JBUON 2019; 24(4): 1538-1543
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

The clinical features associated with mutated BRCA1 and 2 genes in ovarian cancer patients

Paul Kubelac[1,3], Catalin Vlad[2,3], Ioana Berindan-Neagoe[4,5,6], Alexandru Irimie[2,3], Patriciu Achimas-Cadariu[2,3]

[1]Department of Medical Oncology, The Oncology Institute "Prof. Dr. Ion Chiricuta", Cluj-Napoca, Romania; [2]Department of Surgery, The Oncology Institute "Prof. Dr. Ion Chiricuta", Cluj Napoca, Romania; [3]Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania; [4]Research Centre for Functional Genomics,Biomedicine and Translational Medicine,Iuliu Hatieganu University of Medicine and Pharmacy,Cluj-Napoca,Romania; [5]Research Center for Advanced Medicine Medfuture, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania; [6]Department of Functional Genomics and Experimental Pathology, The Oncology Institute "Prof. Dr. Ion Chiricuta", Cluj-Napoca, Romania

Summary

Purpose: *The treatment of ovarian cancer continues to pose a challenge especially through the development of platinum-resistant disease and still has the highest mortality rate among gynecologic cancers. Patients are usually diagnosed with advanced stages where complete cytoreduction and standard platinum-based chemotherapy are the mainstay of treatment. Advances into the molecular underpinnings of DNA damage repair highlighted BRCA 1/2 and other related genes as key regulators of platinum sensitivity. Our study characterizes clinical features and outcomes associated with BRCA mutations in a cohort of ovarian cancer patients.*

Methods: *Patient data from our Institute was retrospectively extracted for all patients that were tested for the presence of BRCA germline or somatic mutations through next generation sequencing between May 2016 and August 2018.*

Results: *Eighty-eight patients were included in the present analysis. Advanced FIGO stage IIIC-IV was common at presentation (71.6%), with 58% of patients undergoing primary debulking surgery. BRCA mutant cases represented 44% and were associated with a significantly higher frequency for complete pathologic response, first and second platinum sensitive relapse and a significantly longer overall survival for advanced stage cases treated with neoadjuvant chemotherapy. Mean age at presentation was significantly lower in the BRCA mutated (52 years) compared to BRCA wildtype patients (54.2 years, p=0.045).*

Conclusions: *Upfront knowledge of BRCA status is encouraged, given the recent advent of targeted therapies and for the decision regarding optimal sequence of available therapeutic strategies.*

Key words: *BRCA 1, BRCA 2, ovarian cancer, neoadjuvant chemotherapy, platinum sensitivity, primary debulking surgery, survival*

Introduction

Ovarian cancer remains one of the major threats for women worldwide with more than 300,000 new cases and 185,000 deaths predicted for 2020, with Romania being among the top 25 countries with the highest rates of ovarian cancer [1]. Epidemiologic studies have linked ovarian cancer risk with age, nulliparity, however the most prominent risk factor is the presence of a BRCA mutation with a lifetime risk of 44% for BRCA1 and about 17% for BRCA2 mutation carriers (BRCAm) [2]. Due to nonspecific symptoms and the lack of any efficient screening programs the majority of

Corresponding author: Paul Kubelac, MD. 34-36 Republicii street, 400015 Cluj-Napoca, Romania.
Tel: +40 746 183 376, Email: kubelac.paul@umfcluj.ro.
Received: 12/12/2018; Accepted: 07/01/2019

This work by JBUON is licensed under a Creative Commons Attribution 4.0 International License.

cases occur with advanced-stage disease where the primary treatment consists in primary debulking surgery (PDS) followed by adjuvant platinum-based chemotherapy to no macroscopic residual disease [3]. However, due to bulky unresectable disease or poor performance status, neoadjuvant chemotherapy (NACT) with the aim of downsizing tumor burden followed by interval debulking surgery (IDS) is regarded as a feasible option which demonstrated similar survival outcomes [4]. Owing to the surgical drawbacks of the current trials on IDS, another trial is under way with results still to be reported [5].

Another issue of NACT is the potentially higher risk of inducing platinum resistance in patients with a large tumor burden [6], as it has also been demonstrated in a subgroup analysis of the EORTC trial. Patients with tumors under 5 cm treated with NACT had a worse survival, probably due to the subsequent development of platinum-resistant disease through the expansion of pretreatment platinum resistant subclones [7,8]. Platinum compounds are DNA damaging agents that exert their effect through nucleophilic reactions inducing the formation of DNA-protein complexes and DNA inter- and intra-strand adducts that block the DNA replication and finally lead to cell death if left unrepaired [9]. Among the DNA damage repair machinery of cells, BRCA 1/2 genes play an important role into the repair of double strand breaks through the homologous recombination (HR) pathway that is frequently deployed into the restoration of platinum-induced damage [10]. Approximatively 20% of ovarian cancer cases harbor a germline or somatic BRCA mutation, rendering ovarian cancer tumor cells homologous recombination deficient (HRD) and more sensitive to platinum agents and PARP inhibitors [11,12]. Hence, in the present study we set out to characterize the clinical features of platinum sensitivity that are associated with BRCA 1/2 mutations in ovarian cancer in both primary debulking and interval debulking surgery.

Methods

Eighty-eight patients from our Institute tested for the presence of BRCA 1/2 mutations between May 2016 and August 2018 were retrospectively included if they met the selection criteria. Patients had histologically confirmed ovarian cancer, irrespective of tumor stage. Patients were included if they had either optimal (residual tumor <10 mm) PDS or IDS, followed by adjuvant platinum-based chemotherapy. Patients without adequate follow-up or missing clinical information were excluded from the present analysis. The present study received favorable approval from the Institutional Ethics Committee. All patients signed the informed consent and all their data were processed anonymously.

For the detection of BRCA 1/2 germline or somatic mutations DNA was extracted using a commercially available BRCA kit for the BRCA1 and BRCA2 genes within a private accredited laboratory. Sequencing was carried out using Illumina technology. Reads were aligned to the reference sequence (hg19), and sequence changes were identified and interpreted in the context of a single clinically relevant transcript. All clinically significant observations were confirmed by Sanger. Unless otherwise indicated, all targeted regions were sequenced with ≥60x depth. The presence of large genomic rearrangements (LRGs) was investigated using a commercial computational algorithm (SeqPilot, JSI Medical Systems) and the presence of LGRs was verified using the MLPA method (Multiplex Ligation-dependent Probe Amplification, BRCA1: P002, BRCA2: P045, MRC Holland; AJHG 67:841-50, 2000).

Statistics

Clinical data was retrieved from patient files and from the Institutional electronic database. Statistical tests were performed using SPSS Statistics v22.0. Chi-square test was used to detect significant associations between selected clinical variables. Independent samples t-test for equality of means were used in conjunction with Levene's Test for equality of variances where appropriate. Tumor progression was defined based on

Table 1. Clinical characteristics of the study group

Characteristics	*n*	%
Histology		
Serous	84	95.5
Other	4	4.5
Histological grade		
Low grade	8	9.1
High grade	80	90.9
BRCA 1/2 mutations	39	44.3
BRCA 1 mutations	26	29.5
Germline mutations	36	40.9
2014 FIGO stage		
I	8	9.1
II	6	6.8
III	67	76.1
IV	7	8
Type of surgery		
Primary debulking surgery	51	58
Interval debulking surgery	37	42
Outcome of surgery		
Complete debulking, 0 mm	78	88.6
Optimal debulking, <10 mm	10	11.4
Secondary debulking surgery	14	15.9
1st relapse	67	76.1
2nd relapse	41	46.6
Death	17	19.3
Total	88	100

RECIST imaging criteria [13]. Platinum-sensitive relapse was defined as tumor relapse that occurred more than six months after completion of the last platinum-based chemotherapy cycle. Overall survival was calculated irrespective of death cause. Median follow-up was calculated using inversed Kaplan-Meier method. Two-sided p value was used in determining significant results with a 0.05 threshold.

Results

Eighty-eight patients were tested for the presence of BRCA 1 and 2 mutations. More than 90% of cases were high grade serous ovarian cancer, and the majority of patients (n=63, 71.6%) were diagnosed with advanced FIGO stage IIIC-IV. Thirty-nine 39 (44.3%) patients had a germline or somatic pathogenic BRCA1/2 mutation. PDS was offered to 51 (58%) patients and was followed by a median of 6 cycles of platinum-based chemotherapy. The remaining 37 (42%) patients had a median of 6 cycles of platinum-based NACT, underwent IDS and were offered a mean number of 3 adjuvant cycles of the same regimen. The outcome of surgery was complete debulking to no macroscopic residual in over 88% of the cases.

A first relapse occurred in 76.1% of cases. Depending on the platinum-free interval, a subsequent line of platinum-based therapy was chosen and secondary debulking surgery was offered to a minority of 14 cases. Forty-one patients had a second relapse and death occurred in 17 cases, given the short follow-up period. Full patients' characteristics are presented in Table 1.

Out of the total number of 37 patients who underwent NACT, 11 (29.7%) patients achieved complete pathological response (CPR) when evaluated during IDS. Following a platinum-sensitive relapse, 17 patients were eligible and received a PARPi for a median of 8 months (range 2-20) with 12 patients

Table 2. Clinical features associated with BRCA status

Features	*BRCA mutated*		*BRCA wildtype*		*OR (95% CI)*	*p*
	n	*%*	*n*	*%*		
NACT with IDS	17	45.9	20	54.1	1.12 (0.47-2.62)	0.793
Complete pathological response after NACT	8	72.7	3	27.3	5.03 (1.06-23.8)	0.033
FIGO IIIC-IV	29	46	34	54	1.27 (0.49-3.27)	0.607
Platinum sensitive 1st relapse	34	50.7	33	49.3	3.29 (1.08-10.0)	0.03
Lymph nodes 1st relapse	17	60.7	11	39.3	2.66 (1.06-6.71)	0.034
Complete or partial response to 1st relapse	25	54.3	21	45.7	3.86 (1.09-13.6)	0.029
Platinum sensitive 2nd relapse	19	59.4	13	40.6	3.06 (1.09-8.61)	0.031
Change from platinum sensitive to platinum resistant relapse	7	29.2	17	70.8	0.21 (0.06-0.69)	0.008

Table 3. Survival outcome according to complete pathological response after NACT

Survival outcome		*Complete pathological response*			*Without complete pathological response*			*p*
Population	*Survival interval*	*N*	*Mean*	*95% CI*	*N*	*Mean*	*95% CI*	
Patients with NACT	PFS1	11	28.6	(22.2-35.0)	26	19.0	(15.0-22.8)	0.021
	PFS2	6	14.4	(9.62-19.2)	18	8.7	(6.48-10.9)	0.054
	OS	11	65.0	(47.1-82.7)	26	60.4	(45.2-75.5)	0.455

Table 4. Survival outcome according to BRCA status

Survival outcome		*BRCAm*			*BRCAwt*			*p*
Population	*Survival interval*	*N*	*Mean*	*95% CI*	*N*	*Mean*	*95% CI*	
All cases	PFS1	39	48.7	(24.1-73.2)	49	27.7	(16.6-38.8)	0.197
	PFS2	29	16.1	(12.4-19.6)	34	11.7	(9.77-13.5)	0.018
	OS	39	178.5	(130.-226.)	49	116.8	(75.8-157.)	0.606
FIGO IIIC-IV	PFS1	17	23.0	(18.2-27.8)	18	17.4	(14.4-20.3)	0.049
patients with NACT	PFS2	12	11.5	(7.91-15.1)	11	7.7	(6.02-9.38)	0.019
	OS	17	67.1	(53.9-80.1)	18	37.9	(28.1-47.5)	0.036

still on treatment (database lock November 2018). The median follow-up was 41 months.

The mean age at diagnosis was 54.2±8.9 years, however when taking into account the BRCA status there was a significant difference (mean difference 3.8±1.8, p=0.045) between BRCA mutated cases (mean 52±8.9) and BRCA wildtype (BRCAwt) cases (mean 55.9±8.6). There was no significant difference with regards to the choice of IDS versus PDS (OR 1.12, p=0.793) or advanced FIGO stage (IIIC-IV versus I-IIIB) at presentation (OR 1.27, p=0.607) that could be attributed to the presence of BRCA mutation.

The presence of BRCA mutation was, however, associated with a series of clinical features that denote platinum sensitivity, presented in Table 2. There was a higher frequency of CPR after NACT (OR 5.03, p=0.033), a higher frequency of a first or second platinum-sensitive (occurrence of relapse after a minimum of 6 months since last administration of platinum-based chemotherapy) relapse (OR 3.29, p=0.003 and OR 3.06, p=0.031, respectively) and a higher frequency of complete or partial responses of first relapse (OR 3.86, p=0.029) for cases harboring a BRCA mutation in comparison with BRCA wildtype cases. There was also a higher frequency of lymph node involvement during the first relapse in the BRCA mutated population (OR 2.66, p=0.034). Looking at the dynamics of platinum sensitivity from the first to the second relapse, we observed that BRCA mutated cases had a lower frequency of changing their clinical phenotype from platinum-sensitive to platinum-resistant relapse (OR 0.21, p=0.008) in comparison with cases without BRCA mutations.

Patients that achieved CPR after NACT had a significantly higher progression-free survival 1 (PFS1) in comparison with patients without CPR (28.6 versus 19 months, p=0.021), however there was no significant difference in progression free survival 2 (PFS2) or overall survival (OS) between the two populations, presented in Table 3.

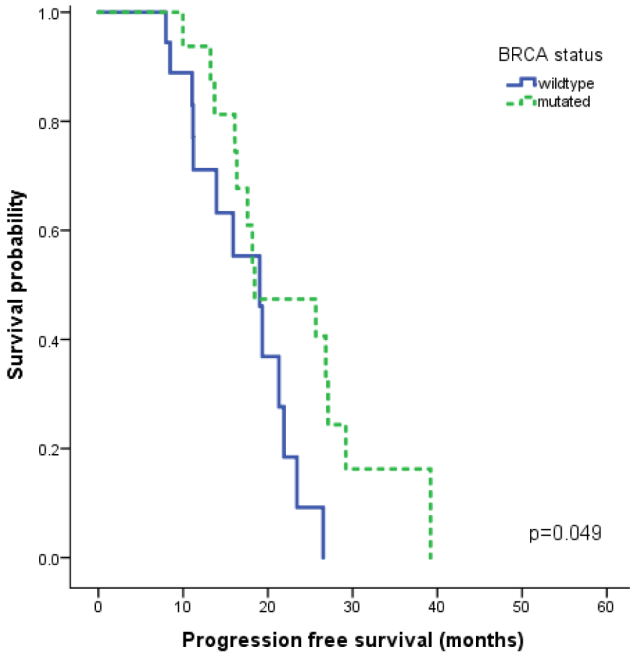

Figure 1. Kaplan-Meier progression free survival 1 of advanced stage patients with NACT.

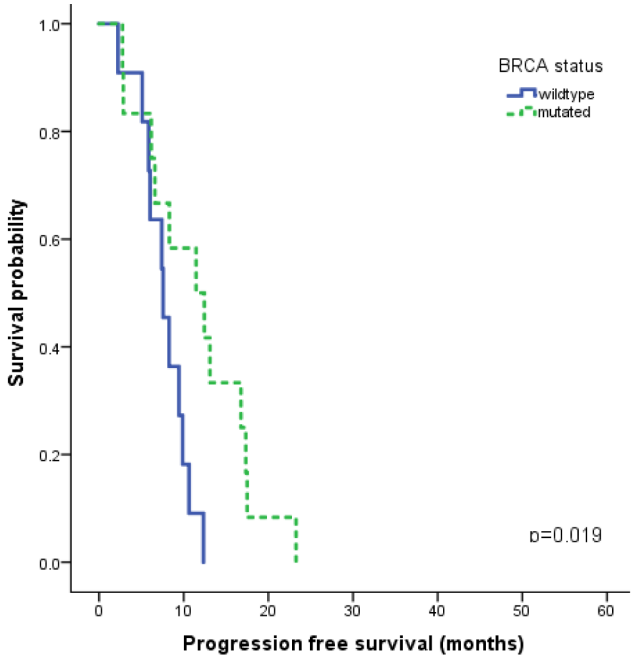

Figure 2. Kaplan-Meier progression free survival 2 of advanced stage patients with NACT.

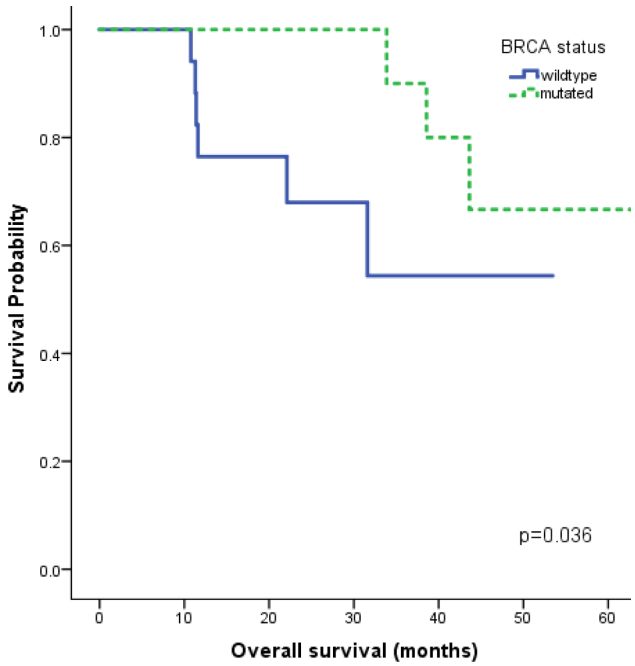

Figure 3. Kaplan-Meier overall survival of advanced stage patients with NACT.

We investigated if the presence of BRCA mutations has any prognostic value. In the overall population, the presence of BRCA mutation was associated with a significantly longer PFS2 (16.1 versus 11.7 months, p=0.018), but there were no significant differences regarding PFS1 or OS (Table 4). However, in advanced-stage disease (FIGO IIIC-IV) subpopulation that underwent NACT, the presence of BRCA mutation was associated with a significantly longer PFS1 (23 versus 17.4 months, p=0.049, Figure 1), PFS2 (11.5 versus 7.7 months, p=0.019, Figure 2) and OS (67.1 versus 37.9 months, p=0.036, Figure 3) in comparison with cases without BRCA mutation.

Discussion

Ovarian cancer brings a global burden of disease and continues to pose a challenge regarding optimal treatment strategies and preventing the development of platinum-resistant disease. Our study population included a balanced proportion or BRCAwt and BRCAm (44.3%) patients, however, given the current reimbursement status for PARPi that was an enrichment for high grade serous histology cancer as it has been previously described [15]. Close to three quarters (71.6%) of patients had extensive intraabdominal disease with tumor nodules over 2 cm in size (FIGO stage IIIC-IV) in accordance with the current clinical presentation [16], however complete debulking was achieved in 88% of cases, either through PDS or NACT followed by IDS, with the former being a more frequent (58%) choice within this population. Secondary debulking surgery was also offered only to a selected minority of patients at relapse, usually in those that had a longer progression free interval and with a good performance status [17]. A first relapse occurred in more than three quarters of the study population (76.1%), however overall survival (OS) data are limited to only 19.3% event occurrences with the rest of the data being censored, given the relatively short follow-up interval.

Taking into account the BRCA status, there were no significant differences regarding FIGO stage at presentation or regarding the choice of IDS versus PDS. BRCA status was usually assessed after the first recurrence. The majority of BRCAm were germline (92%) and were predominantly situated in the BRCA1 (66%) gene. BRCAm affected patients were significantly younger (mean difference 3.8 years) as is has been previously reported [18,19], however this did not impact the choice of primary treatment in our study.

In patients with NACT, the presence of BRCAm was associated with a higher frequency for CPR. Higher response rates to neoadjuvant systemic therapy have also been previously reported but in BRCAm breast cancer patients, with the highest benefit derived from platinum-based therapies [20,21]. Currently, there is compelling evidence that BRCAm tumors exhibit increased sensitivity to platinum-based agents through the loss of critical functions that BRCA genes play, such as HR mediated repair, cell cycle regulation and apoptosis [22]. Regardless the BRCA status, CPR was associated with a significantly longer PFS1; however, in our study this did not translate into a significant OS advantage. There is evidence in the literature that CPR is an independent prognostic factor for both progression-free survival (PFS) and OS, and it probably reflects a BRCAness profile that can also be determined by other genes involved in DNA damage response [23]. Our BRCAm patients exhibited previously described characteristics of platinum sensitivity such as a platinum-sensitive relapse, first relapse and its favorable response to platinum therapy [11,24,25]. Interestingly, we noted that the cohort of BRCAm patients retained its platinum-sensitive phenotype even at the second relapse and by analyzing paired first and second relapses we noted that BRCAm patients were approximatively 5 times less likely to change their phenotype from platinum-sensitive to platinum-resistant than the BRCAwt population. It is also remarkable to note that during the first relapse BRCAm patients were 2.66 more likely to have affected lymph nodes. This could be explained in part by an increased capacity to metastasize more frequently outside the peritoneum given the unstable genome of tumors with defects in DNA damage repair pathways [26]. Although we could not detect an OS advantage in the whole unselected patient population, for patients with advanced-stage disease treated with neoadjuvant chemotherapy BRCAm status was associated with a longer PFS and OS, suggesting that NACT in this patient population would be less detrimental in selecting platinum-resistant subclones and upfront knowledge of BRCA status should be recommended in all patients, however larger prospective studies are warranted for the subsequent development of this topic.

Acknowledgements

This study was supported by the Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca (Internal research grant number 3066/29/01.02.2018).

Conflict of interests

The authors declare no conflict of interests.

References

1. Ferlay J, Colombet M, Soerjomataram I et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer 2019;144:1941-53.
2. Kuchenbaecker KB, Hopper JL, Barnes DR et al. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. JAMA 2017;317:2402-16.
3. Vlad C, Kubelac P, Alexandru I, Achimas-Cadariu P. The role of primary debulking in advanced ovarian cancer patients. JBUON 2016;21:1320.
4. Vergote I, Coens C, Nankivell M et al. Neoadjuvant chemotherapy versus debulking surgery in advanced tubo-ovarian cancers: pooled analysis of individual patient data from the EORTC 55971 and CHORUS trials. Lancet Oncol 2018;19:1680-7.
5. Mahner S, Heitz F, Burges A et al. TRUST: Trial of radical upfront surgical therapy in advanced ovarian cancer (ENGOT ov33 / AGO-OVAR OP7). J Clin Oncol 2017;35:TPS5602-TPS.
6. da Costa A, Valadares CV, Baiocchi G et al. Neoadjuvant Chemotherapy Followed by Interval Debulking Surgery and the Risk of Platinum Resistance in Epithelial Ovarian Cancer. Ann Surg Oncol 2015;22:S971-8.
7. van Meurs HS, Tajik P, Hof MH et al. Which patients benefit most from primary surgery or neoadjuvant chemotherapy in stage IIIC or IV ovarian cancer? An exploratory analysis of the European Organisation for Research and Treatment of Cancer 55971 randomised trial. Eur J Cancer 2013;49:3191-201.
8. Cooke SL, Brenton JD. Evolution of platinum resistance in high-grade serous ovarian cancer. Lancet Oncol 2011;12:1169-74.
9. Galluzzi L, Vitale I, Michels J et al. Systems biology of cisplatin resistance: past, present and future. Cell Death Dis 2014;5:e1257.
10. Alkema NG, Wisman GB, van der Zee AG, van Vugt MA, de Jong S. Studying platinum sensitivity and resistance in high-grade serous ovarian cancer: Different models for different questions. Drug Resist Updat 2016;24:55-69.
11. Pennington KP, Walsh T, Harrell MI et al. Germline and Somatic Mutations in Homologous Recombination Genes Predict Platinum Response and Survival in Ovarian, Fallopian Tube, and Peritoneal Carcinomas. Clin Cancer Res 2014;20:764-75.
12. Varol U, Kucukzeybek Y, Alacacioglu A et al. BRCA genes: BRCA 1 and BRCA 2. JBUON 2018;23:862-6.
13. Eisenhauer EA, Therasse P, Bogaerts J et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-47.
14. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Control Clin Trials 1996;17:343-6.
15. Lakhani SR, Manek S, Penault-Llorca F et al. Pathology of ovarian cancers in BRCA1 and BRCA2 carriers. Clin Cancer Res 2004;10:2473-81.
16. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018;68:7-30.
17. Suh DH, Kim HS, Chang SJ, Bristow RE. Surgical management of recurrent ovarian cancer. Gynecol Oncol 2016;142:357-67.
18. Triantafyllidou O, Vlachos IS, Apostolou P et al. Epidemiological and clinicopathological characteristics of BRCA-positive and BRCA-negative breast cancer patients in Greece. JBUON 2015;20:978-84.
19. Litton JK, Ready K, Chen H et al. Earlier age of onset of BRCA mutation-related cancers in subsequent generations. Cancer 2012;118:321-5.
20. Arun B, Bayraktar S, Liu DD, et al. Response to neoadjuvant systemic therapy for breast cancer in BRCA mutation carriers and noncarriers: a single-institution experience. J Clin Oncol 2011;29:3739-46.
21. Wunderle M, Gass P, Haberle L et al. BRCA mutations and their influence on pathological complete response and prognosis in a clinical cohort of neoadjuvantly treated breast cancer patients. Breast Cancer Res Treat 2018;171:85-94.
22. Yoshida K, Miki Y. Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. Cancer Sci 2004;95:866-71.
23. Petrillo M, Zannoni GF, Tortorella L et al. Prognostic role and predictors of complete pathologic response to neoadjuvant chemotherapy in primary unresectable ovarian cancer. Am J Obstet Gynecol 2014;211:632.e1-8.
24. Boyd J, Sonoda Y, Federici MG et al. Clinicopathologic features of BRCA-linked and sporadic ovarian cancer. JAMA 2000;283:2260-5.
25. Cass I, Baldwin RL, Varkey T, Moslehi R, Narod SA, Karlan BY. Improved survival in women with BRCA-associated ovarian carcinoma. Cancer 2003;97:2187-95.
26. Gee ME, Faraahi Z, McCormick A, Edmondson RJ. DNA damage repair in ovarian cancer: unlocking the heterogeneity. J Ovarian Res 2018;11:50.