case reports

Constitutional Mosaicism: A Critical Issue in the Definition of BRCA-Inherited Cancer Risk

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Introduction

In ovarian cancer, *BRCA* tests have a well-recognized dual role in defining hereditary cancer predisposition and predicting response to treatment.[1-4] The implementation of *BRCA1-BRCA2* next generation sequencing (NGS) somatic testing from tumor tissues is recommended in ovarian carcinoma as the first genetic test,[5,6] following specific guidelines on biological specimen type, preanalytical process, library construction, and sequencing procedures.[6,7] In case of a positive result from cancer tissue, a likely pathogenic or pathogenic variant should be confirmed with an orthogonal technique. Moreover, its constitutional origin and zygosity should be assessed in the matched DNA from peripheral blood. Testing neoplastic tissue samples can, therefore, present a general picture of acquired somatic variants occurring in neoplastic processes. In addition, it sheds light on constitutional variants that could be useful for a definition of personal and familiar hereditary cancer risk.[8]

The detection limit for somatic variants is mainly set at a 5% variant allele frequency (VAF), to recognize small neoplastic clones as well. For germline testing, conversely, when a heterozygous variant is expected, VAFs below 30% are generally filtered out as low-quality data.[9] Nevertheless, low-frequency variants could be present in DNA samples extracted from peripheral blood because of clonal hematopoiesis of indeterminate potential or constitutional mosaicism.[9,10]

Here, we report a case including a patient diagnosed with ovarian carcinoma and her family members. The finding of a somatic variant in the patient's tumor tissue was confirmed in her peripheral blood sample but with an unexpected frequency that led us to clarify the issue of low-frequency constitutional variants and formulate a new algorithm for the interpretation of *BRCA* genetic test results.

Results

A 55-year-old woman with a recent diagnosis of high-grade serous carcinoma of the right ovary (stage III) and a previous diagnosis of triple-negative breast cancer (stage I) at the age of 42 years was referred to the Genetic Oncology Unit. Following the standard procedure for *BRCA* diagnostic testing, a peripheral blood draw and adequate formalin-fixed, paraffin-embedded (FFPE) sections from neoplastic tissue were collected as ovarian cancer surgery was performed.

The DNA from ovarian carcinoma (tumor cell content amounting to 65%) was first sequenced with the Oncomine *BRCA1-BRCA2* amplicon-based NGS panel. The analysis revealed the presence of a single-nucleotide variant in the *BRCA1* coding sequence: the nonsense c.5251C>T, p.(Arg1751*), with a VAF of 50.4% (6,876× depth). This is a loss-of-function mutation classified as pathogenic according to American College of Medical Genetics and Genomics guidelines. To ascertain the constitutional origin of the mutation, the DNA isolated from the patient's blood was sequenced searching for this variant. Unexpectedly, the Sanger corresponding peaks were found, but they reported a nonheterozygous frequency, returning a VAF around 13%.

To exclude a sequencing artifact, we repeated the sequencing in the DNA with new polymerase chain reaction primers that confirmed the low frequency. At the same time, we tested the DNA with a different NGS approach, hybridization capture based (Hereditary Cancer Solution [HCS] by SOPHiA GENETICS, Saint Sulpice, Switzerland). The pathogenic *BRCA1* variant was confirmed along with the low VAF (15.8%, 2,158× depth).

The patient reported no history of hematologic disease. A clinical revision of hematological parameters did not reveal potential undisclosed or undiagnosed hematologic malignancies while chimerism due to a bone marrow transplant was excluded. Therefore, these preliminary results were interpreted as indicative of constitutional mosaicism. Then, amplicon-based NGS was repeated on the DNA samples from a second blood draw, nasal mucosa tissue, excised for unrelated symptoms, and from a buccal swab. All these analyses yielded positive results, with frequencies ranging from 11.6% to 19% (7,135×-8,134× depth). The different origins of the tested samples (mesodermal/ectodermal) suggested the patient's bona fide constitutional mosaicism, rather than a clonal hematopoiesis of indeterminate potential.

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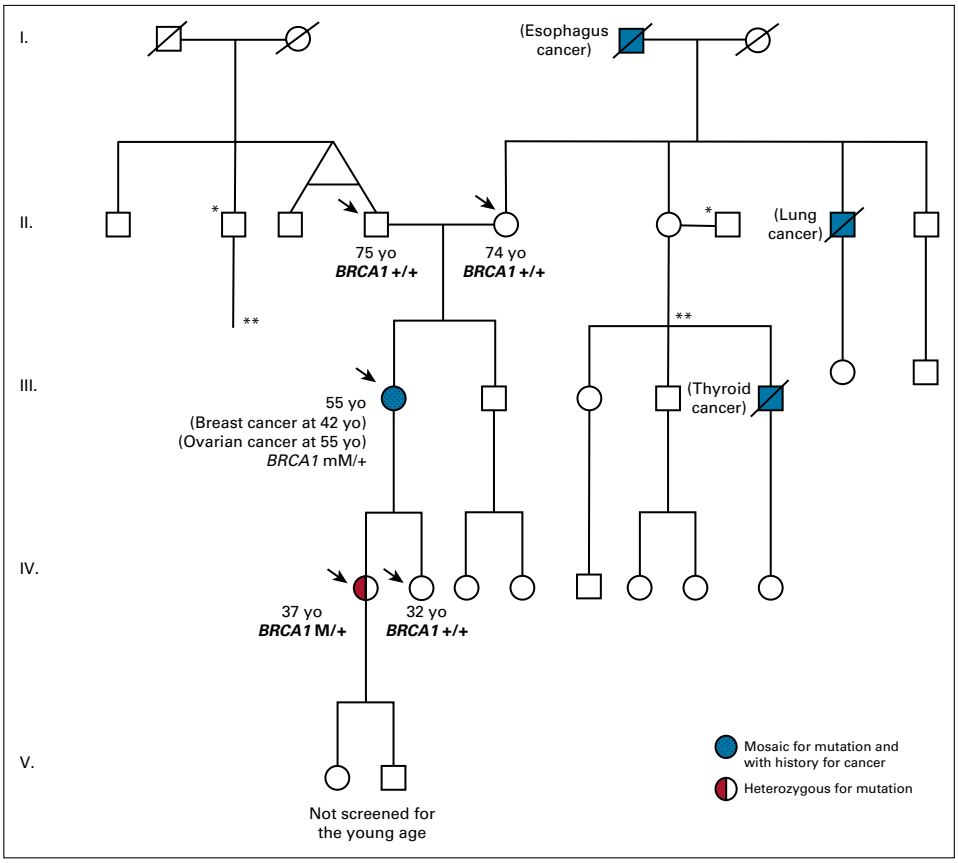

FIG 1. Pedigree chart showing the tested patient and her family. In the diagram, the arrows indicate the patients who were tested in the different generations (roman numerals) and their age expressed in yo. *indicates same male subject. **indicates same pedigree diagram. mM/+, mosaic for mutation; M/+, heterozygous for mutation; +/+, no mutation; yo, years old.

We finally tested the parents, age 75 and 74 years, and the offspring, two daughters age 37 and 32 years, with no personal history of cancer. Both parents tested negative, but the eldest daughter carried the pathogenic variant. This provided definitive proof that the low-frequency variant is a form of constitutional mosaicism present both in the patient's somatic tissues and in her germline, which makes it inheritable (Fig 1).

Next, to further define carcinoma etiology, we searched for the *BRCA1* variant also in the DNA from her earlier breast ductal carcinoma (cancer fraction 100%), where the variant was present with an even higher VAF (64%, 6, 611× depth).

In light of the molecular results, histological slides were critically revised. Ovarian serous carcinoma presented as solid proliferation of pleomorphic epithelial cells with wide necrotic areas. It showed the already described solid, pseudo-endometrioid, transitional-like phenotype alternating solid, transitional-like and endometrioid growth patterns.[11,12] Similarly, breast carcinoma showed some histological features expected in *BRCA1* mutation carriers, mainly a triple-negative phenotype, high nuclear grade, and lymphocytic infiltrate.[13-15]

For her stage III ovarian cancer, the patient was treated after surgery with front line therapy with carboplatin and paclitaxel for six cycles. She then started maintenance therapy with olaparib that is still ongoing after 2 months since chemotherapy discontinuation. For her previous breast cancer diagnosis, after conservative surgery and radiotherapy, she received adjuvant anthracycline and taxane-based chemotherapy. To date, the patient remains free from recurrence.

Discussion

There is general agreement that in patients with ovarian carcinoma, *BRCA* pathogenic variants should first be searched for on tumor tissue[5,6] to predict response to platinum-based agents and PARP inhibitors. Subsequently, pathogenic variants should be searched also on DNA from leukocytes to determine their somatic/constitutional origin, to allow for family screening in case of germinal pathogenic variants. The peculiar case we have described allowed us to define the rightly patient's cancer risk and consequently, through cascade screening, to identify one of the two daughters as a heterozygous carrier for the pathogenic variant. Here, we demonstrate that the low-frequency *BRCA1* pathogenic variant represents a mutational event that arose in the first cellular divisions of the patient's embryonic development, making her tissues a mix of normal and mutated cells that is a mosaic. Indeed, in all of the nontransformed tissues we tested, the pathogenic *BRCA1* variant has no common 50% heterozygotic frequency, but a lower one (11%-19%). In fact, the frequency of this pathogenic variant was much higher in her

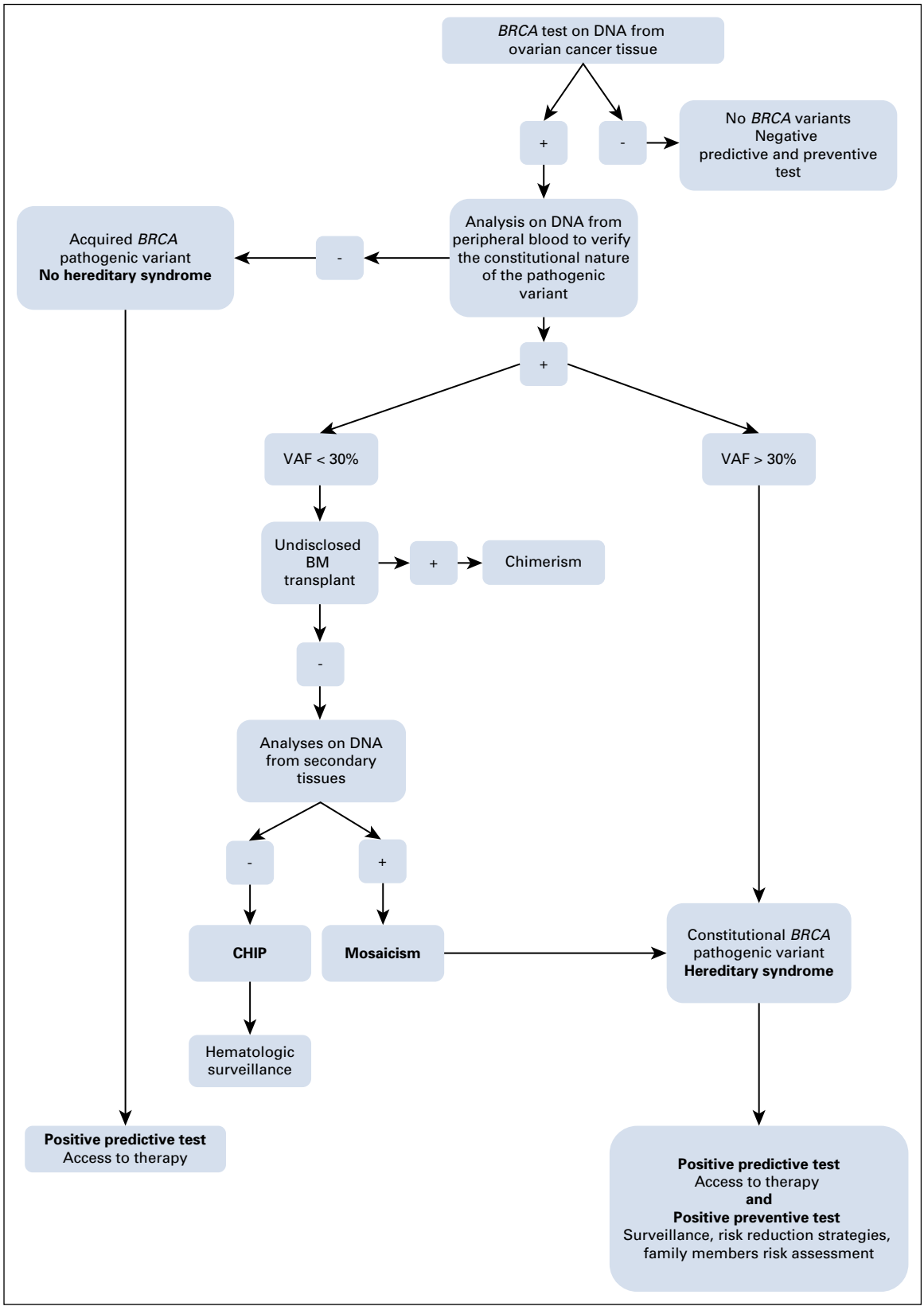

FIG 2. Decisional algorithm to manage low VAF and interpret *BRCA* genetic test results in ovarian cancer. VAF, variant allele frequency.

neoplastic ovarian tissue (50.4%). When we moved to the DNA from her earlier breast carcinoma, we found an even higher VAF (64%). Hence, these findings demonstrate that a pathogenic constitutional variant, even if present at a minor percentage of tissue cells, can make a fundamental contribution to tumor etiology.

Moreover, this case allowed us to further investigate the issue of low frequency germline variants which, although infrequent, must be taken into consideration even when mutational analysis is carried out as a germline test for the definition of hereditary cancer risk. Despite a clinical history of breast carcinoma, the patient was initially not eligible for *BRCA* genetic testing according to former guidelines by the Associazione Italiana di Oncologia Medica. Nevertheless, even if this patient had been eligible for a germline test possibly using an NGS gene panel, she would probably have been reported as negative because the great majority of NGS bioinformatic pipelines for constitutional analysis discard genomic variants with frequencies below 20%-30%.[9]

Potential constitutional mosaicism is a well-known mechanism for multiple hereditary cancer-associated genes.[16-19] The work of geneticists and bioinformaticians should, therefore, be proactive to improve the current standard of constitutional analysis routines raising flags in mutational analysis workflows. Mosaicism may indeed have a profound impact on patients' surveillance, prophylactic surgery, cancer treatment options, and family member risk assessment.[16] With this aim, we propose a new algorithm to represent the diagnostic pathway to increase the diagnostic sensitivity of *BRCA* germinal assessment and decrease the number of false negatives when pathogenic or likely pathogenic variants occur at low frequencies (Fig 2).[20] In particular, with VAF below 30% and when bone marrow transplant is excluded, further DNA analyses on secondary normal tissues should be performed. The identification of a pathogenic variant on these tissues as well leads to diagnosis of mosaicism and enables access to personalized therapies and preventive cancer strategies.

Methods

Ethics. Written informed consent was obtained from each patient to collect and test blood and FFPE samples and report their clinical course.

DNA isolation. Isolation of all DNAs from FFPE and peripheral blood samples was performed as previously described.[21] DNA from buccal cells was extracted using the MagAttract DNA Mini M48 Kit with the BioRobot M48 workstation (Qiagen, Germany).

NGS analysis with a BRCA1-BRCA2 amplicon-based panel. Amplicon-based library setup and sequencing were performed via the *BRCA* Oncomine kit and the IonChef/IONS5 platforms (Thermo Fisher Scientific, Waltham, MA), as already described.[21] Sequencing depth was set according to reach a minimum variant coverage of 50× and detect a minimum variant allele frequency as low as 5%. Data were analyzed through Oncomine Ion Reporter pipeline as previously described.[22] Reporting followed the Human Genome Variation Society nomenclature, according to the American College of Medical Genetics and Genomics criteria.[23]

NGS analysis with a hybridization-capture based multigene panel. The sequencing library was prepared with an automated procedure using the certified for in vitro diagnostic use in the European community hybridization capture based HCS v1.1 kit (SOPHiA GENETICS) and sequenced and analyzed as previously described with certified for in vitro diagnostic use in the European community SOPHiA DDM Software.[22]

Sanger sequencing. Sanger sequencing was performed as already described.[21] Sequencing data were analyzed with SeqScapeSoftware3.0 and/or Minor Variant Finder. The Minor Variant Finder software is designed for the accurate detection and reporting of minor variants in Sanger sequencing traces, with a detection level as low as 5% and a Results Review Indicator estimation of false-negative and false-positive results (Thermo Fisher). Only results with an estimated Results Review Indicator as low-risk were accepted and reported in this work.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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