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The Impact of Pathogenic BRCA1/2 Tumor Mutation Status on Advanced Stage-High Grade Serous Epithelial Ovarian Cancer Survival Outcome

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Abstract
Objective: To evaluate the impact of pathogenic BRCA1/2 tumor mutation on advanced stage-high grade serous epithelial ovarian cancer (HGSOC) survival outcome.
Methods: A total of 68 from 144 patients were diagnosed with FIGO 2014 stage IIB-IV HGSOC between January 1st 2015, until March 31st 2021, at Dr. Cipto Mangunkusumo National Central Referral Hospital, Persahabatan Central Referral Hospital, and MRCCC Central Referral Hospital Siloam Jakarta, underwent NGS tumor BRCA1/2 gene testing and were included in this cohort historical study. We compared patient's overall survival outcomes to pathogenic BRCA1/2 tumor mutational status. The patient's clinicopathological characteristic factors that might affect patient's survival outcomes were also investigated.
Results: In the group with pathogenic BRCA1/2 tumor mutation, the likelihood of dying was 86% lower (adjusted RR 0.149; 95%CI 0.046-0.475; p-value=0.001), and the median survival was better (median 46 months; 95%CI 34.009-57.991; p value=0.001) than the group without pathogenic BRCA1/2 tumor mutations (median 23 months; 95%CI 15.657-30.343; p value=0.001). The multivariate analyses identified pathogenic BRCA1/2 tumor mutation as an independent favorable prognostic factor for survival outcome (adjusted RR 0.149; 95%CI 0.046-0.475; p-value=0.001).
Conclusions: In advanced stage-HGSOC patients, the pathogenic BRCA1/2 tumor mutations group have a better prognosis with longer survival outcomes than those without pathogenic BRCA1/2 tumor mutations.
Keywords: Ovarian Neoplasms, High-Grade Serous Epithelial Ovarian Carcinoma, BRCA1/2, Clinical Outcome, Survival Outcome.

Introduction

Ovarian cancer is the third most common gynecological cancer in the world, after cervical cancer and uterine cancer, with the second highest mortality rate, after cervical cancer. The Globocan 2020 study noted that new cases and mortality cases of ovarian cancer, respectively, reached 313,959 cases and 207,252 cases, which increased compared to 2018, 295,414 cases and 184,799 cases, respectively. he world's 5-year ovarian cancer prevalence is estimated at more than 823,315, which also increased compared to 2018, with 600,000 cases. Asia is the continent with the highest incidence, mortality, and 5-year prevalence rates, respectively, 170,759 (54.4%) cases, 112,936 (54.5%) cases, and 435,574 (52.9%) cases. In Indonesia, the incidence, mortality, and 5-year prevalence rate due to ovarian cancer is the second most common after

cervical cancer, with 14,896 cases, 9,581 cases, and 37,533 cases, respectively, placing it as the country with thethird highest number of cases in Asia, after China and India (Ferlay et al., 2019; IARC, 2020). Cancer Registration of the Indonesian Society of Gynecologic Oncology (INASGO) noted, in the January 1st, 2015-November 20th, 2019 period, there were 1,342 cases of epithelial ovarian cancer, 63% of patients presenting at an advanced stage, with a low 5-year survival rate, 30-55% (HOGI, 2018).

The low survival rates, and high mortality rates in advanced ovarian cancer patients, are associated with low optimal debulking rates. Based on the Cochrane database review report, optimal debulking surgery significantly increased overall and progression-free survival (Martín-Cameán et al., 2016). The median survival in the complete resection (no macroscopic residual disease), optimal debulking, and the sub-optimal debulking group were 99.1 months, 36.2 months, and 29.6 months, respectively (Mahmood et al., 2022). They also reported that optimal and sub-optimal debulking's disease-free interval were 40-48 months and 6-18 months. Indonesia has a low optimal debulking rate, based on a cross-sectional descriptive study at Dr. Cipto Mangunkusumo National Central Referral Hospital, in 2012-2016, which was 46%, compared to developed countries, 60%-70% (Purbadi and Saspriyana, 2021). These factors contribute to the high ovarian cancer case-fatality rate in the developing countries, such as Indonesia, which reached 59.2-63.8%, while developed countries only reached 54.8%. The case fatality rate of ovarian cancer in Indonesia ranks fifth highest in Asia, after India, China, Japan, and Pakistan (Siegel et al., 2015; Razi et al., 2016).

Efforts to reduce the case fatality rate, and increase the survival rate of ovarian cancer patients through increasing optimal debulking rates, Platinum-Taxane chemotherapy administration, and anti-VEGF targeted therapy, have not yielded satisfactory results, as proved by the not decreasing incidence, mortality, and 5-years prevalence rates. This fact is the background for developing of genetic alteration and cellular behavior research in ovarian cancer, as an effort to develop personalized medicine, supported by advances in DNA sequencing technology. An epithelial ovarian cancer, as the most common type (±90% of all ovarian cancers), especially type II (±70% of epithelial types), is a major concern for the development of personalized medicine, due to the high number of cases, aggressive growth, and high mortality. Although the majority of epithelial ovarian cancers represent sporadic disease, associated with TP53 mutations (>95% type II), approximately 15-23% are known to represent a hereditary group, associated with mutations in the BRCA1/2 cancer susceptibility gene (±20-29% of ovarian cancers). The prevalence rate of mutations varies based on histological subtypes, the highest being high-grade serous epithelial ovarian cancer (HGSOC), 20-27% of epithelial ovarian cancer (Ledermann et al., 2016a; Chirasophon et al., 2017; Manchana et al., 2019).

The BRCA1/2 gene contributes to the process of DNA repair, cell-cycle checkpoint control, protein ubiquitylation, and chromatin remodeling. In the DNA-repair process, BRCA1/2 is involved in repairing DNA damage, binds to RAD51, initiates homologous recombination, and repairs double-strand breaks DNA. When a cell undergoes a mutation, the DNA repair process tends to go wrong (error-prone repair). In the checkpoint control process, BRCA1/2 mutations cause inactivation of the BRCT domain, which is a cell cycle regulator. Ubiquitylation is the process of protein assembly, followed by proteasome degradation. BRCA1 assists this process, by forming the BRCA1-BARD1 complex. In the chromatin remodeling process, BRCA1 plays a role in DNA repair, forming multimeric complexes with chromatin-remodeling complexes (SW1 and SNF), and histone deacetylase complexes. Mutations in this gene will interfere with chromatin remodeling in DNA damage. All of these mechanisms, which play a role in the carcinogenesis of HGSOC, are related to the BRCA 1/2 mutation.

The pathogenic BRCA1/2 mutations were not only associated with an increased risk of type II epithelial ovarian cancer, but also good predictors of therapeutic response. According to a meta-analysis, BRCA1/2 mutations respond better to Platinum chemotherapy, have higher complete response rates, lower partial responses, and longer progression-free survival (PFS), compared to the wild-type group. This is associated with inhibition of DNA repair pathways, making tumor cells more sensitive to the DNA-damaging effects of chemotherapy. As personalized medicine, the pathogenic BRCA 1/2 mutations are also associated with developing targeted therapy for poly (ADP-ribose) polymerase (PARP) inhibitors. In the state of homologous recombination deficiency due to BRCA 1/2 mutations, cancer cells are highly dependent on the PARP-mediated base excision repair (BER) mechanism of DNA single-strand breaks to repair DNA damage spontaneously. PARP inhibitors exert a significant anti-tumor effect, related to synthetic lethality in HGSOC patients with pathogenic BRCA1/2 mutations (Evans and Matulonis; 2017).

Based on the description above, ovarian cancer is still a major health problem in Indonesia. An efforts to detect risk factors for hereditary ovarian cancer susceptibility genes, and determine targeted treatment, intending to increase survival rates, are closely related to the pathogenic BRCA1/2 mutations analysis. The Dr. Cipto Mangunkusumo Hospital, as a center of a national referral hospital, Persahabatan Hospital, and MRCCC Hospital Siloam, the largest cancer referral center in Jakarta, need to collect accurate data regarding the effect of pathogenic BRCA1/2 tumor mutations, on the HGSOC survival outcome. The development of BRCA-related personalized medicine to increase the survival outcome of epithelial ovarian cancer patients in Indonesia is expected to be achieved.

Patients and Methods

This study is an observational analytic study, using a historical cohort study design, aiming to determine the effect of pathogenic BRCA1/2 tumor mutations on advanced stage-high grade serous epithelial ovarian cancer patient's overall survival, at Dr. Cipto Mangunkusumo National Central Referral Hospital, Persahabatan Central Referral Hospital, and MRCCC Central Referral Hospital Siloam Jakarta.

The research was carried out after obtaining an Ethical Review from Komisi Etik Penelitian Kesehatan (KEPK) Medical Faculty of Indonesia University/ Dr. Cipto Mangunkusumo National Central Referral Hospital, No.: KET-198/UN2.F1/ETIK/PPM.00.02/2020, February 24th, 2020; and No. ND-291/UN2.F1/ETIK/PPM.00.02/2021; Ethical Review of KEPK Persahabatan Central Referral Hospital, No. 106/KEPK-RSUPP/10/2020, October 1st, 2020; and Research Approval Letter of MRCCC Central Referral Hospital Siloam Jakarta, No. 735/SS/Dir/V/2021. Written informed consent was obtained from the subjects.

The subjects were selected by consecutive sampling from archival data and medical records of Anatomic Pathology Department – Medical Faculty of Indonesia University/ Dr. Cipto Mangunkusumo National Central Referral Hospital, Persahabatan Central Referral Hospital, and MRCCC Central Referral Hospital Siloam Jakarta, with a diagnosis of advanced stage-high grade serous epithelial ovarian cancer, evidenced by the results of histopathological examination, Formalin-Fixed and Paraffin-Embedded (FFPE) tumor tissue blocks and HE slide staining, from January 1st, 2015 until March 31st, 2021 period, started from January 1st, 2015.

Patient selection was carried out based on the inclusion and exclusion criteria of the study, reviewed the HE slide by a single expert Pathologist, confirmed the histopathological type of high-grade serous epithelial ovarian cancer, and selected the best FFPE block with the highest tumor cell content. The Data collection of pathogenic BRCA1/2 tumor mutation sequencing analysis was performed at MedGenome Labs. Ltd. India and KALGen Innolab Indonesia Clinical Laboratory.

The laboratory analysis begins with the Quality Control selection, at least 10% of the viable neoplastic cells – tumor content (>150 tumor cells/HPF), is considered as an acceptable criteria to continue the pathogenic BRCA1/2 tumor mutation analysis. The DNA extraction, library preparation, targeted enrichment, and sequencing: DNA extraction from FFPE tumor tissue block, is used to perform targeted gene capture, using a custom capture kit for the entire coding region of the BRCA1 and BRCA2 genes. The DNA captured library was sequenced on the Illumina HiSeq series to produce 2x150 bp sequence reads at 80 – 100X at the target sequencing depth.

The NGS data analysis and clinical reports: clinically associated mutations, annotated using variants published in the literature and disease database tools – ClinVar, OMIM, GWAS, HGMD, SwissVar, cBioPortal, OncoMD (MedGenome's lab curated somatic database, including TCGA and COSMIC). The variants were most frequently filtered by minor allele frequency (MAF) on 1000 Genome phase 3, ExAC, gnomAD, dbSNP141, 1000 Japanese Genome, and the internal Indian population database. The biological effects of non-synonymous variants were calculated using multiple prediction algorithms such as PolyPhen, SIFT, Mutation Taster2, and LRT. The reportable mutations, prioritized and reported, according to the AMP-ASCO-CAP guidelines. Only non-synonymous and splice site variants were found in the coding regions of the BRCA1 and BRCA2 genes, which will be used for clinical interpretation. The silent variation, which does not cause amino acid changes in the coding region, is not reported. Incomplete annotated variants, and nonsense-mediated decay transcripts, were not reported. Accepting of BRCA1/2 tumor mutation analysis results from the MedGenome laboratory and KALGen Innolab Indonesia Clinical Laboratory. The subjects' monitoring was done until the time subjects experienced a death event, or were declared alive (sensor) until the completion of the research observation period, June 30th, 2021. The subject data is then processed and analyzed using SPSS version 20 for Windows, according to the research objectives and hypotheses.

Based on data from the Cancer Registration Department of Anatomic Pathology, Dr. Cipto Mangunkusumo National Central Referral Hospital, Persahabatan Central Referral Hospital, and MRCCC Central Referral Hospital Siloam Jakarta, during the research period, there were 986 cases of ovarian cancer, consisting of 791 cases of epithelial ovarian cancer, and 195 cases of non-epithelial ovarian cancer. The epithelial ovarian cancer consisted of 299 serous epithelial cases (low grade serous ovarian carcinoma n=73, and high grade serous ovarian carcinoma n=226); and 492 non-serous epithelial cases (clear cell adenocarcinoma n=193; endometrioid adenocarcinoma n=131; mucinous adenocarcinoma n=161; undifferentiated adenocarcinoma n=7), and 195 non-epithelial cases (yolk sac tumor, dysgerminoma, granulosa cell tumor, immature teratomas, sarcomas, sertoli cell tumors, and leydig cell tumors). A total of 487 of 791 cases of epithelial ovarian cancer in the three hospitals (61.57%), first diagnosed at an advanced stage (FIGO 2014 stage IIB-IVB). In this study, the entire study sample used histopathological type high grade serous epithelial ovarian cancer (HGSOC), due to samples homogenity.

Based on the data above, as many as 144 of the 226 sample lists of HGSOC (Dr. Cipto Mangunkusumo National Central Referral Hospital (n = 106), Persahabatan Central Referral Hospital (n = 24), and MRCCC Central Referral Hospital Siloam Jakarta (n = 14), were met the inclusion criteria as advanced stage-high grade serous epithelial ovarian cancer, underwent primary treatment with laparotomy debulking, and adjuvant chemotherapy, complete data available on HE slides and FFPE block preparations. The remaining 82 samples could not be included in the study, due to FIGO stage <IIB (early stage), incomplete HE slide, and/or FFPE block data. A total of 87 from the 144 available samples were complete, fulfill the research inclusion selection criteria by the Pathologist (reassessment of histopathological subtype, degree of differentiation, stage IIB-IV, tumor cell content >20%), as many as 57 samples were declared not fulfill reassessment of the research inclusion criteria (Figure 1).

All 87 samples mentioned above were examined for pathogenic BRCA1/2 gene tumor mutation analysis. 68 FFPE samples were declared to meet the quality control (QC) criteria, followed by DNA isolation, library preparation, template preparation, and DNA sequencing. The remaining 19 FFPE samples were declared not fulfill quality control criteria, due to the low DNA Integrity Index (DIN), due to high DNA fragmentation (<120-150 bp), in suboptimal FFPE quality.

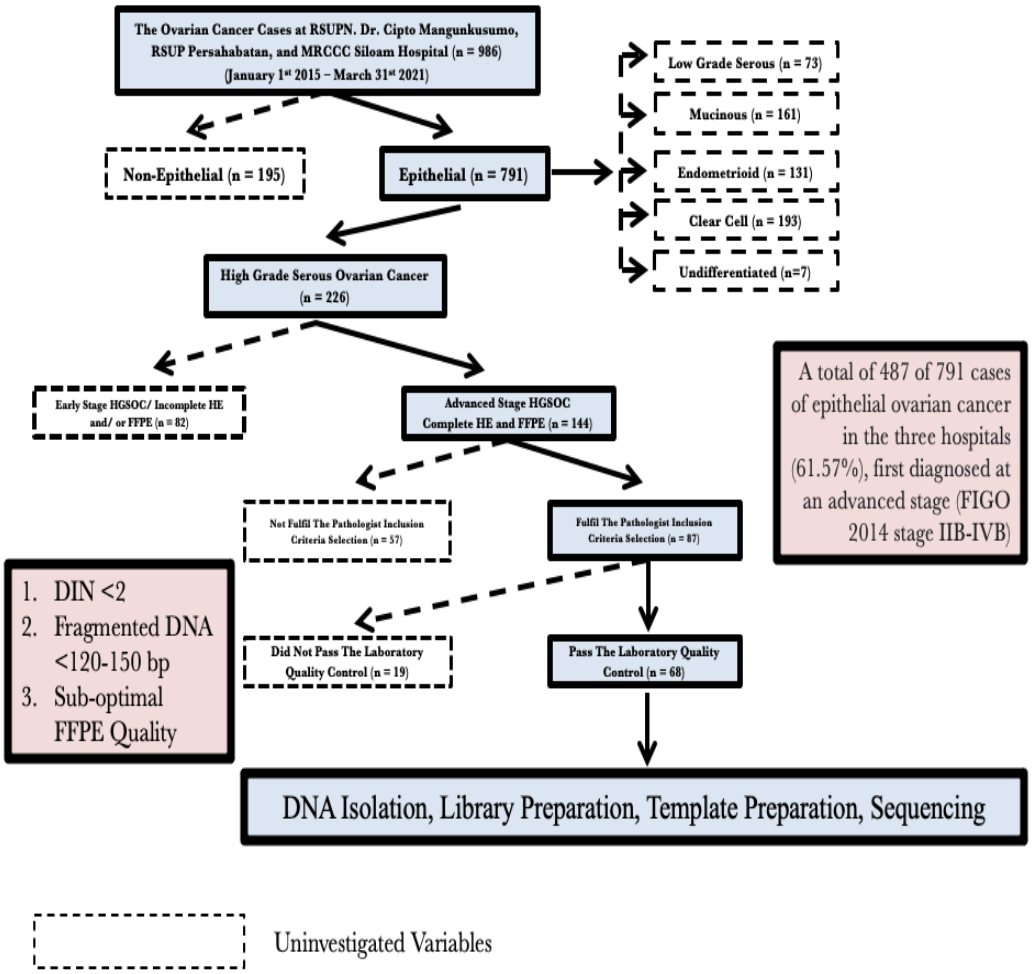

Figure 1. The Research Sample Selection – The Impact of Pathogenic Tumor BRCA1/2 Mutational Status on Advanced Stage-High Grade Serous Epithelial Ovarian Cancer Survival Outcome

Result

Based on univariate analysis, most of the samples were 50 years old, ie 76.47% (52/68); only 23.53% (16/68) of the sample was <50 years old. A total of 72.05% (49/68) of the sample was in the menopausal age group, and 11.76% (8/68) of the sample was nulliparous. A total of 20.58% (14/68) of the sample had a family history of breast cancer and/or ovarian cancer (HBOC). A total of 0.03% (2/68) of the sample had a previous history of breast cancer. Most of the patients were diagnosed at FIGO 2014 stage III, ie 67.64% (46/68), the rest were FIGO IIB stage at 22.05% (15/68), and FIGO IV stage at 10.29% (7/68). A total of 60.29% (41/68) samples had preoperative CA-125 levels ≥500 mIU/mL, and 54.41% (37/68) samples with intra-operative ascites volume ≥500 mL. A total of 27.94% (19/68) of the sample had a cytoreductive residual lesion ≥1 cm (sub-optimal debulking). 11.76% (8/68) of the sample received neoadjuvant chemotherapy. Adjuvant chemotherapy was administered to 88.23% (60/68) of the sample, and 47.05% (32/68) of the sample was recorded to have died in this study.

Table 1. The Pathogenic BRCA1/2 Tumor Mutation, VUS, and *Wild Type* Distribution in *Advanced Stage High-Grade Serous Epithelial Ovarian Cancer*

No	Variables	Pathogenic BRCA1/2 Tumor Mutation (n = 14)	Pathogenic mBRCA1/2 and VUS (n = 5)	VUS (n = 15)	Wild Type (n = 34)	Total (n = 68)
1	Age (Years Old)					
	<40 (0)	2 (28.58%)	0 (0.00%)	0 (0.00%)	5 (71.42%)	7 (100.00%)
	41-49 (1)	0 (0.00%)	1 (11.11%)	1 (11.11%)	7 (77.78%)	9 (100.00%)
	50-59 (2)	4 (15.38%)	2 (7.69%)	6 (23.08%)	14 (53.84%)	26 (100.00%)
	60-69 (3)	5 (33.33%)	2 (13.33%)	2 (13.33%)	6 (40.00%)	15 (100.00%)
	≥70 (4)	3 (27.27%)	0 (0.00%)	6 (54.54%)	2 (18.18%)	11 (100.00%)
2	Parity					
	P0 (0)	0 (0.00%)	1 (12.50%)	2 (25.00%)	5 (62.50%)	8 (100.00%)
	P1 (1)	4 (18.18%	2 (9.09%)	4 (18.18%)	12 (54.54%)	22 (100.00%)
	P2 (2)	3 (15.00%)	1 (5.00%)	4 (20.00%)	12 (60.00%)	20 (100.00%)
	P≥3 (3)	7 (38.88%)	1 (5.55%)	5 (27.77%)	5 (27.77%)	18 (100.00%)
3	Body Mass Index (BMI kg/m^2)					
	<18,5 (0)	0 (0.00%)	0 (0.00%)	1 (33.33%)	2 (66.67%)	3 (100.00%)
	18,5 – 24,9 (1)	7 (17.07%)	3 (7.32%)	8 (19.51%)	23 (56.09%)	41 (100.00%)
	25,0 – 29,9 (2)	5 (25.00%)	2 (10.00%)	4 (20.00%)	9 (45.00%)	20 (100.00%)
	≥30 (3)	2 (50.00%)	0 (0.00%)	2 (50.00%)	0 (0.00%)	4 (100.00%)
4	Breast Cancer History					
	No (0)	14 (21.21%)	5 (7.57%)	15 (22.72)	32 (48.48%)	66 (100.00%)
	Yes (1)	0 (0.00%)	0 0.00%)	0 (0.00%)	2 (100.00%)	2 (100.00%)
5	Hereditary Breast and Ovarian Cancer Family History					
	No (0)	9 (16.66%)	2 (3.70%)	13 (24.07%)	30 (55.55%)	54 (100.00%)
	Yes (1)	5 (35.71%)	3 (21.42%)	2 (14.28%)	4 (28.57%)	14 (100.00%)
6	FIGO 2014 Staging					
	IIB (0)	2 (13.33%)	2 (13.33%)	6 (40.00%)	5 (33.33%)	15 (100.00%)
	III (1)	12 (26.00%)	3 (6.52%)	8 (17.39%)	23 (50.00%)	46 (100.00%)
	IV (2)	0 (0.00%)	0 (0.00%)	1 (14.28%)	6 (85.72%)	7 (100.00%)
7	Pre-Operative CA-125 Level (U/mL)					
	<500 (0)	7 (25.92%)	3 (11.11%)	4 (14.81%)	13 (48.14%)	27 (100.00%)
	≥500 (1)	7 (17.07%)	2 (4.87%)	11 (26.82%)	21 (51.22%)	41 (100.00%)
8	Intra Operative Ascites Volume (mL)					
	<500 (0)	8 (25.80%)	4 (12.90%)	9 (29.03%)	10 (32.25%)	31 (100.00%)
	≥500 (1)	6 (16.22%)	1 (2.70%)	6 (16.22%)	24 (64.86%)	37 (100.00%)
9	Cytoreductive Residual Lesion					
	R0 (0)	7 (20.59%)	3 (8.82%)	10 (29.41%)	14 (41.18%)	34 (100.00%)
	<1 cm (1)	6 (40.00%)	0 (0.00%)	0 (0.00%)	9 (60.00%)	15 (100.00%)

	≥1 cm (2)	1 (5.26%)	2 (10.52%)	5 (26.32%)	11 (57.89%)	19 (100.00%)
10	Neoadjuvant Chemotherapy (NACT)					
	No (0)	12 (20.00%)	5 (8.33%)	14 (23.33%)	29 (48.33%)	60 (100.00%)
	Yes (1)	2 (25.00%)	0 (0.00%)	1 (12.50%)	5 (62.50%)	8 (100.00%)
11	Adjuvant Chemotherapy					
	Yes (0)	13 (21.67%)	4 (6.67%)	10 (16.67%)	33 (55.00%)	60 (100.00%)
	No (1)	1 (12.50%)	1 (12.50%)	5 (62.50%)	1 (12.50%)	8 (100.00%)
12	Survival Outcome Status					
	Alive/ Censor	10 (27.77%)	4 (11.11%)	7 (19.44%)	15 (41.67%)	36 (100.00%)
	Death	4 (12.50%)	1 (3.12%)	8 (25.00%)	19 (59.37%)	32 (100.00%)

Based on Table 1 above, the proportion of pathogenic BRCA1/2 tumor mutation in this study was 27.94%. The pathogenic BRCA1 tumor mutation was identified in 10 samples (14.7%), pathogenic BRCA2 tumor mutation in 10 samples (14.7%), with pathogenic tumor mutations to BRCA1 and BRCA2 at the same sample known in 1 sample (1.47%). The incidence of BRCA1/2 variants of uncertain significance (VUS) in this study was 29.41% (20/68), with 25% (5/20) of them, known to have both BRCA1/2 and VUS pathogenic mutations. The incidence of the wild-type BRCA1/2 gene in this study was 50% (34/68).

Table 2. The Pathogenic BRCA1/2 Tumor Mutation In Advanced Stage-High Grade Serous Epithelial Ovarian Cancer

Patient ID	Age	Parity	Breast Ca	HBOC	FIGO	Codon	Protein Changes	Exon	Mutant Allele Frequency	Variant Description
2002434	59	P3	No	No	IIIA1(ii)	BRCA1; c.2216_2217delAA	p.Lys739fs	Exon 10	94.10%	2 bp Deletion – Frameshift
2003831	63	P2	No	Yes	IIIC	BRCA2; c.5054C>A	p.Ser1685Ter	Exon 11	93.20%	Deletion – Frameshift
2004332	47	P1	No	No	IIIC	BRCA2; c.250C>T	p.Gln84Ter	Exon 3	11.20%	Deletion – Frameshift
2004439	60	P0	No	No	IIIA1(ii)	BRCA2; c.8191C>T	p.Gln2731Ter	Exon 18	CCT=77.24%, CTT=2.64%	Deletion – Frameshift
2006388	74	P3	No	No	IIIC	BRCA1; c.4548-1G>T	p.Cys2817Ter	Exon 15	63.20%	SNV – Splice Acceptor
1804430	68	P4	No	Yes	IIIC	BRCA2; c.8447_8451del		Exon 19	14.50%	Deletion – Frameshift
1901348	71	P5	No	Yes	IIIC (Post NACT 3 Series)	BRCA1 c.134+1G>T	p.Gln139Ter	Intron 3	81.10%	SNV – Splice Donor
1603275	63	P3	No	Yes	IIIC	BRCA1 c.415C>T		Exon 6	6.6.%	SNV – Nonsense
1505166	65	P14	No	No	IIB	BRCA2 c.8636_8637del	p.Thr2880AsnfsTer26	Exon 21	55.60%	Deletion – Frameshift
H210509	50	P1	No	No	IIIC (Post NACT 3 Series)	BRCA2; c.957_958delTCinsAA	p.Asn319_Leu320delinsLysIle	Exon 10	CAAAAAAAA=0.0694, CAAAAAAAAATC=0.0, CAAAAAAAATC=0.0, CAAAAAAATC=0.9105, CAAAATC=0.0201	Deletion – Frameshift
						BRCA2; c.956_957insA	p.Asn319fs			
H210699	69	P2	No	Yes	IIIC	BRCA2; c.5351delA	p.Asn1784fs	Exon 11	AC=0.0167, CAA=0.0, C=0.1041, CATC=0.0	1 bp Deletion – Frameshift
19000673	54	P2	No	No	IIIC	BRCA1; c.3627_3628insA	p.Glu1210fs	Exon 10	80.20%	Deletion – Frameshift
16004057	54	P1	No	Yes	IIB	BRCA2; c.7976G>A	p.Arg2659Lys	Exon 17	11.70%	SNV – Missense
						BRCA1; c.4738G>A	p.Glu1580Lys	Exon 15	13.60%	SNV – Missense Hotspot
						BRCA2; c.8332-1G>A		Exon 19	29.40%	SNV – Splice Acceptor
						BRCA2; c.7753G>A	p.Gly2585Arg	Exon 16	16.90%	SNV – Missense
1800480	53	P1	No	No	IIB	BRCA2;	p.Asn863fs	Exon	CAAAAAA=0.0%,	1 bp Deletion –

6						c.2588delA		11	CAAAAAAG=0.0%, CAAAA=17.07%, CAAAAAAT=10%, CAAA=0.0%, CAAAAAT=0.0%	Frameshift
18000991	52	P3	No	Yes	IIB	BRCA2; c.6937+1G>A			10.40%	SNV – Splice Variant
H181315	36	P1	No	Yes	IIIA1(ii)	BRCA1 c.4817del (ENST00000471181.2)	p.Pro1606Glnfs Ter16	Exon 16	341X / 77.1%	Deletion – Frameshift
H191366	84	P7	No	No	IIIB	BRCA1 c.2726dup	p.Asn909Lysfs Ter6	Exon 10 (ENST00000471181.2	1223X / 83.6%	Insertion Variation – Frameshift
H171499	39	P1	No	No	IIIC	BRCA1 c.709G>T (ENST00000471181.2)	p.Glu237Ter	Exon 10	100X / 83%	SNV – Nonsense
288979	61	P2	No	No	IIIC	BRCA1 c.2964dup (ENST00000471181.2)	p.Phe989Ilefs Ter3	Exon 10	865X / 31.9%	Insertion Variation – Frameshift

Based on Table 3, the total incidence of death in this study was 47.05% (32/68). The highest incidence of death was seen in the age group <50 years (56.3%), parity ≤1 (53.3%), BMI <25 kg/m2 (54.5%), no previous history of breast cancer (48.5%), no HBOC family history (50.0%), FIGO 2014 stage ≥III (52.8%), pre-operative CA 125 level ≥500 IU/mL and intraoperative ascites volume ≥500 mL (63.4% and 62.2%, respectively), post-debulking laparotomy positive residual lesions (73.5%), administration of neoadjuvant chemotherapy (87.5%), no adjuvant chemotherapy (75.0%), and no pathogenic BRCA1/2 tumor mutation (55.1%).

Table 3. Survival Analysis of Advanced Stage High-Grade Serous Epithelial Ovarian Cancer Patients

No.	Variables	Death/ Event		Alive/ Censors		P-value	*RR Crude*	95% CI	
		n	%	n	%			*Lower*	*Upper*
1	Age (Years)								
	<50 (0)	9	56.3	7	43.8	0.062	1	Referensi	
	≥50 (1)	23	44.2	29	55.8		0.463	0.206	1.04
2	Parity								
	P≤1 (0)	16	53.3	14	46.7	0.213	1	Referensi	
	P>1 (1)	16	42.1	12	57.9		0.641	0.318	1.291
3	Body Mass Index (BMI Kg/m2)								
	<25,0	24	54.5	20	45.5	0.06	1	Referensi	
	≥25,0	8	33.3	16	66.7		0.648	0.282	1.489
4	Breast Cancer History								
	Tidak (0)	32	48.5	34	51.5	0.461	1	Referensi	
	Ya (1)	0	0.0	2	100.0		0.046	0.0	164.864
5	HBOC-Related Family History								
	Tidak (0)	27	50.0	27	50.0	0.045	1	Referensi	
	Ya (1)	5	35.7	9	64.3		0.364	0.136	0.978
6	FIGO Stagging 2014								
	IIB (0)	4	26.7	11	73.3	0.015	1	Referensi	
	≥III (1)	28	52.8	25	47.2		3.731	1.291	10.781
7	Pre-Operative CA-125 (IU/mL)								
	<500 (0)	6	22.2	21	77.8	<0.001	1	Referensi	
	≥500 (1)	26	63.4	15	36.6		5.725	2.183	15.014
8	Intra-Operative Ascites Volume (mL)								

	<500 (0)	9	29.0	22	71.0	0.001	1	Referensi	
	≥500 (1)	23	62.2	14	37.8		4	1.771	9.03
9	Post-Cytoreductive Surgery Residual Tumor Lesion								
	R0 (0)	7	20.6	27	79.4	<0.001	1	Referensi	
	No R0/ Positive Residual Tumor Lesion (1)	25	73.5	9	26.5		5.102	2.199	11.837
10	Neoadjuvant Chemotherapy (NACT)								
	No (0)	25	41.7	35	58.3	0.003	1	Referensi	
	Yes (1)	7	87.5	1	12.5		3.795	1.56	9.234
11	Adjuvant Chemotherapy								
	Yes (0)	26	43.3	34	56.7	<0.001	1	Referensi	
	No (1)	6	75.0	2	25.0		14.021	4.612	42.626
12	Pathogenic BRCA1/2 Tumor Mutation								
	No (0)	27	55.1	22	44.9	0.035	1	Referensi	
	Yes (1)	5	26.3	14	73.7		0.354	0.134	0.93

Noted:

[a] *Fisher's Exact*

[b] *Chi-square*

* Significancy (p value <0,05)

Multivariate analysis was continued on the variables with p-value <0.2 by Cox regression analysis. In this study, 5 main variables were considered to have the most influence on survival, Table 4, i.e pathogenic BRCA1/2 tumor mutation, parity >1, pre-operative CA-125 levels ≥500 IU/mL, positive residual tumor lesion, and no adjuvant chemotherapy. The pathogenic BRCA1/2 tumor mutation group's likelihood of dying was 86% lower (adjusted RR 0.149; 95%CI 0.046-0.475; p-value=0.001), than without the pathogenic BRCA1/2 tumor mutation group. In the parity group >1, the likelihood of dying also lower 73% (adjusted RR 0.371; 95%CI 0.168-0.823; p-value=0.015), than parity ≤1 group. In the preoperative CA 125 level group ≥500 mIU/mL, the likelihood of dying increased by 3.2 times higher (adjusted RR 3.189; 95%CI 1.091-9.324; p-value=0.034) than preoperative CA 125 level <500 mIU/mL. In the positive residual tumor lesion group, the likelihood of dying was 6.98 times greater (adjusted RR 6.989; 95%CI 2.523-19.357; p-value<0.001) than R0 group (no macroscopic residual tumor). In the study sample group without adjuvant chemotherapy, the likelihood of dying increased to 46.94 times greater (adjusted RR 46.949; 95%CI 11.114-198.340; p value <0.001) than with the adjuvant chemotherapy group.

Table 4. The Most Influential Variables on Advanced Stage High-Grade Serous Epithelial Ovarian Cancer Patients Mortality Rate

No.	Variables	Beta Value	Error Standard	Significancy	RR/Exp (B) Adjusted	95% CI	
						Lower	Upper
1.	Pathogenic BRCA1/2 Mutation	-1.907	0.593	0.001	0.149	0.046	0.475
2.	Parity >1	-0.991	0.406	0.015	0.371	0.168	0.823
3.	CA-125 Level ≥500 mIU/mL	1.160	0.547	0.034	3.189	1.091	9.324
4.	Positive Residual Tumor Lesion	1.944	0.520	<0.001	6.989	2.523	19.357
5.	No Adjuvant Chemotherapy	3.849	0.735	<0.001	46.949	11.114	198.340

Cox-regression's multivariate analysis ENTER method for p-value <0.2 after bivariate analysis variables

Based on survival analysis, between the pathogenic BRCA1/2 tumor mutation group and without pathogenic BRCA1/2 tumor mutation group, Table 5, using Kaplan Meier-Log Rank Test, the pathogenic mutation group had a better median survival (median 46 months; 95%CI 34.009-57.991; p-value=0.001),

Figure 2, than no pathogenic BRCA1/2 tumor mutation group (median 23 months; 95%CI 15.657-30.343; p-value=0.001).

Table 5. Survival Analysis between the Pathogenic BRCA1/2 Tumor Mutation Group and the Non-Pathogenic BRCA1/2 Tumor Mutation Group Patients with Advanced Stage High-Grade Serous Epithelial Ovarian Cancer

No.	Pathogenic BRCA1/2 Tumor Mutation	Median				
		Survival Estimation (Months)	p-Value	Standart Error	95% Confidence Interval	
					Lower	Upper
1.	No Pathogenic BRCA1/2 Tumor Mutation	23.00	0.001	3.746	15.657	30.343
2.	Pathogenic BRCA1/2 Tumor Mutation	46.00		6.118	34.009	57.991
	Overall	36.00		6.713	22.842	49.158

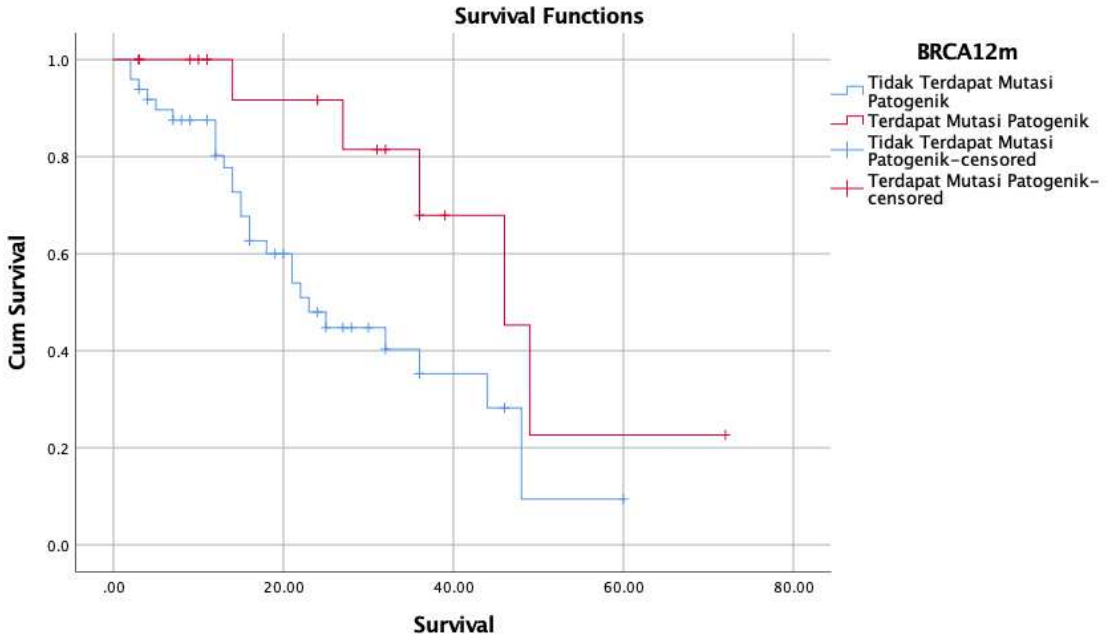

Figure 2. Kaplan-Meier Curve Effect of Pathogenic BRCA1/2 Tumor Mutation on Survival of Advanced Stage-High Grade Serous Epithelial Ovarian Cancer Patients

Discussion

This pathogenic BRCA1/2 tumor mutation detection, based on the Next Generation Sequencing study, both proportion, and evaluation of the pathogenic BRCA1/2 tumor mutation impact, on the survival outcome of epithelial ovarian cancer patients, is the first study in Indonesia.

In several developed countries, the BRCA1/2 mutations in ovarian cancer research are supported by the National Health Insurance System, which has approved and covered the cost of the BRCA1/2 gene detection in all epithelial ovarian cancer patients. Germline BRCA1/2 gene assay has been recommended for all epithelial ovarian cancer patients in these countries. In Indonesia, the detection of these genes, not yet included in the insurance financing system, has not become a routine detection in epithelial ovarian cancer patients, including at Dr. Cipto Mangunkusumo National Central Referral Hospital, Persahabatan Central Referral Hospital, and MRCCC Central Referral Hospital Siloam Jakarta. The expensive examination fees, cultural factors, social stigma, and guilty feelings towards other family members, allow patients to avoid this genetic testing. Therefore, we carried out this study to determine the proportion of pathogenic BRCA1/2

tumor mutation, and the impact of pathogenic BRCA1/2 tumor mutation on the survival outcome of advanced stage-HGSOC patients, at the three national center referral hospitals, as well as to socialize the importance of pathogenic BRCA1/2 tumor mutation's detections on the risk factor detection, management, and survival of epithelial ovarian cancer patients in Indonesia.

The Correlation of Age, Parity, Body Mass Index, Breast Cancer History, and Family History of HBOC with Mortality Incidence

In the Cox regression multivariate analysis of Table 4, parity is one of the variables that is considered to have the most significant effect on samples mortality incidence, but in contrast with age, body mass index, breast cancer history, and HBOC family history. In the parity group >1, the likelihood of dying was lower 73% (*adjusted RR* 0.371; 95%CI 0.168-0.823; p-value=0.015), than parity ≤1 group.

The Correlation of FIGO 2014 Staging, Pre-operative CA 125 Level, Intraoperative Ascites Volume, and Positive Residual Tumor Lesion with Mortality Incidence

In the Cox regression multivariate analysis Table 4, the residual tumor lesion is one of the variables considered to affect survival outcomes significantly. In the positive residual tumor lesion group, the likelihood of dying was 6.98 times greater (*adjusted* RR 6.989; 95%CI 2.523-19.357; p-value<0.001) than R0 group (no macroscopic residual tumor). This correlation was not seen in the FIGO 2014 staging variables, pre-operative CA 125 levels, and intraoperative ascites volume (p-value >0.05, respectively).

In the previous effect of BRCA1/2 mutational status on cytoreductive surgery residual tumor lesion's study, among 69 BRCA1/2 mutation patients, compared with 298 wild-type HGSOC patients (FIGO stage IIIC-IV), multivariate analysis showed that BRCA1/2 mutation status is not related to the residual tumor volume. Briefly, the better survival outcome of the pathogenic BRCA1/2 tumor mutation's group was not associated with cytoreductive residual tumor lesion.

The Correlation of The Neoadjuvant Chemotherapy (NACT), and Adjuvant Chemotherapy with Mortality Incidence

Regarding the administration of neoadjuvant chemotherapy (NACT) variable, in a previous study report, by comparing the survival rates of 49 patients who received NACT, based on the BRCA1/2 mutation status, the NACT (IDS) group had poor OS and PFS, compared to the without NACT (PDS) group, p=0.003 and p<0.001, respectively. There was no OS difference in the BRCA1/2 mutation, and without BRCA mutation groups (median 67.2 and 47.8 months; p value=0.231). The BRCA1/2 germline mutation group has a better PFS (median 17.2 and 14.2 months; p=0.014). Based on multivariate analysis, the BRCA1/2 mutation status was a good prognostic factor, increasing PFS (Huang, 2018).

Several other studies, also compared survival rates, in the primary management outcome of without pathogenic BRCA1/2 tumor mutation's group. Although there were no significant differences in FIGO stage characteristics, and residual tumor lesion, the NACT group had a significantly poor PFS than the PDS group (median 14.2 vs 16.9 months; p=0.003). Similar results were also reported in the retrospective multicenter study of Petrillo et al. In the BRCA1/2 mutations group. However, FIGO stage IV cases were more common in the NACT group, PFS did not differ between the NACT-IDS and PDS groups (P=0.082). However, the NACT group showed poor OS than the PFS group (5-year survival rates; 57.9% vs. 82.8%; p=0.040).

In this study, the highest incidence of mortality was seen in the neoadjuvant chemotherapy group, 87.5% (7/8). According to Table 3, 85.71% (6/7) of the incidence of death in this group, occurred in the group without pathogenic mutations, compared to 14.28% (1/7) in the pathogenic BRCA1/2 tumor mutation group. Based on the bivariate Chi-square/ Fisher's Exact analysis in the neoadjuvant chemotherapy group, the likelihood of dying was 3.79 times greater (crude RR 3.795; 95%CI 1.56-9.234; p-value=0.003), than the group without neoadjuvant chemotherapy. However, based on multivariate and Cox regression analysis, NACT did not significantly affect survival outcome (Table 4).

Based on Table 3, the highest mortality incidence was also seen in the group without adjuvant chemotherapy, which was 75% (6/8). In that group, the likelihood of dying was 14.02 times greater, significant statistically (crude RR 14.021; 95%CI 4.612-42.626; p-value <0.001), than receiving adjuvant chemotherapy group. Based on Cox-regression multivariate analysis, Table 4, the absence of adjuvant chemotherapy significantly increased the risk of death (adjusted RR 46.949; 95%CI 11.114-198.340; p-value<0.001).

The Effect of Pathogenic BRCA1/2 Tumor Mutation on Survival Outcome of Advanced Stage High-Grade Serous Epithelial Ovarian Cancer Patients

The BRCA mutation status may have different survival effects, after undergoing different primary treatments, due to differences in disease patterns or clinical characteristics of HGSOC and differences in chemotherapy response. At the time of diagnosis, HGSOC patients with the BRCA1/2 mutation were reported to have a higher peritoneal tumor load, and increased proportion of lymph node enlargement significantly, compared with the wild-type BRCA gene group. The retrospective study reported that nodular peritoneal disease was closely associated with BRCA mutation status, whereas mesenteric involvement and supra-diaphragmatic lymphadenopathy, were significantly associated with the wild-type BRCA gene (Kim et al., 2019). The high response rate to Platinum-based chemotherapy in the BRCA1/2 mutation group, likely to have a similar effect on the NACT-IDS and PDS groups, led to no difference in survival outcome, as shown in the results of this study.

Based on the survival outcome analysis in Tables 3 and 4, the pathogenic BRCA1/2 tumor mutation, significantly reduced the likelihood of death, up to 86% lower (adjusted RR 0.149; 95%CI 0.046-0.475; p-value=0.001), than without the pathogenic BRCA1/2 tumor mutation group. Based on multivariate analysis, between the pathogenic BRCA1/2 tumor mutations group, and without pathogenic BRCA1/2 tumor mutation, using Kaplan Meier-Log Rank Test analysis, the pathogenic mutation group had a better median survival (median 46 months; 95%CI 34,009-57.991; p value=0.001), when compared to the group without pathogenic mutations (median 23 months; 95%CI 15.657-30.343; p value=0.001). Administration of NACT did not affect survival in this study.

In this study, in addition to the pathogenic BRCA1/2 tumor mutation status, another variable that influenced overall survival outcome was the parity group >1 (adjusted RR 0.371; 95%CI 0.168-0.823; p-value=0.015), the pre-operative CA 125 level group ≥500 mIU/mL (adjusted RR 3.189; 95%CI 1.091-9.324; p-value=0.034) the positive residual tumor lesion group (adjusted RR 6.989; 95%CI 2.523-19.357; p-value<0.001), no adjuvant chemotherapy was given (adjusted RR 46.949; 95%CI 11.114-198.340; value p<0.001).

The Role of BRCA1/2 Gene Structure and Function on Ovarian Carcinogenesis

The clinical characteristics, aggressive disease pattern, and good chemotherapy response are the hallmarks of HGSOC with BRCA1/2 gene mutation, closely related to the gene molecular behavior. The BRCA1 and BRCA2 genes are separate, on two different chromosomes (17q21 and 13q12.3, respectively). Both genes have particular primary sequences, but disturbances in one or both of these genes will give the same pathophysiological effect, in the form of the same spectrum of cancer (Fanale et al., 2021).

The BRCA1 and BRCA2 genes are tumor suppressor genes, that maintain genomic stability and control cell growth (Gorodetska et al., 2019). The BRCA1 and BRCA2 proteins mainly play a role in the repair of DNA double-strand breaks (DSBs) through the homologous recombination (HR) pathway (Liu and Lu, 2020). DSBs are repaired through 2 main pathways, nonhomologous end-joining (NHEJ) and HR. NHEJ will end with changes in the DNA sequence at the site of damage (Pannunzio et al., 2018). In the presence of DSBs, HR allows the exchange of the same genetic sequence, from healthy homologous sister chromatids to the site of damage occurrence, resulting in accurate DNA damage repair, and high genomic stability (Shen and Li, 2022). The roles of the two proteins, BRCA1 and BRCA2, are significant in repairing of DSBs via HR. Deficiency of BRCA1 or BRCA2 function can result in a high degree of chromosomal instability, such as chromosome breaks, severe aneuploidy, and centrosome amplification (Girolimetti et al., 2014), thereby stimulating an alternative pathway for DSBs repair, NHEJ, resulting in mutation accumulation (Kay et al., 2019). Genetic aberrations can occur spontaneously, greatly assisting the mechanism. The action of DNA strand-destroying agents, inducing DSBs, particularly DNA cross-linking agent Platinum (Rycenga and Long, 2018), explains the better response to Platinum therapy in patients with BRCA1/2 mutated ovarian cancer, compared with no BRCA1/2 mutation group.

Currently, several BRCA1 and BRCA2 interactor proteins have been identified. RAD51 plays a role in improving DSBs, the most essential part of the HR process. Its function is critical to the completeness of the protein encoded by the two genes BRCA1/2 (Wassing and Esashi, 2021). Some studies have successfully described the role of BRCA2 in the regulation for RAD51 recruitment and repair of DNA double stand breaks (DSBs) (Mishra et al., 2022). BRCA1 exhibits physical interactions with RAD51, forming the complex responsible for resectioning single-stranded DNA, at the site of the double-strand breakage. Other studies have explained the role of BRCA1 in changing chromatin structure, due to DNA damage, thus allowing access to repair damaged DNA structures (Broering et al., 2014). DNA damage will be followed by extensive phosphorylation of histone H2AX, and form a focus at the site of damage. BRCA1 was recruited into the foci,

before involving other factors, such as RAD51. This explains that H2AX and BRCA1 initiate the DNA repair mechanism, by modifying the local chromatin structure, allowing DNA repair proteins to reach the site of damage. BRCA1 and BRCA2 also function as transcriptional co-regulators and chromatin remodeling functions. BRCA1 has the ability to co-activate p53-dependent endogenous p21 stimulation.

The Platinum and PARP Inhibitor's Promising Therapeutic Response for BRCA1/2 Mutation's Ovarian Cancer Group

Although BRCA mutation-associated ovarian cancers appeared to be more aggressive, compared to sporadic ovarian cancers, the group showed higher sensitivity to Platinum agent and other DNA-damaging regimens. Platinum agent intervention occurs in the DNA cross-links process, causing double-stranded DNA helical damage, which cannot be repaired due to disruption of HR repair mechanisms. Several studies have shown an increased long-term survival rate in women with BRCA mutation-associated ovarian cancer, with Platinum agent chemotherapy, compared to the sporadic group (Foulkes and Shuen, 2013). Intraperitoneal Cisplatin chemotherapy has shown good long-term outcomes, in advanced ovarian cancer associated with the BRCA1/2 mutation (Kwa et al., 2013).

Regarding the management of pathogenic BRCA1/2 mutation ovarian cancer, several previous studies have been conducted to evaluate the effect of BRCA1/2 germline mutation on the prognosis of epithelial ovarian cancer patients. In the BRCA1/2 mutation group, only OS appeared to be significantly longer, compared to the wild type group, but not for PFS (Seo et al., 2019). Another study reported that OS and PFS were significantly longer in the BRCA1/2 mutation group (Huang, 2018). An Israeli national population study reported that BRCA1/2 mutational status increased long-term survival (Magwood et al., 2012). Analysis of The Cancer Genome Atlas found that BRCA2 mutation, but not BRCA1 mutation group, was associated with significantly increased OS and PFS (Neff, Senter, and Salam; 2017).

According to a meta-analysis study, the response to Platinum chemotherapy in the pathogenic BRCA1/2 mutation group was better, compared to wild-type patients (OR 2.64; 95% CI: 1.38-5.05; p=0.003). When compared with the sporadic group, patients with the pathogenic BRCA 1/2 mutation had a higher complete response rate (OR 2.14; 95% CI: 1.49-3.08; $p < 0.001$), lower partial response (OR 0, 60; 95% CI: 0.39–0.91; $p < 0.017$), and longer progression-free survival (PFS). It is known that the increased response to chemotherapy in patients with the BRCA1/2 mutation is possible due to inhibition of the DNA repair pathway, which sensitizes tumor cells to the DNA-damaging effects of chemotherapy (Li et al., 2021).

In recent years, poly (ADP-ribose) polymerase enzyme (PARP) inhibitors, have emerged with promising therapeutic approaches, in the BRCA1/2 mutation group. PARP is a protein class that produces large branched poly (ADP) ribose (PAR) chains from NAD+. PARP is involved in some pathways, including regulation of transcription, DNA replication, and repair of DNA damage (Pujade-Lauraine, 2017). Of several PARP proteins detected, PARP-1 and PARP-2 were found to play the most role in DNA stability (Kutuzov et al., 2021). PARP-1 is a highly potent nuclear enzyme, plays a role in helping repair single-strand breaks (SSB) through the BER pathway, so it has a very important role in genomic integrity. Inhibition of PARP activity causes DNA lesions, due to inefficient repair of SSBs, leading to DSB or replication fork collapse. This damage requires the function of BRCA1 and BRCA2 in DNA repair (Rose et al., 2020). In the presence of BRCA1 or BRCA2 defects, HR is impaired. Therefore, inhibition of PARP may result in the replication process, with DNA lesions that cannot be repaired effectively, leading to decreased chromosomal stability, cell cycle arrest, and/or cell death (Schoonen and van Vugt, 2017). The cells with normal BRCA1 or BRCA2 deficiency and associated tumors are more sensitive to PARP inhibitors than heterozygous mutant or wild-type cells (Moschetta et al., 2016). Wild-type cells, and heterozygous BRCA1 or BRCA2 can repair DSB, maintaining cell viability. Ovarian cancer patients with BRCA mutations a lack of wild-type BRCA1 or BRCA2 in their tumor cells, but normal cells retain one copy of the wild-type gene. Therefore, PARP inhibitors selectively exert a lethal effect on cells with deficient BRCA1 or BRCA2 function, associated with minimal toxicity to normal cells (Moschetta et al., 2016).

The FDA has approved three classes of PARP inhibitors (classes I-III) as maintenance therapy in patients with primary ovarian, fallopian tube, and peritoneal cancer, who show complete or partial response to Platinum-based chemotherapy, regardless of BRCA and HRD mutation status. However, data from 4 RCTs (Ledermann et al., 2016b; Matulonis et al., 2016; Mirza et al., 2016; Coleman et al., 2017; Dougherty et al., 2017), showed that the main benefit of PFS PARP inhibitors over placebo, especially in the germline or somatic BRCA mutation group (HR 0.18-0.3), followed by HRD positive tumors (HR 0.32-0,38) (Ledermann

et al., 2016b; Matulonis et al., 2016), and the weakest effect was seen in the wild-type BRCA group and HRD negative tumors (HR 0.58 ARIEL3 and NOVA studies).

Three other RCTs have evaluated the incorporation of PARP inhibitors, as first-line therapy, VELIA, PRIMA, and PAOLA-1. In all three RCTs, tumors with BRCA mutations showed the greatest benefit of PARP inhibitor therapy, HR 0.4 (maintenance of PARPi class II versus placebo) PRIMA; HR 0.31 (maintenance of PARPi class I and anti-VEGF versus anti-VEGF and placebo) PAOLA-1; and HR 0.44 (chemotherapy-PARPi class II followed by maintenance of PARPi class II versus chemotherapy, and placebo followed by maintenance placebo) VELIA. Stratification of VELIA and PAOLA-1 RCTs based on BRCA mutation status (tumor BRCA mutation status in PAOLA-1, and BRCA germline mutation in VELIA). In this RCT, compared with tumor BRCA mutations, the benefit of adding a PARP inhibitor in patients with BRCA wild-type tumors showed at least clinical benefit, HR 0.8 (95% CI 0.64-1.00) in VELIA127 and HR 0, 71 (95% CI 0.58-0.88) at PAOLA-1 (Ray-Coquard et al., 2019).

The Timing of Pathogenic BRCA1/2 Mutation Detections

Women with epithelial ovarian cancer should be offered genetic testing during histopathological diagnosis. If a BRCA1/2 germline mutation test is unavailable at the time of diagnosis, it should be offered as soon as possible, whenever possible (time, place, and cost). This examination has clinical implications regarding treatment decisions (ASCO Guidelines LoE high; strong recommendation) (Konstantinopoulos et al., 2020).

In women who do not carry a pathogenic germline mutation or a likely pathogenic BRCA1/2 variant, testing for a pathogenic BRCA1/2 somatic mutation or a likely pathogenic variant may be offered. BRCA1/2 somatic mutation examination can be performed until the time of recurrence/relapse, for women who have completed the first complete treatment modality, and in the follow-up period, due to the implications for further management according to the FDA (ASCO Guidelines LoE intermediate; moderate recommendation) (Konstantinopoulos et al., 2020).

Research Limitations and Strengths

This study has several limitations. First, selection bias and other issues can arise regarding the design of historical cohort studies. Second, the monitoring period was short due to the limited research period. Third, in this study, there was no assessment of therapeutic response, and evaluation of the primary disease-free interval, either in the BRCA1/2 tumor pathogenic mutation group, or in the no mutation group, due to the lack of complete post-adjuvant monitoring data (periodic clinical, imaging, and tumor marker CA-125 level monitoring). Based on this study, evaluation of the completeness of medical records is an important factor for the sustainability of the research, especially in teaching hospitals. Fourth, the number of samples of the pathogenic BRCA1/2 tumor mutation group was limited, because not the entire list of target populations could be included as the study sample. A total of 39.58% of the target population did not meet the re-selection of the inclusion criteria by Pathologists, due to a mismatch of histopathological types, and low tumor cell content. Suboptimal FFPE sample quality, low DNA integrity index (DIN), was recorded in 21.84% of research sample candidates, due to high DNA fragmentation (<120-150 bp), so it did not pass the NGS quality control. Both caused the limited number of samples in several variables of clinical characteristics (history of breast cancer, neoadjuvant chemotherapy administration, BRCA gene domain mutation), correlation analysis could not be assessed.

In addition to the study's limitations, the strengths of this study include a more specific study population, only involving advanced stage-high grade serous epithelial ovarian cancer patients. The selection of inclusion criteria for all samples was carried out directly by single expert Pathologist so that interobserver variations could be avoided. The pathogenic BRCA1/2 tumor mutations were examined in an EMQN (European Molecular Genetics Quality Network) certified laboratory. The control of survival bias was carried out by ensuring that the factors that influenced survival were included in the statistical analysis. Research methodologies are clearly defined, produce valuable useful accurate data, applicable in clinical practice.

Conclusion

In advanced stage-HGSOC, patients with pathogenic BRCA1/2 tumor mutations have a better prognosis with longer survival outcome than those without pathogenic BRCA1/2 tumor mutations.

Abbreviations

ASGO : American Society of Gynecologic Oncologists; BRCA: Breast Cancer (susceptibility gene); HBOC: Hereditary Breast and Ovarian Cancer syndrome; HGSOC: High Grade Serous Epithelial Ovarian Cancer; HR: Homologous Recombination; NHEJ: Non-Homologous End Joining; RR: Relative Risk.

Author's contribution

Sutrisno: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing-original draft, writing-review and editing, visualization, supervision, project administration, funding acquisition study concept, data collection, data interpretation, and writing the paper.

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Availability of data and materials

The data used to support the findings of this study are included in the article.

Declarations

Ethics approval and consent to participate

The data used in this study is secondary data from medical records. The authors did not make any contact with the patients. Thus, based on our regulations (Peraturan Menteri Kesehatan NOMOR 269/MENKES/PER/III/2008), the study did not expose any of the patients' data, the medical records could be used for study purposes.

Consent for publication

Not applicable

Competing interest

The authors declare no conflict of interest in preparing this article.

Provenance and peer review

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