BRCA1/2*-associated (58%) and 101 sporadic EOC patients (45%) with FIGO stage III/IV EOC, undergoing primary debulking surgery only, but did not analyse *BRCA1* and *BRCA2* patients separately. [26] Hyman et al showed a higher optimal primary debulking rate in *BRCA*-associated (N=69), compared with sporadic EOC patients (N=298), being 84.1% versus 70.1%, respectively (P=0.03), but these differences disappeared after correcting for age. [25]

In our study, the percentage of optimal debulking after primary surgery was lower (46% in the *BRCA1*, 51% in the *BRCA2*, and 40% in the sporadic group respectively), than in the study of Hyman et al (2012), [25] but comparable with the study of Boyd et al (Boyd, 2000). [26] This might be explained by the fact that 57% of the patients in our study cohorts and 100% of the patients in the study of Boyd et al. were operated before 2000, when it was not the standard procedure to perform debulking surgery by a gynecologic oncologist with the aim to obtain complete or optimal cytoreductive surgery (no residual tumor at all or <1cm residual tumor), which resulted in either less patients with an optimal debulking or it was not documented adequately. When we performed a subanalysis of patients operated after 2000 we observed a slightly higher optimal debulking rate after primary surgery (except for sporadic patients) being 51% (*BRCA1*), 56% (*BRCA2*) and 36% (sporadic) and after definitive surgery as well, being 75% (*BRCA1*), 60% (*BRCA2*) and 71% (sporadic), but percentages were still lower than in the study of Hyman et al. An additional possible explanation is that 58 *BRCA1*, 18 *BRCA2* and 10 sporadic EOC patients with unknown residual tumor after definitive debulking surgery were excluded. It might be that a considerable number of these patients had less than 1 cm residual tumor size.

Our results regarding the significant association between optimal surgical resection after definitive surgery and a longer PFS and OS in sporadic patients are in line with the data of abundant previous studies of EOC patients overall, however, not stratifying for *BRCA* mutation status. [1-5] Our findings concerning the relation of optimal surgical resection with PFS and OS in *BRCA1* and *BRCA2* EOC patients are unique and interesting. While optimal resection was associated with PFS and non-significantly with OS in *BRCA1* patients, optimal resection was not associated with neither PFS nor OS in *BRCA2* patients. A possible speculative explanation for our observations is that *BRCA* patients are particularly sensitive to chemosensitivity, being even better in *BRCA2* patients as previously reported by our group (100% complete response or partial response in our series: data not shown), [21] and that in this way chemotherapy may compensate to a certain degree for suboptimal surgery. With respect to the results of the *BRCA2* group in particular, it is possible that the number of *BRCA2*-associated patients was too small to show differences. Therefore, our results are hypothesis generating, and should first be confirmed in other studies, before firm conclusions can be drawn.

The prognostic value of residual tumor in *BRCA*-associated patients has also been addressed in the study of Alsop et al (88 *BRCA1*, 53 *BRCA2* patients) [12] and Birbak et al (70 *BRCA1/2* patients).[30] Both studies found that residual tumor < 1cm coincided with an improved PFS and OS in *BRCA1/2* patients, although in the study of Birbak et al these findings were not significant. However, none of the studies distinguished between *BRCA1* and *BRCA2* mutation carriers, and both studies differ on several points with our study. We only included patients with FIGO stage IIb and higher, while both Alsop et al and Birbak et al studied a more heterogeneous patient group regarding prognosis, since also patients with low FIGO stages were included. Further, Birbak et al. included 21 patients with a somatic *BRCA* mutation in the *BRCA* group, while it is unknown whether EOC patients with a sporadic mutation have a similar phenotype compared to patients with a germline *BRCA* mutation. In addition, both studies examined the prognostic effect of residual disease after primary debulking, while we examined the prognostic effect of residual disease after definitive surgery. Previous studies demonstrated that optimal debulking after definitive surgery is a similar prognostic factor for OS, compared with optimal debulking after primary surgery.[1,2] Further, neoadjuvant chemotherapy is increasingly used nowadays, so including

patients with neoadjuvant chemotherapy makes the patient groups more representative for the currently treated EOC patient groups. By excluding patients, treated with neoadjuvant chemotherapy, like in the studies of Alsop et al. and Birbak et al., a possible bias might have been introduced by selecting patients with an expected good operability and therefore a better survival. After performing our survival analyses, including only EOC patients who underwent primary debulking surgery, our conclusions about the prognostic effect of residual tumor size on survival remained comparable with the initial analyses, with no association between optimal resection with neither PFS nor OS in *BRCA2* patients (data available on request).

Strengths of our study are the consecutive series of *BRCA1*-associated, *BRCA2*-associated and sporadic EOC patients with FIGO stage IIb and higher, the large sample size of both *BRCA* cohorts and the detailed clinical information. However, we are aware that some limitations have to be considered as well. Firstly, information regarding complete debulking (no macroscopic residual disease) is not available in our study, since this was not commonly documented before 1990. It is currently known that complete cytoreduction is associated with a better prognosis than optimal or incomplete debulking.[2,3,31-33]
Secondly, we had missing data regarding residual tumor size after surgery in 17% of the EOC patients, and these patients were excluded from further analyses, which might have biased our results. When we performed a subanalysis in this group of patients, the median OS for these patients was 47, 54 and 15 months for *BRCA1, BRCA2* and sporadic patients, respectively, which was somewhat lower than in the group with known residual tumor (see Table 4).
Finally, the three patient groups were different regarding some patient- and tumor characteristics. *BRCA2* patients had a higher median age at diagnosis, compared with both *BRCA1* and sporadic patients. This relatively young age of the sporadic EOC patients is the result of the matching procedure with the individual *BRCA*-associated patients used for the previous study (see methods section). Further, tumors in *BRCA1/2* mutation carriers were more commonly poorly differentiated and *BRCA1/2*-associated EOC patients were more often diagnosed between 2000 and 2010. However, in the multivariate analyses we corrected for these variables and survival differences as described remained comparable.

Despite the limitations, we think that our findings about the impact of residual tumor size after surgery in relation to PFS and OS, being different in the three groups, is very interesting and asks for further research on this item by other research groups. Further, our data already have actual implications. Primarily, trials concerning EOC patients should always stratify between sporadic, *BRCA1* and *BRCA2* patients, respectively, since prognosis and, in our opinion, also efficacy of therapy have to be investigated for the different groups separately. Additionally, our observations underscore that it is important to perform genetic testing already at EOC diagnosis, enabling to obtain more knowledge about the various aspects of therapy and prognosis of the different groups of EOC patients.

In conclusion, we observed a higher optimal debulking rate after primary surgery in both *BRCA1*- and *BRCA2*-associated patients compared to sporadic EOC patients, but after definitive surgery the optimal debulking rate was similar in the three groups. Further, we found that optimal resection after definitive surgery was not associated with an improved PFS and OS in *BRCA2*-associated EOC patients, whereas in *BRCA1*-associated EOC patients there was a positive association with PFS, and a non-significant association with OS. Our results require other studies to confirm or refute our data. Possibly an international collaboration should be pursued to include enough patients in a database, making it possible to evaluate the predictive value of complete surgery on survival in *BRCA1/2*-associated EOC patients further.

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4

RISK OF BREAST CANCER AFTER *BRCA* OVARIAN CANCER

4.1

Risk of primary and contralateral breast cancer after ovarian cancer in *BRCA1/2* mutation carriers; implications for counselling?

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ABSTRACT

Background

To assess the incidence of primary (PBC) and contralateral breast cancer (CBC) in *BRCA1/2*-associated epithelial ovarian cancer (OC) patients.

Methods

From the database of the Rotterdam Family Cancer Clinic, *BRCA*-associated OC patients without (at risk of PBC; n=79) or with a history of unilateral BC (at risk of CBC; n=37) were selected. Controls were unaffected *BRCA* mutation carriers (n=351) or mutation carriers with a previous unilateral BC (n=294). Risks of PBC and CBC were calculated using the Kaplan-Meier survival method with death as competing risk event.

Results

BRCA-associated OC patients had a lower 2-, 5- and 10-year risk of PBC (3%, 6% and 11%, respectively), compared with unaffected mutation carriers (6%, 16% and 28% respectively, p=0.03), but had a considerably higher mortality rate at similar time points (13%, 33% and 61% versus 1%, 2% and 2% respectively; p<0.001). In *BRCA* patients with a previous unilateral BC, the 2-, 5- and 10-year risks of CBC were non-significantly lower in OC patients than in patients without OC (0%, 7% and 7% versus 6%, 16% and 34%, respectively, p=0.06), while the mortality rate was higher in OC patients (19%, 34% and 55% versus 4%, 11% and 21%, respectively, p<0.001).

Conclusions

BRCA-associated OC patients have a lower risk of developing a subsequent PBC or CBC than mutation carriers without OC, whereas the risk of dying due to OC is higher than the risk of developing BC. These data may facilitate more tailored counselling of this patient subgroup, while confirmative studies are warranted.

INTRODUCTION

Women with a germline mutation in the *BRCA1* or *BRCA2* gene have substantially elevated risks of developing both breast cancer (BC) and ovarian cancer (OC). The cumulative 10-year risk of primary breast cancer (PBC) has been estimated to range between 6-23% for *BRCA1/2* mutation carriers, with the highest risk between 40-50 years.[1-6]After unilateral BC the 10-year risk of contralateral breast cancer (CBC) has been estimated to be 29-39% in *BRCA1*- and 23-35% in *BRCA2*-associated patients.[1,7-10] Factors potentially affecting the latter include age at first BC diagnosis (especially when diagnosed <50 years),[7,8,11]adjuvant systemic therapy[12] and risk-reducing salpingo-oophorectomy (RRSO).[13]In view of these increased BC risks, *BRCA1* and *BRCA2* mutation carriers are informed about various risk-reducing strategies, consisting of intensive BC surveillance (including MRI),[14,15] chemoprevention,[12,16,17] risk-reducing mastectomy (RRM),[18-22]and/or RRSO.[13, 23-28]

While several publications reported about the PBC risk in unaffected mutation carriers, and CBC risk in *BRCA*-associated BC patients, to our knowledge, no data are available about the BC risks after *BRCA*-associated OC. For this subgroup these risks may be reduced by the treatment given for OC, mainly consisting of surgery (including salpingo-oophorectomy) as well as platinum-based chemotherapy. First, salpingo-oophorectomy in mutation carriers has been associated with a reduced BC risk, being most pronounced if performed at premenopausal age.[24] Further, it has been reported that *BRCA* mutation carriers are highly sensitive to platinum-based chemotherapy, leading to an improved survival for *BRCA*-associated compared with sporadic OC patients.[29,30] However, despite an improved outcome in *BRCA*-associated OC patients, the five- and 10-years survival remains poor (63% and 35%, respectively)[31], which has to be taken into account at counselling. So, precise data concerning the risks of PBC or CBC after *BRCA*-associated OC are warranted enabling optimization of- and more patient-tailored counselling regarding the subsequent strategies for this patient subgroup.

In the current study, therefore, we assessed the incidences of PBC and CBC in *BRCA*-associated OC patients without and with a history of unilateral BC, in comparison with mutation carriers without OC.

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METHODS

Patient selection

From the database of the Rotterdam Family Cancer Clinic (FCC) of the Erasmus University MC-DDHCC we selected all *BRCA1*- and *BRCA2*-associated epithelial OC patients, diagnosed between January 1st, 1980 and January 1st, 2009. DNA testing was performed as has been described before.[32] Excluded were patients who underwent a RRM before OC diagnosis, patients with another malignancy (besides unilateral BC) before OC diagnosis, BC patients with recurrent disease and patients with inadequate data concerning tumor- and treatment characteristics, and follow-up data. In total, 79 *BRCA*-associated OC patients without a history of unilateral BC (at risk of PBC), and 37 *BRCA*-associated OC patients with a previous unilateral BC (at risk of CBC) were included in the current analyses.

As controls for the *BRCA*-associated OC patients (without BC), we selected all unaffected *BRCA1/2* mutation carriers registered at the FCC and being ≥ 35 years (n=351; at risk of PBC). For the OC group at risk of CBC, the controls consisted of *BRCA1/2*-associated unilateral BC patients (without OC), as assessed at the first visit at the FCC (n=294). RRSO was not an exclusion criterion, since this more accurately represents the group of female *BRCA* mutation carriers seen at FCCs.

For all eligible patients, information regarding patient, tumor and treatment characteristics, preventive surgery and follow-up data was retrieved from medical files. The study was approved by the institutional Review Board.

Statistical analyses:

Start of study follow-up for the *BRCA*-associated OC patients was the date of OC diagnosis, and for both control groups the date of first visit at the FCC or the 35th birthday, whichever came last. Endpoint of the study was BC, either primary or contralateral (both invasive cancer and ductal carcinoma in situ (DCIS)). Cumulative BC incidences were calculated using the Kaplan-Meier method. Because OC is associated with a high mortality rate and death is an event precluding the occurrence of BC, death was considered as competing risk event in the analyses.[33] *BRCA1/2* mutation carriers were censored at the date of RRM, date of loss to follow-up or end date of the study (June 1st, 2009). A log-rank test was used to compare the BC incidences and mortality

rates between the different groups. To investigate if age influenced the risk of BC, the regression coefficient of age on BC risk was calculated. To assess if PBC and CBC incidence rates differed between the OC and control patients, independently of the mortality rate in the OC group, Kaplan-Meier analyses were also performed with death as censoring event.

A multivariate Cox proportional hazard model was used to adjust the differences in BC risks between the OC and control groups for potential confounders, considering age at- and year of study entry, and type of mutation (*BRCA1* versus *BRCA2*) as variables. In the analyses for the CBC risk, the time period between the first BC and start of follow-up was considered as additional potential confounder. Further, women were censored at dates of death, RRM, loss to follow-up or end date of the study (June 1st, 2009). Two sided $p<0.05$ was considered statistically significant. The statistical analyses were performed using SPSS version 15.0 and STATA version 11.1 software.

RESULTS

Patient- and treatment characteristics are described in table I. The OC group at risk of PBC consisted of significantly more *BRCA1* mutation carriers and was older than the control group at study entry. In the OC group, at risk of CBC, the primary BC occurred approximately 10 years before the OC diagnosis. Since OC was mostly diagnosed at advanced stage (FIGO stage III/IV in 76%), most patients received chemotherapy (mainly platinum-based) as part of primary OC treatment. The mean follow-up for women at risk of PBC was 6.7 and 6.5 years for patients with and without OC, respectively, while in the groups at risk of CBC this was 6.6 and 8.9 years, respectively.

In total, eight PBCs were detected in the OC group, and 49 PBCs in the control group (table II), including three PBCs in controls (6.1%), detected at RRM. In the patients with a previous unilateral BC, four CBCs were detected in the OC group, and 70 CBCs in the control group (table II) of which six were detected at RRM (OC group: 1; controls: 5). One OC patient in the PBC risk group had metastatic BC at diagnosis, detected before 2000 and one OC patient at risk of CBC was diagnosed with a pT4 tumour, while NM stages were not significantly different between OC (all diagnosed after 2000) and control patients.

As shown in table II and figure I, significantly less *BRCA*-associated OC patients developed a PBC, compared with unaffected mutation carriers. The 2-, 5- and 10-year risk of PBC in OC patients was 3%, 6% and 11%, compared with 6%, 16% and 28% respectively in unaffected mutation carriers (p=0.03). In contrast, at similar time points the risk of death was significantly higher in OC patients than in unaffected mutation carriers (table II). Age at study entry was not statistically significantly associated with BC risk (regression coefficient 0.89, 95% CI 0.72-1.11) indicating that it was no confounder in our study. Of the eight women

Table I: Patient demographics

	BRCA mutation carriers at risk of primary breast cancer			*BRCA* mutation carriers at risk of contralateral breast cancer		
	OC patients	Unaffected (controls)	p-value	OC patients with previous BC	History of BC at FCC registration(controls)	p-value
	N (%)	N (%)		N (%)	N (%)	
Number	79	351		37	294	
BRCA1	72 (91)	252 (72)	<0.001	30 (81)	216 (72)	0.32
BRCA2	7 (9)	99 (28)		7 (19)	78 (27)	
Median age at study entry(range)	50.3yrs (33.4-72.8)	40.1 yrs (35.0-69.2)	<0.001	55.0 yrs (39.5-68.3)	41.3 yrs (35.0-73.9)	<0.001
Median age at diagnosis BC (range)	-	-		45.8 yrs (33.0-59.3)	40.1yrs (24.8-69.4)	<0.001
Year at study entry						
1980-1990	16 (20)	6 (2)	<0.001	8 (22)	39 (13)	0.17
1990-2000	43 (54)	99 (28)		16 (43)	107 (37)	
2000-2009	20 (26)	246 (70)		13 (35)	148 (50)	
FIGO stage of OC						
I	10 (13)			4 (12)		
II	8 (11)			5 (14)		
III	47 (63)			5 (60)		
IV	10 (13)			5 (14)		
Unknown	4			1		

Table I: continued

	BRCA mutation carriers at risk of primary breast cancer			*BRCA* mutation carriers at risk of contralateral breast cancer		
	OC patients	Unaffected (controls)	p-value	OC patients with previous BC	History of BC at FCC registration(controls)	p-value
	N (%)	N (%)		N (%)	N (%)	
Chemotherapy for OC						
No	5 (6)	-		1 (3)	-	
Platinum/ taxol	32 (41)	-		16 (43)	-	
Platinum without taxol	41 (52)	-		18 (49)	-	
Non platinum-based	1 (1)	-		2 (5)	-	
RRSO						
No	-	181 (52)		-	172 (59)	
Yes	-	170 (48)		-	122 (41)	
Median age in yrs (range)	-	47.5 (29.2-71.3)		-	46.7 (34.4-72.1)	
RRM						
No	76 (96)	242 (69)	<0.001	35 (95)	212 (72)	0.003
Yes	3 (4)	109 (31)		2 (5.)	82 (28)	
Median age in yrs (range)	49.8 (44.6-52.8)	40.9 (35.4-60.4)		50.6 (48.0-53.1)	44.5 (35.4-65.1)	

BC: breast cancer; OC: ovarian cancer; FCC: Family Cancer Clinic; RRSO: risk-reducing salpingo-oophorectomy; RRM: risk-reducing mastectomy

diagnosed with a PBC after OC, four patients died (three due to BC, one from an unknown reason) within a mean follow-up time after PBC of 4.1 years.

In the patient groups with a previous unilateral BC, OC patients non-significantly less often developed a CBC than control patients (Table II, figure II). The 2-, 5- and 10-year risk of CBC was 0%, 7% and 7% in OC patients versus 6%, 16% and 34%, respectively, in control patients (p=0.06). At similar time points, the risk of death in OC patients was significantly higher than in controls (table II). Age at study entry, was not statistically significantly associated with CBC risk (regression coefficient 0.81, 95% CI 0.57-1.11). Of the four women developing a CBC after OC, one patient died, but not due to cancer.

In the analyses with death as censoring event (versus competing risk event), the risk of PBC also remained significantly lower in the OC group than in the control group, being 3%, 7% and 19%, compared with 6%, 21% and 53%, respectively, at 2-, 5- and 10 year (p=0.03). Further, we observed a trend for a lower CBC risk in the OC versus the control patients, with 2-, 5- and 10-year incidences being 0%, 11% and 11% versus 6%, 19% and 40%, respectively (p=0.06) (data not shown).

Performing the analyses after exclusion of controls who underwent premenopausal RRSO, significance increased regarding PBC risk, while the CBC risk in OC patients became significantly lower.

In a multivariate analysis, after adjusting for age at and time period of OC diagnosis, and mutation status, the risk of developing a PBC remained significantly lower in *BRCA*-associated OC patients compared with healthy mutation carriers (Hazard Ratio (HR) 0.35; 95% CI 0.15-0.82). The risk of CBC was also lower in OC patients than in control patients without OC, but the confidence interval was wide (HR 0.52; 95% CI: 0.18-1.53, Table II)

Table II: Incidence of primary (PBC) and contralateral breast cancer (CBC), and risk of death

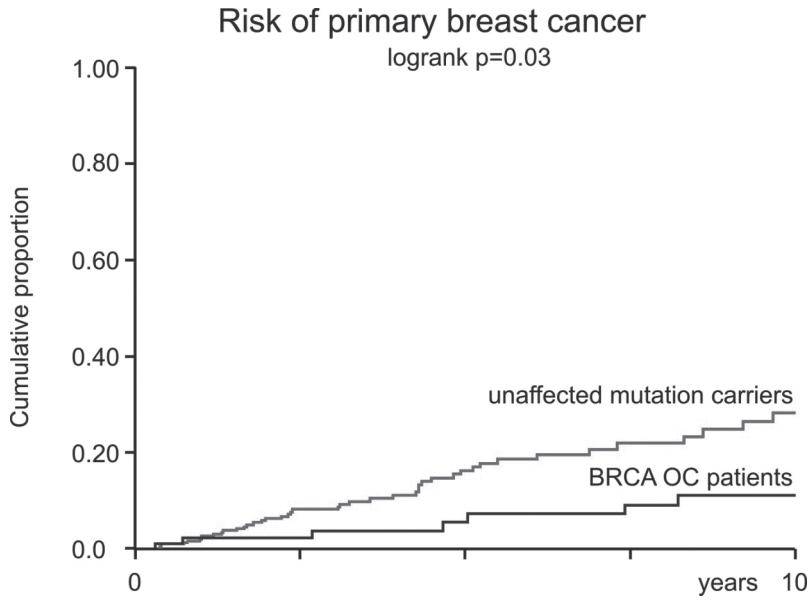
At risk of PBC				
	OC patients		**Unaffected mutation carriers (controls)**	
Number	79		351	
Mean years at risk	5.8 yrs		4.0 yrs	
Number of PBCs (including DCIS)	8		49	
Median age at PBC (range)	57.8 yrs (49.0-65.1)		44.7 yrs (35.3-70.7)	
T size				
Tis	1 (17%)		3 (6%)	
T1	2 (33%)		38(78%)	
T2	3 (50%)		7 (14%)	
T3	-		1 (2%)	
Tx	2		-	
	p=0.106			
Nodal status				
N negative	3 (43%)		35 (74%)	
N positive	4 (57%)		12 (26%)	
	p=0.087			
Metastasis status				
M negative	6 (86%)		46 (100%)	
M positive	1 (14%)		-	
	p=0.01			
Number of deaths	42		4	
	PBC risk	**Risk of death**	**PBC risk**	**Risk of death**
After 2 years	3%	13%	6%	1%
After 5 years	6%	33%	16%	2%
After 10 years	11%	61%	28%	2%
	p=0.03	**P<0.001**		
	HR	**p-value**	**HR**	
Univariate (95% CI)	0.43 (0.20-0.95)	0.04	Reference group	
Multivariate (95% CI)	0.35 (0.15-0.82) *	0.02	Reference group	

Table II: Continued

At risk of PBC				
	OC patients		**Unaffected mutation carriers (controls)**	
Number	37		294	
Mean years at risk	6.4 yrs		5.6 yrs	
Number of CBCs (including DCIS)	4		70	
Median age at CBC (range)	56.9 yrs (46.1-71.9)		46.2 yrs (35.5-77.7)	
T size				
Tis	-		7 (11)	
T1	3 (75%)		45 (68)	
T2	-		13 (20)	
T4	1 (25%)		1 (1)	
T unknown	-		4	
	p= 0.036			
Nodal status				
N negative	3 (100%)		44 (71.0%)	
N positive	-		18 (29.0%)	
N unknown	1		1	
	p= 0.272			
Metastasis status				
M negative	4 (100%)		63 (100%)	
Number of deaths	17		39	
	CBC risk	**Risk of death**	**CBC risk**	**Risk of death**
After 2 years	0%	19%	6%	4%
After 5 years	7%	34%	16%	11%
After 10 years	7%	55%	34%	21%
	p=0.06	**P<0.001**		
	HR	**p-value**	**HR**	
Univariate (95%CI)	0.40 (0.14-1.09)	0.07	Reference group	
Multivariate(95%CI)	0.52 (0.18-1.53) ^	0.24	Reference group	

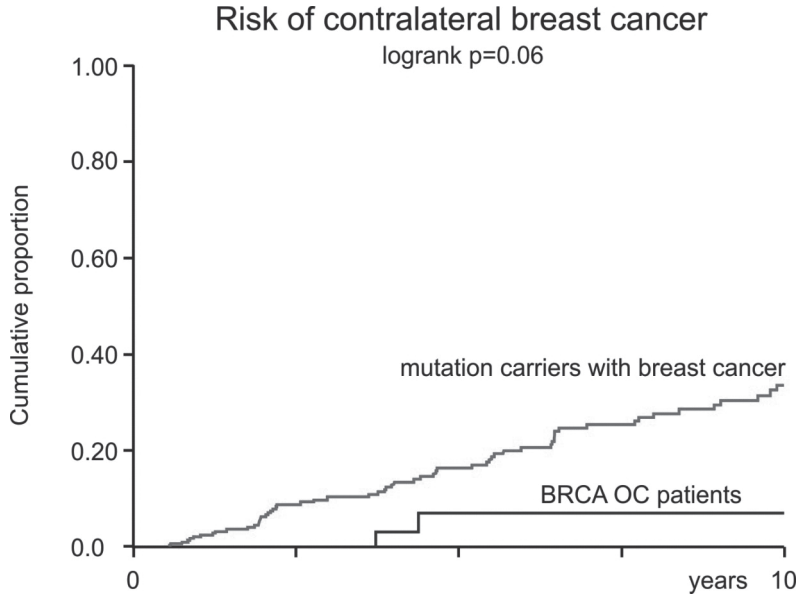
* Adjusted for year at study entry. Age at start study and mutation type has <10% impact on HR
^Adjusted for age start study and time between 1st BC and start study. Year of start study and mutation has <10% impact on HR
HR: hazard ratio; CI: confidence interval; OC: ovarian cancer; BC: breast cancer

Figure I. Primary breast cancer in *BRCA*-associated OC patients and unaffected *BRCA* mutation carriers



Number of women at risk at	*2 yrs*	*5 yr*	*10 yrs*
BRCA-associated OC	*61*	*35*	*13*
Unaffected BRCA	*212*	*107*	*33*

Figure II. Contralateral breast cancer in *BRCA*-associated OC patients and *BRCA* mutation carriers without OC



Number of women at risk at	*2 yrs*	*5 yrs*	*10 yrs*
BRCA-associated OC	*28*	*16*	*7*
BRCA without OC	*208*	*126*	*43*

DISCUSSION

To our knowledge, these are the first estimates on the PBC or CBC risks after *BRCA*-associated OC. Compared with mutation carriers without OC, *BRCA*-associated OC patients had a significantly lower risk of PBC and a non-significantly lower risk of developing a CBC within the first 10 years after OC. Importantly, the mortality rate in *BRCA*-associated OC patients during this time period was substantial, and higher than the risk of developing a subsequent BC.

The observation of a lower BC incidence in *BRCA*-associated OC patients versus controls without OC, persisting in the analyses with death as censoring event, suggests that the primary therapy for OC might influence the occurrence of BC hereafter. The reductive effect of salpingo-oophorectomy on BC risk has repeatedly been shown, probably most pronounced in healthy mutation carriers if performed before 50 years.[13,26] and concerns PBC rather than CBC.[13]It is not clear whether surgery in our OC groups contributed much to the observed lower BC risk, since about 50% of the OC patients was older than 50 years at diagnosis. Further, 97% of the OC patients received first-line chemotherapy, mainly platinum-based. In view of the observation that platinum-based chemotherapy is highly effective in *BRCA*-associated OC patients,[29,31]also reported for *BRCA*-associated BC,[34,35] we hypothesize that chemotherapy given for OC might reduce the risk of a subsequent BC by eradicating occult microscopic disease. In fact, a risk-reductive effect of adjuvant chemotherapy on CBC in *BRCA* mutation carriers has been reported by Reding et al.[36] Since only six OC patients did not receive chemotherapy as part of the primary treatment, it was not possible to separately analyse the risk of developing BC after OC in patients treated with or without chemotherapy.

We are aware that some limitations have to be considered. First, the OC and control patients are significantly different regarding age at and year of study entry, and the majority of the OC patients underwent genetic testing after OC diagnosis. Over time various BC screening schemes have been used, since BC screening for high-risk women at FCCs was introduced around 1994 when genetic testing for *BRCA* became available, while the MRI for high-risk women was introduced from 2000 onwards in the Netherlands.[15,37] Further, population screening for BC (biennial mammography for women 50-75

years) has been introduced in 1990, being highly attended (>80%). Also, follow-up after BC includes a yearly mammography. The mentioned factors potentially may have resulted in a higher BC detection rate in unaffected controls, due to more intensive screening and higher sensitivity of the MRI, as the majority of these women started study follow-up after 2000 (table I). On the other hand, lead time (time between tumor detection and developing symptoms) is very short in *BRCA1/2* mutation carriers due to the high tumor growth rate.[38] Furthermore, >40% of the controls underwent a RRSO <50 years, lowering their BC risk.

Also, increasing the age at study entry in the control groups from 35 to 40 or 45 years, respectively, resulted in more comparable median ages at study entry of the OC and control groups, while differences in BC risks became somewhat smaller, but significance did not disappear (data available on request). Furthermore, age at study entry was not significantly correlated with BC risk, and in the multivariate analyses conclusions did not alter after adjustment for age at and year of study entry. Comparable studies in further nationwide and international studies including larger patient cohorts are warranted enabling to match OC patients with controls for age at and period of diagnosis.

Secondly, our study groups were too small to distinguish between both *BRCA1* and *BRCA2* mutation carriers and OC patients with low versus advanced disease. Since most included OC patients were diagnosed with advanced stage disease, it should be examined further whether our results are also applicable for OC FIGO stage I or II patients.

Finally, in all groups the majority of the patients underwent genetic testing after developing BC and/or OC, and indexpatients were not excluded from the analyses implying that BC risks in the cancer groups might be overestimated. However, if BC risks in the OC groups are overestimated due to ascertainment bias, the unbiased BC risk will even be lower. Repeating the analyses starting at the date of first DNA diagnosis in the family, the PBC risk in the OC group remained non-significantly lower than in the control group (HR 0.40; 95% CI 0.16-1.37).

Despite the mentioned limitations, our data showing that the BC risk after advanced stage *BRCA*-associated OC is lower than for a mutation carrier without OC, while the risk of OC-related death within the first years remains substantial, provide helpful data for involved clinicians allowing a more tailored counselling of the *BRCA*-associated OC patient. First, the BC risk

in the first decade after *BRCA*-associated OC can be communicated more precisely. Second, the BC risks in view of the mortality rates after advanced stage OC indicate that the option of RRM might not be justified for this patient group, also because RRM may be associated with negative effects regarding body image, potential surgical complications, and relevant costs.[40-42] Further research should be stimulated trying to obtain more data concerning BC risks after low-stage *BRCA*-associated OC and for OC patients with a disease-free interval of more than five years, since different risks and strategies may possibly apply for these patients.

Further, our data underscore that the counselling of a *BRCA*-associated OC patient is complex, since counselling should not only address the subsequent BC risk, but also the consideration of this risk against the OC prognosis. Therefore, we would like to propose that a multidisciplinary team, including a clinical geneticist, and a gynaecological, medical and surgical oncologist should be involved in decision-making and counselling, which is now only available at family cancer clinics.

Additionally, the findings suggesting a lower BC risk over the first years after *BRCA*-associated OC raise the question concerning the optimal BC surveillance program for mutation carriers affected with OC. Currently, in most developed countries, the BC surveillance for *BRCA* mutation carriers, irrespective of a previous cancer, consists of yearly mammography and MRI between 30-60 years, being expensive, time-consuming and leading to additional exams due to the relative low specificity of MRI, while cost-effectiveness has only been shown for healthy mutation carriers.[43] Potentially, an alternative regimen of annual imaging deserves consideration for the *BRCA*-associated OC subgroup.

In conclusion, compared to *BRCA* mutation carriers without OC, *BRCA*-associated OC patients had a lower PBC or CBC risk, while the mortality rate within the first years after OC was higher than the risk of developing a subsequent BC. These findings provide additional data enabling more patient-tailored counselling for this patient subgroup, and indicate that risk-reducing mastectomy within the first years after advanced stage OC should not be considered. We propose to refer *BRCA*-associated OC patients for optimal decision making and counselling to a FCC with an adequately equipped multidisciplinary team. Given the heterogeneity of the study cohorts, it is warranted to confirm our data in future studies with larger sample sizes.

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5

RECURRENT *BRCA*-ASSOCIATED EPITHELIAL OVARIAN CANCER

5.1

Recurrent epithelial ovarian cancer in *BRCA1*-associated, *BRCA2*-associated and sporadic patients: disease presentation, treatment response and survival

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Submitted

*Both authors contributed equally to this work.

ABSTRACT

Background

BRCA1/2-associated epithelial ovarian cancer (EOC) has a more favourable outcome, versus sporadic EOC, but it is insufficiently known whether this also accounts for the relapse setting. Therefore, we examined characteristics, type(s) of treatment, response to (chemo-)therapy and outcome of recurrent EOC in *BRCA1*-associated, *BRCA2*-associated and sporadic patients.

Methods

130 *BRCA1*-associated, 44 *BRCA2*-associated, and 138 sporadic patients with recurrent EOC were included. Data of *BRCA1/2* and sporadic patients were retrieved from a nationwide and a local dataset respectively. Analyses were performed using a Pearson's χ^2 test, Kaplan-Meier and Cox-regression methods.

Results

First recurrent EOC mainly presented as multifocal and distant disease. Most of the *BRCA1, BRCA2* and sporadic patients were treated with chemotherapy (92,3%, 84,1% and 89,9% respectively), while surgery was part of the treatment in 16,9%, 20,4% and 22,5% respectively. Objective response (=complete+partial response) to chemotherapy was higher in *BRCA1*(76.9%) and *BRCA2* (84.8%), versus in sporadic patients (43.8%) (both p<0.001). Median progression-free survival (PFS) after first recurrence was significantly longer in both *BRCA1* (12.6 months) and *BRCA2* (13.0 months) versus in sporadic patients (7.5 months). Median overall survival (OS) was longer in *BRCA1* and *BRCA2* than in sporadic patients (33.2, 29.0 and 16.3 months respectively). The objective response to chemotherapy for second recurrence remained high in *BRCA1/2* patients (64.0%, 65.0%, and 46.9%, respectively, p=0,01 and 0.05), while PFS and OS were not different between the groups.

Conclusion:

Both *BRCA1*- and *BRCA2*-associated EOC patients have a better outcome from first recurrence, versus sporadic patients, mainly due to an increased chemosensitivity..

INTRODUCTION

Epithelial ovarian cancer (EOC) is the leading cause of death from gynaecological malignancies in the Western world with a 5 years overall survival (OS) of only 37%.[1] More than 75% of the women with EOC are diagnosed with advanced stage disease (FIGO stage III or IV)[2] and ultimately, the majority of these patients will suffer from recurrent disease, mainly being an incurable entity.[3-5] The presentation of recurrent disease greatly differs between patients, regarding local versus distant and unifocal versus multifocal disease.[6-10] Standard treatment of recurrent EOC mainly consists of chemotherapy, and in selected cases (low tumor burden, localized disease, long treatment-free interval (TFI)) also of surgery (cytoreduction). The clinical benefit of surgery for recurrent disease is insufficiently known, since data from randomised studies are lacking, although it has been suggested that surgery is associated with an improved OS.[11-13]

To date, it is known that a genetic susceptibility due to a germline DNA mutation in the *BRCA1, BRCA2, TP53*, *MLH1, PMS2, MSH2* and *MSH6* genes confers an increased lifetime risk of developing EOC. Mutations in the *BRCA1* or *BRCA2* gene are estimated to account for 8-16% of the EOC cases and contribute to the majority of the hereditary EOC cases.[14-16] In early studies it has been observed that *BRCA1/2*-associated EOC patients have a longer OS and an increased progression-free survival (PFS) after primary therapy versus sporadic patients.[5,17-25] Additionally, more recent studies reported a longer PFS and an improved OS for *BRCA2*- versus *BRCA1*-associated EOC patients.[26-28] It is thought that the improved outcome of *BRCA1/2*-associated EOC is related to an increased chemosensitivity, resulting from a deficient DNA repair mechanism of double-strand DNA breaks by means of homologous recombination.[5,19-21]

Data concerning the characteristics and outcome of recurrent EOC in *BRCA1* and *BRCA2* mutation carriers are very scarce. Two small studies (n=82 and n=19) reported that *BRCA1/2*-associated, compared to sporadic EOC seems to metastasize more frequently to the viscera.[16,29] The issue of chemosensitivity, regarding recurrent *BRCA*-associated EOC, has only been addressed in two small studies (n=17, n=82), both suggesting that the more favourable response to (platinum-based) chemotherapy was maintained in the relapse setting.[16,17] To our knowledge, however, there are no data

regarding chemosensitivity of recurrent EOC in *BRCA1* and *BRCA2* mutation carriers separately.
In the current analyses, therefore, we evaluated the characteristics, type(s) and outcome of treatment, with emphasis on chemosensitivity, of recurrent EOC in sufficiently large groups, enabling to study *BRCA1*-associated, *BRCA2*-associated and sporadic patients separately.

PATIENTS AND METHODS

Patient selection

BRCA1/2-associated patients with recurrent EOC were selected from a nationwide dataset[26], and sporadic patients with recurrent disease from a local dataset[18], both available from previous studies.
For the nationwide study, *BRCA1* and *BRCA2* patients, identified with a deleterious *BRCA1* or *BRCA2* mutation or being a first-degree family member of a proven *BRCA* mutation carrier, were recruited from databases of the Erasmus University MC-Cancer Institute, Radboud University MC and the Netherlands Cancer Institute-Antoni van Leeuwenhoek hospital. Aiming to expand the *BRCA2* group, *BRCA2* patients were also selected from databases of the Leiden University Medical Center, Amsterdam Medical Center, VU University Medical Center, University Medical Center Utrecht, Maastricht University Medical Center and the Netherlands Foundation for the Detection of Hereditary Tumors. Sporadic EOC patients were selected from a local database of the Erasmus University MC Cancer Institute available from a previous study (see reference for details)[18], whereby sporadic patients were matched (ratio 1:2), with individual *BRCA1*- and *BRCA2*-associated cases from the Rotterdam Family Cancer Clinic database for age at (±5 years) and period of primary diagnosis (±5 years).[18] Overall inclusion criteria involved primary EOC (defined according to the FIGO guidelines 2009[30]), diagnosed between January 1st 1980 and January 1st 2011, complete data about patient, tumor and treatment characteristics and available follow-up data and without a history of another malignancy besides breast cancer (BC). Patients with a borderline ovarian tumor or suspicion of primary or recurrent BC at time of primary EOC diagnosis were excluded. Additionally, sporadic EOC patients with a strong family history of BC and/or EOC,

defined as two relatives (first or second degree) with BC, one relative (first or second degree) with BC diagnosed before the age of 55 years and/or one relative (first or second degree) with EOC, irrespective of age, were excluded to minimize the chance of being a *BRCA1/2* mutation carrier.
Recurrent disease activity was detected either by diagnostic imaging, CA125 analysis, gynaecologic examination and/or surgery. First recurrent EOC was defined as recurrent disease activity after first line therapy, excluding patients with progressive disease (PD) during first line chemotherapy. Second recurrence was defined as recurrent/progressive disease activity after first recurrence, excluding patients experiencing PD during chemotherapy for first recurrence.
From the original datasets with 195 *BRCA1*-associated, 89 *BRCA2*-asscociated and 234 sporadic EOC patients, ultimately 130(66.7%) *BRCA1*-associated, 44 (49.4%) *BRCA2*-associated and 138 (59.0%) sporadic patients were identified with recurrent EOC according to the eligibility criteria (see figure 1). The study was approved by the Institutional Review Board of the Erasmus MC, Rotterdam.

Data collection and definitions

Data were retrospectively extracted from the databases, and additionally from medical records of the University Medical Centres, and of community hospitals if relevant additional information was needed. Collected data of primary disease concerned patient- and tumor characteristics, types and outcome of treatment, dates of detection and start of therapy for recurrent disease, and date and cause of death. Gathered data for first (= second line therapy) and second recurrence (= third line therapy) concerned types of disease and treatment, response after treatment (including response to chemotherapy), date of progressive disease/subsequent recurrence and date of start of subsequent treatment. Follow-up information was collected until March 20th 2011.

With regard to first recurrence, disease activity in the pelvis was defined as local recurrence, and outside the pelvis as distant disease. Response to treatment, including chemotherapy, was assessed by means of the WHO-criteria, since 59.3% of our patients were treated before the introduction of the RECIST criteria in 2000[31]. Treatment response was classified in one

of the following categories: no evidence of disease (NED) or complete response (CR), partial response (PR), stable disease (SD) or PD. We defined progression free survival (PFS) as the time between diagnosis of a first or second recurrence respectively, and the date of progressive disease or subsequent recurrence. Treatment-free interval (TFI) was defined as the time between end of respective treatment and start of the following line of treatment. OS after recurrence was defined as the time between the date of first or second recurrence, respectively, until date of death. Ovarian Cancer Specific Survival (OCSS) after recurrence was defined as the time period between date of first or second recurrence, respectively, until the date of death due to EOC.

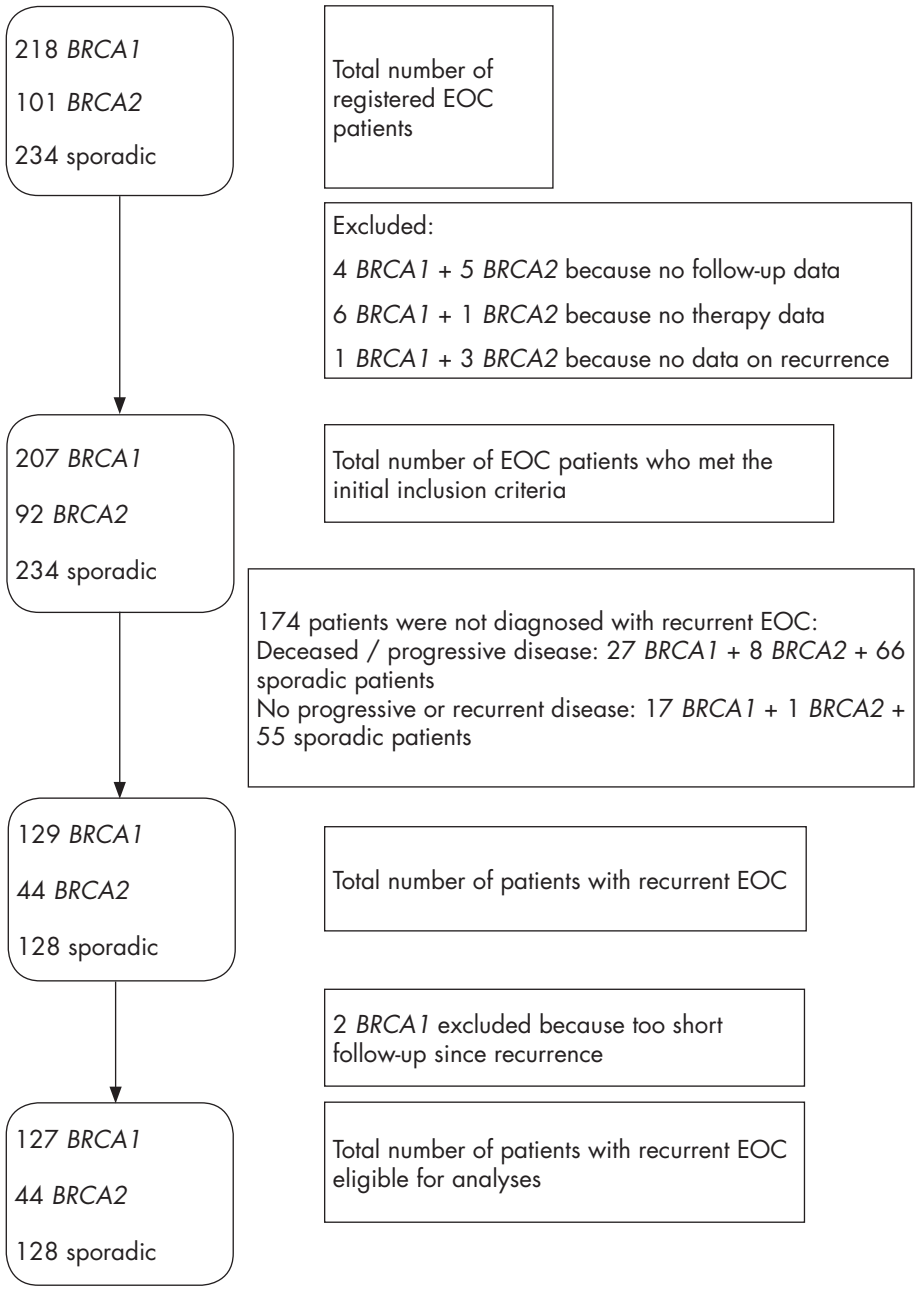
Statistical analysis

The SPSS statistical package version 17.0.2 was used for all statistical analyses.

Differences in patient, tumor and treatment characteristics at primary diagnosis and at first recurrence between *BRCA1*-associated, *BRCA2*-associated and sporadic EOC patients, respectively, were analyzed with the chi-square test or the Fisher's exact test for categorical variables and the Student's t-test or Mann-Whitney U-test for continuous variables. Response to chemotherapy for first and second recurrence was evaluated for all chemotherapy regimens and for platinum-based chemotherapy separately. Differences in response to chemotherapy between *BRCA1*-associated, *BRCA2*-associated and sporadic EOC patients were tested with the Pearson's chi-square test with linear-by-linear association, considering three response categories: objective response (=CR+PR), SD and PD. In addition, odds ratios for objective response were calculated for *BRCA1*- and *BRCA2*-associated versus sporadic patients, and for *BRCA1*- versus *BRCA2*-associated patients in a multivariate logistic regression model, adjusting for possible confounders, including age at primary diagnosis, year of diagnosis of primary EOC, FIGO stage, histology, tumor grade, type of chemotherapy given for primary and recurrent disease, localisation and type of recurrence. PFS, OS and OCSS after first and second recurrence, respectively, were calculated, using the Kaplan-Meier survival method and differences between the three groups were tested with a logrank test. Censoring events were end date of the study (March 20th 2011) or loss of follow-up. In the analysis for OCSS and PFS, patients were also censored

by date of death due to another cause than EOC. Differences in PFS and OS after first recurrence between the three patient groups were also analyzed in a univariate and multivariate Cox proportional hazard model to estimate hazard ratios (HR) and to adjust for possible confounders (see confounders included in the regression model for objective response). The proportional hazard assumption was assessed with an extended Cox model.
All p-values were two tailed. Differences associated with $p<0.05$ were considered statistically significant.

Figure 1. Flowchart selection of patients



RESULTS

Characteristics of recurrent EOC patients:

Table 1 depicts the characteristics of the 312 included recurrent EOC patients. The median age at primary diagnosis was significantly higher for *BRCA2*, versus *BRCA1* and sporadic EOC patients (58.4, 51.3 and 52.7 years, p=0.001 and p=0.01, respectively). The comparable age of sporadic and *BRCA1* patients is mainly the result of the matching procedure performed in our previous study (see methods section). EOC was diagnosed in more recent years in *BRCA1* and *BRCA2* versus sporadic patients (both p<0.001). Consequently, more *BRCA1*- and *BRCA2*-associated patients received platinum/paclitaxel chemotherapy for primary disease (p<0.001 and p=0.001, respectively). However, the percentage of patients receiving platinum-based chemotherapy was not significantly different between the three groups. *BRCA1* and *BRCA2*-associated patients more often had high grade tumors, versus sporadic patients (p=0.01 and p=0.03, respectively). The great majority of patients had NED after primary treatment, not significantly different between the groups.

First recurrence of EOC presented after a longer TFI for both *BRCA1*- and *BRCA2*-associated versus sporadic EOC patients (p=0.001 and p=0.01, respectively) (Table 1). Comparable with primary disease, age at recurrent disease was significantly higher for *BRCA2* patients. Recurrent EOC most frequently presented as distant, and multifocal disease, which was not different between the three groups.

Table 1. Characteristics of epithelial ovarian cancer (EOC) patients with recurrent disease, at primary and recurrent disease presentation respectively

	BRCA1		*BRCA2*		Sporadic		P-values		
	N	%	N	%	N	%	*BRCA1* vs *BRCA2*	*BRCA1* vs sporadic	*BRCA2* vs sporadic
Primary disease									
Total number of patients	130		44		138				
Median age (yrs) at diagnosis (range)	51.3 (32.6-72.8)		58.4 (31.3-78.3)		52.7 (33.9-74.0)		0.001	0.24	0.01
Year of diagnosis									
1980-1989	11	8.5	2	4.5	24	17.4	0.36	<0.001	<0.001
1990-1999	53	40.7	17	38.7	78	56.5			
>2000	66	50.8	25	56.8	36	26.1			
FIGO stage									
I/II	13	10.2	3	7.0	22	16.3	0.95	0.31	0.44
III	84	66.1	31	72.1	83	61.5			
IV	30	23.7	9	20.9	30	22.2			
Unknown	3	-	1	-	3	-			
Tumor grade									
3 (poorly differentiated)	75	70.1	27	71.1	65	50.8	0.72	0.01	0.03
Unknown	23	-	6	-	10				
Tumor size after definitive surgery (*)									
<1 cm	86	75.4	31	83.8	88	68.2	0.29	0.21	0.06
>1 cm	28	24.6	6	16.2	41	31.8			
Unknown	16	-	5	-	9	-			
Type of chemotherapy									
Platinum/paclitaxel	88	69.8	34	77.3	60	45.5	0.49	<0.001	0.001
Platinum/other	31	24.6	7	15.9	61	46.2			
Non-platinum-based	7	5.6	3	6.8	11	8.3			
Unknown	2	-	0	-	1	-			
None	2	-	0	-	5	-			

Table I: Continued

	BRCA1		*BRCA2*		Sporadic		P-values		
	N	%	N	%	N	%	*BRCA1* vs *BRCA2*	*BRCA1* vs sporadic	*BRCA2* vs sporadic
NED at end of primary therapy									
Yes	109	87.2	31	83.8	117	84.8	0.59	0.57	0.88
Unknown	5	-	7	-	0	-			
First recurrence									
Median age (yrs) at recurrence (range)	53.8 (33.8-76.7)		59.8 (32.3-79.8)		54.3 (36.0-74.9)		0.001	0.63	0.002
TFI from end of primary therapy (months) – median (range)	15.1 (1.9-174.2)		17.3 (0.5-85.6)		11.9 (1.1-83.6)		0.84	0.001	0.01
Follow-up after first recurrence (months) – median (range)	24.4 (0.2-210.7)		26.8 (0.3-113.3)		15.4 (1.1-154.0)		1.0	0.02	0.06
Site of first recurrence									
Local	38	30.6	13	32.5	33	25.6	0.83	0.37	0.39
Distant	86	69.4	27	67.5	96	74.4			
Unknown	6	-	4	-	9	-			
Type of first recurrence									
Unifocal	26	21.1	8	20.5	33	25.6	0.93	0.41	0.52
Multifocal	97	78.9	31	79.5	96	74.4			
Unknown	7	-	5	-	9	-			

NED = no evidence of disease, TFI= treatment-free interval
* two BRCA-2 associated patients did not have surgery for unknown reasons

First recurrence

As shown in table 2 no significant differences were observed in the type(s) of treatment administered for first recurrence. The majority of patients were treated with chemotherapy only (72.8%), while chemotherapy was not administered in 7.7%, 15.8% and 10.1% of the *BRCA1*, *BRCA2* and sporadic patients respectively. Surgery (alone or in combination with another therapy type) was performed in 16.9% of *BRCA1,* 20.4% of *BRCA2* and 22.5% of sporadic EOC patients respectively. *BRCA1*- and *BRCA2*-associated patients more often received platinum/paclitaxel chemotherapy, reflecting different time periods of treatment, but the use of platinum-based chemotherapy did not differ between the three groups.

Both *BRCA1*- and *BRCA2*-associated EOC patients had a significantly higher objective response rate (CR+PR) to chemotherapy, versus sporadic patients (both $p<0.001$) (Table 2). After adjusting for possible confounders (see methods) the objective response rate also remained significantly higher for *BRCA1* and *BRCA2*, versus sporadic patients (odds ratio 3.86 and 10.54, respectively, Table 2). If we restricted the analyses to platinum-based chemotherapy, the objective response rate was 86% or higher for both *BRCA1* and *BRCA2* patients, being significantly higher than for sporadic patients (odds ratio 4.70 and 4.83 respectively). *BRCA2* patients more often obtained a CR, and experienced less often PD during chemotherapy than *BRCA1* patients, but differences were not statistically significant.

At the end of treatment (irrespective of type) NED was more often observed in *BRCA2* than in *BRCA1*, as well as in sporadic patients ($p=0.01$ and $p<0.001$, respectively) (Table 2). NED was also more often reached in *BRCA1* compared to sporadic patients ($p=0.04$). Residual tumor size after surgery, if performed in this setting, was not significantly different between the three groups.

The median PFS after first recurrence was significantly longer in *BRCA1* (12.6 months; $p=0.01$) and *BRCA2* (13.0 months; $p=0.03$), versus in sporadic patients (7.7 months) (Table 2, Figure 2a). Also, the median OS was significantly longer in both *BRCA1* and *BRCA2* than in sporadic patients, being 33.2, 29.0 and 16.3 months, respectively ($p<0.001$, and $p=0.01$, Table 2, Figure 3a). Findings for OCSS were comparable with those of OS (data not shown).

Table 2. Type and outcome of therapy administered for first recurrence in *BRCA1*-associated, *BRCA2*-associated and sporadic EOC patients respectively

	BRCA1		*BRCA2*		Sporadic		P-values		
	N	%	N	%	N	%	*BRCA1* vs *BRCA2*	*BRCA1* vs sporadic	*BRCA2* vs sporadic
Type of treatment									
Chemotherapy alone	100	76.9	30	68.2	97	70.3	0.56	0.46	0.43
Chemotherapy + surgery	20	15.4	7	15.9	27	19.6			
Surgery alone	2	1.5	2	4.5	5	2.9			
Radiotherapy	4	3.1	3	6.8	2	2.1			
No treatment	4	3.1	2	4.5	7	5.1			
Unknown	0	-	0	-	0	-			
Type of chemotherapy									
Platinum/paclitaxel	64	55.2	20	62.5	40	33.6	0.70	0.003	0.01
Platinum/other	38	32.7	8	25.0	53	44.5			
Non-platinum based	14	12.1	4	12.5	26	21.9			
Unknown type of chemotherapy	4	-	5	-	5	-			
None	10	-	7	-	14	-			
NED after treatment (all types)									
Yes	45	39.1	22	62.9	36	27.1	0.01	0.04	<0.001
Unknown	15	-	9	-	5	-			
Tumor size after surgery									
No surgery	108	-	35	-	106	-	1.0	0.22	0.41
< 1 cm	13	68.4	5	71.4	13	50.0			
>1 cm	6	31.6	2	28.6	13	50.0			
Unknown	3	-	2	-	6	-			
Response to chemotherapy									
All chemotherapy regimens (#)									
CR	42	38.9	20	60.6	33	27.3	0.16	<0.001	<0.001
PR	41	38.0	8	24.2	20	16.5			
SD	4	3.7	2	6.1	9	7.4			
PD	21	19.4	3	9.1	59	48.8			

Table II: Continued

	BRCA1		***BRCA2***		**Sporadic**		**P-values**		
	N	**%**	**N**	**%**	**N**	**%**	***BRCA1* vs *BRCA2***	***BRCA1* vs sporadic**	***BRCA2* vs sporadic**
Unknown	12	-	4	-	3	-			
	BRCA1 vs *BRCA2*		*BRCA1* vs sporadic		*BRCA2* vs sporadic				
Odds ratio## (95% CI)	0.33* (0.08-1.30)		3.86* (2.02-7.38)		10.54* (3.00-37.01)		0.33	<0.001	<0.001
Platinum-based regimens (#)									
CR	41	44.1	17	70.8	30	33.3	0.71	<0.001	0.002
PR	39	41.9	4	16.7	19	21.1			
SD	4	4.3	2	8.3	8	8.9			
PD	9	9.7	1	4.2	33	36.7			
Unknown	9	-	4	-	3	-			
	BRCA1 vs *BRCA2*		*BRCA1* vs sporadic		*BRCA2* vs sporadic				
Odds ratio## (95% CI)	0.84* (0.20-3.56)		4.70* (2.27-9.75)		4.83* (1.28-18.29)		0.81	<0.001	0.02
PFS all patients (**)									
Median in months (95% CI)	12.6 (11.8-13.3)		13.0 (11.2-14.8)		7.5 (6.2-8.8)		0.71	0.007	0.03
12 months	67	55.6	27	70.8	47	34.4			
24 months	16	14.3	5	13.1	14	10.8			
HR (95% CI) ***	0.71 (0.55-0.91)		0.66 (0.46-0.95)		1			0.006	0.03
OS all patients									
Median in months (95% CI)	33.2 (23.3-43.1)		29.0 (12.2-45.8)		16.3 (12.2-45.8)		0.96	<0.001	0.01
12 months	96	80.1	32	81.3	79	58.2			
24 months	67	60.3	25	66.1	51	38.6			
HR (95% CI) ***	0.62 (0.47-0.81)		0.62 (0.42-0.90)		1			<0.001	0.01

#p = two-sided p-values comparing the distribution OR (=CR + PR), SD and PD between *BRCA1* vs *BRCA2*, *BRCA1* vs sporadic and *BRCA2* vs sporadic EOC patients, respectively.
##Odds ratio was calculated for OR (CR and PR)
*Adjusted for year of diagnosis, location of recurrence and type of treatment given for recurrent disease. Other possible confounders did not change the HR with > 10%.
**1 *BRCA1* and 1 *BRCA2* patient with unknown date of progression were excluded for this analysis

*** Univariate analysis; none of the possible confounders change the HR with >10% (see patients and methods section for the possible confounders included)

Second recurrence

Of the in total 130 *BRCA1* patients with a first recurrence, 21.5% deceased or had PD during therapy, 69.2% had a second recurrence according to our definition and 9.3% had no progression or recurrence yet at the end of study follow-up. In the *BRCA2* group (N=44) this distribution was 9.1%, 77.3% and 13.6%, and in the sporadic group (N=128) 49.3%, 44.9% and 5.8% respectively (data not shown in tables).

As shown in table 3, no significant differences were observed in the type(s) of treatment administered for second recurrence. The great majority of patients (75-85.5%) received chemotherapy, while surgery was performed in 21.5% of the *BRCA1*, 14.7 % of the *BRCA2* and 6.5 % of the sporadic patients respectively.

Objective response rates to chemotherapy were higher in *BRCA1* and *BRCA2* patients, versus sporadic patients (64.0%, 65.0% and 46.9%, respectively, table 3), reaching significance for *BRCA1* versus sporadic patients after correction for year of diagnosis and type of treatment (other variables did not change the hazard model with more than 10%, see methods section). In the group of patients treated with platinum-based chemotherapy, *BRCA1* patients showed a significantly higher response rate versus sporadic patients as well, also after correction for year of diagnosis and location of recurrence (odds ratio 3.01, p=0.02), whereas a trend for a higher response rate was observed in *BRCA2* versus sporadic patients (odds ratio 4.08, p=0.07).

Despite the higher response rates to chemotherapy for *BRCA1/2*-associated patients, we did not observe significant differences in PFS, OS and OCSS after second recurrence between the three patient groups (Table 3/ Figure 2b and 3b; data OCSS not shown).

Table 3. Type and outcome of treatment for second recurrence of EOC in *BRCA1*, *BRCA2* and sporadic patients, respectively

	BRCA1		*BRCA2*		Sporadic		P-value		
	N	%	N	%	N	%	*BRCA1* vs *BRCA2*	*BRCA1* vs sporadic	*BRCA2* vs sporadic
Number of patients	90		34		62				
Type of treatment									
Chemotherapy only	51	58.0	24	70.6	49	79.0	0.38	0.11	0.71
Chemotherapy + surgery	15	17.0	3	8.8	4	6.5			
Surgery alone	4	4.5	2	5.9	0	0			
Radiotherapy	3	3.4	1	2.9	0	0			
No treatment	15	17.0	4	11.8	9	14.5			
Unknown	2	-	0	-	0	-			
Response to chemotherapy									
All chemotherapy regimens (#)									
CR	10	20.0	6	30.0	6	12.2	0.65	0.16	0.66
PR	22	44.0	7	35.0	17	34.7			
SD	7	14.0	0	0	11	22.4			
PD	11	22.0	7	35.0	15	30.7			
Unknown	16	-	7	-	4	-			
	BRCA1 vs *BRCA2*		*BRCA1* vs *sporadic*		*BRCA2* vs *sporadic*				
Odds ratio## (95% CI)	0.90* (0.24-3.33)		3.24* (1.27-8.26)		3.73* (0.99-14.09)		0.88	0.01	0.05
Platinum-based regimen (#)									
CR	10	25.6	6	40.0	5	11.4	1.0	0.04	0.22
PR	19	48.8	6	40.0	17	38.6			
SD	5	12.8	0	0	11	25.0			
PD	5	12.8	3	20.0	11	25.0			
Unknown	10	-	5	-	1	-			

Table 3: Continued

	BRCA1		*BRCA2*		Sporadic		P-value		
	N	%	N	%	N	%	*BRCA1* vs *BRCA2*	*BRCA1* vs sporadic	*BRCA2* vs sporadic
	BRCA1 vs *BRCA2*		*BRCA1* vs sporad		*BRCA2* vs sporad				
Odds ratio## (95% CI)	0.68* (0.15-3.13)		3.01* (1.16-7.82)		4.08* (0.91-18.30)		0.62	0.02	0.07
PFS all patients**									
Median in months (95% CI)	10.0 (7.6-12.4)		7.0 (5.0-9.1)		9.6 (7.2-11.9)		0.09	0.16	0.54
12 months	27	37.1	6	21.8	19	32.3			
24 months	10	15.1	3	10.9	3	5.1			
OS all patients									
Median in months (95% CI)	19.5 (13.9-25.0)		15.7 (0.0-36.1)		15.1 (10.3-19.8)		0.86	0.11	0.18
12 months	59	71.0	18	61.8	38	62.4			
24 months	31	43.2	12	48.1	22	37.6			

#p = two-sided p-values comparing Objective response (CR+PR), SD and PD between *BRCA1* vs *BRCA2*, *BRCA1* vs sporadic and B#RCA2 vs sporadic EOC patients, respectively.
#Odds ratio was calculated for OR (CR and PR)
*Adjusted for year of diagnosis and type of chemotherapy given for first and second recurrence. Other possible confounders did not change the HR with > 10%.
**11 *BRCA1*, 3 *BRCA2* and 2 sporadic patients with unknown date of progression were excluded for this analysis

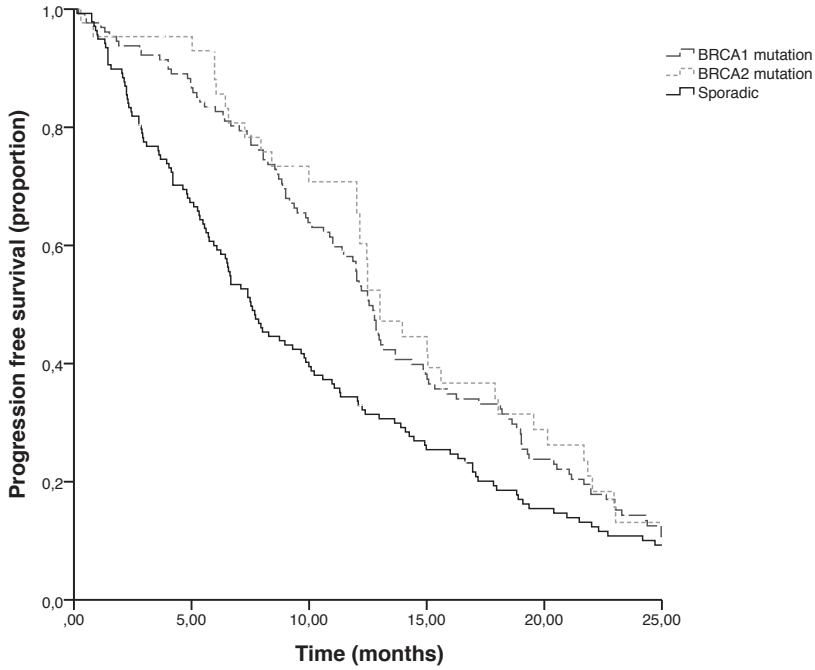


Figure 2A

Number of patients at risk

Time	6 mo	12 mo	24 mo
BRCA1	103	67	16
BRCA2	36	27	5
Sporadic	82	47	14

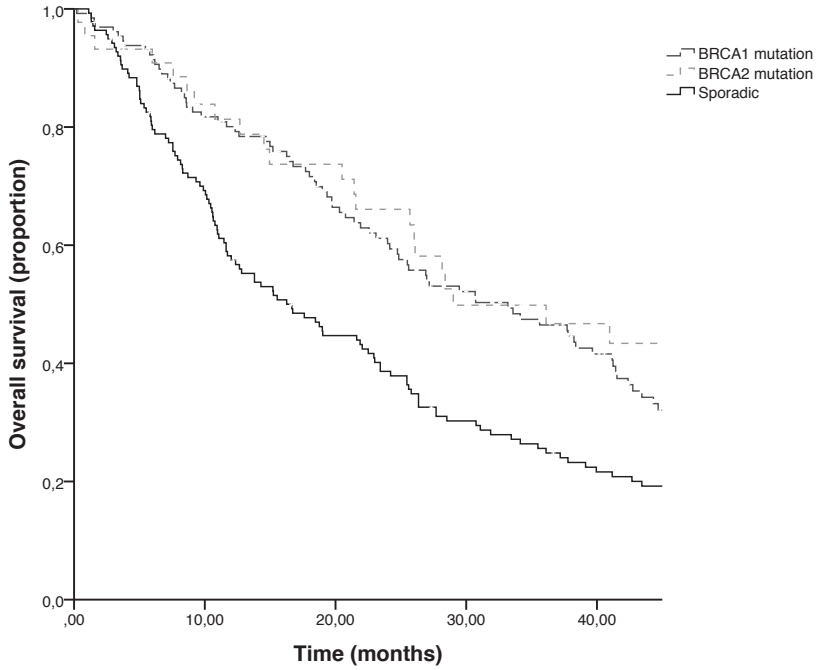


Figure 2B

Number of patients at risk

Time	12 mo	24 mo	48 mo
BRCA1	96	67	27
BRCA2	32	25	11
Sporadic	79	51	23

Figure 2: Progression free survival (figure 2A) and overall survival (figure 2B) after first recurrence in respectively *BRCA1*-associated, *BRCA2*-associated and sporadic EOC patients

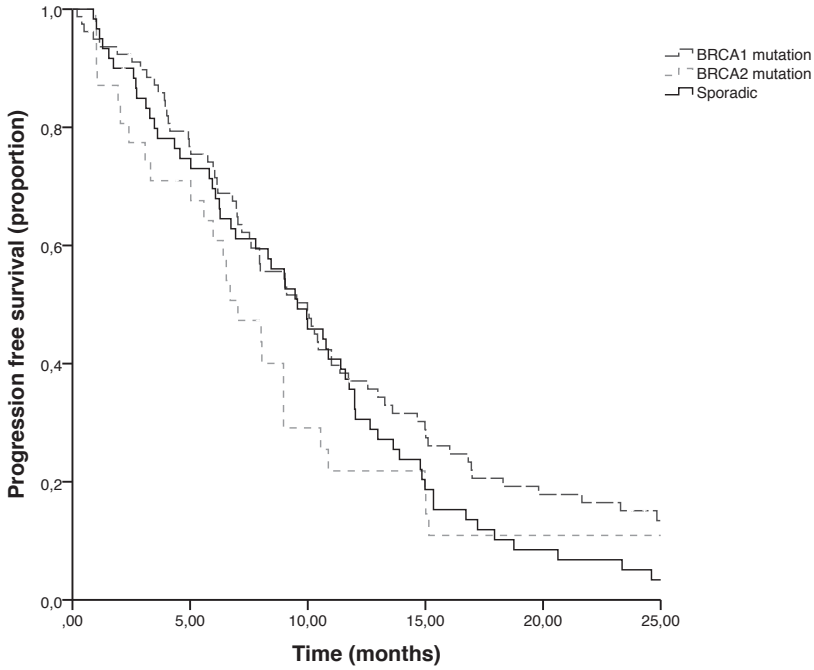


Figure 3A

Number of patients at risk

Time	6 mo	12 mo	24 mo
BRCA1	55	27	10
BRCA2	18	6	3
Sporadic	41	19	3

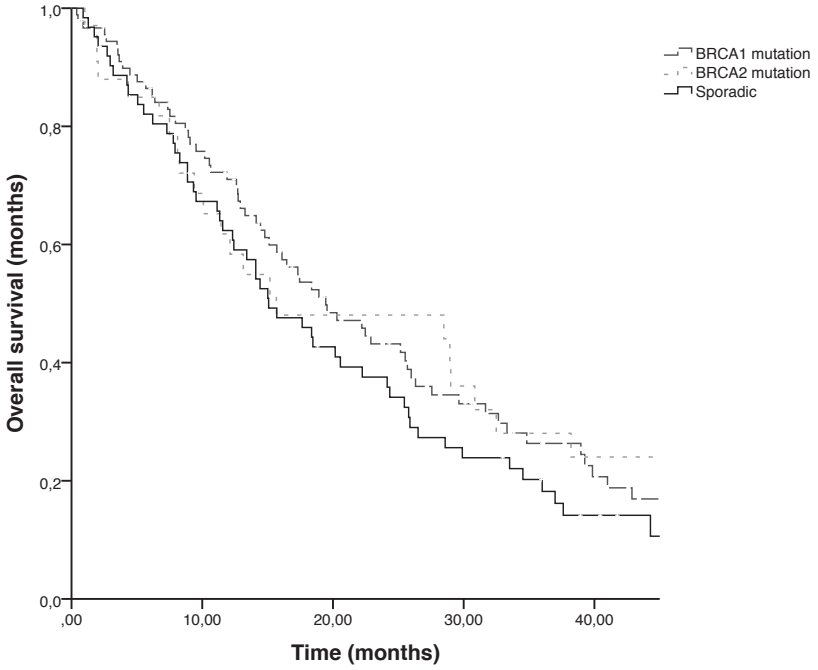


Figure 3B

Number of patients at risk

Time	12 mo	24 mo	48 mo
BRCA1	59	31	8
BRCA2	18	12	5
Sporadic	38	22	3

Figure 3. Progression free survival (figure 3A) and overall survival after second recurrence (figure 3B) in *BRCA1*-associated, *BRCA2*-associated and sporadic EOC patients

DISCUSSION

As far as we know, this is the largest study evaluating in detail the clinical presentation of, type(s) and results of treatment for, and survival after recurrent EOC in *BRCA1- and BRCA2*-associated patients separately, versus sporadic patients. The great majority of recurrent EOC patients was diagnosed with multifocal and distant disease, and treated with chemotherapy, while surgery was performed in approximately 20% of patients at first recurrence, and in 15% of patients at second recurrence. Both *BRCA1*- and *BRCA2*-associated patients showed a significantly better response to chemotherapy, resulting in a longer PFS and OS from first recurrence versus sporadic EOC patients. The objective response rate to (platinum-based) chemotherapy, given for second recurrence, remained higher for both *BRCA*-associated groups, but did not result in a prolonged PFS and OS. Chemosensitivity and survival outcomes were not significantly different between *BRCA1*- and *BRCA2*-associated recurrent EOC patients.

The observed higher response rates to chemotherapy for first recurrent EOC in *BRCA1*- and *BRCA2*-associated, in comparison with sporadic patients, accounted both for all types of chemotherapy and platinum-based chemotherapy. These data are extending on the results of the smaller studies of Alsop et al (n=82 *BRCA1/2*) and Tan et al (n=17 *BRCA1/2*).[16,17] Tan found a higher response rate for all chemotherapy regimens, being highest for platinum-containing chemotherapy in very small groups of *BRCA1/2*-associated (N=12) compared to sporadic EOC patients (N=22), while Alsop also found a better response to both platinum and non-platinum based chemotherapy for first recurrence in *BRCA*-associated patients, even in patients with early relapse after primary treatment. Due to sample sizes, none of these studies discriminated between *BRCA1*- and *BRCA2*-associated patients. Regarding second recurrence, the response rates to chemotherapy in our study remained higher for both *BRCA*-associated groups, being in line with the study of Tan et al, although Tan et al. only included 12 *BRCA1/2* patients with a second recurrence, versus 134 patients in our study.[17]

We observed a significantly longer PFS from first recurrent EOC for both *BRCA* groups versus sporadic patients. Despite the higher response rates to

chemotherapy for second recurrence in the *BRCA* groups, the PFS was not significantly different anymore between the three groups. Possible reasons for the latter are small numbers, and the observation that less sporadic patients were treated for a second recurrence (*BRCA1*: 56.2%, *BRCA2*: 65.9%, and sporadic: 41.4%) suggesting selection of especially chemosensitive patients in the sporadic group. Our data about PFS cannot be compared with any other data, since these are lacking. Tan et al. reported the TFI instead, with a longer TFI from first recurrent disease for *BRCA1/2*-associated compared with sporadic EOC patients, being 11.7 and 4.5 months respectively (p=0.001).[17] The median TFI after first recurrence in our patient groups was 10.2 months for *BRCA1*, 10.0 months for *BRCA2* and 9.2 months for sporadic patients, respectively (not significant). In our opinion, these data reflect that patient groups in both studies are not entirely comparable.

In a subanalysis performed in patients with a TFI of less than six months from primary disease, both *BRCA1* and *BRCA2* patients showed a better response to chemotherapy versus sporadic patients (data not shown). PFS and OS were longer in *BRCA1* and *BRCA2* patients in comparison with sporadic patients as well (8.1, 6.4 and 5.3 months, and 16.5, 21.6, and 10.6 months respectively), reaching significance for *BRCA1* versus sporadic patients. In the patient group with a TFI of more than six months the differences in response rate, PFS and OS between the three patient groups were not significant anymore, possibly since sporadic patients in this setting also are still chemosensitive or due to small numbers. The findings regarding the former cohort are interesting, and in line with results from Alsop et al[16], supporting the suggestion that *BRCA* patients respond better to chemotherapy given for recurrent disease, also in patients with platinum-resistant disease.

The observation of an increased OS from first recurrence in both *BRCA1*- and *BRCA2*-associated in comparison with sporadic patients is in line with the data of the small study from Tan et al, reporting a median OS of 5 versus 1.6 years, and a higher risk of death after first recurrence in sporadic versus *BRCA*-associated EOC patients (HR 4.46 (95% CI 1.54-12.96). The median OS from first recurrence of 5 years for the *BRCA* group in the study of Tan et al., however, is much longer than seen in our *BRCA* groups (33.2

and 29 months respectively), in our opinion suggesting selection of long living patients in the study of Tan et al.

Overall, our results regarding a high response rate to chemotherapy, for both first and second recurrence (at first recurrence also applying for *BRCA* patients with a short TFI (<6 months) after primary disease), and a prolonged PFS and OS from first recurrence indicate that the higher chemosensitivity is maintained for *BRCA1/2*-associated patients with recurrent disease. Improved chemosensitivity leading to a longer PFS and TFI from primary treatment has previously been reported from our and other groups and to our knowledge, in this way, is the main explanation for the improved OS for *BRCA1/2*-associated versus sporadic EOC patients observed in most studies.[14-19,22-25]

PFS and OS from first recurrence were not different between *BRCA1*- and *BRCA2*-associated EOC patients, which is not as expected and not in line with data from recent studies, also from our group, indicating an improved overall outcome after primary disease of *BRCA2*- versus *BRCA1*-associated EOC patients.[26-28] However, the sample size of the *BRCA2* group was not very large, which might possibly explain our results. Therefore, further research on this issue is warranted.

We did not observe significant differences in localisation or type of treatment for recurrent EOC between the three patient groups, whereas two studies described that *BRCA1/2*-associated EOC frequently metastasizes to the viscera, and that sporadic EOC commonly remains confined to the peritoneum.[16,28] In our study, however, we only distinguished between local versus distant and unifocal versus multifocal recurrences, and we have no information about type of affected organs, or visceral versus no visceral disease and therefore were not able to address this item further. In future (prospective) studies, it would be worthwhile to consider a more differentiated registration of localisation of recurrences, since this might influence the decision-making regarding a surgical treatment or not for recurrent disease. Interestingly, surgery was performed in approximately 20% of the patients with first recurrence and 15% of the patients with second recurrence. However, the clinical benefit of surgery in this setting is unknown, especially since data from randomised trials are lacking.[10,11,12,13] Of note, the role of

surgical cytoreduction for recurrent EOC is currently being investigated in a Dutch study, randomizing between surgical cytoreduction followed by chemotherapy, and chemotherapy alone in patients with recurrent platinum-sensitive EOC being accessible for surgical debulking[32], but the patient enrollment is more difficult than expected. Our findings underscore that this question remains relevant.

Strengths of our study are the large sample size of both *BRCA1*- and *BRCA2*-associated EOC patients with recurrent disease, the availability of detailed information regarding type(s) of therapy for and outcomes from primary and second recurrent EOC, and the sufficiently long follow-up period.

Limitations however, should also be considered. Firstly, the three patients groups differed with respect to time period of diagnosis, with significantly more *BRCA1*- and *BRCA2*-associated EOC patients being diagnosed after 1995, although the percentage of patients, receiving platinum-based chemotherapy was not significantly different. When performing analyses in a subgroup of *BRCA1/2* and sporadic patients diagnosed with EOC after January 1st 1995, differences in outcome between *BRCA1*, *BRCA2* and sporadic patients remained (data not shown). Moreover, in the univariate analysis regarding PFS and OS, and the multivariate analysis of response to chemotherapy, correcting for period of diagnosis and location of recurrence, the outcome remained significant as well. Regarding PFS and OS, none of the possible confounders changed the HR with more than 10%. Therefore, differences in year of diagnosis do not appear to significantly influence our results.

Further, we could not exclude the possibility of a survival bias in the *BRCA1*- and *BRCA2*-associated EOC patients, potentially occurring by preferably selecting long-living EOC patients who were referred for genetic testing a long time after the initial EOC diagnosis. Because we expect that potential survival bias is less in *BRCA1* and *BRCA2* patients with a DNA test before first recurrence, we performed analyses regarding PFS and OS in these patient groups (the group with a DNA test before EOC was too small for a separate analyses). We found a comparable median PFS and median OS in *BRCA1* and *BRCA2* patients with a DNA test before recurrent disease, versus the total group of *BRCA1* and *BRCA2* patients (data not shown). Therefore,

we assume that survival bias is not playing an important role, but it cannot entirely be excluded.

Our findings might have several clinical implications. Primarily, the provided additional data about an improved chemosensitivity and survival of both *BRCA*-associated cohorts regarding recurrent EOC facilitates a more accurate information to *BRCA* mutation carriers about the expected chemosensitivity and survival in this setting. Also, our observation that response to chemotherapy and survival in *BRCA* patients with a short TFI after primary disease remain more favorable versus sporadic patients, indicates that platinum-based chemotherapy in this setting may be given again to mutation carriers. Additionally, our observations underscore that it is important to perform genetic testing at EOC diagnosis, since this may have implications for the use of standard chemotherapy, as mentioned above, and regarding the consideration of especially mutation carriers for participation in ongoing studies with novel drugs, for instance PARP (poly ADP-ribose polymerase) or VEGF (vascular endothelial growth factor) inhibitors.[33,34] Further, trials concerning EOC patients should always stratify between sporadic, *BRCA1* and *BRCA2* patients, respectively, since prognosis and, in our opinion, also efficacy of therapy have to be investigated for the different groups separately

In conclusion, we have provided additional data showing that *BRCA1*- and *BRCA2*-associated versus sporadic patients with recurrent EOC have higher response rates to (platinum-based) chemotherapy, resulting in a longer PFS and OS from first recurrence. Also, *BRCA* patients with a short treatment free interval remained sensitive to platinum-based chemotherapy. Chemotherapy sensitivity at second recurrence remained present in *BRCA* patients, but data in larger groups are warranted. Our data indicate that in future trials stratification for *BRCA1* or *BRCA2* mutation status versus sporadic EOC patients is warranted, necessitating genetic testing from the moment of diagnosis. Further, knowledge about the genetic status of EOC patients is important regarding counselling and decision-making with respect to type of therapy in case of recurrent disease, but also regarding the participation of respective patients in studies with new treatment modalities.

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6

GENERAL DISCUSSION

GENERAL DISCUSSION

The knowledge about *BRCA1*- and *BRCA2*-associated epithelial ovarian cancer (EOC) has increased rapidly since the identification of the *BRCA1* and *BRCA2* genes in 1994 and 1995, respectively. Early studies reported that the overall survival (OS) of *BRCA*-associated, compared with sporadic EOC patients was improved. These publications, however, were limited by small numbers and lack of data regarding type of therapy, whereas the majority of the studies were conducted in primarily Ashkenazy Jewish populations, which might not be representative for other more heterogeneous populations.[1-8] Further, it was unknown whether the favorable survival of *BRCA1/2* EOC patients is due to differences in tumor characteristics, disease presentation and/or treatment response. In addition, whereas *BRCA1*- and *BRCA2*-associated breast cancer (BC) features different phenotypes, *BRCA1*- and *BRCA2*-associated EOC have been studied as one group.
The aims of this thesis were to further investigate tumor characteristics, disease presentation, the efficacy of different types of therapy and survival in Dutch *BRCA1*- and *BRCA2*-associated and sporadic EOC patients, regarding both primary and recurrent disease. Furthermore, we focused on potential differences between *BRCA1*- and *BRCA2*-associated EOC. Additionally, the risk of developing a subsequent breast cancer (BC) in *BRCA*-associated EOC patients was another research question, since data hereon for this specific subgroup were not available. The study questions were addressed in cohorts of Dutch patients selected from the Erasmus MC Cancer Institute (both *BRCA*-associated and sporadic EOC patients) and a collaborative effort of all the Dutch university hospitals regarding *BRCA*-associated EOC patients.
In this final chapter, the results of the various studies described in this thesis are discussed and recommendations for future research are suggested.

Survival, tumor characteristics and effects of therapy in *BRCA1*-associated, *BRCA2*-associated and sporadic EOC

In the Rotterdam cohorts, we observed a longer OS in *BRCA1*- and *BRCA2*-associated compared with sporadic EOC patients (Chapter 2.1), which is in line with observations of previous studies.[1-8] We also found that progression-

free survival (PFS) from primary disease was increased in the *BRCA*-associated compared to sporadic EOC patients. Although until now, PFS has only been studied in relatively small sample sizes and heterogeneous patient cohorts, all studies investigating this issue reported a longer PFS for *BRCA*-associated versus sporadic EOC patients.[3,8] In our analyses, we found no evidence that the improved PFS and OS in *BRCA1/2* EOC patients is related to a better operability of *BRCA1*- and *BRCA2*-associated EOC patients (Chapter 3.1) and/or by different tumor characteristics of primary (Chapter 2.1) or recurrent disease (Chapter 5.1).

Importantly, we did observe a better response to especially platinum-based chemotherapy, applying for primary disease (Chapter 2.1) as well as for first recurrent disease (Chapter 5.1) which in our opinion mainly contributes to the improved overall survival of *BRCA*-associated EOC patients.

Data regarding the efficacy of chemotherapy given for recurrent disease were not available at the start of our analyses. Considering first recurrent EOC, we observed that both *BRCA1*- and *BRCA2*-associated EOC patients have a better clinical outcome consisting of a better response to chemotherapy and a longer PFS and OS , compared with sporadic patients. Also, *BRCA1/2* patients with a short treatment free interval (<6 months) remained sensitive to platinum-based chemotherapy Since the great majority of patients in the setting of a first recurrence is treated with chemotherapy, without relevant differences in clinical presentation of and/or types of treatment for recurrent EOC between the groups, the improved outcome is most likely due to an increased chemosensitivity (Chapter 5.1). Our observations hereon are in line with the results of two smaller published studies, also showing a more favourable response to (platinum-based) chemotherapy in the relapse setting.[8,9] Both previous studies, however, included a much smaller number of *BRCA*-associated EOC patients with recurrent disease (N=84 and 17 respectively) than in our analyses (N=171), and did not report detailed data concerning types of therapy for recurrent EOC. Further, in our analyses we also found a high response rate to chemotherapy given for second recurrence, but this did not result in an improved PFS and/or longer OS from second recurrence, possibly due to patient selection and sample size.

Our data regarding a high chemosensitivity in *BRCA*-associated EOC patients, especially concerning platinum-based regimens, provide in vivo data, confirming the results from previous in vitro studies reporting that

BRCA-deficient cells were highly sensitive to cisplatin or carboplatin-based analogues, because of their impaired ability to repair double strand DNA breaks by homologous recombination. Promising new chemotherapeutic agents for *BRCA1/2* EOC patients therefore are DNA-repair pathway inhibitors, in particular the PARP(poly (ADP-ribose) polymerase) inhibitors, which are currently being studied in phase 1/2 and phase 3 trials, expecting the registration of the first PARP inhibitor for *BRCA*-associated EOC patients.[10-12] In the analyses in the context of this thesis we did not investigate the effects of PARP inhibitors in *BRCA1/2*-associated EOC patients, since our follow-up stopped on January the 1th 2011, when PARP inhibitors were not commonly used yet. Because most of the EOC patients will experience recurrent EOC, other new drugs are important. Over the past years the value of vascular endothelial growth factor (VEGF) inhibitors (i.e. bevacizumab) added to platinum-based chemotherapy has been studied in EOC. While the combination regimen (including bevacizumab) has resulted in a modestly increased PFS, OS data are too preliminary to draw clear conclusions.[13] Potentially, bevacizumab may be of greater value in particular subgroups of patients, such as high grade serous cancers, being the most common histology of *BRCA*-associated EOC patients, but this remains to be studied prospectively.

In the current analyses, we also addressed the operability and the impact of optimal resection after primary and/or interval debulking surgery in *BRCA*-associated versus sporadic patients since this is an important independent prognostic factor for OS of EOC patients overall,[14-18] and had not been studied yet for the *BRCA* group separately at the start of our studies. In our analyses we observed that the rate of optimal resection (residual tumor size <1cm versus ≥1cm) after definitive surgery was comparable between *BRCA1, BRCA2* and sporadic EOC patients. Optimal resection after definitive surgery was associated with a more favorable PFS and OS in sporadic patients, a more favorable and non-significantly longer OS in *BRCA1* patients, whereas in *BRCA2* patients optimal resection was not associated with neither PFS nor OS. Reasons for these findings are unclear yet, but a possible explanation could be that *BRCA2* patients have a very high chemosensitivity (100% complete response or partial response) and therefore optimal surgery cannot contribute anymore to an improved survival. Another explanation could

be that the number of *BRCA2*-associated patients was too small to show significant differences. So our results should first be confirmed or refuted in other studies and preferably in prospective trials. Until that time, surgery in *BRCA*-associated EOC patients should be performed as it is being done for sporadic EOC patients aiming at complete debulking. Unfortunately, we have no detailed information about leaving no residual disease (zero mm/complete debulking) after debulking surgery, since this information was not available for patients diagnosed and treated between 1980-2000. It is currently known that complete cytoreduction is associated with a better prognosis than leaving less than 1 cm residual tumor.[14,16,19-21]

In conclusion, the results of our studies suggest that the observed more favourable survival of *BRCA1*- and *BRCA2*-associated EOC patients is probably the result of an improved sensitivity to platinum-based chemotherapy.

BRCA1- and *BRCA2*-associated EOC: different diseases?

At the time that the studies of this thesis started, *BRCA1*- and *BRCA2*-associated EOC were considered as two similar entities, mainly because histology and tumor characteristics in general were similar. In addition, in view of the low incidence of especially *BRCA2*-associated EOC in the Netherlands but also worldwide, it was not easy to evaluate *BRCA1*- and *BRCA2*-associated EOC separately. In our first analyses of the Rotterdam Family Cancer Clinic patients, we observed that *BRCA2*-associated patients (N=13) had somewhat different features than *BRCA1*-associated EOC, like an older age at diagnosis. Through a collaborative effort from all Dutch university hospitals we were able to recruit more *BRCA1*-and *BRCA2*-associated EOC patients (*BRCA1*: N=245, *BRCA2*: N=99) and study differences between *BRCA1*- and *BRCA2*-associated EOC more in detail.
Firstly, *BRCA2* patients were in general older at diagnosis compared to *BRCA1* patients (median 55.5 versus 51.0 years; $P<0.001$; chapter 2.2). These results were also found by other groups.[22-24] Secondly, we described that PFS and OS from primary diagnosis were significantly longer in *BRCA2*- compared with *BRCA1*-associated EOC patients (chapter 2.2). Meanwhile, other research groups have also reported an improved overall survival in

BRCA2- compared with *BRCA1*-associated EOC patients, supporting the validity of our data hereon.[22,24,25] However, our study is the first that reported about a significantly improved PFS after primary EOC treatment in *BRCA2* compared with *BRCA1* patients, being 3.9 years (95%-CI 2.5-5.3) versus 2.2 years (95%-CI 1.9-2.5) respectively (*P*=0.006). So far, it is unclear if this improved PFS and OS of the *BRCA2* group is a result of an increased sensitivity to chemotherapy, since both *BRCA* groups have a high response rate, although on the other hand our data concerning a prolonged TFI (as surrogate measure for chemosensitivity) in the *BRCA2* compared with the *BRCA1* group do suggest this. It would be of interest, to study this further in larger patient groups. In patients with recurrent EOC no significant differences were observed in response and survival between *BRCA1*- and *BRCA2*-associated EOC patients, possibly due to the low numbers and patient selection. Data from other groups hereon are not available yet, but it would be very interesting to study this in larger patient groups.

We were not able to confirm that surgery might play a role in the longer PFS and OS in the *BRCA2*- compared with the *BRCA1*-associated EOC patients, since the optimal debulking rate was comparable in the two groups and optimal resection after definitive surgery was associated with a more favorable and non-significantly longer OS in *BRCA1* patients, whereas in *BRCA2* patients optimal resection was not associated with neither PFS nor OS.

Of note, the risk of developing EOC is lower for *BRCA2* versus *BRCA1* mutation carriers, estimated at 3-19% for *BRCA2* and 18-54% for *BRCA1*, indicating a differential role for the *BRCA2* versus the *BRCA1* gene. Both *BRCA1* and *BRCA2* proteins functionally play key roles in DNA damage repair, but they appear to have distinct functions. *BRCA1* plays a versatile role in tumor suppression through its ability to participate in DNA damage response, checkpoint control, mitotic spindle assembly, centrosome duplication and sister chromatid decantation. *BRCA2* has one main function in double-strand break repair by homologous recombination, namely by regulating the RAD51 protein.[26] Also, it was suggested that *BRCA2* mutations are more instable and that the promotor region is less often hypermethylated compared to *BRCA1*,[22] which might explain the longer duration of response to chemotherapy.

In conclusion, the results of our studies suggest that *BRCA1*- and *BRCA2*-associated EOC are different entities and further research is warranted to confirm our results.

Risk of breast cancer after *BRCA*-associated EOC

At the beginning of our research , no separate data were available about the risk of breast cancer in the specific subgroup of women with a history of *BRCA*-associated ovarian cancer. Therefore, a respective woman was counselled as "unaffected woman" regarding breast cancer risk, whereby the option of prophylactic mastectomy frequently was discussed. In view of the poor prognosis of ovarian cancer and the hypothesized lower risk of breast cancer after treatment for ovarian cancer (by chemotherapy or postmenopausal status), questions arose whether this prophylactic mastectomy is justified after *BRCA*-associated ovarian cancer. We observed that the risk of primary breast cancer after treatment for *BRCA*-associated EOC (chapter 4.1.) , compared with mutation carriers without EOC, was significantly lower risk after 2, 5 and 10 years (3%, 6% and 11% in *BRCA* EOC patients versus 6%, 16% and 28% in unaffected mutation carriers, respectively). In addition, the risk of developing a subsequent contralateral breast cancer was lower in *BRCA*-associated EOC patients with a 2, 5 and 10 years risk of 0%, 7% and 7% versus 6%, 16% and 34% in patients without EOC, respectively. Importantly, the mortality rates in *BRCA*-associated EOC patients were substantial after 2, 5 and 10 years (being 13%, 33% and 61% in *BRCA*-associated EOC patients at risk of primary breast cancer, and 19%, 34% and 55% in *BRCA*-associated EOC patients at risk of contralateral breast cancer), and were much higher than the risk of developing a subsequent BC. Therefore, our study provides important additional knowledge for the subgroup of *BRCA*-associated EOC patients that should be used in counselling and discussion of possible strategies. In view of our data, we propose to discuss optimal breast cancer surveillance as the best option for a *BRCA* mutation carrier with a history of EOC, rather than risk-reducing mastectomy. Whether a similar advice remains the best option for the ovarian cancer patient with low stage disease or a disease fee interval of 5 years or more remains to be studied, since no data hereon are available yet. In view of the complexity of the counselling and decision-making of the *BRCA*-associated EOC patient,

we propose to refer a respective patient to a family cancer clinic with an adequately equipped multidisciplinary team.
Meanwhile, results on this topic have also been published by the group of Domcheck et al. They support our data, indicating that BC surveillance rather than a surgical management in women with *BRCA*-associated EOC should be recommended.[27] However, Domcheck did not use a control group of patients without EOC, as we did, which makes their results less comparable with unaffected mutation carriers.

General conclusions and future research

In this thesis, we observed that:

1. Women with *BRCA1*- and *BRCA2*-associated epithelial ovarian cancer have a longer progression-free survival and overall survival compared with sporadic EOC patients, accounting for primary disease as well as first recurrence. In our opinion, this is due to an increased chemosensitivity of *BRCA1*- and *BRCA2*-associated EOC, rather than a better operability or different tumor characteristics.

2. *BRCA1*-associated and *BRCA2*-associated EOC are two different entities, with a more favourable survival of *BRCA2*-associated EOC patients, possibly due to a better chemosensitivity of the *BRCA2* group (reflected by a longer TFI) . This should be confirmed, however in larger patient groups

3. *BRCA*-associated EOC patients have a lower risk of developing a subsequent breast cancer (both primary or contralateral) and a higher mortality rate, than mutation carriers without EOC. In view of our findings, optimal breast cancer surveillance should be discussed with a respective EOC patient rather than risk-reducing mastectomy.

Although the various studies in *BRCA*-associated EOC patients, performed in the context of this thesis, provided valuable additional information, there are many remaining questions to be answered. For instance, we did not evaluate the breast cancer risk after *BRCA1*-associated and *BRCA2*-associated EOC separately. In view of our own data, indicating that *BRCA1*- and *BRCA2*-

associated EOC are different entities, this should be analysed separately, especially since *BRCA1*- and *BRCA2*-associated breast cancers have different phenotypes. In addition, we recommend future studies, including EOC patients, to stratify for *BRCA1*- and *BRCA2*-associated and sporadic EOC.
In our study, no histopathological differences were observed between *BRCA1, BRCA2* and sporadic EOC patients. However, we did not perform a central pathology review, which is worthwhile to do aiming at further clarification of the histological characteristics of *BRCA1*- versus *BRCA2*-associated EOC in detail.
Additionally, the question regarding the value of surgery in case of recurrent disease remains unresolved and is still of interest. In our analyses, 20% of the patients underwent surgery as part of the therapy for a first recurrence, while this was the case in 15% of the patients being treated for a second recurrence (=third line therapy). However, so far, no prospective trial was able to include enough patients to evaluate the exact value of surgery for recurrent EOC, although we presume this should be possible in a national or international study.
Since the studies, described in this thesis have a retrospective design, future studies should focus on developing a larger (inter)national prospective database, including *BRCA1*- and *BRCA2*-associated and sporadic EOC patients with a long term follow-up of at least ten years, making it possible to study more homogeneous groups and to preclude survival bias. Another interesting research question concerns the impact of location (different functional domains) of the mutation within the *BRCA1* or *BRCA2* gene, the type of mutation (missense/ large deletions) and other types of *BRCA* inactivation (methylation, somatic mutations) regarding tumour characteristics, efficacy of therapy, and survival measures. This will only be possible through international consortia.
Moreover, in view of our data, we underscore that it is important to give information about and initiate genetic testing in women with high grade serous EOC at diagnosis, since this has impact regarding counselling and therapy of EOC. In clinical practice, this concerns counselling about a more favourable outcome, a higher chemosensitivity and longer continuation of platinum-based chemotherapy, information regarding participation in PARP-related studies, and possibly in the future also for other therapies. Additionally, information about the mutation status of a woman with EOC

is important in view of the consequences regarding the subsequent breast cancer risk and optimal surveillance herefore.
In the Netherlands, one step has already been taken with the current concept guideline "hereditary ovarian cancer" of the Dutch Association of Gynecology ("Nederlandse Vereniniging voor Obstetrie en Gynaecologie").[28] This guideline recommends referring all women with epithelial ovarian cancer to a clinical geneticist, irrespective of age and histology at diagnosis. The question is whether this should not be restricted to women with high grade serous EOC, since they have a higher chance of having a mutation in one of the *BRCA* genes, compared with other histological subtypes, but this can be reconsidered in the future.

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7

SUMMARY & SAMENVATTING

Summary

The aims of this thesis were to further investigate tumor characteristics, disease presentation, the efficacy of different types of therapy and survival in Dutch *BRCA1*- and *BRCA2*-associated and sporadic epithelial ovarian cancer (EOC) patients, regarding both primary and recurrent disease. Furthermore, we focussed on potential differences between *BRCA1*- and *BRCA2*-associated EOC. Additionally, the risk of developing a subsequent breast cancer (BC) in *BRCA*-associated EOC patients was another research question, since data hereon for this specific subgroup were lacking.
The study questions were addressed in a retrospective study. *BRCA1/2*-associated patients were primarily selected from the database of the "Family Cancer Clinic" of the Erasmus University MC-Cancer Institute (former Erasmus MC-Daniel den Hoed Cancer Centre), and later expanded into a national cohort with *BRCA*-associated EOC patients recruited from all university hospitals in the Netherlands, the Netherlands Foundation for the Detection of Hereditary Tumors and the Netherlands Cancer Institute-Antoni van Leeuwenhoek hospital. Sporadic patients were retrieved from the cancer registry of the institution or the Comprehensive Cancer Center Rotterdam. All clinical data were collected from existing databases and medical files.

In **Chapter 1** we give a general introduction to the research described in this thesis. Yearly, approximately 1250 new patients are diagnosed with ovarian cancer in the Netherlands. EOC is the leading cause of death from gynaecological malignancies in the Netherlands with about 1000 deaths annually. Due to the non-specific symptoms at the beginning of the disease, the majority (70%) of the patients with ovarian cancer are diagnosed with advanced stage disease (FIGO stage IIb-IV). Standard treatment for patients with high stage ovarian cancer (stage IIb-IV) consists of surgery in combination with chemotherapy (carboplatin/paclitaxel). Five year survival of high stage (stage IIb-IV) epithelial ovarian cancer is only 5-60%.
Approximately 10-20% of all EOC cases are due to a genetic predisposition and mutations in the *BRCA1* and *BRCA2* genes are responsible for more than 90% of the hereditary ovarian cancer cases. Women with a mutation in one of the *BRCA*-genes have a considerably increased risk of breast and/or ovarian cancer. Female *BRCA1* mutation carriers have a 18%-54%

cumulative lifetime risk of developing EOC, whereas for *BRCA2* mutation carriers the estimated cumulative lifetime risk is lower, ranging between 2.4%-19%, becoming relevant from the age of 35-40 and 40-45 years respectively. Since no safe and effective screening method for EOC exists, women in the Netherlands are counselled to decide for a risk reducing salpingo-oophorectomy from the age of 35-40 years for *BRCA1* mutation carriers and from the age of 40-45 years for *BRCA*2 mutation carriers respectively to reduce the risk of ovarian cancer. After this procedure the residual risk of peritoneal cancer is estimated to be 1-2%.
BRCA1- and *BRCA2*-associated EOCs are mostly of serous high-grade histology and often present at a younger age than sporadic EOC. Early studies have described that the overall survival of *BRCA*-associated EOC patients was better compared to sporadic EOC patients. It has been suggested that the longer survival may be caused by a better sensitivity to (platinum-based) chemotherapy, what could be explained by an impaired ability to repair double-stranded DNA breaks via homologous recombination.
Most available data from clinical studies at the start of our research were limited by small numbers of patients, few data about specific tumor characteristics and treatment (concerning both primary as recurrent disease) and no or few data about potential differences between *BRCA1*- and *BRCA2*-associated EOC. Further, no information was available about the impact of optimal surgical debulking for *BRCA1/2*-associated compared with sporadic EOC patients. Moreover, data about the risk of developing a subsequent breast cancer (either primary or contralateral) after *BRCA*-associated EOC was lacking, whereas in some hospitals these women are being counselled concerning BC risks and advised as if they did not have a history of ovarian cancer.

Chapter 2.1. describes tumor characteristics, response to first-line chemotherapy (including surgery/chemotherapy), progression-free survival and overall survival in 99 *BRCA1*-associated and 13 *BRCA2*-associated, in comparison with 222 sporadic EOC patients. The *BRCA1/2*-associated EOC patients were selected from the database of the Rotterdam Family Cancer Clinic of the Erasmus University MC-Cancer Institute and were matched (1:2) with sporadic EOC patients for age at (±5 years) and period of diagnosis (±5 years). The sporadic patients were selected from the cancer registry

of the institution or from the Comprehensive Cancer Center Rotterdam. All patients received, mainly platinum-based, chemotherapy as part of primary treatment. Differentiation grade, FIGO stage and histology were equally distributed in *BRCA1/2* and sporadic patients. Patients with *BRCA1- (*87%) and *BRCA2*-associated (92%) EOC more often had a complete response/ no evidence of disease after first-line treatment, including (platinum-based) chemotherapy, in comparison with patients with sporadic EOC (71%). Median progression-free survival for *BRCA1*-associated patients was 2.1 years (p=0.006), in *BRCA2*-associated patients 5.6 years (p=0.008), compared to 1.3 years in sporadic patients. Median overall survival was as well significantly better in *BRCA1*-associated and *BRCA2*-associated EOC patients, in comparison with sporadic EOC patients (5.9 years (p<0.001), >10 years (p=0.008), and 2.9 years, respectively). *BRCA2*-associated, compared with *BRCA1*-associated EOC patients, showed a trend for a longer progression-free survival and overall survival (p=0.05). Overall, in this study we observed a better outcome after primary therapy, including (platinum-based) chemotherapy, for *BRCA1/2*-associated in comparison with sporadic EOC patients. Surprisingly, we found a trend for a better outcome of *BRCA2*-associated, compared to *BRCA1*-associated EOC patients, potentially not significant due to the small number of *BRCA2* patients.

Aiming to further explore potential differences between *BRCA1*- and *BRCA2*-associated EOC, we examined tumor characteristics and outcome after primary therapy in a large nationwide cohort of *BRCA1*- and *BRCA2*-associated EOC patients **(Chapter 2.2.)**. From all eight Dutch university hospitals, the Netherlands Cancer Institute (NKI) and the Netherlands Foundation for the Detection of Hereditary Tumors (STOET) we selected 245 *BRCA1*-associated and 99 *BRCA2*-associated EOC patients for the study, described in this chapter. In some centers we only collected *BRCA2*-associated cases, aiming to expand the *BRCA2* group especially.
EOC was diagnosed at a younger age in *BRCA1*-associated compared with *BRCA2*-associated patients (median 51.0 versus 55.5 years; *P*<0.001). Tumor characteristics did not significantly differ between the two patient groups. EOC was generally diagnosed at advanced stage (FIGO-stage III/ IV), was mainly poorly differentiated and of serous histology. Approximately 30% of the EOC patients had a history of breast cancer. The majority of the

patients (94%) were treated with a combination of surgery and platinum-based chemotherapy; only 5 *BRCA1*-associated EOC patients received non platinum-based chemotherapy. At the end of primary treatment, including chemotherapy, complete response/no evidence of disease was obtained in 86% of the *BRCA1*- versus 90% of the *BRCA2*-associated patients (P=0.36), while progressive disease during first-line chemotherapy was not observed in any of the *BRCA2* patients, but in five (2%) *BRCA1* patients. Treatment-free interval after first line chemotherapy was significantly longer in *BRCA2*-associated (median: 2.8 years*)* than in *BRCA1*-associated EOC patients (median: 1.7 years; P=0.009). Also, progression-free survival and overall survival were significantly longer in *BRCA2*-associated compared with *BRCA1*-associated EOC patients. Median progression-free survival was 2.2 years in the *BRCA1* group, compared with 3.9 years in the *BRCA2* group (*P*=0.006). Median overall survival in the *BRCA1* and *BRCA2* group was 6.0 years and 9.7 years (*P*=0.04) respectively. In this study we found evidence for a more favourable outcome for *BRCA2*-associated compared to *BRCA1*-associated EOC patients, suggesting that this are different entities.

Optimal debulking is a known prognostic factor for survival in sporadic EOC, but data hereon regarding *BRCA1/2*-associated EOC were lacking. In **Chapter 3.1.** we assessed the optimal debulking rate after primary and definitive (being primary and/or interval debulking) surgery in a large cohort of 158 *BRCA1*, 68 *BRCA2* and 181 sporadic EOC patients with FIGO stage IIb and higher. Further we examined the impact of optimal debulking on survival in the three groups. Residual tumor after surgery was categorized as optimal or incomplete resection (residual tumor <1cm or ≥ 1cm). *BRCA1/2* patients were selected from the national database and sporadic patients from the available Rotterdam dataset used for our first study (Chapter 2.1). Of the selected patients 141 (89%) *BRCA1*-associated, 57 (84%) *BRCA2*-associated and 169 (93%) sporadic EOC patients underwent primary debulking surgery, while 17 *BRCA1* (11%), 11 *BRCA2* (16%) and 12 sporadic patients (7%) were treated with neoadjuvant chemotherapy, followed by interval debulking surgery. Some of the patients who underwent primary debulking subsequently underwent interval debulking surgery (after chemotherapy), which was the case for 52 *BRCA1*-associated, 22 *BRCA2*-associated and 55 sporadic EOC patients. The optimal debulking rate after

primary surgery was higher in *BRCA1* and *BRCA2* than in sporadic patients (46%, 51% and 40%, respectively), but after definitive surgery this rate was not different anymore between the three groups. Optimal versus incomplete resection was associated with a more favorable progression-free survival in sporadic (17 versus 11 months; HR 1.98; 95% CI1.34-2.92) and *BRCA1* patients (25 versus 19 months; HR 2.01; 95% CI1.07-3.75), but remarkably not in *BRCA2* patients (35 versus 92 months; HR 0.36; 95% CI 0.12-1.08). Also, optimal versus incomplete resection was associated with a longer overall survival in sporadic patients (40 versus 23 months; HR 1.88; 95% CI1.26-2.81), and a non-significantly longer overall survival in *BRCA1* patients (69 versus 63 months, HR 1.46; 95%CI 0.68-3.15). In contrast, for *BRCA2* patients optimal debulking was not associated with a longer overall survival (93 versus 145 months, HR 0.53; 95% CI 0.15-1.93). The results of this study indicate that the optimal debulking rate after definitive surgery was not different between the three groups, and suggest that optimal debulking is a less strong prognostic factor for survival in *BRCA*-associated EOC patients and especially for *BRCA2* patients. Whether this is a reflection of an improved chemosensitivity warrants further investigation.

In **Chapter 4.1.** we investigated the incidence of primary and contralateral breast cancer after *BRCA*-associated EOC in 79 mutation carriers without a history of breast cancer (=at risk of primary breast cancer) and 37 *BRCA*-associated BC patients (=at risk of contralateral breast cancer) respectively. Control patients were 351 unaffected mutation carriers (without any cancer (=at risk of primary breast cancer) and 294 *BRCA*-associated breast cancer patients without EOC (=at risk of contralateral breast cancer). The patients were selected from the database of the Family Cancer Clinic of the Erasmus University MC-Cancer Institute, Rotterdam. Excluded were patients who underwent a risk-reducing mastectomy before EOC diagnosis or first visit to the family cancer clinic, patients with another malignancy (besides unilateral breast cancer) before EOC diagnosis or first visit to the family cancer clinic, breast cancer patients with recurrent disease and EOC patients with inadequate data concerning tumor and treatment characteristics, and follow-up data. In the control group risk-reducing salpingo-oophorectomy was not an exclusion criterion, since this more accurately represents the group of female *BRCA* mutation carriers seen at Family Cancer Clinics. We

found that *BRCA*-associated EOC patients had a lower 2-, 5- and 10-year risk of primary breast cancer (3%, 6% and 11%, respectively), compared with unaffected mutation carriers (6%, 16% and 28% respectively, p=0.03), whereas EOC patients had a considerably higher mortality rate at similar time points. In *BRCA*-associated breast cancer patients, the 2-, 5- and 10-year risks of contralateral breast cancer were non-significantly lower in EOC patients than in *BRCA* patients without EOC (0%, 7% and 7% versus 6%, 16% and 34%, respectively, p=0.06). Again, the mortality rate was higher in the EOC patients, due to EOC. In view of the patient numbers, it was not possible to distinguish between *BRCA1* and *BRCA2* patients separately. The results of our study, showing that *BRCA*-associated EOC patients have a lower risk of developing a subsequent primary or contralateral breast cancer than mutation carriers without EOC, and an increased risk of dying due to EOC, implies that mutation carriers with EOC should be counselled individually, both regarding the risk of breast cancer, and the appropriate BC strategy favouring surveillance instead of risk-reducing mastectomy. Further, in view of the complexity of this counselling process the input of several specialists, including oncologists besides the clinical geneticist, and working in a multidisciplinary team is needed to formulate an optimal advice.

Despite that initial response rates to chemotherapy are mostly high, unfortunately the majority of the EOC patients will eventually develop recurrent disease. In **Chapter 5.1.** we report the results concerning characteristics, type(s) and outcome of treatment of patients treated for first and second recurrent EOC. First recurrent EOC was defined as recurrent disease activity after first line therapy, excluding patients with progressive disease during first line chemotherapy. Second recurrence was defined as recurrent/progressive disease activity after first recurrence, excluding patients experiencing progressive disease during chemotherapy for first recurrence. From the databases (national concerning *BRCA1/2* patients, and Rotterdam concerning sporadic patients), 130 *BRCA1*-associated, 44 *BRCA2*-associated, and 138 sporadic patients with a first recurrence of EOC were identified. First recurrent EOC mainly presented as multifocal and distant disease. The great majority of the *BRCA1, BRCA2* and sporadic patients were treated with (mainly platinum-based) chemotherapy for recurrent EOC (92%, 84% and 90% respectively), while surgery was part of the treatment in 17%, 20% and 23% respectively. Objective response (=complete+partial response)

to chemotherapy was higher in *BRCA1* (76.9%) and *BRCA2* (84.8%), versus sporadic patients (43.8%) (both p<0.001). Median progression-free survival after first recurrence was significantly longer in both *BRCA1* (12.6 months) and *BRCA2* (13.0 months) versus sporadic patients (7.5 months). Median overall survival was longer in *BRCA1* and *BRCA2* than in sporadic patients (33.2, 29.0 and 16.3 months respectively). In a subanalysis performed in patients with a treatment-free interval of less than six months from primary disease, both *BRCA1* and *BRCA2* patients showed a better response to chemotherapy versus sporadic patients, while progression-free survival and overall survival were longer in *BRCA1* and *BRCA2* patients than in sporadic patients as well.
Of the in total 130 *BRCA1* patients with a first recurrence (according to our definition), 21.5% deceased or had PD during therapy, 69.2% had a second recurrence and 9.3% had no progression or recurrence yet at the end of study follow-up. In the *BRCA2* group (N=44) this distribution was 9.1%, 77.3% and 13.6%, and in the sporadic group (N=128) 49.3%, 44.9% and 5.8% respectively. The great majority of patients (75-85.5%) received (mainly platinum-based) chemotherapy, while surgery was performed in 22% of the *BRCA1*, 15 % of the *BRCA2* and 7 % of the sporadic patients respectively.

The objective response to chemotherapy for patients treated for a second recurrence remained high in *BRCA1/2* patients (64.0%, 65.0%, and 46.9%, respectively, p=0.01 and 0.05), but did not result in significant differences in progression-free survival and overall survival from second recurrence between the three patient groups. The latter results potentially partially reflect selection of sporadic patients remaining chemosensitive. Overall, we provided additional data on the treatment for recurrent EOC indicating that chemotherapy was the mainstay of therapy, although surgery remained to be performed in selected patients. Importantly, both *BRCA1*- and *BRCA2*-associated EOC patients performed better from first recurrence, versus sporadic patients, mainly due to an increased chemosensitivity.

Finally, **Chapter 6** provides a general discussion on the results, and the strengths and limitations of our findings, as well as recommendations for future research.

SAMENVATTING

Het doel van dit proefschrift was om meer inzicht te krijgen in tumorkarakteristieken, ziekte presentatie, uitkomsten van verschillende soorten therapie (voor zowel primair als recidief ziekte) en overleving bij zowel primair als recidief *BRCA1*-geassocieerd, *BRCA2*-geassocieerd en sporadisch epitheliaal ovariumcarcinoom (EOC). Verder hebben we onderzocht of er verschillen zijn tussen *BRCA1*- en *BRCA2*-geassocieerd EOC. Tevens werd onderzocht of het risico op borstkanker na een *BRCA*-geassocieerd EOC verschilt van dat van een *BRCA* mutatiedraagster zonder EOC omdat hierover geen data bekend waren terwijl het wel van invloed zou kunnen zijn op counseling en advisering. De onderzoeksvragen werden uitgewerkt in retrospectieve analyses. *BRCA*-geassocieerde EOC patiënten werden geïdentificeerd vanuit het databestand van de "Familiepoli" van het Erasmus MC Kankerinstituut (voormalig Erasmus MC-Daniel den Hoed Kankerinstituut). Verder werden vanuit alle academische centra van Nederland, de Stichting opsporing erfelijke tumoren (STOET) en Het Nederlands Kanker Instituut-Antonie van Leeuwenhoek ziekenhuis *BRCA*-geassocieerde EOC patiënten gerekruteerd om met name de groep *BRCA2* patiënten te vergroten. Sporadische patiënten werden via het IKR (Integraal kankercentrum Rotterdam; tegenwoordig IKNL) geïdentificeerd. Gegevens van geselecteerde patiënten werden vanuit bestaande databases en uit de medische dossiers verzameld.

In **Hoofdstuk 1** wordt een algemene introductie over de achtergronden van het onderzoek gegeven. Het EOC is de belangrijkste oorzaak van sterfte als gevolg van gynaecologische maligniteiten. In Nederland worden jaarlijks 1250 vrouwen gediagnosticeerd met ovariumcarcinoom en jaarlijks overlijden 1000 vrouwen hieraan. De meeste patiënten (70%) presenteren zich met een gevorderd (FIGO IIb-IV) stadium omdat de ziekte pas laat klachten geeft. De standaard behandeling van het EOC bestaat meestal uit een combinatie van chirurgie en chemotherapie (taxol/carboplatin). De 5-jaars overleving van patiënten met gevorderd stadium epitheliaal ovariumcarcinoom is slechts 5-60%, afhankelijk van het stadium van de ziekte.

Ongeveer 10-20% van de epitheliale ovariumcarcinomen wordt veroorzaakt door een genetische predispositie, en meest frequent door een mutatie in het *BRCA1* of *BRCA2* gen. Vrouwen met een mutatie in één van de *BRCA* genen hebben een sterk verhoogd risico op mamma- en/of ovariumcarcinoom. Het cumulatieve levenslange risico op ovariumcarcinoom is 18-54% voor *BRCA1* mutatiedraagsters en 2.4-19% voor *BRCA2* mutatiedraagsters, wat relevant wordt vanaf respectievelijk het 35-40ste jaar en 40-45ste. Vrouwen met een *BRCA* mutatie worden in Nederland geadviseerd om (na voltooiing van de kinderwens) preventief hun eierstokken en eileiders te laten verwijderen om het risico op ovariumkanker te verlagen, mede omdat er geen veilige en effectieve screeningsmethode is. Voor *BRCA1* mutatiedraagsters wordt dit aangeraden vanaf het 35-40ste levensjaar en voor *BRCA2* mutatiedraagsters vanaf het 40-45ste levensjaar. Het risico op peritoneaal/extra-ovarieel ovariumcarcinoom na een dergelijke preventieve operatie is ongeveer 1-2%. *BRCA*-geassocieerd EOC wordt meestal gekenmerkt door een slecht gedifferentieerde, sereuze tumor, die zich vaak presenteert op een jongere leeftijd dan niet erfelijk (sporadisch) ovariumcarcinoom.

In eerdere studies werd een langere overleving gevonden voor patiënten met een *BRCA*-geassocieerd ovariumcarcinoom vergeleken met die van sporadische ovariumcarcinoom patiënten. Er werd gesuggereerd dat deze gunstigere overleving het gevolg zou kunnen zijn van een betere respons op chemotherapie, wat weer verklaard zou worden door een inadequaat DNA herstelmechanisme aangezien "homologe recombinatie" bij patiënten met een *BRCA* mutatie insufficiënt functioneert.

De beschikbare literatuur, bij aanvang van ons onderzoek, kende beperkingen o.a. kleine aantallen patiënten, weinig data over specifieke tumorkenmerken en behandeling (zowel betreffende primaire behandeling, als therapie bij recidief ziekte), geen tot weinig gegevens over potentiële verschillen tussen *BRCA1*- en *BRCA2*-geassocieerd EOC, geen data over de rol van optimale chirurgische cytoreductie in verhouding tot de betere overleving van *BRCA*-geassocieerd EOC, en evenmin gegevens over de kenmerken en uitkomsten van recidief *BRCA*-geassocieerd EOC. Tevens waren er geen gegevens beschikbaar over het risico op borstkanker na *BRCA*-geassocieerd EOC, terwijl deze vrouwen in de praktijk soms gecounseld en geadviseerd worden alsof ze geen ovariumcarcinoom hebben gehad.

In **Hoofdstuk 2.1** worden de tumorkenmerken, de respons op eerstelijns behandeling (inclusief chirurgie/chemotherapie), en de progressievrije overleving en totale overleving beschreven van 99 *BRCA1*-geassocieerde en 13 *BRCA2*-geassocieerde in vergelijking met 222 sporadische EOC patiënten. De *BRCA1/2* patiënten werden geselecteerd uit de database van de familiepoli van het Erasmus MC Kankerinstituut en werden gematcht (1:2 ratio) met sporadische EOC patiënten op leeftijd van diagnose (± 5 jaar) en periode van diagnose (± 5 jaar). De sporadische patiënten werden geselecteerd vanuit de kankerregistratie van het IKR (Integraal kankercentrum Rotterdam; tegenwoordig IKNL) Rotterdam. Alle patiënten kregen, met name platinum-bevattende, chemotherapie als onderdeel van primaire therapie. Differentiatiegraad, FIGO stadium en histologie waren vergelijkbaar in de groepen. Patiënten met een *BRCA1/2*-geassocieerd EOC hadden een betere respons op eerstelijns behandeling, inclusief (platinum-bevattende) chemotherapie, in vergelijking met patiënten met een sporadisch EOC. Patiënten met *BRCA1*- en *BRCA2*-geassocieerd EOC hadden vaker een complete respons/no evidence of disease na eerstelijns behandeling, inclusief platinum-bevattende chemotherapie, in vergelijking met sporadische EOC patiënten (respectievelijk 87%, 92% en 71%).
De mediane progressievrije overleving was significant beter voor *BRCA1*- en *BRCA2*- geassocieerde in vergelijking met sporadische EOC patiënten en bedroeg voor *BRCA1* patiënten 2.1 jaar (P=0.006), voor *BRCA2* patiënten 5.6 jaar (p=0.008), en voor sporadische patiënten 1.3 jaar. De mediane totale overleving was ook significant beter voor *BRCA1*- en *BRCA2*-geassocieerde EOC patiënten in vergelijking met sporadische EOC patiënten, namelijk respectievelijk 5.9 jaar (p<0.001), >10 jaar (p=0.008), en 2.9 jaar. Er werd een trend gevonden voor een langere progressievrije overleving en totale overleving voor *BRCA2*- ten opzichte van *BRCA1*-geassocieerde EOC patiënten (p=0.05). Samengevat vonden we in deze studie dat patiënten met een *BRCA1/2*-geassocieerd EOC betere uitkomsten hebben na de primaire therapie, inclusief (platinum-bevattende) chemotherapie, in vergelijking met patiënten met een sporadisch EOC. Tevens vonden we een trend voor een betere uitkomst voor *BRCA2*- versus *BRCA1*-geassocieerd EOC, waarschijnlijk niet significant als gevolg van de kleine groep *BRCA2* patiënten.

Om de eventuele verschillen tussen *BRCA1*- en *BRCA2*-geassocieerd EOC verder te exploreren hebben we in een nationaal cohort onderzocht of er verschillen zijn in tumorkarakteristieken, behandeling en uitkomsten na primaire behandeling tussen *BRCA1*- en *BRCA2*-geassocieerd EOC **(Hoofdstuk 2.2)**. Uit alle universitaire centra in Nederland, het Nederlands Kanker Instituut-Antonie van Leeuwenhoek ziekenhuis en de STOET hebben we 245 *BRCA1*- en 99 *BRCA2*- geassocieerde EOC patiënten geselecteerd voor deze studie. In sommige centra hebben we alleen *BRCA2* patiënten geselecteerd met het doel om met name de *BRCA2* groep te vergroten. *BRCA1*-geassocieerde EOC patiënten waren significant jonger bij diagnose dan *BRCA2*-geassocieerde patiënten (51 versus 55 jaar). Tumorkarakteristieken waren vergelijkbaar in de twee groepen. De meeste tumoren waren slecht gedifferentieerde sereuze tumoren en de meeste patiënten hadden FIGO stadium III/IV. Ongeveer 30% van de EOC patiënten had al eerder borstkanker gehad. Het overgrote merendeel van de EOC patiënten (94%) werd behandeld met chirurgie en chemotherapie, wat grotendeels een platinum-bevattend regime betrof; slechts vijf *BRCA1* patiënten kregen niet platinum-bevattende chemotherapie. In de groep patiënten die zowel chirurgie als chemotherapie kregen, had 86% van de *BRCA1* en 90% van de *BRCA2* patiënten aan het einde van de primaire behandeling een complete respons/no evidence of disease, terwijl progressieve ziekte tijdens eerstelijns chemotherapie niet werd gezien bij de *BRCA2* patiënten maar wel bij vijf (2%) *BRCA1* patiënten (ongeacht chemotherapie regime) (P=0.36). Het behandelvrije interval was significant langer voor patiënten met *BRCA2*-geassocieerd (mediaan: 2.8 jaar) ten opzichte van *BRCA1*-geassocieerd (mediaan: 1.7 jaar. P=0.009) EOC. Ook waren de progressievrije en totale overleving significant langer bij *BRCA2* in vergelijking met *BRCA1* patiënten, namelijk respectievelijk 3.9 jaar en 2.2 jaar (*P*=0.006), en respectievelijk 9.7 jaar en 6 jaar (P=0.04). Uit dit onderzoek kan worden geconcludeerd dat *BRCA2*-geassocieerde EOC patiënten een betere prognose hebben dan *BRCA1*-geassocieerde EOC patiënten, wat suggereert dat dit verschillende entiteiten zijn.

Optimale debulking is een belangrijke prognostische factor voor overleving bij sporadische EOC patiënten, maar er zijn geen data hierover bekend voor *BRCA1/2* patiënten. In **Hoofdstuk 3.1.** hebben we het percentage optimale debulkingen na primaire of definitieve (gedefinieerd als primaire

en/of interval debulking) chirurgie geëvalueerd in een groot cohort van 158 *BRCA1*, 68 *BRCA2* en 181 sporadische EOC patiënten met FIGO stadium IIb of hoger. Verder hebben we onderzocht wat de invloed is van optimale debulking op de overleving voor de drie groepen EOC patiënten. Resttumor na chirurgie werd gescoord als optimale (< 1cm) of incomplete resectie (≥ 1cm). *BRCA1/2* patiënten werden geselecteerd uit de eerder beschreven nationale database en sporadische patiënten uit de beschikbare Rotterdamse dataset, die we eerder voor onze eerste studie gebruikten (Hoofdstuk 2.1). Van de geselecteerde patiënten ondergingen 141 (89%) *BRCA1*-geassocieerde, 57 (84%) *BRCA2*-geassocieerde en 169 (93%) sporadische EOC patiënten een primaire debulking, terwijl 17 *BRCA1* (11%), 11 *BRCA2* (16%) en 12 sporadische patiënten (7%) werden behandeld met neoadjuvante chemotherapie, gevolgd door een interval debulking. Sommige patiënten ondergingen na een primaire debulking en chemotherapie nog een interval debulking, wat het geval was voor 52 *BRCA1*-geassocieerde, 22 *BRCA2*-geassocieerde en 55 sporadische EOC patiënten. Optimale debulking na primaire chirurgie werd vaker gevonden bij *BRCA1* en *BRCA2* patiënten, dan bij sporadische patiënten (respectievelijk 46%, 51% and 40%). Na definitieve chirurgie was het percentage optimale debulkingen niet meer verschillend tussen de drie groepen (respectievelijk 68%, 60% and 60%). Optimale, in vergelijking met incomplete, resectie was geassocieerd met een langere progressievrije overleving bij sporadische (17 versus 11 maanden; HR 1.98; 95% CI1.34-2.92) en bij *BRCA1* patiënten (25 versus 19 maanden; HR 2.01; 95% CI1.07-3.75), maar verrassend genoeg niet bij *BRCA2* patiënten (35 versus 92 maanden; HR 0.36; 95% CI 0.12-1.08). Ook was een optimale (versus incomplete) resectie geassocieerd met een langere totale overleving bij sporadische patiënten (40 versus 23 maanden; HR 1.88; 95% CI1.26-2.81), en een niet significante langere totale overleving bij *BRCA1* patiënten (69 versus 63 maanden, HR 1.46; 95% CI 0.68-3.15). Optimale debulking was niet geassocieerd met een langere totale overleving bij *BRCA2* patiënten (93 versus 145 maanden, HR 0.53; 95% CI 0.15-1.93). Uit deze studie blijkt dat het percentage optimale debulkingen na definitieve chirurgie niet verschillend was tussen de drie onderzoeksgroepen, en suggereert dat een optimale debulking een minder sterke prognostische factor voor een betere overleving is bij *BRCA*-geassocieerd EOC, en met name bij *BRCA2*-geassocieerd EOC patiënten.

Of dit een weerspiegeling is van een verhoogde chemosensitiviteit zal verder onderzoek moeten bevestigen dan wel weerleggen.

In **hoofdstuk 4** wordt de incidentie van een primaire en contralaterale borstkanker beschreven bij respectievelijk *79 BRCA*-geassocieerde EOC patiënten zonder mammacarcinoom in de voorgeschiedenis (= risico op primaire borstkanker) en *37 BRCA*-geassocieerde EOC patiënten met een voorgeschiedenis van unilateraal mammacarcinoom (= risico op contralaterale borstkanker). Controlepatiënten waren *BRCA* mutatiedraagsters zonder EOC en respectievelijk geen (n= 351) of wel (n= 294) een voorgeschiedenis van unilaterale borstkanker. De patiënten werden geselecteerd vanuit de database van de familiepoli van het Erasmus MC-Kankerinstituut. Exclusiecriteria waren: preventieve mastectomie vóór de EOC diagnose of het eerste bezoek aan de familiepoli, andere maligniteit (behoudens unilateraal mammacarcinoom) vóór de EOC diagnose of eerste bezoek aan de familiepoli, mammacarcinoom patiënten met recidief ziekte, en EOC patiënten met inadequate informatie over tumor- en behandelkarakteristieken en follow-up. In de controlegroep was een preventieve adnexextirpatie geen exclusie criterium omdat dit meer representatief is voor de vrouwen die een familiepoli bezoeken.
Patiënten met een *BRCA*-geassocieerd EOC zonder voorgeschiedenis van borstkanker hadden na 2, 5 en 10 jaar een lager risico op een eerste borstkanker (respectievelijk 3%, 6% en 11%) in vergelijking met mutatiedraagsters zonder EOC en zonder borstkanker in de voorgeschiedenis (respectievelijk 6%, 16% en 28%, P=0.03). Verder was de mortaliteit significant hoger bij patiënten met *BRCA*-geassocieerd EOC. *BRCA*-geassocieerde EOC patiënten met een eerdere unilaterale borstkanker hadden een niet significant lager 2, 5 en 10 jaars risico op contralaterale borstkanker dan *BRCA*-geassocieerde borstkanker patiënten zonder EOC (respectievelijk 0%, 7% and 7% versus 6%, 16% and 34%, p=0.06). In dit cohort werd ook een hogere mortaliteit gevonden in de groep EOC patiënten versus in de groep patiënten zonder EOC, meestal ten gevolge van ovariumcarcinoom sterfte. Met het oog op het aantal patiënten was het niet mogelijk om afzonderlijke analyses te doen voor *BRCA1* en *BRCA2* patiënten. Concluderend vonden we dat patiënten met een *BRCA*-geassocieerd EOC een lager risico hebben op borstkanker, geldend voor zowel primaire borstkanker als contralaterale borstkanker, in

vergelijking met *BRCA* mutatiedraagsters zonder EOC. Tevens vonden we dat de 2, 5 en 10 jaars mortaliteit telkens hoger was voor EOC patiënten ten gevolge van sterfte aan ovariumcarcinoom. Deze resultaten geven aan dat de counseling bij mutatiedraagsters met een ovariumcarcinoom in de voorgeschiedenis geïndividualiseerd moet worden betreffende het risico op borstkanker, en dat in de meeste gevallen optimale borstkanker surveillance de voorkeur heeft boven risicoreducerende mastectomie. Een optimaal advies dient geformuleerd te worden in een multidisciplinair overleg waarbij zowel klinisch genetici, internist oncologen, gynaecologen als chirurgen vertegenwoordigd zijn.

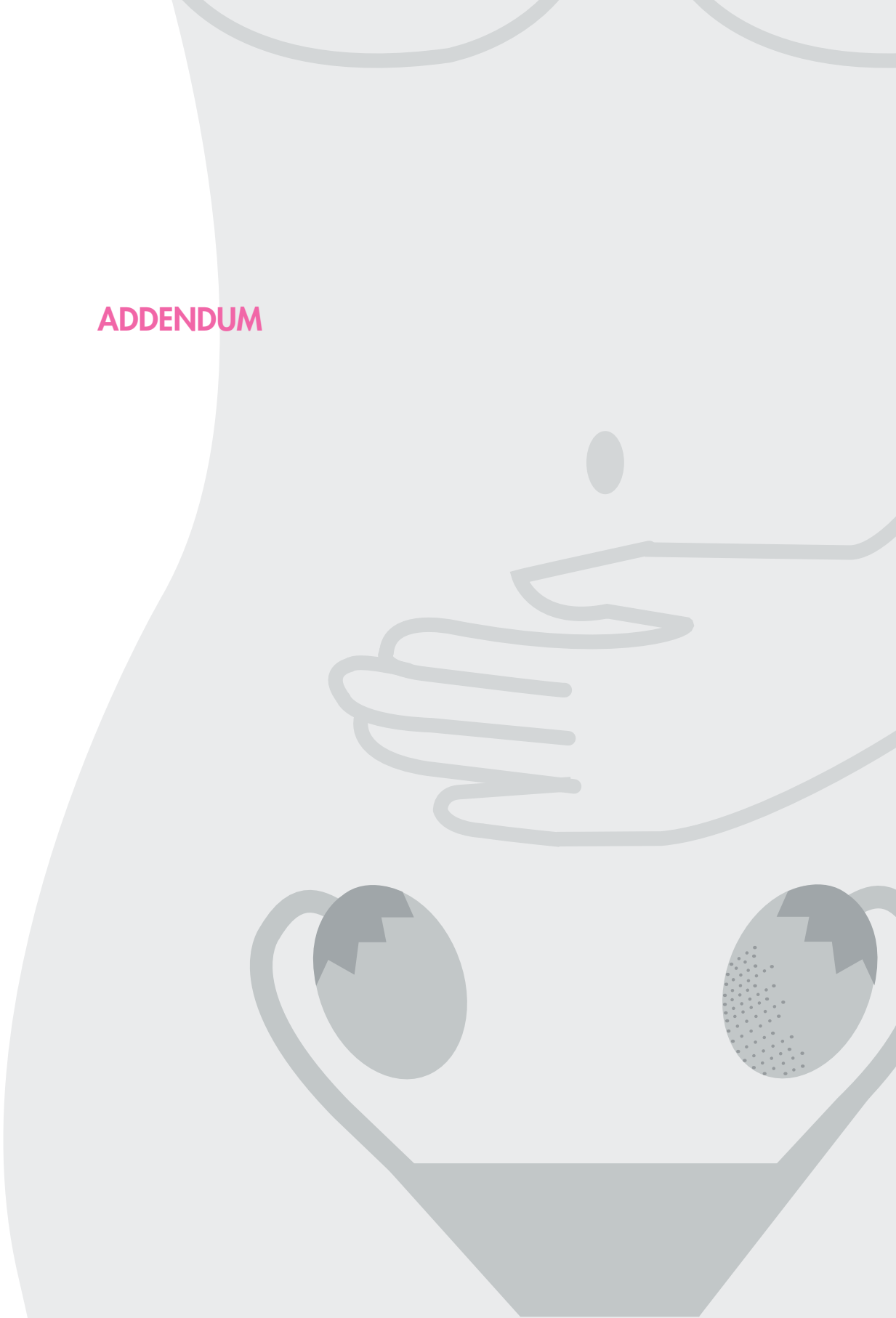
Ondanks dat de initiële respons op chemotherapie vaak hoog is krijgt helaas de meerderheid van de EOC patiënten na kortere of langere tijd een recidief. In **hoofdstuk 5.1.** hebben we de kenmerken van en het type en de uitkomst van de behandeling van patiënten met een recidief EOC bestudeerd. Een eerste recidief EOC werd gedefinieerd als recidief ziekte activiteit na eerstelijns therapie, waarbij patiënten met progressieve ziekte tijdens de eerstelijns behandeling werden geëxcludeerd. Een tweede recidief werd gedefinieerd als recidief ziekte activiteit na een eerste recidief, waarbij patiënten met progressieve ziekte tijdens de behandeling voor het eerste recidief werden geëxcludeerd. Uit de databases hebben we 130 *BRCA1*-geassocieerde, 44 *BRCA2*-geassocieerde, en 138 sporadische patiënten met een eerste recidief EOC geselecteerd.

Een eerste recidief EOC presenteerde zich met name als multifocale ziekte buiten het bekkengebied. De meeste *BRCA1, BRCA2* en sporadische patiënten werden behandeld met voornamelijk platinum-bevattende chemotherapie (respectievelijk 92%, 84% en 90%), terwijl chirurgie onderdeel van de behandeling was bij respectievelijk 17%, 20% en 23% van de patiënten. Een objectieve respons (=complete en partiële respons) op chemotherapie werd vaker geobserveerd bij *BRCA1* (76.9%) en *BRCA2* (84.8%), versus sporadische patiënten (43.8%) (beiden $p<0.001$). De mediane progressievrije overleving na het eerste recidief was significant langer, zowel bij *BRCA1* (12.6 maanden) als bij *BRCA2* patiënten (13.0 maanden) versus sporadische patiënten (7.5 maanden). De mediane totale overleving vanaf het eerste recidief was langer bij *BRCA1* en *BRCA2* in vergelijking met sporadische patiënten (respectievelijk 33.2, 29.0 en 16.3 maanden). In een subanalyse, uitgevoerd in de groep patiënten met een behandelvrij interval

van minder dan zes maanden na einde eerstelijns behandeling, toonden zowel *BRCA1* als *BRCA2* patiënten een betere respons op chemotherapie dan sporadische EOC patiënten. De progressie-vrije overleving en totale overleving waren ook langer bij *BRCA1* en *BRCA2* patiënten in vergelijking met sporadische patiënten.
Van de 130 *BRCA1* patiënten met een eerste recidief had 21.5% van de patiënten hetzij progressieve ziekte of overleed ten gevolge van de ziekte, 69.2% had een tweede recidief en 9.3% had nog geen progressie of recidief aan het einde van de studie follow-up. In de *BRCA2* groep (N=44) was deze verdeling respectievelijk 9.1%, 77.3% en 13.6%, en in de sporadische groep (N=128) respectievelijk 49.3%, 44.9% en 5.8%. De meerderheid van de patiënten met een tweede recidief (75-85.5%) werd behandeld met chemotherapie, bestaande uit voornamelijk platinum-bevattende regimes, terwijl chirurgie nog bij 22% van de *BRCA1*, 15% van de *BRCA2* en 7% van de sporadische patiënten als onderdeel van de behandeling werd uitgevoerd. De objectieve respons op chemotherapie bij een tweede recidief bleef hoog bij *BRCA1/2* patiënten (respectievelijk 64.0%, 65.0%, en 46.9%, p=0.01 en 0.05), maar dit resulteerde niet in significante verschillen betreffende progressie-vrije overleving en totale overleving in de drie patiëntengroepen. Deze bevindingen, en met name de afwezigheid van verschil in chemosensitiviteit tussen *BRCA*-geassocieerde en sporadische patiënten, zou mogelijk het gevolg kunnen zijn van patiënten selectie van met name de sporadische groep. Samengevat hebben we gevonden dat bij het recidief ovariumcarcinoom chemotherapie de voornaamste behandeling was, maar dat chirurgie in geselecteerde gevallen een onderdeel van de behandeling bleef uitmaken, ook voor een tweede recidief. De uitkomsten na een eerste recidief zijn gunstiger voor zowel *BRCA1*- als *BRCA2*-geassocieerde EOC patiënten, versus sporadische patiënten, wat met name wordt veroorzaakt door een hogere chemosensitiviteit.

Tot slot bevat **hoofdstuk 6** de algemene discussie over de resultaten, inclusief de beperkingen en sterktes van de studies, en worden aanbevelingen voor toekomstig onderzoek gedaan.

ADDENDUM



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LIST OF ABBREVIATIONS

BC	Breast cancer
BRCA	BReast CAncer genes
CBC	Contralateral breast cancer
CI	Confidence interval
CLTR	Cumulative lifetime risk
CR	Complete response
DCIS	Ductal carcinoma in situ
DNA	Desoxyribo Nucleic Acid
EOC	Epithelial ovarian cancer
EORCT	The European Organisation for Research and Treatment of Cancer
ER	Estrogen receptor
FCC	Family Cancer Cinic
FIGO	International Federation of Gynecology and Obstetrics
HR	Hazard ratio
MRI	Magnetic Resonance Imaging
NED	No evidence of disease
OC	Ovarian cancer
OCSS	Ovarian cancer specific survival
OR	Objective response
OR	Odss ratio
OS	Overall survival
PARP	poly(ADP-ribose) polymerase
PBC	Primary breast cancer
PD	Progressive disease
PFS	Progression-free survival
PR	Partial response
PR	Progesterone receptor
RECIST	Response Evaluation Criteria In Solid Tumors
RRSO	Risk reducing salpingo-oophorectomy
RRM	Risk reducing mastectomy
SD	Stable disease
SPSS	Statistical Package for the Social Sciences
STIC	Serous tubal intraepithelial carcinoma
TIC	Tubal intraepithelial carcinomas

TFI	Treatment-free interval
TNM	Tumour, Nodes, Metastasis
VEGF	Vascular endothelial growth factor (intro)
WHO	World Health Organisation

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General courses

2011	Cursus medische statistiek in de oncologie, IKNL, Amsterdam	1
2011	Gynaecologische Oncologie, Landelijke WOG-nascholingscursus	1
2010	Evidence Based Medicine, Erasmus MC	1
2010	Biomedical English Writing, Erasmus MC	2

National and international oral presentations:

Dutch oral presentations:

2011	HEBON dag, *BRCA1*- and *BRCA2*-associated Ovarian Cancer: Dutch data, Amsterdam	2
2010	Refereeravond cluster Rotterdam, Betere uitkomsten na primaire behandeling bij *BRCA1* en *BRCA2* mutatiedraagsters met een ovariumcarcinoom in vergelijking met patiënten met een sporadisch ovariumcarcinoom, Erasmus MC, Rotterdam	2
2010	IKR bijeenkomst, *BRCA* ovariumcarcinoom	2

2009	Gynaecongres, Betere uitkomsten na primaire behandeling bij Gynaecongres, Betere uitkomsten na primaire behandeling bij *BRCA1* en *BRCA2* mutatiedraagsters met een ovariumcarcinoom in vergelijking met patiënten met een sporadisch ovariumcarcinoom (oral en poster)	2

English oral presentations:

2012	14th Biennial Meeting of the International Gynecologic Cancer Society (IGCS), Outcome of *BRCA1*- compared with *BRCA2*-associated ovarian cancer: a nationwide study in the Netherlands, Vancouver, Canada	2
2011	Scientific meeting medical oncology, Rotterdam: Risk of primary and contralateral breast cancer after ovarian cancer in *BRCA1/2* mutation carriers; is a risk-reducing mastectomy justified? The Netherlands	2
2011	ESGO, 17th International Meeting Of The European Society Of Gynaecological Oncology, Risk of primary and contralateral breast cancer after ovarian cancer in *BRCA1/2* mutation carriers; is a risk-reducing mastectomy justified? Milan, Italy	2
2010	13th Biennial Meeting of the International Gynecologic Cancer Society (IGCS)) Chemosensitivity and outcome of *BRCA1*- and *BRCA2*-associated ovarian cancer, patients after first line chemotherapy compared with sporadic ovarian cancer patients, Prague, Chech republic (oral and poster)	2

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ABOUT THE AUTHOR

Peggy van Ineveld-Vencken was born on November 14th 1977 in Heerlen, The Netherlands. In 1996 she started medical school at the University of Maastricht where she graduated cum laude. The last three months of medical school she worked at the emergency department of the San José hospital, Monterrey, Mexico. From 2002-2004 she worked as a resident gynecology ("ANIOS") at the Orbis medisch centrum, Sittard. Since she was very dedicated to the specialization gynecology she decided to leave Limburg and work as a resident ("ANIOS") gynecology in the Erasmus Medical Centre, Rotterdam from 2002-2004. She was very delighted when she heard she could start her training in obstetrics and gynaecology in May 2006 in the Sint Franciscus Gasthuis, Rotterdam, and the Erasmus University Medical Centre Rotterdam. In the second year of her specialization (2008) she started with her PhD study at the Erasmus Medical Centre/ Daniel den Hoed Cancer Centre in combination with her full-time specialization. The last year of her specialization (2011) consisted of a differentiation year oncology which took place at the Daniel den Hoed Cancer Centre Rotterdam and the Erasmus University Medical Centre. She finished her training on December 31th 2011. In February 2012 she started to work as a gynecologist in the partnership Bergen op Zoom/ Roosendaal with oncology as her field of interest. On June 1th 2013 she married Siebe van Ineveld, the love of her life. Together they live in Rotterdam, The Netherlands.

DANKWOORD

Met enige huivering verhuisde ik in 2004 van het bourgondische Maastricht naar het "harde" Rotterdam, waar ik in opleiding tot gynaecoloog hoopte te komen.
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