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Implementation of Molecular Profiling in the Diagnosis and Treatment Planning of Patients With Advanced Ovarian Cancer

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Introduction. Early diagnosis and personalized treatment of patients with malignant ovarian tumors based on molecular changes in the tumor of a specific patient is a priority research area in gynecological oncology. However, the clinical informativeness of certain genetic signatures remains unclear. Molecular profiling based on the next-generation sequencing (NGS) method, which allows multigenomic research of ovarian tumors, is not widely used among clinicians in routine clinical practice in Ukraine.

The aim of this study was to evaluate the informativeness of molecular genetic testing using a panel that detects damage to genes of signaling pathways and the homologous recombination system (HRR) for the final diagnosis and determination of the treatment plan for patients with ovarian cancer (OC).

Methods and materials. 30 patients with OC at stages III-IV undergoing inpatient treatment at the Lviv Regional Oncology Treatment and Diagnostic Center (LROTDC) during 2019–2023 were examined. The presence of germinal and somatic mutations in 32 genes was investigated using the NGS method, including genes of the HRR system, genes of signaling pathways (BRAF, ERBB2, KRAS, NRAS, PIK3CA) and the TP53 gene.

Results. Mutational changes were identified in the tumors of 23 (76.7%) examined patients and in the peripheral blood of 2 (6.7%) patients. Out of 25 cases, DNA repair deficiency by homologous recombination (HRD status) was detected in 14 samples (56%), distributed by tumor morphotype as follows: high-grade serous carcinoma (HGSC) – 9 samples (64.3%), endometrial cancer – 3 samples (21.4%) and clear cell carcinoma – 2 samples (14.3%). TP53 mutation was detected in 10 cases (40%), of which seven patients had HGSC (70%). The presence of a KRAS mutation was found in 3 patients (12%) with the morphology of endometrial cancer (2 cases, 66.7%) and HGSC (1 case, 33.3%). A relatively rare AR mutation was also detected in one patient (4%).

HRD status correlated with high sensitivity to platinum-based chemotherapy (85.7% – chemosensitive, 14.3% – chemoresistant). Conversely, the presence of KRAS mutation made it possible to attribute the patient to another morphogenetic type, namely, low-grade serous ovarian cancer, and to apply adjuvant hormone therapy.

Conclusions: Molecular genetic profiling allows for clarifying the morphogenetic type of ovarian cancer and adjusting the patient's treatment strategy, considering that it is advisable to carry it out at the stages of primary diagnosis of common OC forms.

Keywords: ovarian cancer, mutation, genes, molecular profiling, next-generation sequencing.

Introduction

Based on data from the International Agency for Research on Cancer (IARC), an intergovernmental organization within the World Health Organization (WHO), as part of the Globocan project, 324,603 new cases of ovarian cancer (OC) were registered worldwide in 2022.[1] According to the National Cancer Registry of Ukraine (operational data), the incidence of OC was 14.3 cases per 100,000 women in 2021, while the mortality rate was 6.7 cases per 100,000 women (standardized indicator, Ukrainian standard)[2].

Given the lack of effective screening programs for detecting pre-cancer and early stages of OC and the specifics of the pathogenesis of this tumor type, about 75% of OC cases are identified at late stages. The five-year survival rate of patients with stage III of the disease is only about 24%, with stage IV at 4.6%.[3]

Until recently, classic biopsy and determination of the disease stage were the main criteria used to decide on the treatment strategy for patients with OC. However, research in recent decades has shown that OC is a heterogeneous group of diseases that differ not only morphologically but also in the molecular genetic parameters of tumors.

Each tumor is unique and contains an individual combination of genetic abnormalities that determine its aggressiveness and response to therapy. Based on molecular profiling, which determines the functional activity of particular genes, the tumor's potential sensitivity to a certain type of systemic therapy may be identified to predict the disease's further course.

According to the NCCN's recommendations (2024), patients should be assessed for genetic risk by examining hereditary (germinal) mutations in peripheral blood and somatic mutations in tumor tissue at the stage of diagnosis through morphological verification of OC. At the same time, the guidelines for testing for the presence of hereditary and somatic mutations are deliberately broad enough to allow the attending physician to choose any molecular tests necessary to assess the family history of cancer and individual genetic damage in the tumor. In particular, testing for the presence of BRCA1/2 gene mutations, investigating loss of heterozygosity (LOH), and determining the presence of homologous recombination deficiency (HRD) in the absence of hereditary BRCA mutations is recommended already at the initial diagnostic stage. In case of OC recurrence, it is proposed to expand the panel of molecular studies by adding Her2 Neu, MSI, BRAF, FRc, RET, and NTRk [4].

Molecular testing of histological material is carried out using the NGS method. This method provides a comprehensive picture of the tumors' molecular profiles by detecting multiple genetic changes, including point mutations, deletions, insertions, fusions, gene copy number changes, and other structural rearrangements, with high sensitivity and specificity.[5–9]

Molecular testing is performed on 32 genes belonging to the group of repair genes by homologous recombination (HRR) and signaling pathway genes, namely: AR, ATM, ATR, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CDK12, CHEK1, CHEK2, ESR1, FANCA, FANCL, HDAC2, HOXB13, MRE11, NBN, PALB2, PPP2R2A, PTEN, RAD51B, RAD51C, RAD51D, RAD54L, STK11, and TP53 (in coding exons and exon/intron boundaries) and in the hot spots of BRAF, ERBB2, KRAS, NRAS, RIKSSA genes.[10–13]

HRR is a group of genes responsible for the complex process of DNA repair by homologous recombination. Genes involved in this system are engaged in the repair of severe DNA damage, including double-strand breaks. Such repair occurs by involving the material of the homologous chromosome. In the case of mutations in the HRR system genes, the ability to repair double-strand breaks and significant DNA damage is limited. Errors (mutations) accumulate, increasing the risk of developing malignant tumors.[14–17]

At the same time, mutations in the genes of the HRR system may sensitize tumors to platinum-based chemotherapy and inhibitors of poly(adenosine diphosphate [ADP]-ribose) polymerase (PARP inhibitors), where the point of application is the alternative PARP pathway of DNA repair. Thus, it is impossible to repair tumor DNA after damage caused by chemotherapeutic agents [18–20]. However, there is currently no standardized diagnostic method for determining and assessing HRD status in clinical settings in patients with OC. Implementing such a method may help optimize the use of this biomarker in selecting patients for both systemic and targeted therapy. This will allow for personalizing the approach to treating patients with OC, particularly those in the late disease stages, and significantly increase the effectiveness of the applied treatment.[3]

Materials and methods

We conducted a retrospective study, which included 30 patients with III-IV stage OC undergoing inpatient treatment at Lviv Regional Oncology Treatment and Diagnostic Center (LROTDC) from 2019 to 2023. They consented to using their clinical data and operative material for research purposes.

Inclusion criteria: age ≥ 18 years, histopathologically confirmed diagnosis of OC, availability of high-quality biopsy material, and general condition of the patient, assessed on the scale of functional status according to the Eastern Cooperative Oncology Group (ECOG): 0 (normal functional condition, ability to perform daily activities without limitations) or 1 (presence of disease symptoms, ability to walk and perform light work).

The average patient age was 61.3 ± 0.9 (from 45 to 78). 66.7% of patients ($n=20$) were established to have ECOG 0 status, and 33.3% ($n=10$) had ECOG 1 status.

All patients underwent laparoscopic staging and determination of the peritoneal carcinomatosis index (index of peritoneal carcinomatosis, PCI). Twenty-six patients (86.7%) were found to have stage IIIC of the disease, and four patients (13.3%) had stage IV.

Based on the above clinical and instrumental data, the following patient management tactics were chosen: primary cytoreduction was performed in 17 (56.7%) cases, 12 (40%) patients received 3–4 cycles of platinum-based neoadjuvant chemotherapy (NACT) (paclitaxel + carboplatin) with delayed cytoreduction, and in 1 case (3.3%), a decision was made to prescribe platinum-based polychemotherapy (PCT) without further surgical treatment. In the postoperative period, 29 patients (96.7%) received a course of platinum-based adjuvant chemotherapy (ACT).

If a disease relapse was diagnosed within 12 months after treatment, the tumor was considered resistant to the prescribed PCT; if the relapse was recorded after more than 12 months or was not registered, it was considered -sensitive to PCT.

Most tissue samples were obtained from the primary tumor (53.3%, 16 samples), and the remaining 46.7% (14 samples) were obtained from metastatic sites. According to the results of histological examination of tumor samples, the following morphotypes of OC were identified: in 20 (66.7%) patients, the diagnosis of high-grade serous carcinoma was confirmed, 7 (23.3%) patients- had endometrial carcinoma, and 3 (10%) patients- had clear cell carcinoma of the ovaries (Table 1).

Table 1

Clinical and morphological characteristics of patients

Characteristic	Subset	Number of patients ($n=30$)
ECOG status	0	10 (33.3%)
	1	20 (66.7%)
Material	Primary tumor	16 (53.3%)
	Metastasis	14 (46.7%)
Histological subtype	High-grade serous carcinoma	20 (66.7%)
	Endometrial carcinoma	7 (23.3%)
	Clear cell carcinoma	3 (10%)
Studied sample type	Tissue	28 (93.3%)
	Peripheral blood	2 (6.7%)
Disease stage	IIIC	26 (86.7%)
	IV	4 (13.3%)
Received treatment	NACT	11 (36.7%)
	Surgery	29 (96.7%)
	ACT	29 (96.7%)
	PCT	1 (3.3%)

For the purpose of molecular profiling, tumor samples obtained during surgery (laparoscopy or cytoreduction) were sent to the CSD medical and genetic laboratory (Kyiv). Using the NGS method on the Illumina platform (USA), an

extended NGS panel was studied to determine somatic and hereditary mutations in HRR system genes and other genes.[21–23] A genetic panel of mutations in 32 genes (NGS) was used to identify mutations in the coding sequences of AR, ATM, ATR, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CDK12, CHEK1, CHEK2, ESR1, FANCA, FANCL, HDAC2, HOXB13, MRE11 NBN, PALB2, PPP2R2A, PTEN, RAD51B, RAD51C, RAD51D, RAD54L, STK11 and TP53 (in coding exons and exon/intron boundaries) and in hot spots of BRAF, ERBB2, KRAS, NRAS, PIK3CA genes.

Histological material underwent pre-registration and compliance control (NCCN, ESMO) prior to performing the NGS study. Each sample was marked with a specific Q-code, which allowed tracking of the patient's material at all stages of the study. At the initial stage, the pathologist evaluated the histological preparations. The next stage of research is the isolation of nucleic acids. Nucleic acids (DNA and RNA) were extracted in separate working areas in designated equipped boxes.

Stages of nucleic acid extraction:

Deparaffinization allows for extracting paraffin from block sections and obtaining purely tissue material for further processing.

Lysis is the process aimed at the destruction of cell membranes.

Washing guarantees obtaining pure nucleic acid without impurities and inhibitors.

Elution involves separating DNA or RNA from the column filter.

Quality control means measuring the concentration of DNA or RNA on a spectrophotometer.

After receiving isolated nucleic acids, the library preparation process takes place. This chain of successive nucleic acid modifications aims to shape relatively short DNA fragments with individual identifiers. Specific tags in the adapters are attached to the ends of each fragment. Each tag has a unique molecular barcode that identifies samples from different patients. In contrast, adapters allow these fragments to bind with the analyzer's surface where the reaction occurs. The next step is sequencing.

The Illumina NGS sequencing method is based on such innovative technologies as “sequencing by synthesis” with labeled nucleotides, bridge amplification, and DNA molecule clustering technology. The geneticist loaded a cartridge with agents for sequencing and a chip where the detection takes place into the NGS device. The entire sequencing process took 20 to 32 hours. Reaction indicators were displayed on the monitor screen, allowing for analysis of the sequence of nucleotides in the composition of nucleic acids. Detected genetic disorders were interpreted according to the ACMG, ENIGMA Consortium and ACGS recommendations.[5,8,24]

The molecular profiling results were reviewed and discussed during multidisciplinary meetings with the participation of a gynecologist-oncologist, a chemotherapist, a radiation therapist, a pathomorphologist and a medical geneticist.

Research results were statistically processed using Microsoft Excel and Statistica 12 statistical analysis package (StatSoft, USA).

During the statistical processing of obtained data, relative and average values were analyzed. Study results are represented by the arithmetic mean value and the standard error of the arithmetic mean value ($M \pm m$).

A correlation analysis was conducted using the parametric correlation method to identify and assess relationships between quantitative indicators, with Pearson's linear correlation coefficient (r -Pearson) determined with a confidence interval of 95%. A negative value of the coefficient was interpreted as an inverse (negative, negative) relationship between the studied values, a positive one – as a directly proportional (direct, positive) relationship, and a value of 0 – as no relationship. According to the strength of the relationship, the correlation dependence was considered close (strong) at $r=0.70-1$, medium at $r=0.30-0.69$, and weak at $r=0.01-0.29$.

Results

The distribution of mutations identified using the molecular diagnostic panel is presented in Fig. 1.

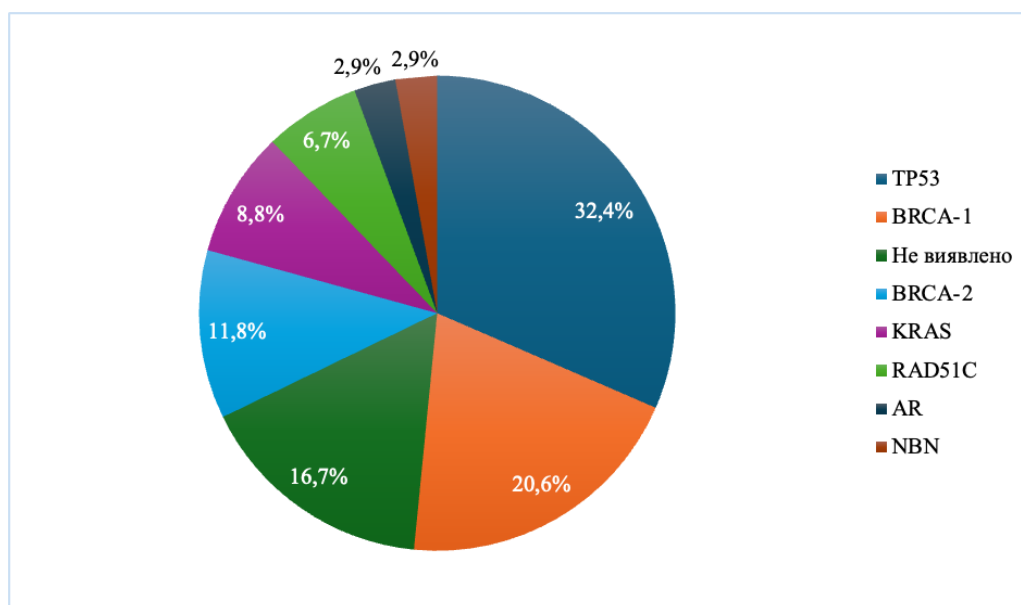


Figure 1. The structure of mutations identified during molecular profiling of the tumor in the studied cohort of patients

When analyzing the results of molecular profiling of tumors (Fig. 1), mutations of the genes TP53 (34.2%, in 11 tumor samples), BRCA-1 (20.6%, in 7 samples), and BRCA-2 (11.8%, in 4 samples) were found. In 16.7% of cases (in 6 tumor samples), no mutations were identified in any of the panel genes. In addition, two patients (6.7%) were found to have mutations in two genes (TP53 and RAD51C, as well as TP53 and AR), and one patient (3.3%) had mutations in three genes (TP53, BRCA1 and KRAS).

Among the 30 samples examined, mutational changes were detected in 23 cases (76.7%) of histological material and in 2 cases (6.7%) of peripheral blood. Out of 25 cases, DNA repair deficiency by homologous recombination (HRD status) was detected in 14 samples (56%), distributed by tumor morphotype as follows: high-grade serous carcinoma – 9 samples (64.3%), endometrial cancer – 3 samples (21.4%) and clear cell carcinoma – 2 samples (14.3%) (Table 2).

Table 2

The structure of the frequency of mutations, morphotypes and sensitivity of tumors to PCT in the studied cohort of patients

Mutation	Histological tumor type			Sensitivity to PCT	
	High-grade serous carcinoma	Endometrial carcinoma	Clear cell carcinoma	Sensitive	Insensitive
TP53	7	3	1	8	4
BRCA1	5	2	-	6	1
BRCA2	4	-	-	3	1
KRAS	1	2	-	2	1
RAD51C	-	1	1	2	-
NBN	-	-	1	-	1
AR	1	-	-	1	-
Not Found	3	2	-	4	1
Total	20	7	3	22	8

The application of molecular profiling of tumor samples made it possible to identify the inconsistency of the tumor's mutational profile with pathomorphological findings and to correct the treatment plan. In particular, the presence of HRD status was established in patients with high-grade serous OC but also in three patients with endometrial carcinoma and one patient with clear cell morphology.

According to correlation analysis results, we found that the frequency of detecting sensitivity to platinum-based PCT was reliably combined with the presence of a BRCA-2 gene mutation ($r=0.45$, $p<0.05$).

The obtained data made it possible to justify the use of PCT with the inclusion of platinum-derivative drugs as an anti-relapse treatment. In addition, supportive therapy with a drug from the PARP inhibitor group applied to the 2nd patient with endometrioid ovarian cancer had a pronounced clinical effect.

It should be noted that the detection of a mutation in the KRAS gene in a patient with a primary morphological diagnosis of high-grade serous OC substantiated the feasibility of reviewing the histological material in a reference morphological laboratory and the additional use of an immunohistochemical study (determining the expression of WT-1, PR receptors, napsin A and p53). The pathomorphological diagnosis (low-grade serous OC) was clarified in the future. Based on this conclusion, changes were made to the adjuvant therapy plan (hormonal therapy).

Discussion

Today, the standard treatment for patients with epithelial OC is surgical cytoreduction and systemic PCT, which uses platinum-based drugs and taxanes. If complete cytoreduction is not possible, treatment begins with neoadjuvant PCT. Unfortunately, for the majority of patients with late stages of OC, there is no sufficiently effective treatment option to reduce the risk of disease progression after first-line chemotherapy due to a number of factors, including general condition, concomitant somatic pathology, duration of the initial response to treatment, adverse events, histological features of the tumor, localization, as well as molecular genetic factors.[8,25] However, the implementation of targeted therapy can change the traditional paradigm.

Drugs included to the PARP inhibitor groups have demonstrated their effectiveness as monotherapy in the treatment of recurrent OC and maintenance therapy in patients sensitive to platinum-based therapy.[15,18] Homologous recombination deficiency (HRD) is a typical characteristic of high-grade serous OC.[15] Recent clinical studies have demonstrated its prognostic potential for evaluating patient response to platinum-based therapy and PARP inhibitors.[18–20] According to The Cancer Genome Atlas, HRD-positive status is the most common change in OC (69%),[12] particularly high-grade serous OC. Noteworthy, HRD is significantly less common but can still be detected in tumors of a different morphological structure (endometrial, clear cell carcinoma).[15] Most often, HRD-positive status is associated with loss-of-function mutations and epigenetic modifications of BRCA1/2 or other genes that also play a significant role in HRR, including RAD51C/D, PALB2, ATM, H2AX, MRE11, RPA, BRIP1, BARD1, RAD51.[15,16]

Mutation of the TR53 gene was found in 34% of patients from all studied subtypes of ovarian cancer, which is less common than in other studied groups.[26] Mutations of the TR53 gene are one of the most common changes in human cancer. Differences in our study's results may be related to the opportunity to investigate a larger number of genes involved in carcinogenesis that have not been investigated before.

The mutation of the BRCA-1 gene was detected with a frequency of 25% in the HGSOC group, which corresponds to the observations of other researchers in this morphological subtype.[15]

The distinction between functionally HRD-positive and HRD-negative tumors is crucial from a clinical perspective. Determining patients' HRD status can help stratify them, identifying those more likely to benefit from a maintenance strategy with PARP inhibitors or bevacizumab as the first-line treatment and certainly those for whom it would be appropriate to reconsider primary therapy, too.

Based on the available data, universal HRD testing by molecular profiling of tumor samples would be extremely useful in understanding individualized maintenance therapy options for patients with late-stage OC after response to first-line platinum-based PCT. Unfortunately, access to HRD testing in many countries, including Ukraine, is currently limited, particularly for economic and technological reasons. However, the usefulness of such testing is obvious, as can be seen in the presentation of our clinical case.

Presentation of the clinical case

Patient S., born in 1950, came to LROTDC in August 2020 with complaints of general weakness, increased abdomen size, and constipation. Clinical laboratory and instrumental examinations were carried out according to local protocols, and the diagnosis of ovarian cancer pT1srN1M1 stage IV was made.

On August 4, 2020, a laparoscopy, omentectomy, and biopsy of pelvic lymph nodes were performed. Metastasis of serous papillary carcinoma G3 was confirmed histologically.

An additional immunohistochemical study was performed: the immunophenotype of the tumor confirmed the endometrial adenocarcinoma. Based on the results, surgical intervention consisting in abdominal hysterectomy with radical excision of pelvic lymph nodes and cytoreduction of the intra-abdominal lesion was planned and performed on September 3, 2020.

When examining the postoperative material in the tissues of the left fallopian tube, morphological signs of G3 (high-grade) endometrial adenocarcinoma with areas of high-grade erosive carcinoma were found; carcinoma metastases (with a predominance of the high-grade serous carcinoma structure) were found in pelvic lymph nodes on the right (conglomerate); carcinoma metastases were identified in the material obtained from the peritoneum.

In addition, the patient underwent molecular genetic testing. Using the NGS method, 32 genes of the HRR system and other genes were investigated, and a pathogenic mutation of the RAD51C gene and the TP53 gene was detected.

On September 30, 2020, the patient began a course of ACT according to the standard regimen of taxane group drugs and platinum in standard doses. The patient received six cycles. Treatment efficacy was assessed by contrast-enhanced computed tomography with response evaluation criteria for solid tumors (RECIST), version 1.1 (RECIST 1.1).[27] After six cycles of ACT, the patient started maintenance therapy with a drug from the PARP inhibitor group orally once a day.

At the moment, the patient continues supportive therapy. According to computer tomography data for February 27, 2024, the condition after the combined treatment of ovarian cancer. Lumbar and inguinal lymphadenopathy with signs of regression. There are no new foci.

The patient feels well, tolerates the treatment satisfactorily, and improves the quality of life.

In conclusions:

The proposed molecular testing panel demonstrated high clinical efficiency and allowed for the adjustment of the treatment plan of patients with advanced OC.

Genetic testing should be performed at the initial stages of the treatment and diagnostic process. This will allow for the application of personalized patient management and the optimization of the choice of systemic and/or targeted therapy, which may improve treatment outcomes.

The discrepancy between the tumor's morphological characteristics and the profile of genetic damage requires additional examination and corrections in each patient's treatment strategy.

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