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BRCA1/2 Testing Landscape in Ovarian Cancer: A Nationwide, Real-World Data Study

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Simple Summary: Nowadays, tumor tests to analyze DNA in tumor cells from epithelial tubal/ovarian cancers (EOC) are performed in many centers to detect tumor pathogenic variants (TPVs) in the *BRCA1/2* genes. Information on the presence of these TPVs guides treatment options and further genetic testing in patients and relatives. However, there is no standardization of testing procedures, and information about how testing is performed is limited. Therefore, we described how *BRCA1/2* tumor testing is performed in 999 EOC patients in the Netherlands in 2019 using real-world clinical data. Tumor tests were performed for 502 patients (50.2%) and TPVs were detected in 14.7% of the tests. This study shows that there is variability in the execution of *BRCA1/2* tumor tests, but there were no indications for quality differences. Adequate reporting and quality monitors are essential to ensure that all centers perform reliable tumor tests, to ultimately identify all patients with *BRCA1/2* TPVs.

Abstract: Analyzing *BRCA1/2* tumor pathogenic variants (TPVs) in epithelial tubal/ovarian cancers (EOC) has become an essential part of the diagnostic workflow in many centers to guide treatment options and genetic cascade testing. However, there is no standardization of testing procedures, including techniques, gene assays, or sequencers used, and data on the execution of tumor tests remains scarce. Therefore, we evaluated characteristics of *BRCA1/2* tumor testing in advanced-stage EOC with real-world national data. Pathology reports of patients diagnosed with EOC in 2019 in the Netherlands were obtained from the Dutch Pathology Registry (PALGA), and data regarding histological subtype and *BRCA1/2* tumor tests were extracted. A total of 999 patients with advanced-stage EOC were included. Tumor tests were performed for 502 patients (50.2%) and *BRCA1/2* TPVs were detected in 14.7%. Of all tests, 48.6% used hybrid capture techniques and 26.5% used PCR-based techniques. More than half of the tests (55.0%) analyzed other genes in addition to *BRCA1/2*. Overall, this study highlights the heterogeneity in the execution of *BRCA1/2* tumor tests. Despite a lack of evidence of quality differences, we emphasize that adequate reporting and internal and external quality monitors are essential for high-quality implementation and execution of reliable *BRCA1/2* tumor testing, which is crucial for identifying all patients with *BRCA1/2* TPVs.

Keywords: *BRCA1/2* pathogenic variants; ovarian carcinoma; tumor test; next generation sequencing; real-world data

1. Introduction

In the last years, testing on *BRCA1/2* pathogenic variants (PVs) in patients diagnosed with epithelial tubal/ovarian cancer (EOC) has become increasingly important. While germline testing for *BRCA1/2* PVs has been available for EOC patients in most medical centers for over a decade, the

introduction of the poly (ADP-ribose) polymerase inhibitor (PARPi) therapy has created the need to also identify patients with somatic PVs, as tumors with *BRCA1/2* PVs (somatic and germline) exhibit superior sensitivity towards this therapy [1-5]. The American Society of Clinical Oncology (ASCO) and the European Society of Medical Oncology (ESMO) recommend performing both somatic and germline testing in EOC patients [6, 7]. To efficiently perform both tests, many centers analyze DNA from tumor samples first using next-generation sequencing (NGS) and subsequently analyze germline pathogenic variants (GPVs) only in those with a PV in the tumor (also referred to as tumor PV (TPV)). Patients with a positive family history and/or no or inconclusive tumor test results are also eligible for germline testing. This sequential workflow reduces the number of referrals for genetic counseling and testing, as well as the associated patient burden, and is considered cost-effective [8-10].

In the Netherlands, this tumor-first workflow is fully implemented in specialized centers. However, there is no standardization of testing procedures, including the techniques, gene assays, or sequence machine used for the analyses. In addition to a lack of national guidelines on testing procedures, data on the performance of the tumor tests, as well as test outcomes, throughout the Netherlands remain scarce. For these reasons, we evaluated the execution of *BRCA1/2* TPV testing in the Netherlands with real-world data from 2019 and provided insight into the number of *BRCA1/2* TPVs detected, and the techniques used in Dutch testing centers.

2. Materials and Methods

Patients diagnosed with EOC in 2019 in the Netherlands were identified with help of the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA) [11]. This database contains excerpts of all pathology reports from pathology departments in The Netherlands and has full national coverage since 1991. The pathology laboratories in the Netherlands are ISO-15189 certified and quality of accredited laboratories is evaluated through various ways, including internal and external audits as well as interlaboratory quality comparisons.

Anonymous pathology reports regarding the execution of *BRCA* tumor tests were retrieved from all patients diagnosed with EOC in 2019. These pathology reports were linked to data from the Netherlands Cancer Registry [12] to obtain the FIGO stage for each patient. Patients were excluded if diagnosed with FIGO stage I or II EOC, as these patients have no indication for adjuvant PARPi therapy [13].

For all included patients, data regarding histological subtype and *BRCA1/2* tumor NGS analyses (including amongst other variables: tumor NGS analysis performed (yes/no); tumor NGS results; technique used; and genes analyzed) were obtained from the pathology reports. Additionally, it was checked whether the tumor test was complemented with a *BRCA1* multiplex ligation-dependent probe amplification (MLPA) analysis (yes/no) and MLPA test result was collected (*BRCA1* PV: yes/no). Information on the detection of variants of unknown clinical significance (VUS), was also collected when reported. Dutch pathology laboratories follow national guidelines for establishing the classification and relevance of detected variants, which include close collaboration with genetics departments of medical centers [14].

In case of a detected *BRCA1/2* TPV in EOC of ambiguous or unspecified histological subtype (e.g., carcinoma not otherwise specified (NOS)), an expert pathologist reviewed the pathology reports and further classified the tumor, if possible, based on information from the corresponding pathology reports and according to the World Health Organization classification of female genital tumors of 2020 [15].

The following endpoints were analyzed in this cohort of advanced stage EOC patients: 1) number of diagnosed EOC by histological subtype; 2) prevalence of *BRCA1/2* tumor variants by histological subtype, including TPVs and VUS; 4) (reporting of) techniques and platforms used in *BRCA1/2* tumor NGS analyses, including the specific genes analyzed; and 5) lead times of *BRCA1/2* tumor analyses.

Data were reported as frequencies and percentages and lead times as mean, standard deviation and minimum and maximum values. Information on the type of technique used for target enrichment

was collected and classified as hybrid capture techniques or PCR-based amplicon techniques. The number of TPVs detected by *BRCA1* MLPA analysis were reported separately from those detected by NGS. Differences in *BRCA1/2* yield and histological subtype between hybrid capture and PCR-based techniques were compared using Chi-square exact test or Fisher's exact test. Lead time of the *BRCA1/2* tumor analyses was defined as the number of days between the date of receival of tumor material in pathology centers and the reported date of the tumor test results. Lead time could only be calculated when both dates were reported.

3. Results

The PALGA search identified 1308 women who were diagnosed with EOC in 2019 in The Netherlands (Figure 1). After exclusion of patient with early-stage disease (FIGO I/II) (*n*=309, 23.6%), a total of 999 EOC patients were included. Most EOC patients were diagnosed with high-grade serous carcinoma (HGSC) (*n*=682; 68.3%), followed by low-grade serous carcinoma (*n*=46, 4.6%) (Table 1). The histological subtype was not reported in the pathology report for 5.8% of the patients.
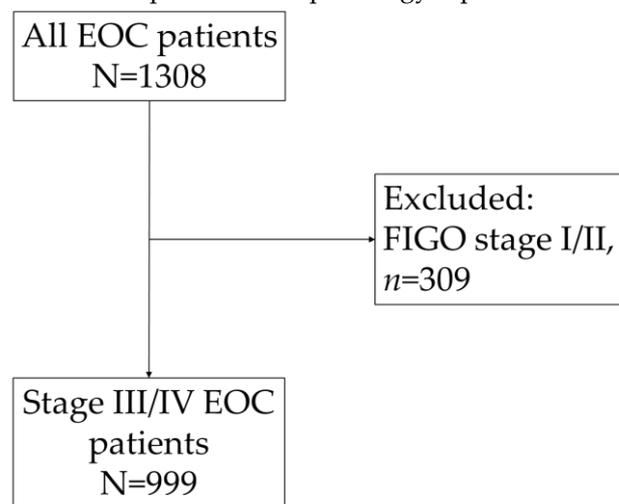
Figure 1. Flowchart of stage III/IV EOC patient selection.

Table 1. Number of EOC diagnoses by histological subtype in The Netherlands in 2019.

Histological subtypes	Stage III/IV EOC patients N=999 *n (%)*
High-grade serous	682 (68.3)
Low-grade serous	46 (4.6)
Endometrioid	12 (1.2)
Clear cell	23 (2.3)
Mucinous	11 (1.1)
Carcinosarcoma	27 (2.7)
Carcinoma NOS	65 (6.5)
Other[1]	75 (7.5)
Unknown	58 (5.8)

[1] Including: Malignant Brenner tumor, mixed type histology and undifferentiated carcinoma. Abbreviations: EOC, epithelial ovarian cancer; NOS, not otherwise specified.

BRCA1/2 tumor NGS analyses were performed in 502 patients (50.3%), and 62 TPVs were detected; 31 TPVs in *BRCA1* and 31 TPVs in *BRCA2* (Table 2). A complementary MLPA analysis was performed for 344 patients (34.4%) and detected an additional 12 *BRCA1* TPVs. Combining the TPVs detected through NGS and MLPA, most *BRCA1/2* TPVs were detected in HGSC (*n*=67, 90.5%)

(Supplementary table 1). The remaining seven TPVs were detected in low-grade serous carcinoma (*n*=1; 1.4%), endometrioid carcinoma (*n*=1; 1.4%), clear cell carcinoma (*n*=1; 1.4%), carcinosarcoma (*n*=3; 4.1%) and carcinoma NOS (*n*=1; 1.4%). In addition, the detection of six VUS was reported in the pathology reports. Caution must be taken when interpreting the latter, since reporting a detected VUS is not universally adopted by all testing centers.

Table 2. Number of *BRCA1/2* tumor tests performed and the prevalence of *BRCA1/2* TPVs and VUS[1.]

		Total
		n (%)
BRCA1/2 tumor NGS performed		502 (50.3)
	BRCA1/2 TPV	62 (12.4)
	BRCA1	31 (6.2)
	BRCA2	31 (6.2)
	BRCA1/2 VUS[1]	6 (1.2)
	BRCA1	4 (0.8)
	BRCA2	2 (0.4)
Complementary *BRCA1* MLPA		344 (34.4)
	BRCA1 TPV	12 (3.5)

[1] Detection of VUS is not routinely reported by all testing centers. Abbreviations: MLPA, Multiplex Ligation-dependent Probe Amplification; TPVs, tumor pathogenic variants; VUS, variance of unknown significance.

Figure 2 visualizes the distribution of different techniques applied for the target enrichment. Of the 502 *BRCA1/2* tumor analyses performed in our cohort, a total of 244 analyses (48.6%) were performed using the hybrid capture technique and 133 analyses (26.5%) using the PCR-based technique (Figure 2). Information on the target enrichment technique applied was missing for a substantial proportion of the performed *BRCA1/2* tumor analyses (unknown technique: *n*=125, 24.9%). The proportion of *BRCA1/2* TPVs detected did not significantly differ between the hybrid capture and PCR-based techniques (12.3% and 21.1%, respectively, P-value=0.078), neither did the distribution of histological subtypes between the type of technique (P-value=0.882) (Supplementary Table S2).

All tests performed using the hybrid capture technique used the single-molecule molecular inversion probes (smMIP) method: *n*=244 (Table 3). A total of four different assays were used for the PCR-based techniques: custom Ampliseq BRCAv5 assay (*n*=59,44.4%); Multiplicom BRCA Tumor MASTR Plus assay (*n*=62,46.6%); Oncomine BRCA Research assay (*n*=11, 8.3%) and Agilent SureMASTR HRR assay (*n*=1, 0.8%). Moreover, most tumor tests analyzed more genes than only *BRCA1/2* (*n*=276, 55.0%). For 2.4% of all tumor tests (*n*=12), the specific genes analyzed were not reported. Assays used and genes analyzed in analyses not reporting target enrichment techniques are reported in Supplementary Table S3.
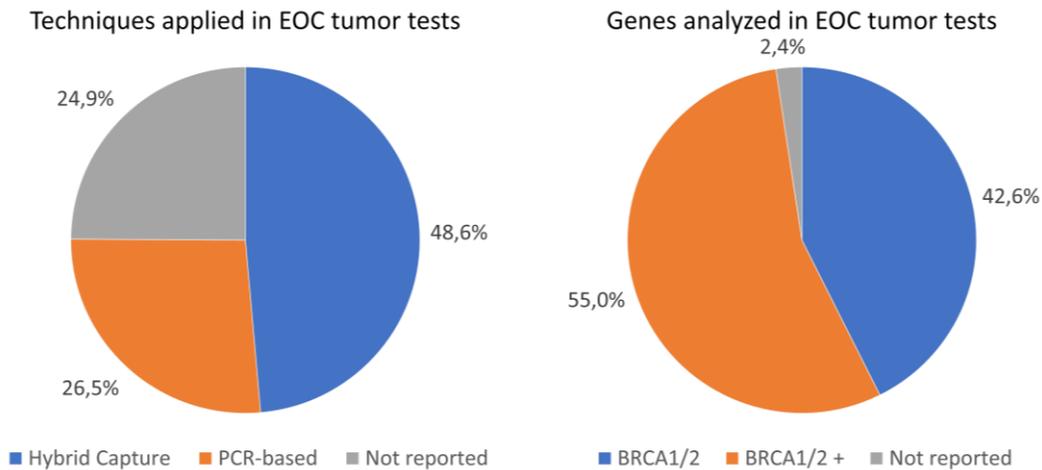
Figure 2. Distribution of techniques applied and genes analyzed in EOC tumor tests.

More than half of the *BRCA1/2* tumor analyses were performed on an Illumina platform (*n*=262; 52.2%), 15.1% on an Ion Torrent platform, and in more than 30% of the *BRCA1/2* tumor analyses the platform used was not specified (Supplemental Table S4). Lead times were analyzed for the cases with reported dates of receival of tumor material and test result (*n*=376). Mean lead time was 38.3 days (SD=64.2 days), ranging from 0 days to 525 days (Supplementary Table S5).

Table 3. Assays used and genes tested in epithelial ovarian tumor analyses using hybrid capture (*n*=244) or PCR-based techniques (*n*=133).

Assays and genes	*n* (%)[1]
Hybrid Capture Technique	
Custom smMIP-based assay	244 (100.0)
BRCA1/2	85 (34.8)
BRCA1/2, RAD51C/D, BRIP1	159 (65.2)
PCR-based Technique	
Custom Ampliseq BRCAv5 assay	59 (44.4)
BRCA1/2	1 (1.7)
BRCA1/2, ATM, BARD1, CDK12, CHEK1/2, FANCL, PALB2, PPP2R2A, RAD51B/C/D, RAD54L	56 (94.9)
BRCA1/2, ATM, BARD1, CDK12, CHEK1/2, FANCL, PALB2, PPP2R2A, RAD51B/C/D, RAD54L, RIF1, TP53, TP53BP1, WRN, XRCC2/3	1 (1.7)
Genes not reported	1 (1.7)
Multiplicom BRCA Tumor MASTR Plus assay	62 (46.6)
BRCA1/2	60 (96.8)
ATM, ATR, BAP1, BARD1, BLM, BRCA1/2, BRIP1, CDK12, CHEK1/2, FANCA/C/D2/E/F/L, MAD2L2, MRE11A, NBN, PALB2, PPP2R2A, RAD51B/C/D, RAD54L, RIF1, TP53, TP53BP1, WRN, XRCC2/3	2 (3.2)
Oncomine BRCA Research assay	11 (8.3)

doi:10.20944/preprints202404.0636.v1

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BRCA1/2	11 (100.0)
Agilent SureMASTR HRR assay	1 (0.8)
BRCA1/2, ATM, CHEK2, PALB2, RAD51C/D, BRIP1	1 (100.0)

[1] Denominator used for calculating percentages for assays is the type of technique applied, denominator used for calculating percentages for genes is the assay used. Abbreviations: smMIP, single-molecule molecular inversion probes; PCR, polymerase chain reaction.

4. Discussion

The current study provides insight into the execution and outcomes of *BRCA1/2* tumor analyses in patients diagnosed with EOC in 2019 in the Netherlands. Of the 999 advanced stage EOC patients included in this study, *BRCA1/2* tumor NGS analyses were performed for 502 patients (50.3%). This study shows that substantial variety exists in the execution of tumor analyses in EOC regarding the techniques and assays used, and the (types of) genes analyzed.

To the best of our knowledge, this study is the first to provide insight into the nationwide landscape of *BRCA1/2* tumor testing in EOC. Besides providing insight into the applied techniques, assays and analyzed genes, this study also highlights the lack of uniform reporting, despite high-quality centralized care and utilization of a national registration database. A great proportion of pathology reports lacked information on used techniques and assays, analyzed genes, and dates. For this reason, lead times could only be analyzed for a subset of 376 tests. Importantly, lead time is included in the criteria of national quality control standards that are currently being implemented [16]. Inadequate reporting limited quality assessment in the current study and could limit the exchange of diagnostic information between clinicians and tailoring of a patient's treatment in clinical practice. Fortunately, studies show that pathology reporting in oncology is changing from a narrative approach to standardized synoptic reporting, leading to significantly increased completeness of the pathology reports [17-19].

A complementary *BRCA1* MLPA analysis was performed in 344 patients. In these patients, the MLPA analysis detected an additional 3.5% of *BRCA1* TPVs. Estimates of the prevalence of large genomic rearrangements in *BRCA1* in EOC specifically remain limited, which makes it challenging to estimate the number of TPVs missed when not performing an MLPA analysis alongside the NGS. A Slovakian study performed MLPA analyses in 39 tumor samples of high-grade serous ovarian cancer and detected one pathogenic *BRCA1* deletion (2.6%) [20]. Pathogenic large rearrangements were also analyzed in 20,000 ovarian tumors with NGS and were detected in 0.7% of the cases, which reflected a total of 6.3% of all *BRCA1/2* TPVs detected in the cohort [21]. This lower percentage could be explained by the lower sensitivity of NGS in detecting large deletions and duplications. Furthermore, the presence of founder mutations, as established in *BRCA1* in The Netherlands [22], increases the number of TPVs to be detected by MLPA analysis and should be considered when making direct comparisons.

The proportion of *BRCA1/2* TPVs detected did not significantly differ between the hybrid capture technique, which constituted solely of smMIP-based assays [23], and the PCR-based techniques and does therefore not indicate significant performance differences between the techniques regarding *BRCA1/2* TPV yield. It must be noted, that for a thorough comparison of *BRCA1/2* yield in hybrid capture versus PCR-based technique, a more diverse inclusion of hybrid capture techniques is preferred. Few studies have compared the overall performance of hybrid capture and PCR-based approaches in detecting pathogenic variants. A better overall performance was reported for the hybrid capture technique in detecting *BRCA1/2* PVs from FFPE EOC tumor samples compared to the PCR-based technique [24], and similar findings were reported in lymphoma [25]. In general, these studies linked the PCR-based technique to a lower sensitivity, due to amplicon dropout and insufficient cover. On the other hand, PCR-based techniques are also reported to be suitable for accurate detection of *BRCA1/2* PVs [26]. Moreover, it requires a lower quality and quantity of DNA and is significantly less time consuming, which are important parameters for a laboratory to consider when choosing between methods [27-29]. Results of the current study do not

show quality differences between the techniques and thereby justify the use of both techniques in *BRCA1/2* TPV detection. In the Netherlands, pathology laboratories are free to choose techniques, given the technique is validated, and the national quality control standard for molecular diagnostics ensures that these techniques meet high-quality criteria [16]. This subsequently guarantees high-quality diagnostics and care for all patients.

A total of 74 *BRCA1/2* TPVs were detected in this EOC cohort (14.7% of all tests) of which 90.5% were detected in HGSC. Our overall proportion of *BRCA1/2* TPVs in EOC unselected for histotype is similar compared to the proportion we reported previously of 13% in a Dutch multi-center study including a consecutive series of EOC patients [30]. The proportion is slightly lower compared to proportions in EOC reported by another Dutch study of 16.7%, which included a complementary MLPA analysis for all cases, and also lower compared to a proportion of 19% reported in the United States [8, 31].

This study shows that EOC tumor tests for *BRCA1/2* TPV detection were already performed for 50% of all patients before this was officially recommended by national and international guidelines[6, 7, 32]. Currently, the tumor testing is implemented nationwide, testing is centralized mostly in academic hospitals, and comprehensive gene panels are more frequently applied. It should be noted that the *BRCA1/2* tumor test rate of 50.3% reported in this study does not imply that only half of all patients received *BRCA1/2* testing. The timeframe analyzed here was before national guidelines recommended tumor testing in EOC, and therefore it is likely that medical centers followed former guidelines and referred patients for genetic counseling and germline testing instead [33].

This study has several strengths and limitations. Strengths include the analysis of real-world clinical data with full nationwide coverage of all EOC pathology reports. Linking the data from narrative pathology reports to clinical characteristics such as FIGO stage allowed us to tailor this evaluation to the population of interest, namely FIGO stage III/IV patients. Nevertheless, data requests from national registries are subject to prespecified timeframes and the possibility exists that tumor tests were requested beyond this timeframe for patients in our population. This may have led to an underestimation of the proportion of EOC patients who received a tumor test. Finally, this study analyzed the execution of tumor tests before full completion of the nationwide implementation of the tumor testing and therefore it is likely that not all centers who are currently performing the tumor testing are included.

5. Conclusions

This study highlights the heterogeneity in the execution of EOC tumor testing in The Netherlands in 2019 despite centralization of testing in specialized centers. Findings of this study are not indicative of quality differences between techniques used and nationally implemented quality control standards ensure high-quality implementation of reliable *BRCA1/2* tumor testing. This is crucial for identifying all patients with *BRCA1/2* TPVs to provide high quality of care as well as for guiding genetic cascade testing to ultimately prevent cancer in unaffected relatives with *BRCA1/2* GPVs.

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