The detection of germline and somatic *BRCA1/2* genetic variants through parallel testing of patients with high-grade serous ovarian cancer: a national retrospective audit

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Objective To determine the frequency of germline and somatic pathogenic *BRCA1* and *BRCA2* variants in patients with high-grade serous ovarian cancer tested by next-generation sequencing (NGS), with the aim of defining the best strategy to be implemented in future routine testing.

Design National retrospective audit.

Setting The All Wales Medical Genomics Service (AWMGS).

Population Patients with high-grade serous ovarian/fallopian tube/peritoneal cancer referred by oncologists to the AWMGS between February 2015 and February 2021 for germline and/or tumour testing of the *BRCA1* and *BRCA2* genes by NGS.

Methods Analysis of NGS data from germline and/or tumour testing.

Main outcome measures Frequency of *BRCA1* and *BRCA2* pathogenic variants.

Results The overall observed germline/somatic pathogenic variant detection rate was 11.6% in the 844 patients included in this study, with a 9.2% (73/791) germline pathogenic variant detection rate. Parallel tumour and germline testing was carried out for 169 patients and the overall pathogenic variant detection rate for this cohort was 14.8%, with 6.5% (11/169) shown to have a somatic pathogenic variant. Two *BRCA1* dosage variants were found during germline screens, representing 2.0% (2/98) of patients with a pathogenic variant that would have been missed through tumour testing alone.

Conclusions Parallel germline and tumour *BRCA1* and *BRCA2* testing maximises the detection of pathogenic variants in patients with high-grade serous ovarian cancer.

Keywords BRCA, germline, mainstreamed, oncology-led, ovarian cancer, pathogenic variant, somatic.

Tweetable abstract Parallel germline and tumour testing maximises *BRCA* pathogenic variant detection in ovarian cancer.

Linked article This article is commented on by C Gourley, p. 443 in this issue. To view this mini commentary visit https://doi.org/10.1111/1471-0528.16978.

Introduction

The identification of a pathogenic variant in either the *BRCA1* or *BRCA2* gene (hereafter collectively referred to as *BRCA*) can have prognostic and predictive implications for patients with ovarian cancer and their families. Poly (ADP-ribose) polymerase inhibitor (PARPi) treatment significantly improves progression-free survival in patients with advanced ovarian cancer, particularly in those with germline and somatic *BRCA* pathogenic variants.[1–6] Additionally, family members that are subsequently found to have a germline pathogenic *BRCA* variant through pre-symptomatic cascade testing are at an increased risk of developing breast and/or ovarian cancer.[7] The identification of individuals with pathogenic *BRCA* variants is therefore crucial to be able to guide treatment and inform at-risk individuals about appropriate

clinical management strategies, which may involve increased surveillance and/or risk-reducing procedures.[8]

Recent UK studies estimate the prevalence of germline pathogenic *BRCA* variants at 7.8–18.5% in patients with various types of epithelial ovarian cancer (Table 1).[9–14] Studies in patients with serous ovarian cancer have shown that a further 4.1–7.1% of patients' tumours harbour an acquired *BRCA* pathogenic variant (Table 2).[15–17] This highlights a significant proportion of this patient population that may be more responsive to PARPi maintenance therapy and demonstrates the clinical need to have effective genetic screening strategies in National Health Service (NHS) laboratories to identify these patients in a timely manner.

BRCA genetic testing in NHS laboratories is currently performed through germline and/or tumour next-generation sequencing (NGS). Acquired somatic variants can only be detected through tumour testing whereas germline variants can be detected by both testing methods. However, most NGS techniques currently employed by NHS laboratories to test tumour tissue are unable to detect large rearrangements and dosage variants. Since 2015, *BRCA* testing through the All Wales Medical Genomics Service (AWMGS) has been offered to all patients diagnosed with high-grade serous ovarian/fallopian tube/peritoneal cancer in Wales through an oncologist-led, mainstreamed referral pathway.[18] A concurrent dual-testing strategy, where germline and tumour testing is requested in parallel, has been employed in Wales since 2019, in-line with current British Gynaecological Cancer Society guidelines.[19] The purpose of this study is to audit the outcomes of these testing strategies to compare the detection of *BRCA* pathogenic variants. This is with the aim of determining the most effective strategy for the timely identification of women who may benefit most from PARPi maintenance therapy to adopt in future routine testing.

Methods

Patient population
This was a retrospective audit of the records of patients with high-grade serous ovarian/fallopian tube/peritoneal cancer who were referred by oncologists across Wales to the AWMGS (Cardiff, Wales) between February 2015 and February 2021 for germline and/or tumour testing of the *BRCA1* and *BRCA2* genes by NGS. There was no patient/public involvement in the design of this audit. Consent for testing was obtained by the referring oncologist. Counselling and results were also delivered by the referring oncologist.

Evolution of the testing strategy
Germline *BRCA* NGS analysis for patients diagnosed with high-grade serous ovarian/peritoneal/fallopian tube cancer through the mainstreamed, oncology-led diagnostic referral pathway began in early 2015. The *BRCA* tumour testing pathway for the same patient population was launched in 2017. Initially tumour samples were only tested in relapsed patients with a normal germline result in a two-step testing strategy. Since the start of 2019, it has been possible to request parallel tumour and germline tests concurrently for newly diagnosed patients. Figure 1 displays the key changes to the testing strategy in relation to the number of germline and tumour tests that were requested since the introduction of this novel, mainstreamed pathway in 2015.

Germline testing
DNA was extracted from blood samples. Analysis of the *BRCA1* and *BRCA2* genes was carried out using the Illumina TruSight® Cancer Sequencing Panel which uses the Illumina TruSight® Rapid Capture protocol for library preparation and target enrichment. Targeted gene NGS was performed using an Illumina® NextSeq 550 sequencing system using the Illumina® TG NextSeq 500/550 Mid Output Kit v2.5 (300 Cycles). The minimum read depth was 20× over the region of interest, which was coding exons ±5 flanking intronic bases (although some variants outside this region were also detectable if the minimum quality thresholds were met). A custom bioinformatics pipeline was used to call and annotate single nucleotide polymorphisms, insertions/deletions and dosage (whole exon/gene deletions/duplications) variants. Sanger sequencing or multiplex ligation-dependent probe amplification where appropriate were used to confirm variants. Referral to the AWMGS clinical genetics service was recommended in cases where a germline pathogenic variant was identified.

Tumour testing
DNA was extracted from formalin-fixed, paraffin-embedded tumour samples with a minimum tumour nuclei content of 10%. Multiplex PCR-based target enrichment of *BRCA1* and *BRCA2* was performed using the Qiagen GeneRead™ DNAseq Targeted Panels V2 kit and the NGHS-102X primer panel (Qiagen, Hilden, Germany). NGS was carried out using an Illumina® MiSeq® sequencer (Illumina, San Diego, CA, USA). Minimum read depth over the region of interest (coding exons ±5 flanking intronic bases) was 500× unless the coverage at this depth was less than 80% for either *BRCA1* or *BRCA2*, in which case the minimum read depth was 100× (as long as the tumour content of the sample was more than 50%). A custom bioinformatics pipeline was used to call and annotate single nucleotide polymorphisms and small insertions/deletions. This methodology does not allow the detection of dosage/structural variants and the sensitivity for deletions/insertions larger than a few bases is not well characterised.

Table 1. Comparison of germline testing results from this study with similar recent UK-based studies

	This study	Rust, 2018[9]	Rahman, 2019[10]	Rumford, 2020[11]	George, 2016[12]	Plaskocinska, 2016[13]	Flaum, 2020[14]
Number of patients	791	236	122	255	207	232	480
Data collection dates	02/2015–02/2021	2013–NR	02/2015–04/2016	04/2016–04/2018	07/2013–11/2014	07/2013–06/2015	11/2017–11/2019
Setting	AWMGS, Wales	Four centres across Scotland	UCLH, England	Imperial College Hospital, England	The Royal Marsden Hospital, England	East Anglian Genetics Services, England	North West of England
Testing criteria	High-grade serous ovarian/fallopian tube/peritoneal cancer	Non-mucinous ovarian cancer	High-grade non-mucinous ovarian cancer	Epithelial ovarian cancer	Non-mucinous ovarian cancer	Newly diagnosed epithelial ovarian cancer	Non-mucinous epithelial cancer of ovary/fallopian tube/peritoneum
Age (years) at diagnosis (or referral where appropriate) of patients with versus without pathogenic variant	58.1 vs 67.7 (mean)	NR	58 vs 62 (median)	57.8 vs 62.9 (mean)	53.9 vs 57.8 (mean)	49.5 vs 66.1 (mean)	53.1/60.5 (*BRCA1/BRCA2*) vs 60.5 (mean)
Germline pathogenic *BRCA* variant detection rate	73 (9.2%)	31 (13.1%)	18 (14.8%)	34 (13.3%)	33 (15.9%)	18 (7.8%)	89 (18.5%)
Dosage variant rate	2 (2.7%)	NR	NR	2 (5.9%)	4 (12.1%)	NR	NR
Founder variant rate	2 (2.7%)	NR	2 (11%)	6 (17.6%)	NR	0 (0%)	AKJ population excluded

AKJ, Ashkenazi Jewish; NR, not reported.
Categorical data presented as number of patients (%).

Table 2. Comparison of parallel tumour and germline testing results from this study with similar recent studies

	This study	Koczkowska, 2016[15]	Eoh, 2020[16]	Peixoto, 2020[17]
Number of patients	169	97	98	95
Setting	AWMGS, Wales	Medical University of Gdansk, Poland	Severance Hospital, South Korea	Portuguese Oncology Institute of Porto, Portugal
Testing criteria	High-grade serous ovarian/fallopian tube/peritoneal cancer	Serous ovarian carcinoma	High-grade serous ovarian cancer	High-grade serous ovarian cancer
Overall (somatic and/or germline) pathogenic variant detection rate	25 (14.8%)	27 (27.8%)	24 (24.5%)	22 (23.2%)
Acquired pathogenic variant rate	11 (6.5%)	4 (4.1%)	7 (7.1%)	6 (6.3%)

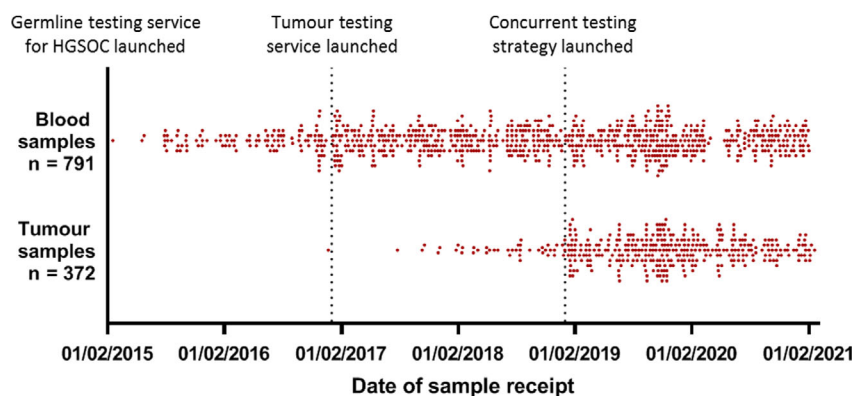Data presented as number of patients (%).

Figure 1. Distribution of dates of arrival of samples in AWMGS of blood and tumour samples referred for germline and tumour *BRCA1/2* tests, respectively. HGSOC, high-grade serous ovarian cancer.

Tumour testing allows the detection of both germline and acquired somatic variants but it is not possible to distinguish between these without carrying out a paired germline analysis. Where a pathogenic variant was identified in the tumour, germline testing was recommended to investigate the possibility of germline origin.

Variant analysis

Variants were analysed using the current American College of Medical Genetics and Genomics (ACMG) and Association for Clinical Genomic Science (ACGS) variant classification guidelines.[20,21] The Cancer Variant Interpretation Group UK (CanVIG-UK) specifications for the interpretation of germline variants in cancer susceptibility genes were also applied.[22] Reference sequences: NM_007294.3 (*BRCA1*) and NM_000059.3 (*BRCA2*). Pathogenic variants in this study refer to those classified as 'likely pathogenic' (class 4) or 'pathogenic' (class 5) according to ACMG guidelines. Variants classified as a variant of uncertain significance are not clinically actionable in terms of guiding treatment

pathways for this patient population and have not been reported in this audit.

Statistical analysis

Categorical data are described using counts and percentages. For the percentages of patients with a pathogenic variant, 95% confidence intervals were calculated. Simple descriptive statistics (mean, standard deviation [SD]) were computed for the age at referral of patients and the range and the time between referrals. GraphPad Prism 8 was used to produce graphs (GraphPad, San Diego, CA, USA).

Results

Testing pathways

Data were available for a total of 844 women (mean age at referral 67.0 years, SD 10.2) who underwent a germline and/or tumour *BRCA* test. A total of 472 patients only received germline testing and 53 patients only received tumour testing. The remaining 319 patients received dual

testing (both a germline and a tumour test) with a subset of these (n = 169) receiving dual testing in parallel, where testing of the tumour sample and the germline sample for that individual overlapped and the referral into each testing pathway was therefore independent from the outcome of the other test. Figure 2 and Table 3 summarise the number of patients according to the testing strategy they received.

Prevalence of pathogenic variants

Irrespective of the testing strategy, a pathogenic variant was detected in 11.6% (98/844) of patients. The pathogenic variants detected and the method of their detection are detailed in Table S1. Twelve variants were identified in more than one patient. The most common pathogenic variants were *BRCA1* c.4065_4068del and *BRCA1* c.2475del with each being identified in 4.1% (4/98) of those with a
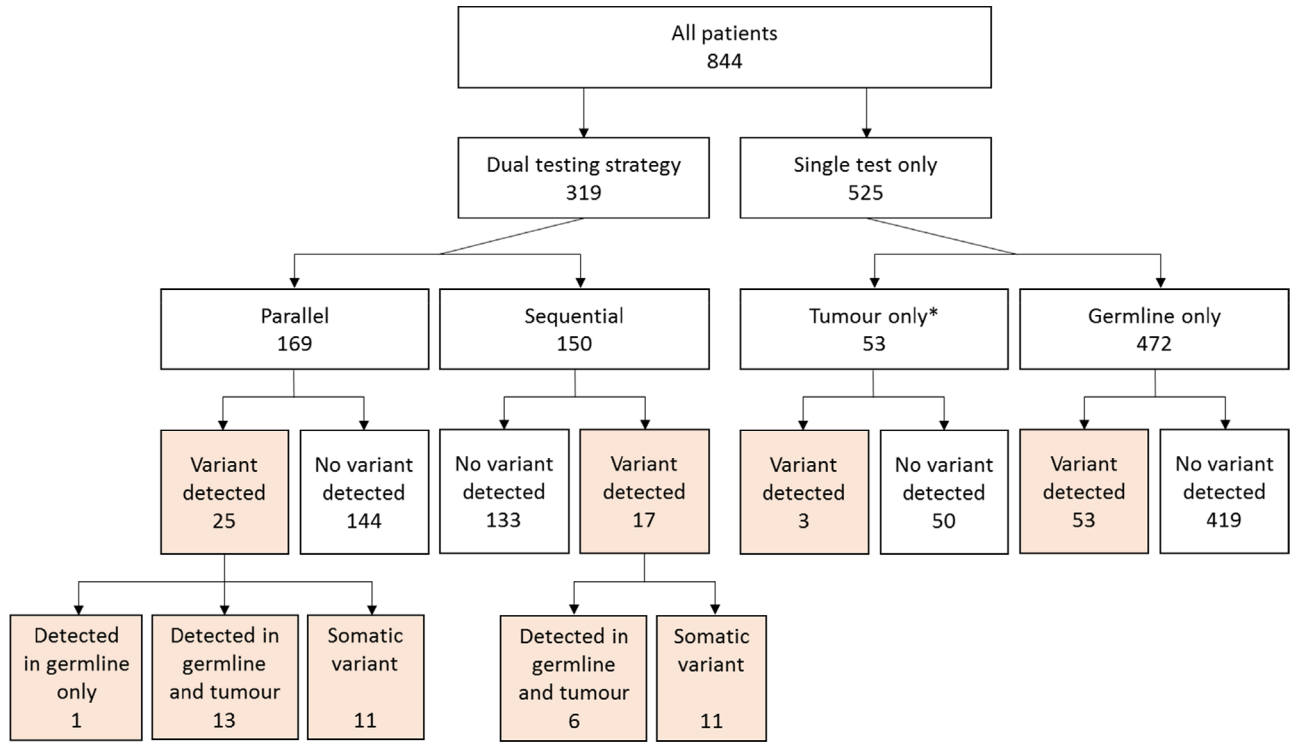

Figure 2. Flow diagram detailing the number of patients according to testing received and outcome (pathogenic variant detection). *Please note: testing of a tumour sample only is not a standalone testing strategy. It is anticipated that further germline testing will be carried out for the majority of these patients.

Table 3. Prevalence of pathogenic *BRCA1/2* variants according to testing strategy

| | Germline testing* | Tumour testing* | Dual testing | | | All patients included in study, regardless of testing strategy |
			Tested sequentially	Tested in parallel	All dual-tested	
Number of patients	791	372	150	169	319	844
Mean age at referral (years)	66.8	66.5	65.8	64.6	66.9	67.0
SD	10.2	9.6	9.4	8.3	10.2	10.2
Number of patients with a pathogenic variant detected	73	44	17	25	42	98
%	9.2%	11.8%	11.3%	14.8%	13.2%	11.6%
95% confidence interval	(7.2%, 11.2%)	(8.5%, 15.1%)	(6.3%, 16.4%)	(9.4%, 20.2%)	(9.5%, 16.9%)	(9.4%, 13.8%)

*An overlapping subset of both of these cohorts form part of the dual-tested cohort.

pathogenic variant. The prevalence of pathogenic variants according to testing strategy is detailed below and in Figure 2 and Table 3.

Germline testing

Germline testing was carried out in 791 patients. Of these, 319 patients also underwent a tumour test so were included in the dual-tested cohort and the remaining 427 patients only underwent germline analysis. A germline pathogenic *BRCA* variant was detected in 73/791 (9.2%) patients; 60.3% (44/73) of variants were in *BRCA1* and 39.7% (29/73) were in *BRCA2*. Two dosage variants (2.7% of all 73 germline variants) were identified: a *BRCA1* deletion of exon 20 was found in one patient who only received a germline test and a duplication of *BRCA1* exon 13 was found in one patient who underwent dual testing. The age of patients at the date of their referral to the AWMGS laboratory for testing was significantly younger in patients where a germline pathogenic *BRCA* variant was identified (mean age at referral 58.1 years, SD 9.4) compared with those where no germline pathogenic variant was detected (mean age at referral 67.7 years, SD 9.9) (Figure S1). Founder variants known to be of high prevalence in Ashkenazi Jewish populations were found in 2.7% (2/73) of patients with a germline pathogenic variant.

Tumour testing

Tumour testing was performed for 372 patients. At the time of this audit, 53 (14.2%) of the 372 patients that had received a tumour test had not yet had a germline test requested, but the majority (319/372, 85.8%) also had germline analysis so are included in the dual-testing cohort. A pathogenic variant was detected in 11.8% (44/372) tumour samples. Two relatively large deletions, *BRCA1* c.1607_1632del and *BRCA2* c.4393_4438del, of 26 and 46 bases respectively, were identified through tumour testing.

Dual testing

Of the 319 patients that had both a germline and tumour *BRCA* test, 42 (13.2%) were found to have a pathogenic variant in the tumour and/or germline. Twenty (47.6%) of these pathogenic variants were germline and the additional 22 (52.4%) pathogenic variants were only identified through tumour testing. Therefore, 6.9% (22/319) of dual-tested individuals had an acquired somatic variant. Eighteen (81.8%) of the 22 somatic variants were in *BRCA1* and four (18.2%) were in *BRCA2*. One dosage variant (2.4% of 42 pathogenic variants), a duplication of *BRCA1* exon 13, was only detected through germline analysis and not by tumour testing. The methods of detection of the pathogenic variants identified in the dual-tested cohort are shown in Figure 3A.

A total of 150 (47.0%) of the 319 patients that had dual testing received germline and somatic screening sequentially, where the result of the first test was issued before the second test being requested. The germline result was reported before the arrival of the tumour sample in the majority of these (86.7%, 130/150) in keeping with historical referral criteria. The mean time between the reporting of the germline result and the initiation of the tumour sample was 371.3 days (SD 347.6).

Parallel dual testing

A total of 169 individuals received a germline and tumour *BRCA* test in parallel and a pathogenic variant was detected in 25 (14.8%) of them. Fourteen of the 25 pathogenic variants (56.0%) identified were present in the germline. The additional 11/25 (44.0%) variants were only detected in the tumour, so acquired somatic variants were found in 11/169 (6.5%) of the parallel dual-tested cohort, with eight (72.7%) of these in *BRCA1* and three (27.3%) in *BRCA2*. As was the case in the full dual-tested cohort, tumour testing was able to detect the majority (24/25, 96.0%) of the pathogenic variants with one dosage variant (1/25, 4.0%) not detected on a tumour test. The methods of detection of the pathogenic variants identified in the parallel dual-tested cohort are shown in Figure 3(B). The mean time between the arrival of the first sample and when the results for both tests were available was 48.9 days (SD 19.5).

Discussion

Main findings

This is one of the largest UK studies to publish data examining the outcomes of *BRCA* genetic testing in patients with high-grade serous ovarian cancer and the first to report outcomes from parallel germline and tumour testing. The AWMGS has been offering germline and tumour *BRCA* testing for this patient population in Wales since 2015 and 2017, respectively, through an oncology-led testing pathway. This mainstreamed approach allows the large-scale delivery of testing to patients who may rely on a rapid genetic result to guide treatment decisions. It has been shown to be effective in other centres and is now recommended in the British Gynaecological Cancer Society national guidelines.[19]

Germline pathogenic *BRCA* variant detection

The prevalence of germline *BRCA* pathogenic variants in this study was 9.2% (73/791), with more variants reported in *BRCA1* than *BRCA2* (44, 60.3% versus 29, 39.7%, respectively), and only 2.7% of patients (2/73 of those with germline variants) had *BRCA* founder variants associated with an Ashkenazi Jewish ancestry. The observed prevalence of germline pathogenic variants was consistent with a study conducted in a largely homogeneous population with regards to ethnicity that also lacked founder variants.[13]
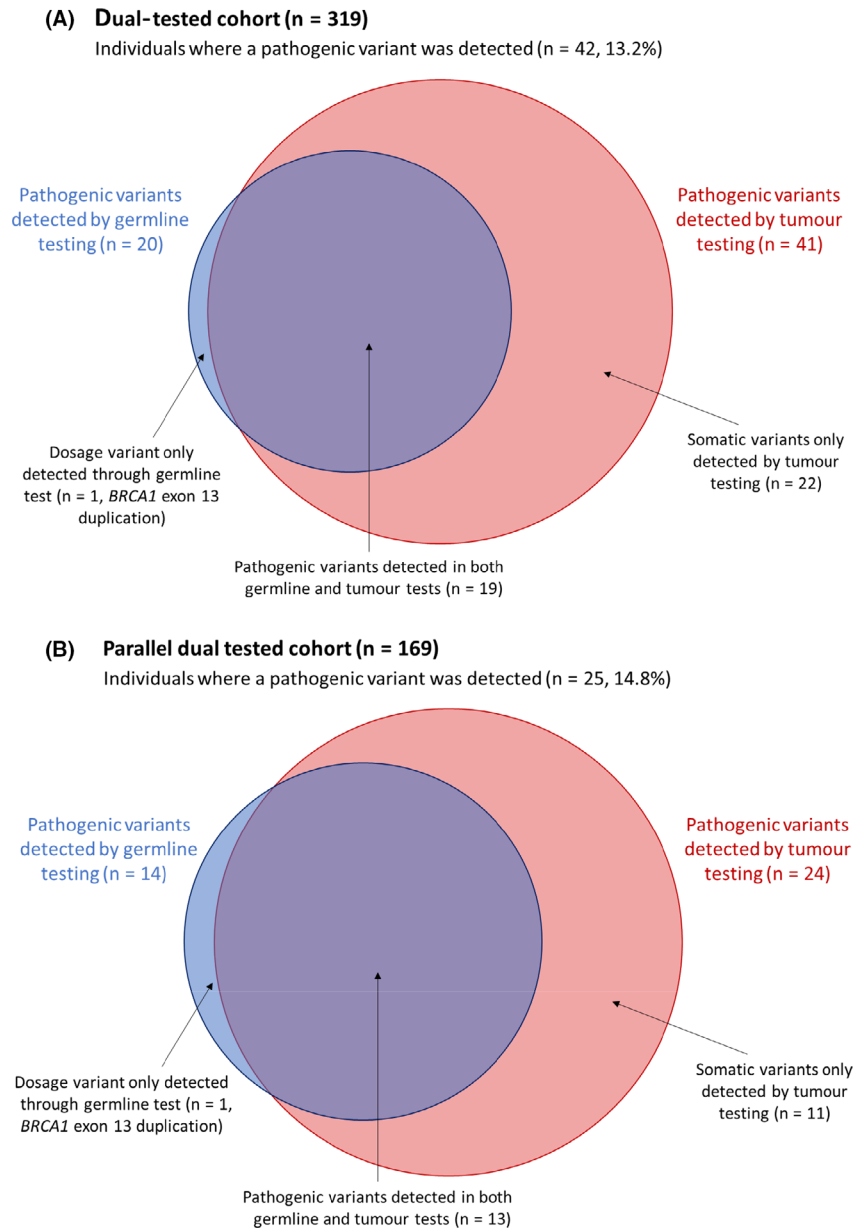
(A) Dual-tested cohort (n = 319)

Individuals where a pathogenic variant was detected (n = 42, 13.2%)

Pathogenic variants detected by germline testing (n = 20)

Pathogenic variants detected by tumour testing (n = 41)

Dosage variant only detected through germline test (n = 1, *BRCA1* exon 13 duplication)

Somatic variants only detected by tumour testing (n = 22)

Pathogenic variants detected in both germline and tumour tests (n = 19)

(B) Parallel dual tested cohort (n = 169)

Individuals where a pathogenic variant was detected (n = 25, 14.8%)

Pathogenic variants detected by germline testing (n = 14)

Pathogenic variants detected by tumour testing (n = 24)

Dosage variant only detected through germline test (n = 1, *BRCA1* exon 13 duplication)

Somatic variants only detected by tumour testing (n = 11)

Pathogenic variants detected in both germline and tumour tests (n = 13)

Figure 3. Method of detection of pathogenic *BRCA1/2* variants identified in dual-tested patients (A) and in parallel dual-tested patients (B).

However it was lower than several reports in the literature from other centres (13–18.5%) with higher rates of founder variants (11–17.6%) that included more diverse tumour types (Table 1).[10,11] Wales was the least ethnically diverse area according to the Office for National Statistics 2011 Census data for England and Wales, and we expect the ethnicities of the individuals included in this study to be in-line with this. We therefore consider it reasonable to suggest that tumour type, the prevalence of founder variants and variation in the ethnic composition of patients may have an association with germline *BRCA* variant detection.

Furthermore, germline pathogenic *BRCA* variants, are known to be associated with individuals with a strong family history of *BRCA*-related cancers. According to reports in the literature, around half (45.5–53.2%) of ovarian cancer patients with *BRCA* pathogenic variants have been shown to have a significant family history.[12,23] In Wales, these patients with ovarian cancer and a family history or secondary personal diagnosis of breast and/or ovarian cancer may have been referred for germline *BRCA* testing through the clinical genetics service, not through the oncologist-led pathway.[18] It is therefore possible to assume

that this study may not have captured the data from some of these individuals with a strong family history of *BRCA*-related cancers, who are more likely to have a germline *BRCA* pathogenic variant. It will be interesting to see the outcomes of larger UK studies, such as the SIGNPOST study, which may be able to fully elucidate the correlation between family history, founder variant rate, ethnicity and/or tumour type with germline pathogenic *BRCA* variant detection rate, as has been suggested by this audit and other regional studies.

Paired germline and tumour testing

The pathogenic variant pick-up rate was 13.2% (42/319) for patients who underwent both a germline and a tumour *BRCA* genetic test. However, before 2019, *BRCA* tumour testing was only available to patients with a germline normal result. The referral of patients for a germline and tumour test through this strategy is therefore not independent and the whole dual-tested cohort may be enriched for germline normal patients (as illustrated in Figure 4). To remove bias from this dual-tested cohort, the outcomes from patients who received germline and tumour analysis in parallel (where testing overlapped and referral for each test was therefore independent) were examined. There were 169 patients in this concurrently tested cohort and the pathogenic *BRCA* variant rate was 14.8% (25/169). This is higher than either of the single-test strategies as pathogenic dosage and acquired variants would both be detectable through dual testing but would be missed using a tumour-only or germline-only testing strategy, respectively. It is also higher than in the cohort where dual testing was not independent, as expected, because individuals with a germline pathogenic variant, that may have not progressed to have a tumour test based on historical referral criteria, would still be included in the parallel-tested group (Figure 4). An acquired somatic *BRCA* variant (not present in the germline) was detected in 11/169 (6.5%) of concurrently dual-tested patients in this study with the majority (72.7%, 8/11) of the variants identified in *BRCA1*. This is in-line with other studies (Table 2) that have reported similar somatic variant detection rates of 4.1–7.1%.[15–17] The acquired somatic variant rate represents a significant proportion of

patients that are eligible for PARPi treatment that would not be identified through germline screening alone and highlights the importance of access to tumour testing services for this population. Furthermore, access to PARPi treatment may have been delayed for these individuals if the historical two-stage referral pathway was employed, highlighting the value of carrying out parallel testing to ensure timely access to the most appropriate treatment for patients with high-grade serous ovarian cancer.

Dosage variants

Two *BRCA* dosage variants (constituting 2.7% of all 73 germline variants) were detected during germline testing in this study: a recurrent and well-documented *BRCA1* exon 13 duplication[24] and a *BRCA1* exon 20 deletion. This means a possible 2.0% (2/98) of patients found to have a pathogenic variant in this study would not have been eligible for PARPi if a tumour testing-only strategy was followed. Dosage variants have been reported to represent 5.9–12.1% of germline pathogenic variants in other studies (Table 1), but also in a low number of patients, and unpublished data from the SIGNPOST study and evidence from Public Health England suggest that this could be as much as 10% or more in various populations that have undergone *BRCA* genetic screening.[4,11,12,19] Given the astonishing potential clinical benefit for patients who receive PARPi treatment and the implications for family members of those with germline pathogenic variants, it would therefore not be appropriate to follow a tumour-testing-only strategy and risk not identifying patients/families harbouring large rearrangements in the *BRCA* genes.[25]

Limitations

Information on screening uptake, patient satisfaction, tumour characteristics, family history, ethnicity, previous treatment regimens, response and follow up was not within the scope of this retrospective audit and has not been examined.

The region of interest examined in the *BRCA* genes in this study is limited to the coding exons and immediate flanking intronic regions that are covered to a minimum read depth threshold. Therefore, variants that lie outside
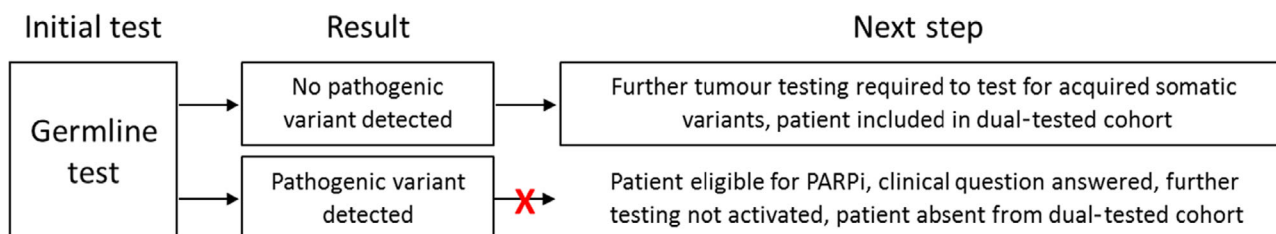

Initial test	Result	Next step
Germline test	No pathogenic variant detected	Further tumour testing required to test for acquired somatic variants, patient included in dual-tested cohort
	Pathogenic variant detected ✗	Patient eligible for PARPi, clinical question answered, further testing not activated, patient absent from dual-tested cohort

Figure 4. Potential source of bias in dual-tested cohort due to historical two-step referral criteria.

this region of interest or that fall in regions not covered by sufficient read depths would not be detected by either tumour or germline *BRCA* testing.

Future directions

The indication for the use of PARPi is rapidly evolving. All patients with high-grade, non-mucinous ovarian cancer in Wales are now eligible for *BRCA* testing, as is done in other NHS laboratories, and some types of PARPi will soon be accessible to all these patients regardless of *BRCA* status. The testing strategy in Wales is also due to expand to include concurrent *BRCA* and genomic instability testing using a tumour sample alongside germline *BRCA* testing to help assess how well these patients may benefit from PARPi treatment and which PARPi to consider. The extension of testing strategies to include other homologous recombination repair genes may also become relevant considering the active research ongoing into the use of PARPi for cancer patients with pathogenic variants in genes such as *PALB2*.[26,27]

The results of this study will be important for informing on the optimal testing strategies for other disease areas that may employ paired germline and tumour testing in the future to identify patients eligible for various treatments. For instance, the utility of PARPi in *BRCA*-positive patients with prostate cancer or breast cancer is currently being investigated in the UK. Furthermore, the application of NGS and bioinformatics technologies that have the capability to detect large genomic rearrangements in tumour samples is on the horizon in routine testing in NHS laboratories and will make significant strides towards the streamlining of this testing pathway.

Conclusions

In this study, parallel tumour and germline testing was carried out for 169 patients with high-grade serous ovarian cancer and pathogenic variants were detected at a frequency of 14.8%, with 6.5% of patients shown to have an acquired pathogenic variant that would not be detected through germline testing alone. Germline NGS techniques can identify large dosage variants that are often undetectable by the NGS methods currently employed to test tumour tissue. Consequently, this study identified a possible 2.0% of patients with a pathogenic variant that would not have been eligible for PARPi if a tumour-only testing strategy was followed.

Based on the findings from this national study, we suggest that the availability of germline and tumour *BRCA1* and *BRCA2* NGS testing services, requested in parallel, would ensure that all eligible patients with non-mucinous ovarian, fallopian tube or peritoneal cancer are afforded the opportunity to access the significant benefits of PARPi treatment in a timely way.

Disclosure of interests

Full disclosure of interests forms are available to view online as supporting information.

Contribution to authorship

The study was designed by SM, RW and SPS. Data were acquired by BF, AM, RJ, LH, CF, EH, AM and AQ. Data analysis and manuscript preparation were by BF. All authors reviewed the manuscript before submission.

Details of ethics approval

Ethical approval was not needed to undertake this audit. Where appropriate, all principles of the Declaration of Helsinki were adhered to.

Data availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

Consent

Consent for testing was obtained by the referring oncologist. No patient identifying information has been disclosed in this audit.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Age of patients at date of referral to AWMGS by germline *BRCA1/2* testing outcome. Tukey box plot; shown are minimum, lower quartile, median, upper quartile and maximum ages for each data set. Mean age at referral 58.1 years (SD 9.4) for group with a germline pathogenic variant identified versus 67.7 years (SD 9.9) for the group with no pathogenic germline variant identified.

Table S1. Pathogenic *BRCA1* and *BRCA2* variants identified in this study according to testing strategy. ∎

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