doi:10.4149/neo_2021_210226N256

High-grade serous ovarian carcinoma and detection of inactivated BRCA genes from biopsy material of Slovak patients

Katarína JANÍKOVÁ[1,2,*], Barbora VÁŇOVÁ[3], Marián GRENDÁR[4], Marek SAMEC[5], Alenka LÍŠKOVÁ[5], Dušan LODERER[2], Ivana KAŠUBOVÁ[2], Anna FARKAŠOVÁ[6], Karla SCHEEROVÁ[6], Pavol SLÁVIK[1], Zora LASABOVÁ[7], Zuzana DANKOVÁ[2], Lukáš PLANK[1,2,6]

[1]Department of Pathological Anatomy, Comenius University in Bratislava, Jessenius Faculty of Medicine and University Hospital in Martin, Martin, Slovakia; [2]Biomedical Center, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Martin, Slovakia; [3]Martin's Immunology Center, Ltd., Martin, Slovak Republic; [4]Bioinformatic Center, Biomedical Center Martin, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Martin, Slovakia; [5]Clinic of Obstetrics and Gynecology, Comenius University in Bratislava, Jessenius Faculty of Medicine and University Hospital in Martin, Martin, Slovakia; [6]Martin's Biopsy Center, Ltd., Martin, Slovakia; [7]Department of Molecular Biology and Genomics, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Martin, Slovakia

**Correspondence: janikova.katarina13@gmail.com*

Received February 26, 2021 / Accepted May 3, 2021

Ovarian cancer is the leading cause of mortality among all gynecological cancers in developed countries and its most common and most lethal type is the high-grade serous ovarian carcinoma (HGSC). At the molecular level, nearly half of all HGSCs exhibit ineffective homologous DNA recombination and disruption of DNA damage/repair pathway inactivation caused often by BRCA1 and BRCA2 gene mutation. Recently, the detection of BRCA1/2 mutations became important for personalized treatment of HGSC patients with the PARP-inhibitors in the defined clinical setting of relapse after positive adjuvant platinum-based chemotherapeutic response. Based on the selection of patients by regional oncologists, we attempted to verify the possibilities of BRCA1/2 mutation testing on archival formalin-fixed paraffin-embedded (FFPE) biopsy material from regional hospitals. In the study we used: a/ FFPE tumor resections of 97 patients sent to our laboratory, originally stored in archives of regional departments for a period of 1–3 years and retrieved on the principle to contain a maximum of non-necrotic tumor tissue, b/ next-generation sequencing (NGS) assay covering all known mutations in the BRCA1/2 genes on MiSeq (Illumina® platform), and c/ Sophia DDM® bioinformatics platform. After processing of FFPE samples, 5 cases were excluded due to the insufficient genomic DNA quantity. Bioinformatics results of NGS analyses of 92 patients' samples indicated 17.39% pathogenic mutations and 32.61% potentially pathogenic mutations in genes BRCA1/2. Overall, 50% pathogenic and potentially pathogenic mutations were detected in the patient's cohort. The relatively high incidence of BRCA1/2 mutations in our series may be influenced by various indicators including the selection of patients based on adjuvant therapy response as well as regional or population heterogeneity in their frequency. Based on the interdisciplinary cooperation, the use of archival biopsy material processed primarily and stored for a longer period in different laboratories without uniformly defined pre-analytical conditions allows identifying the HGSC patients who might better respond to the PARP-inhibition therapy.

Key words: HGSC, BRCA1, BRCA2, PARP-inhibitor, SOPHIA DDM

Ovarian carcinomas, the majority of which are high-grade serous carcinomas (HGSC), represent one of the most common causes of cancer death in women worldwide [1–3]. The same is observed in the Slovak population showing the trend of increasing incidence of HGSC in recent years [4]. The mortality of HGSC seems to be related to the low effectiveness of the traditional treatment of patients undergoing in the first line an aggressive surgical cytoreduction, followed by platinum and taxanes-based chemotherapy [5]. Moreover, relapse and subsequent chemoresistance occur in about 80% of all HGSC patients [6]. Recently, the molecular biology behind the HGSC carcinogenesis became to be crucial for the utilization of principles of personalized medicine allowing to change the paradigm of HGSC therapy.

The most prominent molecular changes in HGSC include alterations in the *TP53* gene, nearly always mutations (virtually all), and inactivation of *BRCA1* and *BRCA2* genes caused by mutations in nearly half of the cases [3, 7]. Biologically, the activity of *BRCA1/2* genes and BRCA proteins is essential for the process called homologous recombination (HR), which is responsible for the repair of double-strand DNA breaks within the so-called DNA damage/repair (DDR)

pathway. The *BRCA1/2* gene mutations cause alterations of BRCA protein function leading to an ineffective DNA repair by HR and accumulation of mutated DNA. These changes may result in genomic instability, carcinogenesis, or cell death [8]. However, an impaired repair system in the DDR pathway ensures higher sensitivity of carcinoma response to platinum-derived chemotherapeutics [9, 10]. The action of these drugs results in a more pronounced effect on tumor cells deficient in HR and relatively less damage to non-tumor cells [11]. The complex HR process also involves a proper activity of the enzyme poly-ADP/adenosine-diphosphate/ribose polymerase (PARP). Nowadays, pharmacological PARP-inhibition is used as a new molecular targeted therapy effective especially for HGSC patients exhibiting *BRCA1/2* mutations associated with HR deficiency [12]. The PARP-inhibitors disable the proper function of PARP in the cells and cause accumulation of damaged DNA with single-strand as well as double-strand breaks followed by apoptosis of tumor cells [13].

The properly adjusted treatment of individual HGSC patients in terms of personalized medicine requires molecular diagnostic in a form of complex tumor DNA genomic profiling by next-generation sequencing (NGS) [14, 15]. However, the detection of *BRCA1/2* gene mutations is associated with difficulties in routine diagnostics that can cause complications of primer design for a polymerase chain reaction, including autosomal genes with a wide range of mutations rich in homopolymer domains and highly polymorphic [14]. In our pilot study, we attempted to verify the possibilities and limits of *BRCA1/2* mutation NGS testing using the routine archival formalin-fixed paraffin-embedded (FFPE) biopsy material of the patients with HGSC as a possible contribution to oncological practice in the Slovak Republic.

Patients and methods

Subjects-patient population and samples. Genetic testing was performed in a series of consecutive resection biopsies of female patients aged 39 to 82 years at the time of testing with a median age of 63 years. At the time of the primary diagnosis of ovarian cancer, patients were examined in various Slovak hospitals in the period from April 2016 to May 2019. After surgical resection of the tumor, the baseline diagnosis of HGSC was histologically confirmed in local pathology laboratories. The patients were then treated in regional oncology centers. The selection of patients for molecular testing of *BRCA1/2* gene mutations administrated by oncologists required a clinical setting of relapsed HGSC after the previous two lines of cytotoxic chemotherapy. Participants were included in the study without regard to age at diagnosis and breast or ovarian cancer family history. The study was approved by the Ethical Committee of the Jessenius Faculty of Medicine in Martin (EC 1755/2015).

After the oncologist's indication to perform the molecular analysis, FFPE blocks of HGSC samples were retrieved from the archives of regional departments of pathology. The responsible pathologists were asked to select the FFPE blocks that contain a maximum of the non-necrotic "viable" tumor tissue. These FFPE blocks were sent to Biomedical Center Martin, Jessenius Faculty of Medicine in Martin and were re-analyzed by two independent pathologists (LP, PS) in agreement with WHO classification criteria [3]. The second biopsy reading included a confirmation of the HGSC diagnosis and evaluation of the viable tumor cells percentage in tissue sections cut from the FFPE blocks. The molecular diagnostics performed in Biomedical Center Martin followed a) the ESMO/ESGO consensus conference recommendations for the molecular pathologic testing of biopsy samples of patients with ovarian carcinoma [16] and b) national guidelines of standards for testing germline mutations in hereditary syndromes [17] modified for the examination of FFPE tissue samples.

DNA isolation. Genomic DNA was isolated from FFPE samples sections using blackPREP FFPE DNA Kit (Analytik Jena AG, Germany) according to the supplier's instructions.

Qualitative and quantitative analysis. In accordance with recommended isolation and sequencing protocols, the following kits for quantitative and qualitative DNA analyses were used: Qubit™ ds DNA BR Assay Kit, Qubit™ ds DNA HS Assay Kit (both Invitrogen™, ThermoFisher Scientific, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., USA). The quantification and qualification of DNA were performed completely according to the manufacturer's instructions. The protocol limit requirements for the quantity of genomic DNA was ≥20 ng/μl.

Preparation of libraries and sequencing. The next step was a preparation of DNA libraries by BRCA MASTR Plus DX with drMID Dx for Illumina® NGS systems (Multiplicom, N.V., Agilent Technologies, Inc., USA) without any change of the manufacturer's protocol IFU494, v.170906. The protocol was approved by directive 98/79/EC as In Vitro Diagnostic Medical Devices (CE-IVD). For the sequencing reactions, the MiSeq reagent Kit v2 – 500 cycles chemistry on the MiSeq platform (Illumina®, USA) was used. The sequencing met the requirements for testing with coverage of all regions of exons and introns with coverage of all known pathogenic *BRCA1/2* variants.

The workflow. The workflow of our study consisted of 21 sequencing runs, three of them represented repeated runs of the previous ones showing low accuracy of reads as rated by Q-score. Importantly, the number of samples in one run varied from 2 to 12 patients' samples depending on the actual arrival dates of the samples.

Bioinformatics analyses. For data analyses and interpretation, we used the Sophia DDM® platform (Sophia Genetics™, Switzerland) recognizing 5 pathogenic categories of the DNA variants according to the ACMG recommendations [18]. The platform includes a software filter powered by artificial intelligence, which filters variants based on their impacts on gene and frequency. Only pathogenic and potentially pathogenic

variants were evaluated for the variant prediction analyses. The set-up filter criteria included the read depth of interpreted variants being ≥1,000 and the required frequency of detected variants being ≥5%.

Final reports. In agreement with the Position Paper of the European Society of Pathology [19], the molecular results were delivered in the context of an integrated biopsy diagnosis. For the patients with proved *BRCA1/2* pathogenic mutation, the report also included a recommendation for genetic counseling for the patient.

Results

FFPE blocks that we retrieved from the registers of pathology departments and used for the analysis were stored in the archives for the interval of 1–3 years. Histologically, all 97 cases fulfilled the criteria of the HGSC subtype of ovarian carcinoma. The viable carcinoma cells percentage in the given FFPE block section of each HGSC case was higher than 70%; therefore, the microdissection of the paraffin sections used for DNA isolation was not applied.

In 92 cases, the DNA concentration was sufficient, reaching the average value of 170 ng/µl. In 5 cases (5.2% of all cases submitted for analysis), DNA concentration was lower than the protocol limit requirements for the quantity of genomic DNA, and these cases were excluded from further sequence analyses. During 18 sequencing runs, we monitored elementary values of quality, which reached percentual average of QC – 82%, a percentual average of PF – 89%, and an average of clusters density – 700 k/mm^2.

The sequencing analyses of DNA isolated from FFPE recognized *BRCA1* and/or *BRCA2* mutations. The *BRCA1* gene variants classified as pathogenic mutations were identified in 10 patients (10.9% of all tested); in addition to a mutation of deletion type, a coincidental potentially pathogenic mutation of deletion type in the *BRCA2* gene was recognized in one of them. The variants classified as pathogenic mutations of the *BRCA2* gene were identified in samples of 6 patients (6.5% of all tested biopsies). Altogether, the pathogenic mutations in both examined genes were identified in biopsy samples of 16 HGSC patients (17.4% of all tested biopsies). Potential pathogenic mutations in *BRCA1/2* genes were identified in 30 (32.6%) HGSC patient samples, particularly *BRCA1* potential pathogenic mutations in 14 (15.2%) patients and *BRCA2* potential pathogenic mutation in 16 (17.4%) patients. In the biopsy samples of 46 (50% of all) HGSC patients, neither pathogenic nor potentially pathogenic *BRCA1/2* gene mutation was found (Figure 1). The results (including the relative incidence of substitution, deletion, and duplication pathogenic mutation types) are summarized in Table 1 and graphically represented in Figure 1 and Figure 2.

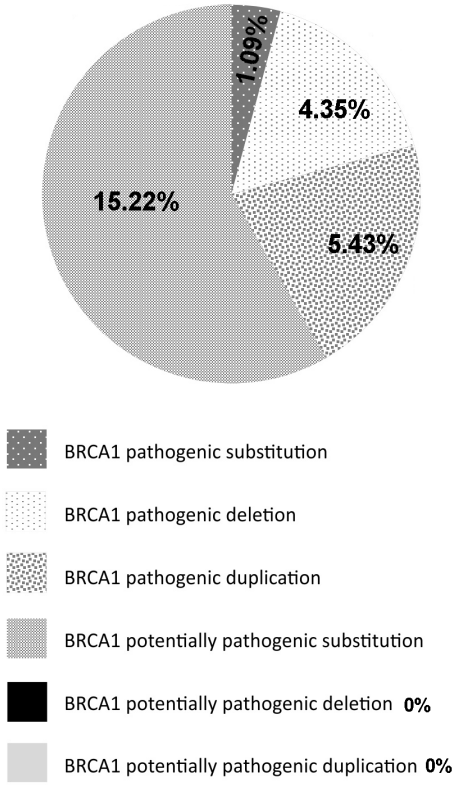

Figure 1. Detected BRCA1 mutations after NGS. Approximately 26% of BRCA1 variants were identified in total.

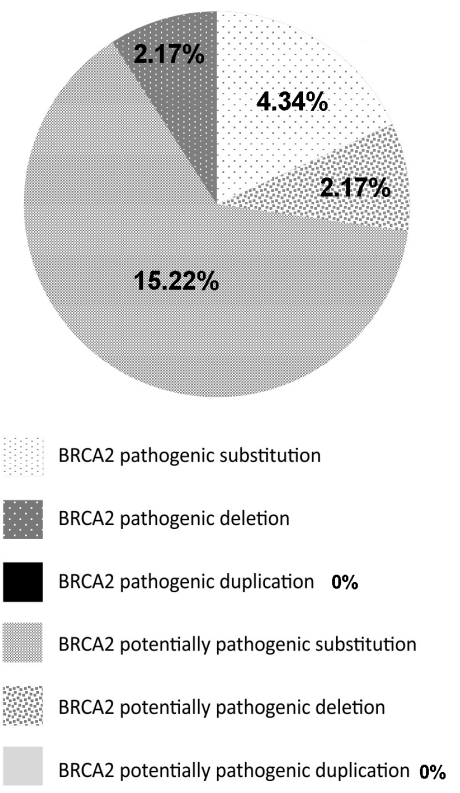

Figure 2. Detected BRCA2 mutations after NGS. Approximately 24% BRCA2 variants were identified in total.

Table 1. The summary of NGS analyses: detected pathogenic and potentially pathogenic BRCA1/2 mutations. The classification is based on the type of mutations: substitution, deletion, duplication.

Mutation	*BRCA1* gene n/% of all cases			*BRCA2* gene n/% of all cases			Total
	Substitution	Deletion	Duplication	Substitution	Deletion	Duplication	
Pathogenic	1/1.09	4/4.35	5/5.43	4/4.35	2/2.17	0/0	16/17.39
Potentially pathogenic	14/15.22	0/0	0/0	14/15.22	2/2.17	0/0	30/32.61
Total	15/16.31	4/4.35	5/5.43	18/19.57	4/4.35	0/0	46/50.00

Discussion

The comprehension of genetic alterations, which impair the process of HR and DNA damage repair mechanisms, contributes to a better understanding of carcinogenic complexity as well as cancer biology and therapy [8]. These alterations include not only inactivating mutations of the *BRCA1/2* genes but also their epigenetic silencing via hypermethylation and alterations of other genes, e.g., *ATM, BARD1, BRIP1, CDH1, CDKN2A, CHEK2,* etc. However, *BRCA1* and *BRCA2* mutations account for the majority of pathologic changes in the DDR pathway [16]. Overall, 50–60% of ovarian cancers are characterized by some alterations in the HR pathway [16], and virtually all "*BRCA*-associated ovarian cancers" exhibit high-grade serous morphology [3].

For many years, the biological impact of these mutations was used in diagnostic oncology and therapeutic practice. The effect of *BRCA1/2* mutations on higher sensitivity of the cytotoxic chemotherapeutical regimes has been utilized for the adjuvant therapy of HGSC patients [9, 10]. From the diagnostic point of view, the identification of pathogenic germline *BRCA1/2* mutations in the patient's blood allowed to detect the carriers of the HBOC/HBOPC (hereditary breast, ovarian, and pancreatic cancer) syndrome [16]. Recently, the identification of the HR deficiency via the detection of *BRCA1/2* mutations in DNA isolated from tumor tissue of HGSC patients starts to be clinically relevant. It may allow the identification of women who may benefit from targeted therapy using PARP inhibitors that represent a new therapeutical modality for HGSC patients [20]. The clinical studies have demonstrated that PARP inhibition of recurrent HGSC with pathogenic *BRCA1* or *BRCA2* mutations improves the patient's progression-free survival. Therefore, the implementation of molecular pathological analyses of the *BRCA1/2* gene status complementing the workflow of the patient's biopsy is necessary for targeted and individualized treatment of HGSC [16, 19].

The typical screening techniques for *BRCA* testing used for the identification of germline mutations traditionally include Sanger sequencing of coding exons and multiplex ligation-dependent probe amplification (MLPA) using high-quality DNA obtained from blood. However, these methods have considerable limitations when rapid predictive testing of genetic aberrations including somatic mutations is required to ensure the best therapy for compromised ovarian cancer patients. Sanger sequencing and MLPA are both time-consuming, require high-quality DNA obtained from blood, and fail to detect somatic tumor mutations that account for approximately 10–40% of mutated cases in platinum-sensitive, high-grade serous recurrent ovarian carcinomas [21, 22].

Due to increased demands for *BRCA* gene mutation detection, rapid and comprehensive diagnostic methods are required. Using an easily accessible HGSC biopsy material, the testing of *BRCA* mutation through NGS permits the simultaneous assessment of all types of mutations [23]. In this study, we used DNA samples isolated from resection FFPE biopsy material of HGSC patients and BRCA MASTR Plus DX kit as a comprehensive solution for detection of the inactivated *BRCA* genes by NGS analyses. This high-throughput method allows the detection of genetic aberrations in a more complex way by harnessing its massively parallel sequencing capability to analyze several genes for multiple mutation types simultaneously in a single run and in a relatively short time [24]. Sophia DDM® software was selected for the bioinformatics analysis, in agreement with recommendations of the diagnostic kit manufacturer and in the context of incompatibility of data interpretation of the *BRCA* mutations in publicly available databases [25]. This tool offers data analysis and variant filtering based on a set of criteria and at the same time, it predicts the possible pathological effect of variants [https://www.sophiagenetics.com/hospitals/sophia-ddm/sophia-ddmr-details.html].

In the past, the studies on the molecular pathology of sporadic HGSC were hampered also by technical challenges in performing molecular studies on FFPE tissues [16]. In our study, tested HGSC biopsy samples represented archival material retrieved from the pathology register in the relapsed settings after surgical resection at baseline followed by adjuvant chemotherapy. Analyzed biopsy materials were obtained from regional hospitals without previous standardization of pre-analytical factors e.g., use of buffered formalin fixation only, standard cold ischemia time, etc. In addition, samples were stored in local pathology register files for at least 1 to 3 years. Nevertheless, due to the selection of the most proper tissue blocks for the analysis, most of them might be used for the isolation of tumor DNA in sufficient quality and quantity allowing subsequent NGS analyses.

Altogether, we used Sophia DDM® software to identify *BRCA1/2* variants classified as pathogenic mutations in 17.4% and variants classified as potentially pathogenic mutations in

32.6% of HGSC patients' samples. In the subset of pathogenic variants, all mutation types including substitutions, deletions, and duplications were identified, while the potentially pathogenic mutations were represented almost exclusively by substitutions. The relatively high incidence of *BRCA1/2* mutations in our series might results partly from the oncologist's preferential selection of the patients responding sufficiently to the cytotoxic chemotherapy, partly due to the limited number of patients in the study. However, the regional and/or population heterogeneity of *BRCA* mutations frequency is obvious and analyses performed in various geographic regions revealed significant differences not only in frequencies but also in types of mutations in the *BRCA1/2* genes [26]. For example, Gornjec et al. [27] reported 39% and Krivokuca et al. [28] 21% incidence of the *BRCA1/2* mutations among patients with HGSC from Slovenia and Serbia, respectively.

Although HGSC patients with unmutated *BRCA* status might be indicated for the PARP inhibition therapy, the mutated *BRCA* status is associated with a better therapeutical response of the patient [16]. Therefore, it remains to be answered whether mutations identified in the tissue biopsy samples by NGS should be verified by consecutive Sanger and/or liquid biopsy DNA sequencing. The same is true for the indication of patients with other cancers for the personalized therapy using PARP inhibitors.

Concerning our study, *BRCA* mutations revealed a high incidence in the analyzed cancer tissue samples among the population of Slovak women. Genetic changes in *BRCA* genes represent a positive predictive biomarker for targeted therapy with PARP inhibitors. The aim of the study was an improvement in the stratification of female patients suitable for this type of therapy. Due to the progress in new methods capable of stratifying patients based on the genetic profile of their tumors and identifying particular mutated genes falling under the criteria of administration of these inhibitors, we are on the right way to ameliorate diagnostic practice in Slovakia and also to improve the overall survival of patients with HGSC according to the principles of the precision medicine in the 21st century.

Acknowledgments: We thank the Institute of Molecular Biology and Genomics of Jessenius Faculty of Medicine in Martin for scientific and technical support, Biomedical Centre Martin (IMTS26220220187), which is co-financed from EU sources, Astra Zeneca for education grant supporting the implementation of NGS analyses, and to Comenius University Grants for Doctoral Students (UK/12/2016).

References

[1] BRAY F, FERLAY J, SOERJOMATARAM I, SIEGEL RL, TORRE LA et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394–424. https://doi.org/10.3322/caac.21492

[2] REICHERT RA (EDS.). Diagnostic gynecologic and obstetric pathology an atlas and text, 1st Edition. Philadelphia, Wolters Kluwer Lippincott Williams & Wilkins; 2012, p 648. ISBN 978-1-60831-077-7.

[3] KURMAN RJ, CARCANGIU ML, HERRINGTON CS, YOUNG RH (Eds.). WHO Classification of Tumours of Female Reproductive Organs. WHO Classification of Tumours,4th Edition. IARC, Lyon, 2014. ISBN 978-92-832-4487-5.

[4] [National Health Information Center] [National Oncological Register of the Slovak Republic – presentation of outputs]. https://iszi.nczisk.sk/nor.sr/incidencia/vyvoj/1997-2011/diagnozy, 2020. [As accessed on May 12, 2020]

[5] MARTÍN-CAMEÁN M, DELGADO-SÁNCHEZ E, PIÑERA A, DIESTRO MD, DE SANTIAGO J et al. The role of surgery in advanced epithelial ovarian cancer. Ecancermedicalscience 2016; 10: 666. https://doi.org/10.3332/ecancer.2016.666

[6] LUVERO D, MILANI A, LEDERMANN JA. Treatment options in recurrent ovarian cancer: latest evidence and clinical potential. Ther Adv Med Oncol 2014; 6: 229–239. https://doi.org/10.1177/1758834014544121

[7] KÖBEL M, KALLOGER SE, HUNTSMAN DG, SANTOS JL, SWENERTON KD et al. Differences in tumor type in low-stage versus high-stage ovarian carcinomas. Int J Gynecol Pathol 2010; 29: 203–211. https://doi.org/10.1097/PGP.0b013e3181c042b6

[8] PRAKASH R, ZHANG Y, FENG W, JASIN M. Homologous Recombination and Human Health: The Roles of BRCA1, BRCA2, and Associated Proteins. Cold Spring Harb Perspect Biol 2015; 7: a016600. https://doi.org/10.1101/cshperspect.a016600

[9] BOLTON KL, CHENEVIX-TRENCH G, GOH C, SADETZKI S, RAMUS SJ et al. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. JAMA 2012; 307: 382–390. https://doi.org/10.1001/jama.2012.20

[10] CHAOYANG S, LI N, DING D, WENG D, MENG L et al. The Role of BRCA Status on the Prognosis of Patients with Epithelial Ovarian Cancer: A Systematic Review of the Literature with a Meta-Analysis. PloS One 2014; 9: e95285. https://doi.org/10.1371/journal.pone.0095285

[11] LESNOCK JL, DARCY KM, TIAN C, DELOIA JA, THRALL MM et al. BRCA1 expression and improved survival in ovarian cancer patients treated with intraperitoneal cisplatin and paclitaxel: a Gynecologic Oncology Group Study. Br J Cancer 2013; 108: 1231–1237. https://doi.org/10.1038/bjc.2013.70

[12] KEUNG MYT, WU Y, VADGAMA JV. PARP Inhibitors as a Therapeutic Agent for Homologous Recombination Deficiency in Breast Cancers. J Clin Med 2019; 8: 435. https://doi.org/10.3390/jcm8040435

[13] BIXEL K, HAYS JL. Olaparib in the management of ovarian cancer. Pharmgenomics Pers Med 2015; 8: 127–135. https://doi.org/10.2147/PGPM.S62809

[14] ZHOU X, REN L, MENG Q, LI Y, YU Y et al. The next-generation sequencing technology and application. Protein Cell 2010; 1: 520–536. https://doi.org/10.1007/s13238-010-0065-3

[15] BASHO RK, ETEROVIC AK MERIC-BERNSTAM F. Clinical Applications and Limitations of Next Generation Sequencing. The American Journal of Hematology/Oncology 2015; 3: 17–22. https://www.gotoper.com/publications/ajho/2015/2015mar/clinical-applications-and-limitations-of-next-generation-sequencing [As accessed on September 10, 2020]

[16] COLOMBO N, SESSA C, DU BOIS A, LEDERMANN J, MCCLUGGAGE WG et al. ESMO-ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease. Ann Oncol 2019; 30: 672–705. https://doi.org/10.1093/annonc/mdz062

[17] ČERMÁK M, BEHULOVÁ LOHAJOVÁ R, HIKKEL I, CISÁRIK F et al. [Standard diagnostic procedure for complex genetic laboratory diagnostics in hereditary breast, ovarian and pancreatic carcinoma syndrome 2020.] https://www.sslg.sk/images/Dokumenty/OdborneUSM/SDTP_HBOPC_LAB_2020.pdf [As accessed on February 22, 2021]

[18] RICHARDS S, AZIZ N, BALE S, BICK D, DAS S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in Medicine 2015; 17: 405–423. https://doi.org/10.1038/gim.2015.30

[19] MATIAS-GUIU X, STANTA G, CARNEIRO F, RYSKA A, HOEFLER G et al. The leading role of pathology in assessing the somatic molecular alterations of cancer: Position Paper of the European Society of Pathology. Virchows Arch 2020; 476: 491–497. https://doi.org/10.1007/s00428-020-02757-0

[20] LEDERMANN J, HARTER P, GOURLEY C, FRIEDLANDER M, VERGOTE I et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol 2014; 15: 852–861. https://doi.org/10.1016/S1470-2045(14)70228-1

[21] LUTHRA R, CHEN H, ROY-CHOWDHURI S, SINGH RR. Next-Generation Sequencing in Clinical Molecular Diagnostics of Cancer: Advantages and Challenges. Cancers (Basel) 2015; 7: 2023–2036. https://doi.org/10.3390/cancers7040874

[22] ENDRIS V, STENZINGER A, PFARR N, PENZEL R, MÖBS M et al. NGS-based BRCA1/2 mutation testing of high-grade serous ovarian cancer tissue: results and conclusions of the first international round robin trial. Virchows Archiv 2016; 468: 697–705. https://doi.org/10.1007/s00428-016-1919-8

[23] MAFFICINI A, SIMBOLO M, PARISI A, RUSEV B, LUCHINI C et al. BRCA somatic and germline mutation detection in paraffin embedded ovarian cancers by next-generation sequencing. Oncotarget 2016; 7: 1076–1083. https://doi.org/10.18632/oncotarget.6834

[24] LEE YJ, KIM D, KIM HS, NA K, LEE JY et al. Integrating a Next Generation Sequencing Panel into Clinical Practice in Ovarian Cancer. Yonsei Med J 2019; 60: 914–923. https://doi.org/10.3349/ymj.2019.60.10.914

[25] BALMAÑA J, DIGIOVANNI L, GADDAM P, WALSH MF, JOSEPH V et al. Conflicting Interpretation of Genetic Variants and Cancer Risk by Commercial Laboratories as Assessed by the Prospective Registry of Multiplex Testing. J Clin Oncol 2016; 34: 4071–4078. https://doi.org/10.1200/JCO.2016.68.4316

[26] MATEJU M, STRIBRNA J, ZIKAN M, KLEIBL Z, JANATOVA M et al. P. Population-based study of BRCA1/2 mutations: Family history based criteria identify minority of mutation carriers. Neoplasma 2010; 57: 280–285. https://doi.org/10.4149/neo_2010_03_280

[27] GORNJEC A, NOVAKOVIC S, STEGEL V, HOCEVAR M, POHAR MARINSEK Z et al. Cytology material is equivalent to tumor tissue in determining mutations of BRCA 1/2 genes in patients with tubo-ovarian high grade serous carcinoma. BMC Cancer 2019; 19: 296. https://doi.org/10.1186/s12885-019-5535-2

[28] KRIVOKUCA A, BOLJEVIC I, JOVANDIC S, MAGIC Z, MANDIC A et al. Germline mutations in cancer susceptibility genes in high grade serous ovarian cancer in Serbia. Journal of Human Genetics 2019; 64: 281–290. https://doi.org/10.1038/s10038-019-0562-z