

Homologous recombination deficiency testing in ovarian cancer: the diagnostic experience of a referral Italian institution

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Summary

Aims. Recently, precision medicine has drastically modified clinical paradigm for the clinical stratification of high-grade serous ovarian cancer (HGSOC) patients. International societies approved poly (ADP-ribose) polymerase (PARP) inhibitors (PARPIs) to treat platinum-sensitive *BRCA1/2* defective HGSOC patients. Beyond *BRCA1/2*, functional defects in homologous recombination repair (HRR) proteins laid the basis for genomic instability evaluation in HGSOC patients. Given that measurement of homologous recombination deficiency (HRD) is extremely complex molecular analysis is outsourced. Of note, this diagnostic algorithm is affected by inconclusive results and high rejection rates. Here, we review the molecular results of *BRCA1/2* and HRD analysis from referral institution in predictive molecular pathology.

Methods. From May 2023 to Jan 2024 molecular records from 147 HGSOC patients simultaneously tested for *BRCA1/2* and HRD measurement were inspected. A commercially available next-generation sequencing (NGS) panel (Amoy Diagnostics Co Ltd, Xiamen, Fujian, China) was adopted to internally perform molecular analysis on formalin-fixed paraffin-embedded (FFPE) samples. In a subset of patients clinical records were matched with molecular results.

Results. Overall, 2 out of 147 (1.3%) cases were morphologically classified as inadequate. Simultaneous *BRCA1/2* - HRD analysis was successfully assessed in 112 out of 145 (77.2%) patients. Molecular analysis revealed 7 out of 112 (6.2%) and 2 out of 112 (1.8%) pathogenetic or likely pathogenetic (class I-II) and variants of uncertain significance (VUS) (class III) *BRCA1/2* molecular alterations, respectively. HRD score was positive in 48 out of 112 (42.8%) HGSOC patients.

Conclusions. HRD testing is a reliable method for the clinical management of HGSOC patients.

Key words: ovarian neoplasms, point of care testing, molecular pathology

Introduction

To date, ovarian cancer (OC) consists of most leading cause of death, worldwide, among gynecological malignancies ¹. In particular, high-grade serous ovarian cancer (HGSOC), mostly diagnosed in advanced stage (II-IV) in accordance with The International Federation of Gynecology and Obstetrics (FIGO) staging system.

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cology and Obstetrics (FIGO) classification system, is affected by unfavorable prognosis and worst clinical outcome². In this scenario, optimized therapeutic strategies play a pivotal role in the clinical management of HGSOC patients. Nowadays, platinum-based chemotherapy in combination with surgery is considered the standard of care for the routine management of OC patients³. In the last years, precision medicine has revolutionized the landscape of clinically available treatments for HGSOC patients⁴. Among these, poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi) emerged as novel targeted approach for HGSOC patients defective in homologous recombination repair (HRR) system⁵⁻⁷. Firstly, PARPi were approved by international societies for the treatment of platinum-sensitive HGSOC patients showing germline or somatic *BRCA1/2* pathogenetic alterations^{8,9}. Of note, activity of the HRR depends on a plethora of equally crucial actors involved, like *BRCA1/2*, in maintenance of DNA integrity^{10,11}. In this regard, functional defects in HRR proteins, classified as homologous recombination deficiency (HRD) status, activate error-prone DNA repair mechanisms laying the basis for genomic instability and evolutionary neoplastic cell transformation¹². Clinical trials investigated the role of HRD status for the stratification of HGSOC patients electing to PARPi administration. It has been certainly showed that HRD positive HGSOC patients had a favorable clinical outcome in comparison with HRR-proficient tumors¹²⁻¹⁴. Remarkably, HRD status is measured by bioinformatic tools able to calculate “genomic scar” from next generation sequencing (NGS) data integrating loss of heterozygosity (LOH), telomeric allelic imbalance (TAI) and large-scale state transitions (LST)¹⁵⁻¹⁷. Regarding technical aspects, NGS platforms that allow molecular analysis of comprehensive genomic regions, measure HRD score integrating optimized bioinformatic algorithm¹⁵. In fact, PARPi registrative clinical trials evaluated *BRCA1/2* molecular alterations and HRD score adopting an outsourcing approach based on centralized molecular testing^{13,14}. However, a not-negligible rejection rate (5-25%), extensive turnaround time (TAT) and the lack of standardized preanalytical procedures dramatically impacts on the molecular testing of HRD score on diagnostic routine samples^{18,19}. In this scenario, commercially available diagnostic assays have been developed to implement in-house HRD testing as a partially resolutive strategy to improve the success rate of HRD molecular analysis¹⁹. Being affected by technical and analytical pitfalls, harmonized analytical workflows and data interpreting system are required to definitively implement this diagnostic strategy in clinical practice¹⁸⁻²⁰. Not surprisingly, our Molecular Predictive Pathology Laboratory

at the Department of Public Health of the University of Naples Federico II routinely adopts in-house NGS assays for *BRCA1/2* and HRD evaluation on diagnostic routine specimens from HGSOC patients. Here, we review our molecular records from NGS analysis of HGSOC patients inspected for clinically relevant *BRCA1/2* variants and HRD status evaluation. To clinically validate our diagnostic workflow, molecular data were also matched with clinical outcomes in a representative series of HGSOC patients.

Study design

From May 2023 to January 2024 molecular records from 147 HGSOC patients simultaneously tested for *BRCA1/2* pathogenetic alterations and HRD measurement were retrieved from internal archive of University of Naples Federico II. For each patient a formalin fixed paraffin embedded (FFPE) representative of tumor lesion was available for molecular analysis adopting a commercially available NGS assay (Amoy Diagnostics Co Ltd, Xiamen, Fujian, China) integrating *BRCA1/2* clinically informative molecular alterations and HRD measurement. Written informed consent was acquired from all patients and documented according to “The Italian Data Protection Authority” (<http://www.garanteprivacy.it/web/guest/home/docweb/-/docweb-display/export/2485392>). All information regarding human material was managed using anonymous numerical codes and all samples were handled in compliance with the Helsinki Declaration (<http://www.wma.net/en/30publications/10policies/b3/>).

Material and methods

For each patient, a series of four unstained slides (5 µm), matched with hematoxylin/eosin (H/E) stained slide representative of tumor lesion, were prepared to perform nucleic acid purification. An expert pathologist internally reviewed each case counting neoplastic cell percentage available to extract nucleic acids. Briefly, DNA extraction was realizing using QiAmp Mini Kit (Qiagen, Hilden, Germany) following manufacturer's instructions. Two platforms were used to evaluate DNA concentration: Qubit fluorimeter 2.0 (ThermoFisher Scientifics, Waltham, Massachusetts, USA) and TapeStation 4200 (Agilent, Santa Clara, California, USA) in accordance with technical guidelines²². In particular, TapeStation 4200 (Agilent, Santa Clara, California, USA) also enabled to calculate DNA integrity number (DIN), reflecting fragmentation index of nucleic acids²². NGS analysis was carried out

adopting the Amoy HRD focus panel (Amoy Diagnostics Co Ltd, Xiamen, Fujian, China) on NextSeq 550 Dx system (Illumina, San Diego, USA). This panel includes SNVs and indels in coding/noncoding regions from 27 HRR-related genes plus hot spot mutations in 5 tumor informative genes among solid tumor patients. Halo-shape Annealing and Defer-Ligation Enrichment systems, derived from Molecular Inversion Probe (MIP) technology, were used for selecting target regions on genomic DNA. An optimized workflow was implemented batching 8 samples in each run to improve the success rate of molecular analysis on scant diagnostic samples. After DNA extension, circular DNA was repaired by manufacturer ligase enzyme and then cleaved by exonucleases in order to create single strand DNA for templating and sequencing procedures on NextSeq 550 Dx platform (Illumina, San Diego, USA). Data processing, *BRCA1/2* variants and HRD scoring (GSS score) was approached on proprietary analysis software. In particular, GSS score greater than or equal to 50 revealed HRD positive status, in accordance with manufacturer procedures. Technically relevant quality metrics (median coverage, alignment percentage, *BRCA* medium effective depth, SNP medium effective depth, SNP uniformity) were manually inspected stratifying samples in accordance with the technical requirement of molecular testing. Briefly, if samples failed in a single quality metrics, the molecular report was successfully accepted; if $2 \geq$ quality metrics < 4 were failed molecular results were classified as “suboptimal” whereas samples affected by 4 not surpassing quality metrics were diagnosed as “failed”.

Results

Overall, 2 out of 147 (1.3%) diagnostic specimens were diagnosed as inadequate after morphological evaluation. In the remaining cases, our internally optimized NGS based workflow was able to successfully carry out $n = 112$ out of 145 (77.2%) molecular analyses both for HRD scoring and *BRCA1/2* in diagnostic routine HG-SOC patients. By considering technical parameters, a neoplastic cell percentage median value of 56.0 % (ranging from 10.0 to 90.0%) were calculated to recover nucleic acids for molecular testing. In addition, nucleic acid purification yielded a median value of 40.0 ng/ μ L (ranging from 1.3 to 60.0 ng/ μ L). Moreover, DNA concentration was also estimated by adopting TapeStation 4200 (Agilent) in 96 out of 112 (85.7%). In this series, nucleic acids showed a median value of 28.5 ng/ μ L (ranging from 3.2 to 120.0 ng/ μ L). DIN was successfully calculated in each instance highlighting a median value

of 3.2 (ranging from 1.4 to 6.3) (Tab. I). Overall, a median coverage of 99.8 % (ranging from 98.4 to 100.0%), alignment percentage of 96.2% (ranging from 78.1 to 99.0 %), *BRCA* medium effective depth of 1329.7 (ranging from 124.0 to 4699.0) *BRCA* uniformity of 98.6% (ranging from 92.5 to 100.0%), SNP medium effective

Table I. Histological characteristics and DNA quantity and quality evaluated by using Qubit and TapeStation 4200 systems.

ID	NC%	DIN	ng/ μ L (TapeStation 4200)	ng/ μ L (Qubit)
1	20%	1.9	7.3	0.9
2	60%	2.8	29.8	60.0
3	60%	1.7	5.3	1.3
4	70%	2.8	45.8	60.0
5	20%	2.3	8.2	3.2
6	30%	2.4	20.8	26.2
7	20%	1.6	4.0	0.5
8	30%	2.2	12.7	12.9
9	70%	2.3	13.5	19.8
10	60%	2.2	19.4	39.4
11	20%	2.1	6.5	15.9
12	40%	3.1	21.1	58.0
13	70%	2.3	11.8	9.2
14	40%	3.4	17.7	35.6
15	30%	2.4	16.6	25.0
16	30%	1.7	4.4	1.3
17	60%	2.4	9.7	34.9
18	70%	2.5	28.2	59.0
19	70%	4.2	96.0	60.0
20	60%	2.8	19.9	38.5
21	50%	2.6	5.5	4.3
22	30%	3.4	11.4	16.7
23	70%	2.6	61.9	60.0
24	20%	2.7	7.3	7.2
25	80%	2.7	7.8	13.3
26	60%	3.1	20.7	32.0
27	60%	1.9	12.7	2.9
28	40%	2.0	8.9	3.0
29	30%	1.5	4.9	6.0
30	60%	2.4	9.1	24.3
31	60%	2.6	88.3	51.0
32	60%	3.0	45.7	40.9
33	30%	1.4	6.8	5.8
34	80%	2.7	51.0	36.4
35	60%	2.3	48.9	42.0
36	60%	2.9	15.7	25.3
37	90%	1.5	4.6	17.0
38	70%	2.6	40.0	44.2
39	60%	2.6	9.7	5.9
40	60%	1.8	2.7	9.2
41	50%	2.5	25.3	39.0
42	80%	3.0	94.5	60.0

Table I. Continues from previous page

ID	NC%	DIN	ng/μl (TapeStation 4200)	ng/μl (Qubit)
43	60%	2.0	9.0	5.1
44	20%	2.2	9.4	2.9
45	30%	2.4	19.9	11.7
46	30%	3.0	20.5	29.3
47	20%	2.4	16.2	11.4
48	60%	2.1	24.8	40.9
49	50%	2.7	49.4	45.4
50	70%	1.8	36.1	37.2
51	70%	3.0	60.2	56.0
52	40%	1.5	5.4	1.8
53	70%	2.4	20.8	25.2
54	50%	2.4	33.0	43.5
55	60%	2.0	13.5	28.2
56	60%	2.0	7.7	18.6
57	70%	1.9	8.5	39.0
58	60%	2.0	25.7	51.0
59	30%	3.2	3.8	3.3
60	20%	-	0.8	0.6
61	10%	2.5	13.7	11.0
62	50%	3.7	16.4	21.6
63	20%	1.4	1.5	0.6
64	50%	1.4	7.3	30.1
65	15%	1.4	4.3	2.0
66	30%	2.0	4.1	1.4
67	15%	1.7	2.9	0.2
68	20%	1.6	4.3	1
69	50%	1.1	3.2	0.6
70	75%	2.5	17.4	19.9
71	20%	1.9	5.7	1.5
72	70%	Inadequate		
73	40%	2.0	6.5	4.6
74	75%	2.0	13.2	19.2
75	50%	1.3	3.7	0.2
76	90%	2.9	5.1	7.5
77	40%	1.5	4.4	4.1
78	90%	2.7	24.1	38.4
79	25%	1.4	4.9	1.5
80	30%	2.5	4.2	2.4
81	40%	1.0	2.5	2.6
82	80%	1.5	6.1	2.1
83	80%	1.4	6.4	1.4
84	90%	1.4	3.3	11.4
85	5%	Inadequate		
86	25%	1.0	3.3	0.6
87	70%	NA	NA	37.9
88	80%	NA	NA	15.5
89	70%	NA	NA	5.8
90	70%	NA	NA	14.0
91	60%	3.7	42.6	60.0
92	70%	3.8	33.4	48.0
93	70%	NA	NA	60.0
94	60%	3.6	10.6	39.5

95	40%	2.7	87.8	60.0
96	80%	NA	NA	60.0
97	60%	NA	NA	34.4
98	20%	6.1	19.8	11.8
99	70%	NA	NA	60.0
100	40%	3.7	15.0	16.4
101	10%	NA	NA	39.5
102	30%	5.5	20.4	11.6
103	80%	NA	NA	37.3
104	40%	3.3	28.8	25.0
105	50%	NA	NA	25.0
106	70%	2.5	3.2	30.7
107	20%	NA	NA	5.0
108	60%	6.0	11.6	2.2
109	70%	4.1	2.8	2.2
110	60%	2.9	34.3	47.9
111	60%	NA	NA	45.9
112	70%	-	1.3	0.8
113	50%	NA	NA	60.0
114	70%	4.7	37.4	8.7
115	50%	4.4	41.1	35.3
116	70%	NA	NA	0.5
117	60%	5.7	28.8	60.0
118	90%	4.4	12	43.2
119	60%	NA	NA	31.5
120	60%	5.0	105.0	60.0
121	60%	NA	NA	38.7
122	60%	6.1	14.0	24.0
123	70%	NA	NA	43.8
124	50%	6.0	14.6	19.4
125	50%	3.0	85.5	24.9
126	30%	NA	NA	0.6
127	60%	3.3	22.0	6.9
128	90%	6.3	88.3	60.0
129	60%	6.3	30.2	60.0
130	30%	4.6	21.4	23.1
131	70%	3.2	30.6	30.0
132	40%	3.1	3.7	5.8
133	40%	5.5	29.6	28.2
134	70%	3.5	14.7	30.3
135	70%	2.7	17.2	40.7
136	80%	3.0	47.9	43.8
137	70%	4.0	108.0	60.0
138	30%	3.3	11.8	6.64
139	70%	3.7	9.5	11.1
140	40%	2.6	7.9	8.6
141	70%	3.1	120.0	58.0
142	80%	3.4	19.9	33.2
143	90%	4.7	94.2	60.0
144	70%	4.3	10.1	22.4
145	70%	4.7	10.3	28.2
146	70%	2.2	10.7	14.7
147	60%	2.6	3.1	2.6

Abbreviations: DIN (DNA Integrity Number); HGSOC (High-Grade Serous Ovarian Cancer); NA (Not Assessed); NC% (Neoplastic Cells Percentage); ng (nanograms); μl (microliters).

depth of 187.7 (ranging from 16.0 to 570.0) and SNP uniformity of 93.4% (ranging from 86.7 to 97.0%) were identified (Tab. II). As showed by NGS analysis, 7 out

of 112 (6.2%) and 2 out of 112 (1.8%) HGSOc patients harbored pathogenetic or likely pathogenetic (class I-II) and Variants of Uncertain Significance (VUS) (class

Table II. Technical parameters in successfully analyzed samples.

ID	Production	Total Reads	Coverage	BRCA Effective Depth	BRCA Uniformity	SNP Effective Depth	SNP Uniformity
2	2.16G	19913472	99.9%	817	100.0%	164	96.1%
4	2.01G	18571616	99.9%	979	100.0%	189	96.4%
5	1.73G	16474232	99.8%	435	99.2%	66	92.1%
6	2.03G	18367404	99.9%	778	99.6%	100	94.5%
8	1.74G	17425778	99.4%	174	95.7%	33	90.3%
9	1.72G	21884288	99.8%	388	95.7%	98	91.3%
10	1.75G	21398172	99.9%	582	99.6%	80	93.7%
11	5.52G	50918844	99.9%	511	99.2%	79	94.1%
12	4.62G	41335728	99.9%	2827	100.0%	427	96.9%
13	3.99G	37815908	99.6%	152	97.6%	28	93.7%
14	4.33G	38975284	99.9%	2661	98.8%	323	95.8%
15	3.84G	34455110	99.9%	1154	99.6%	148	95.2%
16	3.94G	36940658	98.4%	124	96.5%	19	89.8%
17	4.58G	41698228	99.9%	871	99.6%	219	95.8%
18	4.34G	39156410	99.9%	2158	100.0%	277	96.1%
19	3.72G	33514812	99.9%	2587	99.6%	295	96.2%
20	4.09G	37142410	99.9%	1067	100.0%	177	95.9%
21	4.31G	38692248	99.9%	725	96.9%	96	90.8%
22	4.72G	41886534	99.9%	1259	99.2%	133	91.3%
23	4.52G	40647830	99.9%	1364	100.0%	229	96.5%
24	4.76G	42715040	99.8%	660	96.9%	92	89.9%
25	4.64G	41669086	99.9%	921	98.4%	164	93.0%
26	4.58G	41224262	99.9%	1359	99.6%	191	94.5%
27	4.31G	38829700	99.8%	502	97.2%	75	91.6%
28	4.51G	41530520	99.7%	270	96.1%	65	89.1%
29	4.55G	42401678	99.8%	422	99.2%	50	95.7%
30	4.53G	40806372	99.9%	1422	99.2%	191	93.6%
31	4.70G	42825632	99.9%	1203	99.2%	170	95.2%
32	4.96G	44837466	99.9%	1330	100.0%	178	95.1%
33	4.44G	43733852	99.5%	171	99.6%	26	92.7%
34	4.58G	41035748	99.9%	1046	98.0%	155	94.3%
35	4.42G	40583668	99.9%	773	99.6%	117	93.7%
36	4.64G	42201522	99.9%	817	100.0%	70	94.7%
38	4.43G	40157424	99.9%	838	99.6%	120	94.4%
39	3.90G	35460700	99.7%	698	96.5%	76	90.3%
41	4.82G	43868824	99.9%	813	99.2%	126	95.5%
42	4.42G	40131068	99.9%	1100	100.0%	208	95.8%
43	4.36G	40299812	99.8%	398	98.4%	51	94.0%
45	4.84G	43556400	99.9%	840	97.2%	144	93.9%
46	4.56G	40586888	99.9%	1299	98.8%	116	92.4%
47	4.91G	44813608	99.8%	402	96.1%	81	91.4%
48	4.84G	44602962	99.9%	393	98.0%	86	92.4%
49	4.80G	43460562	99.9%	916	99.6%	152	94.8%
50	4.55G	42126022	99.9%	183	99.6%	62	95.9%
51	4.84G	43737052	99.9%	1289	98.8%	210	93.8%
53	3.89G	35529636	99.9%	695	99.6%	121	92.9%
54	3.47G	31529008	99.9%	1197	98.8%	145	93.9%
57	4.12G	37473492	99.9%	1209	99.6%	119	95.1%
58	4.28G	39907842	99.9%	241	99.6%	57	94.4%
59	3.96G	37690392	99.7%	446	97.2%	60	89.7%
61	3.94G	36170532	99.8%	766	92.5%	123	86.8%
62	3.95G	35957586	99.9%	1144	94.5%	145	89.0%

Table II. Continues from previous page.

ID	Production	Total Reads	Coverage	BRCA Effective Depth	BRCA Uniformity	SNP Effective Depth	SNP Uniformity
70	4.20G	39425048	99.9%	1079	99.2%	140	94.5%
73	4.16G	45260928	99.7%	227	97.2%	45	90.5%
74	3.85G	37880930	99.8%	201	99.6%	48	95.7%
76	3.81G	40714262	99.6%	308	98.8%	46	90.1%
78	4.00G	37271824	99.9%	818	99.6%	113	95.7%
87	5.14G	49678042	99.9%	2512	98.4%	426	91.4%
88	4.51G	49024584	99.9%	657	94.1%	205	86.7%
90	5.87G	64179792	99.9%	1027	100.0%	218	96.0%
91	5.49G	48952474	99.9%	3709	100.0%	394	95.4%
92	5.35G	48318194	99.9%	2158	99.6%	358	93.2%
93	4.98G	45098256	99.9%	2689	98.0%	401	95.7%
94	5.31G	47609376	99.9%	2228	100.0%	302	94.7%
95	5.33G	47799930	99.9%	4533	100.0%	570	96.5%
96	4.34G	38893156	99.9%	2326	100.0%	328	95.4%
97	4.62G	41774942	99.9%	1173	99.6%	205	95.6%
98	4.64G	41099162	99.9%	2559	97.2%	243	90.9%
99	4.41G	41545088	99.8%	158	100.0%	41	94.0%
100	4.55G	41198850	99.9%	1818	96.5%	231	88.8%
101	4.08G	36706560	99.9%	3064	99.2%	381	94.7%
102	4.25G	38271362	99.9%	1974	98.0%	280	92.6%
103	4.01G	36258236	99.9%	2374	99.6%	350	95.6%
104	4.32G	39507416	99.9%	2375	100.0%	295	95.8%
105	4.14G	37751412	99.9%	455	98.8%	88	93.9%
106	3.24G	33789398	99.3%	135	100.0%	16	93.6%
107	3.94G	35958628	99.9%	961	98.8%	93	94.7%
108	3.81G	35128618	99.6%	299	99.2%	28	93.3%
110	4.41G	39832638	99.9%	869	99.6%	120	93.8%
111	4.23G	38265164	99.9%	1167	98.8%	184	93.7%
113	4.47G	40114626	99.9%	4699	99.2%	459	94.1%
114	4.15G	37610246	99.9%	1030	98.4%	165	90.2%
115	4.06G	36741122	99.9%	1538	97.6%	316	91.0%
117	5.08G	46050092	99.9%	1876	99.2%	357	91.7%
118	4.61G	40677240	99.8%	991	96.5%	134	89.3%
119	3.57G	32267578	99.9%	1321	98.4%	173	93.8%
120	5.12G	46101768	99.9%	2055	96.9%	283	92.2%
121	5.02G	44891200	99.9%	1063	97.2%	162	92.7%
122	4.35G	38847036	99.9%	2741	96.9%	328	90.2%
123	4.84G	43528842	99.9%	2614	98.8%	358	93.0%
124	5.23G	46709206	99.9%	3116	94.5%	342	88.8%
125	3.44G	31432526	99.9%	1323	99.2%	233	93.3%
127	3.77G	34286630	99.7%	746	94.9%	87	89.0%
128	4.91G	44385696	99.9%	2716	100.0%	401	95.7%
129	5.11G	45898028	99.9%	3621	99.6%	424	94.0%
130	6.52G	58638886	99.9%	3283	99.2%	346	95.5%
131	5.00G	45451438	99.9%	1054	100.0%	161	94.5%
132	4.91G	45782918	99.7%	553	96.5%	59	90.5%
133	4.11G	37079280	100.0%	2798	99.6%	283	94.7%
134	4.19G	37691494	99.9%	1912	99.6%	225	92.8%
135	5.06G	45640186	99.9%	980	93.7%	110	94.0%
136	5.14G	46451684	99.9%	1514	100.0%	199	95.8%
137	5.55G	50133706	99.9%	3069	100.0%	475	97.0%
138	5.17G	46407322	99.9%	1748	97.6%	223	92.8%
139	4.91G	44130552	99.9%	1188	98.8%	188	94.2%
140	4.68G	42457358	99.9%	1024	99.2%	223	93.9%
141	5.37G	48704618	99.9%	2282	100.0%	420	95.9%
142	4.34G	39721426	99.9%	909	100.0%	127.5	94.0%
143	4.04G	36819558	100.0%	1355	99.6%	278.94	94.4%
144	4.20G	39062230	99.9%	748.3	98.0%	115.66	92.7%
145	4.16G	38568320	99.9%	1229	99.2%	211.52	92.9%
146	4.41G	42489920	99.8%	600	97.2%	81.23	89.2%

III) *BRCA1/2* molecular alterations, respectively, in accordance with AMP/CAP/ASCO/ACMG international guidelines²¹. Among *BRCA1/2* mutated patients, 4 out of 9 (44.4%) and 5 out of 9 (55.6%) mutated displayed *BRCA1* and *BRCA2* molecular alterations, respectively. In detail, 4 out of 9 (44.4%) and 5 out of 9 (55.6%) single nucleotide variants (SNVs) and small deletions or insertions were detected. A single alteration was detected in a *BRCA1* non-coding region. In addition, HRD measurement highlighted a positive GS score in 48 out of 112 (42.8%) HGSOC patients (Tab. III).

Sixteen out of 147 patients who underwent testing

Table III. List of *BRCA* molecular alterations found in successfully analyzed samples and GSS score evaluation.

ID	BRCA Alteration	GSscore
2	WT	5.2
4	WT	5.7
5	WT	2.5
6	WT	28.4
8	WT	13.2
9	WT	99.5
10	WT	7.9
11	WT	4.3
12	WT	28.0
13	WT	5.1
14	WT	10.9
15	WT	19.3
16	WT	66.6
17	WT	95.9
18	WT	26.2
19	WT	98.6
20	WT	35.8
21	WT	99.5
22	WT	9.6
23	WT	71.6
24	WT	3.4
25	WT	66.3
26	WT	90.5
27	WT	3.0
28	WT	81.5
29	BRCA1 intron 13 c.4358-31_4358-27del:p.?	3.0
30	WT	34.2
31	WT	21.1
32	WT	84.9
33	WT	3.1
34	WT	95.0
35	WT	79.2
36	WT	72.9
38	WT	9.5
39	WT	3.2
41	BRCA 2 exon 17 c.7887G > A:p.(W2629*);p.(Trp2629Ter)	18.1

42	WT	11.1
43	WT	95.5
45	WT	88.6
46	WT	71.0
47	WT	2.1
48	WT	53.3
49	WT	30.3
50	WT	77.8
51	WT	99.7
53	WT	75.4
54	WT	30.0
57	WT	96.9
58	WT	94.1
59	WT	7.5
61	WT	2.0
62	WT	1.7
70	WT	41.2
73	WT	6.5
74	BRCA2 exon 11 c.5433_5434delinsTT:p.(E1811_E1812delinsD*);p.(Glu1811_Glu1812delinsAspTer)	26.6
76	WT	7.0
78	WT	99.4
87	WT	95.4
88	WT	98.7
90	WT	2.2
91	WT	43.5
92	WT	34.8
93	WT	93.8
94	WT	23.9
95	WT	95.1
96	WT	66.8
97	WT	5.3
98	WT	1.0
99	VUS BRCA1 exon 9 c.964G > A p.Ala322Thr 19,84%	10.0
100	WT	99.7
101	WT	4.0
102	WT	98.8
103	WT	23.3
104	BRCA1 exon 9 c.3229_3229delAG p.Gly1077Ala fs Ter8	95.8
105	WT	6.6
106	BRCA2 exon 23 VUS c.9006A > T; p.Glu3002Asp	4.7
107	WT	21.8
108	WT	0.9
110	WT	13.8
111	WT	72.0
113	WT	93.9
114	WT	25.2
115	WT	99.0
117	WT	98.1
118	WT	99.4
119	WT	35.2
120	WT	23.5
121	WT	75.3

Table III. Continues from previous page.

ID	BRCA Alteration	GSScore
122	WT	64.3
123	WT	99.5
124	WT	94.6
125	VUS BRCA1 exon 23 c.5504G > A, p.R183Q	91.5
127	WT	3.4
128	WT	1.7
129	WT	34.2
130	WT	0.9
131	WT	31.5
132	WT	3.6
133	WT	77.1
134	BRCA2 exon 21 p.A2915Vfs*12;p. Ala2915ValfsTer12	96.7
135	WT	11.0
136	WT	1.4
137	WT	30.3
138	WT	11.0
139	WT	8.5
140	WT	17.4
141	WT	81.0
142	WT	20.5
143	WT	86.2
144	WT	80.8
145	WT	100.0
146	BRCA2 exon 18 c.8184del;p. (K2729Rfs*4);p.(Lys2729ArgfsTer4)	85.7

Abbreviations: BRCA (Breast Related Cancer Antigens); GSS (Genomic Scar Score); VUS (Variants of Uncertain Significance); WT (Wild-Type).

for *BRCA1/2* pathogenic alterations and HRD measurement were referred to the Oncology Department of our University Hospital for clinical care. The baseline characteristics of these patients are detailed in Table IV. The median age at diagnosis was 61 years. All patients were diagnosed with HGSOc. One patient had FIGO stage I disease, while 11 patients had stage III, and four had stage IV. In the context of surgical treatment, 9 patients received primary debulking surgery (PDS), while 4 underwent interval debulking surgery (IDS) after receiving 3 cycles of neoadjuvant platinum-based chemotherapy and 3 after 6 cycles of platinum-based chemotherapy. Among the six patients whose chemotherapy response scores (CRS) were evaluated, three exhibited a minimal tumor response (CRS1), one displayed a partial tumor response (CRS2), and two achieved a complete tumor response (CRS3). The surgical outcomes were as follows: no residual disease (R0) in twelve patients, tumor residue of less than one centimeter (R1) in two, and tumor residue of greater than one centimeter (R2) in two others. All patients received chemother-

Table IV. Characteristics of the 16 of 147 patients who underwent testing for *BRCA1/2* pathogenic alterations and HRD measurement and referred to the Oncology Department of our University Hospital for clinical care.

Characteristics	N = 16
Median age	61.0 (45-82)
ECOG	
· 0	13
· 1	3
Primary tumor location	
· Ovary	16
· Fallopian tube	0
· Peritoneum	0
FIGO stage	
· I-II	1
· III	11
· IV	4
Histological Subtype	
· Serous	16
· Endometrioid	0
Surgery	
· PDS	9
· IDS after 3 cycles	4
· IDS after 6 cycles	3
CRS	
· 1	3
· 2	1
· 3	2
· Unknown	1
Residual disease	
· R0	12
· R1	2
· R2	2
Serum CA125 level	
· Normal	7
· Elevated	9
BRCA1/2 status	
· Wild type	15
· BRCA1	1
· BRCA2	0
HRD status	
· Positive (GSS > 50)	8
· Negative (GSS < 50)	8
Maintenance therapy	
· Bevacizumab	3
· Olaparib	5
· Niraparib	6
· Olaparib + Bevacizumab	1
· None	1
BMI	
· < 18.5	1
· 18.5-24.99	7
· 25.0-29.99	3
· ≥30	5

apy comprising carboplatin AUC5 + paclitaxel 175 mg/m² every 21 days for a total of six cycles. Among the 16 patients, 15 underwent maintenance therapy, with three receiving bevacizumab, five receiving olaparib, six receiving niraparib, and one receiving olaparib in combination with bevacizumab. Currently, the maturation of survival events has not yet reached a level that would allow for the identification of relationships between particular clinical features and oncological results.

Discussion

The rapidly evolving scenario of predictive biomarkers lay the basis for novel therapeutic algorithm in the management of HGSOC patients in clinical practice^{2,23}. *BRCA1/2* pathogenic alterations select HGSOC patients to PARPi administration stratifying patients that could benefit from a targeted approach³⁻⁹. In the last years, HRD status has been elected as integrating biomarker able to identify HGSOC patients administrable with PARPi. Several clinical trials highlighted a statistically significant favorable outcome in HGSOC patients under PARPi administration demon-

strating the clinical efficacy of *BRCA1/2*-HRD molecular analysis in a diagnostic routine setting. To date, HRD status is calculated by externalized NGS based assays able to measure genomic scar integrating molecular hallmarks behind DNA instability. In particular, outsourced diagnostic strategies are affected by high turnaround time (TAT), low sustainable technical cost and a non-negligible rejection rate (30.0-40.0%) drastically reducing the widespread diffusion of externalized systems in diagnostic routine practice. In this regard, a plethora of commercially available NGS assays measuring HRD score may be adopted to internally carry out HRD analysis in institutions involved in precision medicine. Despite the lack of clinical validation, the implementation of in-house HRD testing strategies overcomes technical and logistic issues of externalized testing approaches reducing rejection rate and TAT¹⁹. Start new paragraph we overviewed our diagnostic workflow for detecting *BRCA1/2* clinically informative alterations and calculating HRD score in a retrospective series of HGSOC patients by a commercially available NGS assay. Interestingly, 112 out of 145 (77.2%) HGSOC patients were successfully analyzed for both *BRCA1/2* and HRD status suggesting an adequate successful rate of molecular

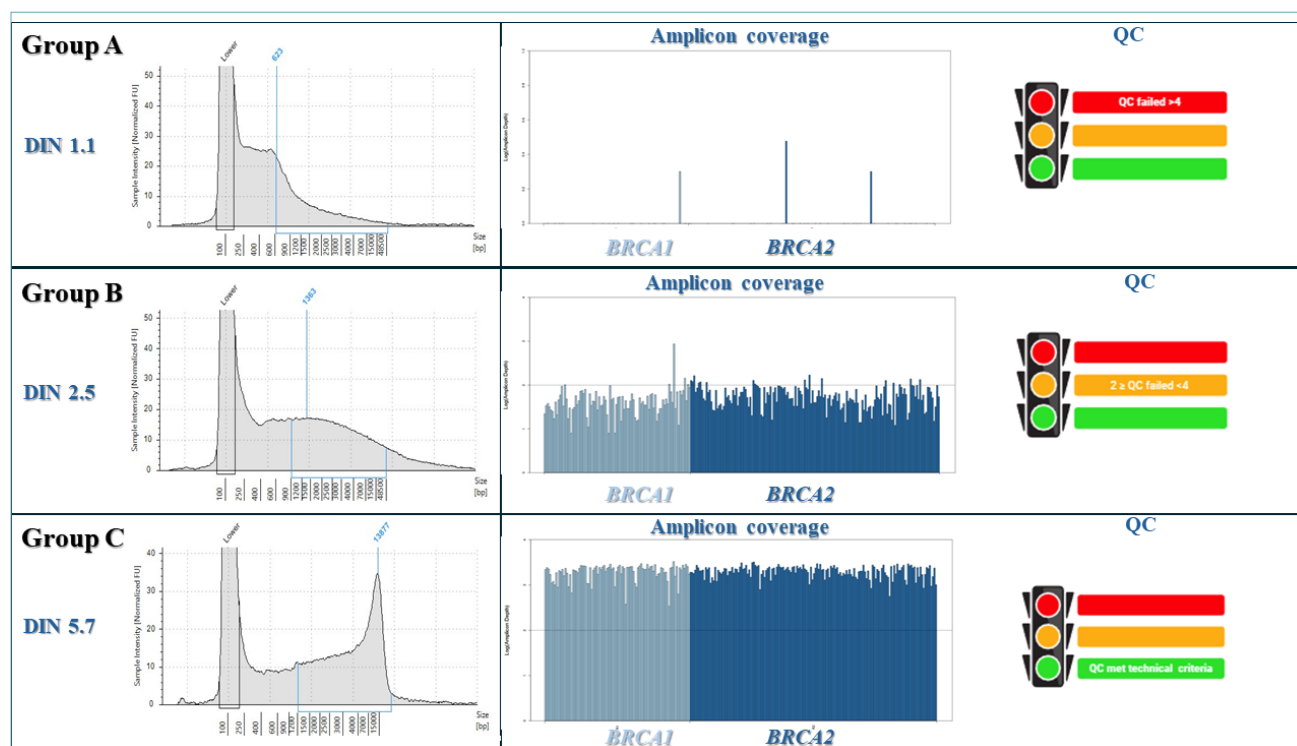


Figure 1. Exemplificative grouping (A, B, C) system to distinguish samples on the basis of technical parameters: DNA fragmentation index and NGS quality check drastically impacts the failure rate of molecular testing.

profiling in a series of real-world samples. In addition, our approach successfully detected clinically impactful BRCA1/2 mutations in 8.0% of HGSOc patients. Among them, 6.2% (7 out of 112) and 1.8% (2 out of 112) cases revealed BRCA class I-II and class III molecular alterations, respectively. Moreover, HRD status was detected in 42.8% (48 out of 112) patients. In this scenario, pre-analytical procedures of managing diagnostic specimens dramatically impact their suitability for molecular profiling. Regarding median DIN value, microfluidic system listed successfully tested HGSOc patients in A ($1 < \text{DIN} < 2$), B ($2 \leq \text{DIN} < 3$) and C ($3 \leq \text{DIN}$) groups (Supplementary Table I). Interestingly, samples affected by > 4 did not surpass quality metrics; $2 \geq \text{quality metrics} < 4$ failed matched with group A and B, respectively, while diagnostic samples with optimal technical parameters were listed in group C (Supplementary Table I) (Fig. 1). The latest point strongly suggests the central role of optimized pre-analytical management of diagnostic routine samples to improve the success rate in of HGSOc patients. Interestingly, NGS failed samples highlighted a median DIN value of 1.7 (ranging from 1.0 to 4.1) demonstrating the central role of pre-analytical procedures to maintain DNA integrity (Supplementary Table II) (Supplementary material)²⁴.

In conclusion, we evaluated a referral laboratory experience on integrated BRCA1/2-HRD molecular analysis in unselected HGSOc patients from our diagnostic routine practice supporting the key role of in-house comprehensive NGS analysis to decrease the rejection rate and TAT to optimize the clinical stratification of HGSOc patients. Further studies are recommended to clinically validate these technical strategies matching molecular and clinical records from a large cohort of HGSOc patients eligible for molecular analysis for PARPi administration.

CONFLICT OF INTEREST STATEMENT

F.P. has relevant relationship (advisory fees, honoraria, travel accommodation and expenses, grants and non-financial support) with Menarini, Roche unrelated to the current work. U.M. has received personal fees (as consultant and/or speaker bureau) from Boehringer Ingelheim, Roche, MSD, Amgen, Thermo Fisher Scientifics, Eli Lilly, Diaceutics, GSK, Merck and AstraZeneca, Janssen, Diatech, Novartis and Hedera unrelated to the current work. G.T. reports personal fees (as speaker bureau or advisor) from Roche, MSD, Pfizer, Boehringer Ingelheim, Eli Lilly, BMS, GSK, Menarini, AstraZeneca, Amgen and Bayer, unrelated to the current work. M.B. has received honoraria for consulting, advisory role, speakers' bureau, travel, accommodation, expenses from MSD Oncology, Ro-

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ETHICAL CONSIDERATION

All information regarding human material was managed using anonymous numerical codes, and all samples were handled in compliance with the Helsinki Declaration.

AUTHORS CONTRIBUTION

Conceptualisation: FP, GR, GT, UM. Methodology: all authors. Software: all authors. Validation: all authors. Formal analysis: all authors. Investigation: all authors. Resources: all authors. Data curation: all authors. Writing—original draft preparation: FP, GR, Writing—review and editing: all authors. Visualisation: all authors. Supervision: UM, GT. Project administration: UM and GT. Funding acquisition: GT, UM

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