In the last 20 years, following the identification of the \textit{BRCA1} and \textit{BRCA2} genes, preventive pathways have been developed for the identification and clinical management of individuals at high risk for ovarian cancer. This review discusses the implementation of \textit{BRCA} testing in the care and treatment pathways of ovarian cancer patients.

\textbf{KEYWORDS}\linebreak • \textit{BRCA1} • \textit{BRCA2} • genetic testing • germline mutations • ovarian cancer • PARP inhibitors • somatic mutations

\textbf{COMMENTARY}\linebreak
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\textbf{Recommendations for the implementation of \textit{BRCA} testing in the care and treatment pathways of ovarian cancer patients}

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of developing breast and ovarian cancer due to the presence of a pathogenic variant in either of these genes. These pathways are aimed at educating high-risk subjects on programs targeted toward early diagnosis and cancer risk reduction.

The approval of a novel class of drugs, the PARP enzyme inhibitors, for the treatment of ovarian cancer patients carrying high-risk BRCA pathogenic variants has changed this scenario. BRCA testing, in addition to providing information on the risk of disease, has become also a predictive marker of drug response in ovarian carcinoma patients. These recommendations prepared by Associazione Italiana di Oncologia Medica (AIOM), Società Italiana Genetica Umana (SIGU), Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBIOC) and Società Italiana di Anatomia Patologica e Citologia Diagnostica – Italian Division of the International Academy of Pathology (SIAPEC-IAP) are focused on the implementation of BRCA testing in the care and treatment pathways of ovarian cancer patients.

**BRCA testing for the prediction of treatment efficacy**

Recent population studies have highlighted that the prevalence of constitutional BRCA pathogenic variants in ovarian cancer patients is >10%, independently of age of diagnosis and of family history of breast/ovarian cancer [1,2]. The prevalence of pathogenic variants progressively increases in patients with serous ovarian carcinoma (17–20%), high-grade serous carcinoma (23–25%) and in platinum-sensitive patients (30–40%) [3–6]. Furthermore, approximately 25% of women carrying pathogenic BRCA variants have a diagnosis of ovarian carcinoma over 60 years of age [1–3].

Retrospective studies suggest that heterozygosity for a BRCA hereditary pathogenic variant in ovarian cancer patients is associated with a significantly more favorable prognosis and is predictive of sensitivity to combination therapies containing platinum derivatives [3,7].

Somatic mutations of the BRCA genes have been identified in approximately 6% of serous ovarian carcinomas [4,6], thus suggesting that inactivation of these genes can also occur during cancer progression.

Importantly, it has been shown that BRCA gene mutations, whether constitutional or somatic, are a biomarker of sensitivity to treatment with PARP inhibitors, a class of pharmacological agents involved in the repair of single-strand DNA breaks, in patients with advanced ovarian cancer [8–9]. The therapeutic effect of PARP inhibitors in ovarian cancer is due to ‘synthetic lethality’, which occurs in cells with an inactive double-strand DNA repair mechanism mediated by homologous recombination (HR). The BRCA1/2 proteins play an essential role in HR [8–13], and their loss of function due to constitutional or somatic BRCA gene mutations can be one of the major causes of HR dysfunction [6,14–15]. Following clinical trials, the PARP inhibitor olaparib has been registered in October 2014 by EMA as a maintenance therapy in patients with relapsed high-grade serous platinum-sensitive ovarian cancer, Fallopian tube cancer and primary peritoneal cancer [5,9,16].

Hence, BRCA testing is now a prerequisite for the indication of PARP inhibitor therapy. However, it is advisable to consider offering the test at the time of initial diagnosis to all patients with nonmucinous and nonborderline ovarian epithelial carcinoma, fallopian tube carcinoma and primary peritoneal carcinoma. This approach would allow us to obtain early the information on the potential sensitivity to PARP inhibitors and at the same time it would also pave the way for cancer genetic counseling and prevention. The timing of BRCA testing should be chosen with the patient in order to respect her needs for the decision-making process. In addition, the patient must be provided with appropriate and thorough information regarding all issues associated with a positive test outcome.

**Types of BRCA testing**

The BRCA test on peripheral blood detects constitutional/hereditary variants — that is, those which can be transmitted to the offspring (50% probability for each child). On the other hand, the test conducted on tumor tissue can also identify the variants acquired by somatic mutations during tumor development and progression, in addition to constitutional defects. This implies that when a variant is identified in tumor DNA, its origin (constitutional or somatic) must be established by analyzing normal tissue (blood or other tissue).

According to available data, 2/3 of the pathogenic BRCA variants identified in patients affected by ovarian cancer are constitutional (present in every cell of the individual) and
Recommendations for BRCA testing in the care & treatment pathways of ovarian cancer patients

1/3 somatic (confined to tumor tissue) [4,6]. Constitutional BRCA variants are nearly always inherited from one parent; less than 1% of cases are due to de novo mutations, occurring in the parental germ cells or in utero.

In the clinical setting, mutation screening of BRCA1 and BRCA2 is usually carried out by sequencing of all coding exons (n = 22 and n = 26, respectively) and corresponding exon/intron junctions. This allows detection of point variations (single nucleotide substitutions and insertions/deletions of one or a few bases) in the DNA sequence. These alterations encompass more than 90% of BRCA pathogenic variants occurring in the coding regions of both genes. In order to maximize sensitivity, the test must include the search for large genomic rearrangements (i.e., deletions or duplications of one or more exons, or of the whole gene), which account for a variable proportion of constitutional BRCA variants across populations, usually not exceeding 10%. These types of changes can be investigated by multiplex ligation probe dependent amplification or multiplex amplicon quantification [17,18].

Currently, sequencing analyses for the detection of constitutional pathogenic BRCA gene variants on peripheral blood is performed in most laboratories using different methods. These include well-established techniques, namely Sanger sequencing, as well as novel technologies – that is, next-generation sequencing, which are under validation [19–24].

There are as yet no standardized tests for BRCA analysis in tumor samples. BRCA testing in tumor tissue is more complex than other molecular somatic tests already included in routine cancer diagnosis. This is due to the high degree of heterogeneity of potentially pathogenic variants, which can be observed along the entire sequence of BRCA1 and BRCA2 genes. Furthermore, in addition to the already mentioned different types of DNA variations (point variations, large deletions/duplications), epigenetic alterations (methylation of the regulatory regions) can also occur [4,15]. This makes it difficult to assess the predictive value, in terms of response to treatment, of the different BRCA variants, in particular those identified at somatic level. Indeed, the available classification algorithms have been devised to identify constitutional variants associated with a high risk of developing breast and ovarian cancer (see below). While BRCA mutation test in blood DNA is routinely applied in the search of constitutional pathogenic variants, the actual feasibility of the somatic test by the diagnostic laboratory should be verified in advance, in consideration of the inherent methodological difficulties. Laboratories should carry out technical assessment procedures and take part in external quality control programs of the tests they offer (constitutional and/or somatic) [25].

Interpretation of BRCA genetic variants
The mutation spectrum of the BRCA genes is very broad, and the interpretation of the clinical significance of identified variants is not always straightforward. Indeed, variant classification is an important aspect of the BRCA testing process, particularly when considering that quite often clinical testing detects genetic alterations not reported in the scientific literature [26]. Therefore, although no consolidated standards exist for the classification of constitutional BRCA variants, it is important that laboratories use updated classification criteria, and that reports be written up in accordance with good laboratory practice recommendations.

The Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) has recently developed specific criteria for interpreting the clinical significance, in relation to cancer risk, of constitutional variants of BRCA genes. These are available on the consortium website [27]. However, it must be considered that studies in mouse models have highlighted that not all BRCA gene variants associated with high risk of developing cancer are predictors of response to anti-PARP therapy [8].

It is therefore necessary that laboratory reporting protocols, including the interpretation process, be made publicly available. In addition, the clinical significance of the BRCA gene variants identified should be clearly stated, along with a list of the essential information used for the classification [26,28]. In this regard, laboratories, in addition to taking part in external quality control programs, should contribute to a systematic and centralized collection of all BRCA variants observed with the aim to improve their classification [25].

BRCA testing in the care & treatment pathway
The use of BRCA testing as a treatment decision tool implies that it should be readily accessible...
to all those patients who may benefit from it and that the test results be made available within a time compatible with the clinical needs.

The models of cancer genetic counseling developed in the preventive setting are currently insufficient to meet the increasing number of BRCA test requests, particularly when the test for genetic predisposition also has a predictive value for treatment which needs to be determined in a short time. The optimal model of genetic counseling within the prevention pathway involves detailed information and discussion of the genetic aspects right from the pretest stage. On the other hand, the need to obtain test results in a timely manner in order to implement treatment planning implies that also oncologists and gynecologists experienced in oncology can directly request the BRCA test to the laboratory. Even when the test is performed in the cancer treatment setting, the arrangement of full care pathways is mandatory, to ensure the correct interpretation of the results for clinical purposes, the correct way to manage family members at risk if a hereditary pathogenic variant is identified and the correct genetic assessment of cases with a noninformative BRCA test result [25–26,28].

Physicians who request the BRCA test must receive appropriate updated training in genetic oncology. Since prescribers bear final responsibility for the use of the test result, they must verify that the laboratory uses appropriate protocols for correct clinical interpretation. Furthermore, protocols must be defined for the referral of patients with inherited pathogenic variant to a clinical cancer genetics team so that family members can receive appropriate care, as well as for particular cases warranting further genetic investigation [28].

Each center must provide clear indications of the management pathways to the patients and their relatives, outlining the duties and responsibilities of the oncology team, of the laboratory and of the clinical cancer genetics team across the different phases of the defined care pathway. Should recognized standards not be available, one should consider submitting these pathways to verification via planned audits, with the aim to improve service quality.

Essential items of the informed consent

The BRCA test for prediction of response to PARP inhibitors may be prescribed by clinical geneticists, oncologists and gynecologists with oncologic expertise. The prescriber has the responsibility to provide appropriate information to the patients on the genetic aspects associated with the results in order to enable them to make informed choices. The information provided to the patient should cover the potential therapeutic benefits of treatment with a PARP inhibitor, together with the possibility of detecting a high cancer risk condition for the patient and her relatives. The timing at which informed consent to genetic testing is obtained, as well as the modalities, must respect the will of the patient, who should be given the possibility to discuss all the different implications of genetic testing, such as whether or not to tell other family members about the test results, before taking the decision.

Physicians who prescribe a BRCA test should abide to an appropriate communication and written informed consent collection protocol, using specific information material and consent forms. Oncologists and gynecologists with oncologic expertise who do not have experience in cancer genetics must follow a training program which includes ethical aspects of BRCA testing. Finally, the care pathway must clearly identify the cancer genetics team to be contacted, should the patient require a closer examination of the genetic aspects before deciding whether or not to undergo the test, as well as for those cases which present particular issues.

Conclusion

In conclusion, BRCA mutations represent a biomarker predictive of sensitivity to treatment with PARP inhibitors, in addition to cancer risk assessment. As highlighted in this recommendation, several issues are associated with diagnostic BRCA testing: the screening methods vary across laboratories, and yet no standardized tests exist; BRCA analyses can reveal changes of unclear clinical significance, in addition to variants with a pathogenic or neutral role; finally, there is a need to devise the most appropriate report models, to avoid misinterpretations among clinicians and confusion among the patients. In this respect, the combined goal of this recommendation by the Italian scientific societies is to improve the outcome of therapy, to promote prevention and finally to reduce mortality of ovarian cancer.

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