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Peritoneal washing is an adequate source for somatic BRCA1/2 mutation testing in ovarian malignancies

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ABSTRACT

Genetic screening for BRCA mutations should be offered to all women diagnosed with epithelial ovarian, fallopian tube, and/or peritoneal cancers given the implications for treatment options and cancer risk assessments. Yet, while germline breast cancer susceptibility gene 1 (BRCA1) and breast cancer susceptibility gene 2 (BRCA2) testing is commonly performed, BRCA1/2 somatic mutations testing is rather challenging since the poor quality of DNA extracted from formalin fixed paraffin embedded (FFPE) samples can significantly impair this process. Peritoneal lavage is routinely performed in surgeries of suspected ovarian malignancies. We have analyzed fresh tumor, peritoneal lavage and blood samples from ten patients and we have found an excellent agreement (88%) between fresh tumor and peritoneal lavage for BRCA mutation testing. Importantly, 112 of the 114 genomic alterations detected in fresh tumor samples were also found in peritoneal lavage fluids. Our data suggest that peritoneal washings can indeed streamline BRCA genes mutation testing procedures.

1. Introduction

Ovarian cancer is the leading cause of death from gynecologic malignancies, ranking the fifth in cancer deaths among women in developed countries [1,2]. Therapeutic strategies against these tumors rely on surgical cytoreduction followed by adjuvant platinum and taxane-based chemotherapy [3,4]. In this regard, during the last years, the role of BRCA1/2 mutations as prognostic and predictive factors has gained increasing attention. Loss of BRCA1/2 functionality is associated with sensitivity to platinum-based chemotherapy as well as poly-ADP ribose polymerase (PARP) inhibitors [5,6]. Given the pivotal role of BRCA genes for risk assessment, prognosis estimation and treatment guidance, there is a general consensus that BRCA1/2 mutation testing should be offered to all women with histologically confirmed non-mucinous epithelial ovarian cancer [7]. Despite recommendations, implementation of BRCA1/2 testing remains challenging, especially in the cases of somatic mutations. With the advantage of being more accurate, sensitive, faster and cheaper than other methodologies, Next Generation Sequencing (NGS) is the technique of choice for BRCA1/2 mutation testing. Nevertheless, NGS is not exempt from technical limitations. In this way, DNA quality has been shown to be a limiting step. As an example, cytosine deamination contributes to background noise for DNA sequencing of formalin fixed paraffin embedded (FFPE) samples [8]. In addition, DNA extracted from FFPE is often degraded. In contrast, DNA from fresh frozen tumors yields better quantity and quality; however, it is seldom available for molecular analysis. Abdominopelvic washings are routinely performed as a part of the protocol in ovarian surgeries. Indeed, peritoneal washings represent important specimens for cytologic examination in ovarian malignancies for prognosis prediction [9]. However, it is discarded for further molecular analysis. In order to increase the number of samples suitable for full BRCA genes analysis and interpretation, we have evaluated the feasibility of this specimen as an alternative to FFPE samples.

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2. Material and methods

Ten patients diagnosed with high grade serous ovarian carcinoma were recruited after signing the corresponding informed consent (Local Ethics committee internal code PI_95-16). Tumor samples (8 fresh frozen and 2 FFPE samples) and matched peritoneal lavage and blood samples were obtained from every patient. Germline DNA was extracted using Maxwell® Blood DNA Purification Kit (Promega). DNA from peritoneal lavage and tumor samples was isolated using the Maxwell® RSC ccfDNA kit (Promega), following a modified protocol (Qiagen DNeasy Blood & Tissue kit and QIAamp DNA FFPE Tissue Kit, respectively. Libraries were prepared with Multiplicom BRCA MASTR™ Dx kit in case of germline DNA, and Multiplicom BRCA Tumor MASTR™ Plus Dx kit for somatic DNA and sequence on a MiSeq (Illumina). Sequence analysis was performed on Sophia Genetics platform. Only variants with a variant frequency ≥4% were considered.

3. Results

Germline pathogenic mutations, namely c.5123C > A (p.Ala1708Glu) and c.1799_1803delATAAA (p.Tyr600Trpfs*14) were found in two patients. In both cases, the mutation was also found both in the tumor as well as in the peritoneal lavage sample. No somatic pathogenic mutation was found in the tumor samples. Overall, 114 variants were detected in tumor DNA. The observed agreement between fresh tumor DNA and peritoneal derived DNA for the detection of somatic variants was 88.2%, indicating an excellent agreement between both methodologies. Specifically 112 (98%) of the 114 genomic alterations detected in fresh tumor samples were also found in peritoneal lavage fluids. On the other hand, of the 17 variants found in the peritoneal washing undetected in the tumor samples, 12 corresponded to germline mutations. The remaining five corresponded to variants with allele frequency below 7%.

Regarding minor allele frequency (MAF), we found a good correlation (Pearson coefficient: 0.91) and good concordance between tumor DNA and peritoneal derived DNA (concordance correlation coefficient: 0.906; $P < 0.001$). Regarding to mutations detected in both tumor DNA and peritoneal derived DNA, MAF values were very similar between both approaches, as depicted on Bland-Altman Plot (Fig. 1), although it was detected that values were slightly lower (minor than one percentage unit) in peritoneal washings compared to tumor samples.

4. Discussion

Our data indicates that peritoneal lavage is an adequate source for BRCA genes testing as preservation of these samples can assist to overcome some limitations derived from the low quantity or poor quality DNA isolated from FFPE samples, increasing successful sequencing and therefore the number of successfully conducted BRCA test. Remarkably, the demand for BRCA testing has steadily augmented in recent years and there is a growing pressure for many diagnostic laboratories to reduce processing time as well as the number of invalid test. Noteworthy, invalid analysis can limit therapeutic options in ovarian cancer patients.

In addition, our data indicates that the discarded fluid after cytological examination of peritoneal washings is of potential use in molecular research. In this way, somatic mutations that restore BRCA1 or BRCA2 function have been associated to resistance to platinum-based chemotherapy or PARP inhibition in germline BRCA1/2 mutation carriers with ovarian cancer or breast cancer [10,11]. Therefore, BRCA1/2 analysis at disease progression is of particular interest in order to identify patients that would not benefit from these therapies. Malignant ascites frequently occurs upon disease progression in ovarian cancer. However, molecular analysis of this specimen is seldom performed although it might have significant implications for treatment selection in BRCA1/2 mutation-associated cancer. It is important to point out that the ease of availability of peritoneal liquid is usually higher than a biopsy of the solid metastasis.

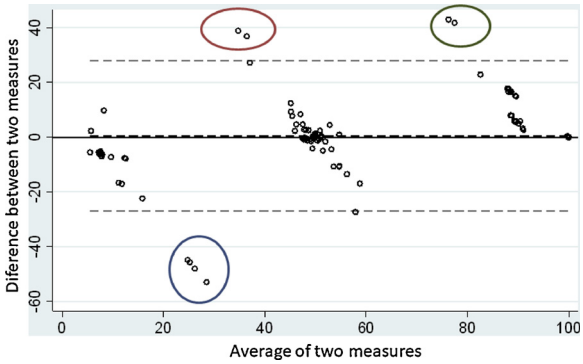

Fig. 1. Bland–Altman plot, evaluating the agreement between MAF obtained from tumor NGS and peritoneal washing NGS. Y axis shows the difference between the two paired MAF measurements (tumor and peritoneal washing). X axis shows the average of these measurements. The horizontal line in the middle of the plot represents the mean difference and the outer lines shows the 95% CI of limits of agreement which are defined as the mean difference plus and minus 1.96 times the standard deviation of the differences. Coloured ovals correspond to discrepancies in MAF. Blue oval corresponds to germline variants (present in blood samples) detected in the peritoneal washings and identified in the tumor samples but at a low allele frequency. Red oval englobes germline variants detected in the tumor samples (MAF ≈ 50%) and identified in peritoneal washings but at a low allele frequency (below 18%). Green oval contains germline variants detected at allele frequencies of ≈ 100% in tumor samples but identified in peritoneal washings at MAF ≈50% (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

5. Conclusions

To our knowledge, this is the first study showing the suitability of peritoneal washings for BRCA1/2 somatic testing. Indeed, peritoneal washings can decrease the number of invalids BRCA1/2 test which might limit therapeutic options for ovarian patients. Moreover, our data supports the preservation and analysis of malignant peritoneal effusions upon disease progression for further molecular research, specifically in BRCA mutation carriers in which identification of patients with acquired resistance to PARP inhibitors or platinum-based chemotherapy can be performed.

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Conflict of interest

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this

manuscript.

Author contributions

CM and AR conceived and designed the experiments. MS and CM recruited the patients. MGE, ETG, LG and ACSR provided the samples. MB, CPB, MS, MC and ESH performed the experiments. MB, CPB and AR analyzed the data. AR and MT wrote and edited the manuscript. AR and MP supervised every aspect of the study.

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