Critical Reviews in Oncology / Hematology 140 (2019) 67–72

Contents lists available at ScienceDirect

Critical Reviews in Oncology / Hematology

journal homepage: www.elsevier.com/locate/critrevonc

Recommendations for the implementation of BRCA testing in ovarian cancer patients and their relatives

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https://doi.org/10.1016/j.critrevonc.2019.05.012
Received 8 April 2019; Received in revised form 21 May 2019; Accepted 22 May 2019

ARTICLE INFO

Keywords:
BRCA1
BRCA2
Ovarian cancer
Genetic testing
PARP inhibitors
Germline mutations
Somatic mutations

ABSTRACT

The current availability of new Poly(ADP-ribose) Polymerase (PARP)-inhibitors for the treatment of ovarian cancer patients independently of the presence of a BRCA pathogenic variant, together with the validation of somatic test for the analysis of *BRCA1/2* genes, involves the need to optimise the guidelines for BRCA testing.

The AIOM-SIGU-SIBIOC-SIAPEC-IAP Italian Scientific Societies, in this position paper, recommend the implementation of BRCA testing with 2 main objectives: the first is the identification of ovarian cancer patients with higher probability of benefit from specific anticancer treatments (test for response to therapy); the second goal, through BRCA testing in the family members of ovarian cancer patients, is the identification of carriers of pathogenic variant, who have inheredited predisposition to cancer development (test for cancer risk). These individuals with increased risk of cancer, should be encouraged to participate in dedicated high-risk surveillance clinics and specific risk-reducing measures (primary and/or secondary prevention programs).

1. Introduction

The AIOM-SIGU-SIBIOC-SIAPEC-IAP Recommendations represent a position paper for the implementation of *BRCA1/2* testing in ovarian cancer patients and their relatives.

This paper, based on expert opinion, reflects the need to update the previous recommendations published in 2016 (Pinto et al., 2016) and to optimise the guidelines for BRCA testing, taking into account the current availability of new Poly(ADP-ribose) Polymerase (PARP)-inhibitors that have been approved for the treatment of ovarian cancer patients independently of the presence of a BRCA pathogenic variant, and following the validation of somatic test for the analysis of *BRCA1/2* genes.

These recommendations are focused on the implementation of BRCA testing for the identification of:

a) ovarian cancer patients with higher probability of response to specific anticancer treatments (test for response to therapy);
b) family members of ovarian cancer patients, carriers of pathogenic variant, who have inheredited predisposition to cancer development (test for cancer risk). These individuals with increased risk of cancer, should be encouraged to participate in dedicated high-risk follow-up clinics and specific risk-reducing measures (primary and/or secondary prevention programs).

2. The need of implementation of BRCA testing

2.1. *BRCA1/2* predictive testing to estimate the cancer treatment efficacy

Retrospective studies have highlighted that ovarian carcinoma patients, carrying a constitutional (germline) *BRCA* pathogenic variant, show an increased pharmacological sensitivity to combination therapies containing platinum derivatives (Alsop et al., 2012; Bolton et al., 2012; George et al., 2017), even when administered at high doses, as usually during intraperitoneal chemotherapy, as well as sensitivity to pegylated liposomal doxorubicin and trabectedin (Safra et al., 2011; Monk et al., 2016). Moreover, it has been shown that BRCA gene pathogenic variants, whether constitutional or somatic, represent a predictive biomarker of higher sensitivity to treatment with PARP inhibitors, a class of pharmacological agents involved in the single-strand DNA breaks repair, in patients with advanced ovarian cancer. The efficacy of PARP inhibitors as therapeutic option 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), where the BRCA1/2 proteins play an essential role (Drost and Jonkers, 2014; George et al., 2013; Cancer Genome Atlas Research Network, 2011; Wiggans et al., 2015; Cortesi et al., 2018). The loss of function due to constitutional or somatic BRCA gene pathogenic variants can be one of the major, although not exclusive, causes of HR dysfunction (Curtin, 2012; Hilton et al., 2002).

The prevalence of constitutional BRCA pathogenic variants in ovarian cancer patients is > 10%, independently of age of diagnosis and family history (Soegaard et al., 2008). Actually, approximately 25% of women carrying pathogenic *BRCA* variants have a diagnosis of ovarian carcinoma over 60 years of age. The prevalence of pathogenic variants progressively increases to 17–20% in patients with serous ovarian carcinoma (Alsop et al., 2012; Cancer Genome Atlas Research Network, 2011), to 23–25% if high-grade (Rust et al., 2018) and to 30–40% if the disease is platinum-sensitive.

Following clinical trials, the PARP inhibitor olaparib has been registered in October 2014 by EMA *(European Medicines Agency)* "as maintenance therapy of patients with platinum-senstive relapsed BRCA-mutated (with germinal and/or somatic mutation) high-grade serous epithelial ovarian, Fallopian tube or primary peritoneal cancer who are in a complete or partial response to platinum-based chemotherapy" (Ledermann et al., 2014; Anon., 2019a). The Italian *Medicines* Agency (AIFA) has approved the drug with the same indication, stating that "the treatment with olaparib must be started and be monitored under the supervision of a doctor experienced in the use of anti-cancer medicines. Patients must have a confirmed breast cancer susceptibility gene mutation (BRCA) (within the germline or the tumor tissue) before starting olaparib treatment. The evaluation of BRCA mutational status must be performed by a laboratory with expertise using a validated test method" (Anon., 2015).

Niraparib, another PARP inhibitor, is currently available in a few countries, including Italy, as "monotherapy for the maintenance treatment of adult patients with platinum-sensitive relapsed high-grade serous epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in response (complete or partial) to platinum-based chemotherapy" (Anon., 2018), independently of the presence of BRCA mutations.

EMA has recently approved the use of:

- olaparib, independently of BRCA mutational status, (on the basis of randomized prospective clinical trials that have demonstrated the benefit of these drugs as maintenance therapy in the overall population of patients with relapsed platinum-sensitive disease);

- another PARP inhibitor, rucaparib, as monotherapy treatment of platinum-sensitive patients with germinal and/or somatic BRCA pathogenic variant, associated with advanced high-grade ovarian cancer, fallopian tube, or primary peritoneal cancer, who have been treated with at least two prior platinum-based chemotherapy, and who are not candidates for further platinum-based chemotherapy (Anon., 2019b), on the basis of the ARIEL 2 study results. Furthermore, the EMA's Committee for Medicinal Products for Human Use (CHMP) adopted a positive opinion on rucaparib (December 2018) for an additional indication as maintenance treatment of adult patients with platinum-sensitive relapsed high-grade epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in response (complete or partial) to platinum-based chemotherapy (on the basis of the ARIEL 3 study

results).

Although these new therapeutic options have demonstrated their efficacy even in patients not carrying BRCA gene pathogenic variants (wild type), it is nevertheless important to investigate the BRCA status in all ovarian cancer patients (with the exception of mucinous and borderline tumors) for the following reasons:

a) patients with test positive for *BRCA1/2* pathogenic variants show a greater benefit from the treatment with PARP inhibitors when compared to patients not carrying such variants (Staropoli et al., 2018);
b) the use of olaparib as maintenance treatment after a first-line chemotherapy in patients with BRCA pathogenic variants has been associated, in a phase III randomized clinical trial, with a statistically significant improvement in progression-free survival (PFS); indeed, the median PFS was 13.8 months in patients receiving placebo and has not been reached after 3 years in those patients receiving olaparib (HR 0.30; 95% CI 0.23- 0.41; $p < 0.001$). Thus, it will be crucial to be informed on the BRCA mutational status at the time of initial diagnosis, leading to the most appropriate first-line treatment approach (Moore et al., 2018);
c) BRCA-positive patients affected by ovarian cancer should undergo active surveillance for the risk of developing secondary cancers (breast cancer and other heredo-familial cancers associated with BRCA gene pathogenic variants);
d) the inability to repair chemotherapy-induced DNA damage confers a significantly better prognosis in BRCA-positive patients with advanced disease when compared to wild type patients;
e) the important implications of cancer risk-assesment and prevention among family members, especially in the case of a positive test result (Anon., 2019a; Mirza et al., 2016; Anon., 2019c; Pujade-Lauraine et al., 2017).

Based on these observations, it is important to offer the BRCA test at the time of initial diagnosis to all patients with non-mucinous and non-borderline ovarian epithelial carcinoma, fallopian tube carcinoma and primary peritoneal carcinoma (Fig. 1).

BRCA testing should be proposed at the time of initial diagnosis, providing appropriate information on all aspects associated with the results and respecting the will of the patients (Table 1).

2.2. *BRCA1/2 testing for the identification of hereditary cancer predisposition*

As above mentioned, a positive test result for a BRCA pathogenic variant in ovarian cancer patients enables the access of their relatives to cancer genetic counseling and to risk- assessment tests, aimed to verify the presence of the familial genetic alteration. In the case of a positive test result, programs for risk reduction and/or early detection of heredo-familial cancers associated with BRCA gene pathogenic variants are implemented (Fig. 1).

In the United States, where BRCA testing has become universal for all ovarian cancer patients over the last few years, epidemiologists estimated that risk reduction strategies (medical or surgical) applied to healthy family members tested positive could lead to a reduction in ovarian cancer incidence by 40% in 10 years (Bayraktar and Arun, 2017). This accomplishment, in a tumor for which still nowadays simple and effective prevention and screening methods are not

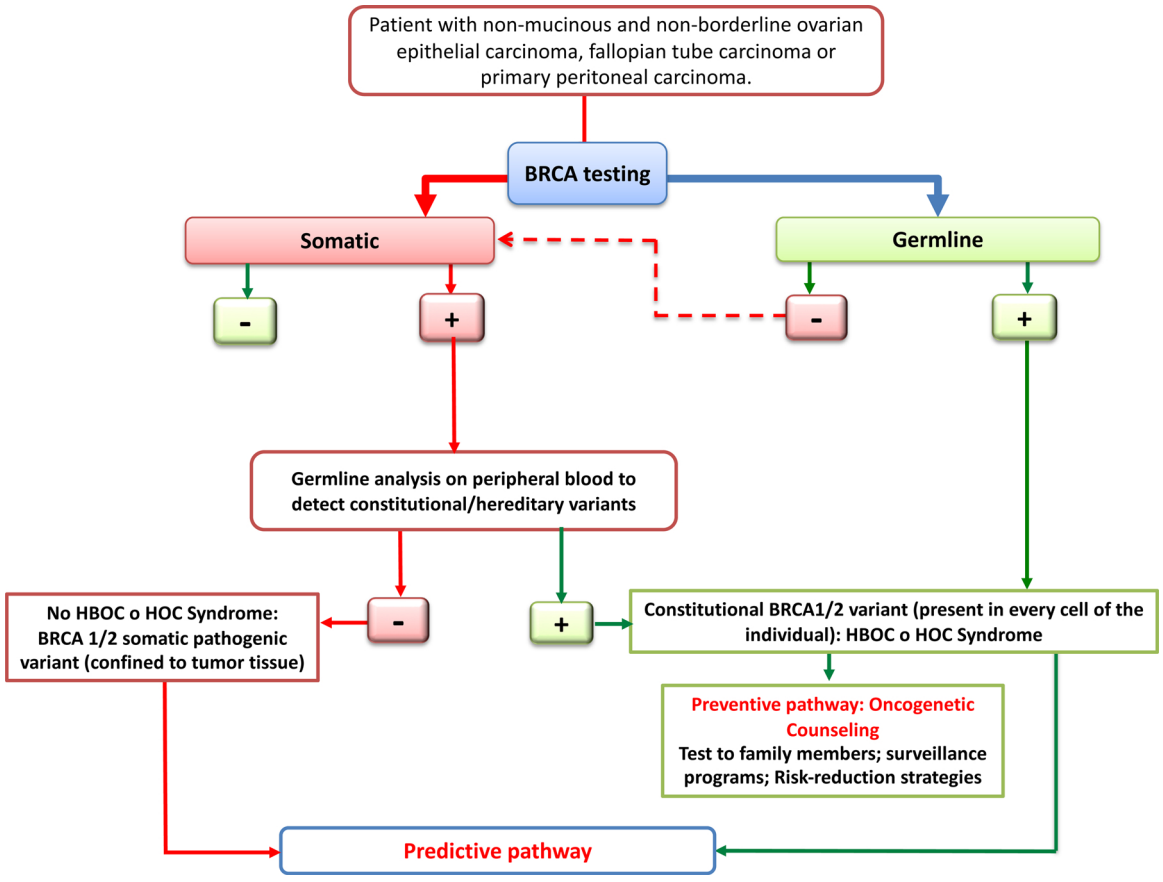

Fig. 1. It is initially preferred to search the BRCA1/2 pathogenic variants on tumor tissue, because the BRCA testing on peripheral blood is able to detect only constitutional/hereditary variants. The identification of a pathogenic variant, somatic or germline, allows to identify the ovarian cancer patients with higher probability of response to specific anticancer treatments. In the case of a constitutional variant, in addition to predictive informations, the patient will gain the access, through the cancer genetic counseling, to test for cancer risk (surveillance programs and risk reduction strategies).

Table 1
Summary of recommendations for the implementation of BRCA testing in ovarian cancer patients and their relatives.

It is important to offer the BRCA testing to all patients with non-mucinous and non-borderline ovarian epithelial carcinoma, fallopian tube carcinoma and primary peritoneal carcinoma.
BRCA testing should be proposed at the time of initial diagnosis, providing appropriate information on all aspects associated with the results and respecting the will of the patients.
Reasons:
a) Patients with test positive for BRCA1/2 pathogenic variants show a greater benefit from the treatment with PARP inhibitors when compared to patients not carrying such variants;
b) BRCA-positive patients affected by ovarian cancer should undergo active surveillance for the risk of developing secondary cancers (breast cancer and other heredo-familial cancers associated with BRCA gene pathogenic variants);
c) The inability to repair chemotherapy-induced DNA damage confers a significantly better prognosis in BRCA-positive patients with advanced disease when compared to wild type patients;
d) BRCA-positive test in ovarian cancer patients has important implications of cancer risk-assesment and prevention among relatives.

Table 2
Summary of recommendations for the interpretation of BRCA genetic variants and management of results in the care & treatment pathway.

a) It is initially preferred to search the BRCA1/2 pathogenic variants on tumor tissue. In one third of cases the BRCA pathogenic variants are exclusively somatic. Thus, the BRCA test conducted on tumor tissue ("somatic test") can also identify the variants acquired as somatic mutations in addition to constitutional defects.
b) 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%.
c) The surgical sample submitted for somatic molecular analysis must be assumed as appropriate (≥20% of neoplastic cells in the specimen) by the pathologist, who must evaluate the characteristics of the tissue block under examination and consider, if necessary, manual macrodissection to select areas with higher tumor cellularity within the specimen.
d) NGS methods allow to detect germline variants also in tumor tissue, without any major problems, while on the other hand fail to identify medium- or large-sized rearrangements as well as low frequency allelic variants in the specimen under examination.
e) It is necessary that laboratories have proven experience of test validation and take part in approved external quality control programs.
f) If the workflow begins with the test in peripheral blood, in case of non-informative result in a patient eligible to receive the treatment based on PARP inhibitor only when the presence of a pathogenic variant is demonstrated in one BRCA gene, it is necessary to refer the tumor tissue to a qualified laboratory for the quest of somatic variants.
g) For the interpretation of BRCA genetic variants it is important that laboratories use the updated criteria developed by the Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA), available on the consortium website.

available, is exceptionally important.

If the reference laboratory exclusively performs the test on peripheral blood, for patients with a negative constitutional/germline test result, it will be necessary to refer the tumor tissue to another laboratory for the quest of somatic BRCA1/2 variants.

3. Types of BRCA testing

Currently, the BRCA test in peripheral blood ("germline or constitutional test") for the detection of constitutional pathogenic variants is performed in most laboratories using well-established techniques, namely by next-generation sequencing (NGS) eventually followed by Sanger sequencing for variant validation.

The sequencing analysis of the coding portion and exon/intron junctions of *BRCA1* and *BRCA2* genes allows the detection of point variations (single nucleotide substitutions and insertions/deletions of one or a few bases) in the DNA sequence and encompasses about 90% of *BRCA* pathogenic variants. 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%. Analyses using NGS methods allow to predict with a certain degree of reliability large rearrangements in *BRCA1/2*, which are usually confimed by techniques such as *Multiplex Ligation Probe dependent Amplification* (MLPA) or *Multiplex Amplicon Quantification* (MAQ). Generally, MLPA and MAQ should be complementarily used, in order to circunvent technical faults (Scaglione et al., 2018; Concolino et al., 2018).

According to available data, it is expected that two thirds of pathogenic *BRCA* variants identified in patients affected by ovarian cancer are constitutional (present in every cell of the individual), inherited from one parent or arisen *de novo* (less than 1% of cases) and, therefore, transmissible to the offspring (50% probability for each child). On the other hand, in one third of cases the pathogenic variants are exclusively somatic and thus confined to tumor tissue (George et al., 2013; Cancer Genome Atlas Research Network, 2011).

The BRCA test conducted on tumor tissue ("somatic test") can also identify the variants acquired as somatic mutations in addition to constitutional defects. Hence, in the event of a positive result, the alteration must be verified in peripheral blood in order to ascertain its constitutional origin. The somatic analysis enables physicians to identify a fraction of around 7% of ovarian cancer patients with a pathogenic BRCA variant that would remain unknown if only test in peripheral blood is performed (Ellison et al., 2018). Nonetheless, in spite of several existing commercial systems that are CE-IVD (In Vitro Diagnostics) certified for the *BRCA1/2* analysis on tumor tissue, many of them are currently not equipped with bioinformatic software applications dedicated to the implementation and interpretation of BRCA analysis. Such systems are not accurate for the assessment of large rearrangements, while resulting to be as reliable as blood tests for point variant identification (Ellison et al., 2018; Capoluongo et al., 2017).

When prescribing the somatic test the following issues are to be considered.

1 The surgical sample submitted for molecular analysis must be assumed as appropriate (≥20% of neoplastic cells in the specimen) (Capoluongo et al., 2017) by the pathologist, who must evaluate the characteristics of the tissue block under examination and consider, if necessary, manual macrodissection to select areas with higher tumor cellularity within the specimen.
2 NGS methods allow to detect germline variants also in tumor tissue, without any major problems (Ellison et al., 2018), while on the other hand fail to identify medium- or large-sized rearrangements as well as low frequency allelic variants in the specimen under examination.
3 Lastly, at present, only few labs conduct the test in tumor tissue, while an ever increasing number of them perform the test in peripheral blood.

In terms of a proper implementation of BRCA testing, it is necessary that laboratories:
a have proven experience of test validation;
b take part in approved external quality control programs.

In any event, there are specific methodological recommendations for the development of an NGS data analysis workflow on ovarian tumor tissue for the detection of BRCA variants (Ellison et al., 2018; Capoluongo et al., 2017).

Furthermore, the use of *ad hoc* standards for each type of analytical process is crucial also in terms of a proper bioinformatics analysis (Zhong et al., 2018). Not least, it is recalled that there is a need of an appropriate tissue storage according to pre-analytical procedures that may provide the best preservation of DNA (Capoluongo et al., 2017; Zhong et al., 2018; Anon., 2019d).

The panel does consider that both the BRCA tests, in tumor tissue

and blood, may be used, but it is preferrable, whenever possibile, to perform the somatic test in the first instance, taking into consideriation, in any event that, independently of the type of sample used, quality standards are to be met along and data analysis and interpretation expertise are required.

If the workflow begins with the test in peripheral blood, in case of non-informative result (no pathogenic variant detected) in a patient eligible to receive the treatment based on PARP inhibitor only when the presence of a pathogenic variant is demonstrated in one BRCA gene, it is necessary to refer the tumor tissue to a qualified laboratory for the quest of somatic variants.

4. Interpretation of BRCA genetic variants

The spectrum of allelic variations of the *BRCA1* and *BRCA2* genes is very broad. 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. Therefore, although several standards exist for the classification of constitutional *BRCA* variants (Richards et al., 2015), it is important that laboratories use the updated criteria developed by the *Evidence-based Network for the Interpretation of Germline Mutant Alleles* (ENIGMA), available on the consortium website (Anon., 2019e), which are gene specific and based on a broad consulation of international experts. ENIGMA classifies variants in five groups, according to IARC recommendations (Plon et al., 2008): benign, likely benign, uncertain, likely pathogenic and pathogenic.

It is important to underline that the mentioned criteria have been developed in order to define the meaning of the variants of BRCA genes as predictive of hereditary risk. At this time, information on the effect of the different BRCA variants in terms of response to treatment is much more limited and specific classification criteria for this purpose have not yet been developed.

It is therefore necessary that laboratory make their protocols, including the interpretation process, available, and indicate in the test report the clinical significance of the *BRCA* gene variants identified along with a list of the essential information used for the classification (Claustres and KožichV, 2014). In this regard, laboratories should take part in external quality control programs and contribute to a systematic and centralized, national and international, collection of all *BRCA* variants observed with the aim to improve their classification (Wallis et al., 2019), as concerned both the definition of hereditary risk and the prediction of response to anticancer treatments.

Furthermore, it is also recommended that the variants classification is periodically verified. Each reclassification must be notified to the referring physician, who is required to inform the person to whom the test has been given (Table 2).

5. Availability of BRCA testing and management of results in the care & treatment pathway

The traditional pre-test models of cancer genetic counseling developed in the risk-assessment setting are currently insufficient to meet the increasing number of BRCA test requests, particularly when the test for genetic predisposition has also a predictive value for treatment, which needs to be determined in a short time.

Although the optimal model of genetic counseling for cancer risk assessment involves detailed information and discussion of the genetic aspects right from the pre-test stage, 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 comprehensive 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 identifed and the correct genetic assessment of cases with a noninformative BRCA test result (Claustres and KožichV, 2014; Wallis et al., 2019).

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.

In the absence of recognized standards, one should consider submitting these pathways to verification via planned audits, with the aim to improve service quality. It is desirable that local health authorities render the BRCA test free of charge for healthy relatives of patients carrying an identified *BRCA1/2* pathogenic variant and, eventually, offer for free the prevention programs to subjects harboring the pathogenic variant, through the introduction of a code exempt for hereditary genetic diseases.

6. Essential items of the informed consent

The BRCA test for prognosis and prediction of response may be prescribed by clinical geneticists, oncologists and gynecologists with oncologic expertise who have the responsibility to provide appropriate information to the patients on the genetic aspects associated with the results.The information provided to the patient should cover the potential benefits in terms of prognostic and therapeutic significance, together with the possibility of detecting a high secondary cancer risk and the presence of a cancer predisposition in 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 a decision.

Physicians who prescribe a BRCA test should abide to an appropriate communication and protocols for collection of specific written informed consent, possibly using *ad hoc* information material. 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 which the patient is referred to, when requiring a deeper evaluation of the genetic aspects, before deciding whether or not to undergo the test, as well as in particular circumstances, such as families with nonspecific hereditary predisposition to cancer.

7. Conclusion

In conclusion, the knowledge of the prognostic and predictive role of individual BRCA mutational status is a rapidly evolving field.

In light of the recent technological advances and therapeutic developments, these recommendations by the Italian scientific societies, highlight the importance of the implementation of BRCA testing in the management and treatment pathways of ovarian cancer patients and their relatives. The identification of *BRCA1/2* pathogenic variants have therapeutic implications in addition to cancer risk assessment and allow us to obtain the information to improve the outcome of medical treatments, to promote specific strategies of risk reduction and finally to improve the survival of ovarian cancer patients and the incidence of the disease in the population.

Financial disclosure

The authors report no financial support.

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