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Prevalence of germline pathogenic *BRCA1/2* variants in sequential epithelial ovarian cancer cases

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41 **Competing interests:** None declared.

Patient consent: All women included in this study provided informed verbal consent to undergo germline *BRCA1/2* testing.

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ABSTRACT

Introduction: Poly(ADP-ribose) polymerase inhibitors significantly improve progression-free survival in platinum-sensitive high-grade serous and endometrioid ovarian carcinoma, with greatest benefits observed in women with a pathogenic *BRCA1/2* variant. Consequently, the demand for germline *BRCA1/2* testing in ovarian cancer has increased substantially, leading to screening of unselected populations of patients. We aimed to determine the prevalence of pathogenic germline *BRCA1/2* variants in women diagnosed with epithelial ovarian cancer, categorised according to the established risk factors for hereditary breast and ovarian cancer syndrome and the Manchester BRCA Score, in order to inform risk stratification.

Methods: A cohort of sequential epithelial ovarian cancer cases recruited between June 2013 and September 2018 underwent germline *BRCA1/2* testing by next-generation sequencing and multiplex ligation-dependent probe amplification.

Results: Five hundred and fifty-seven patients were screened. Of these, 18% had inherited a pathogenic *BRCA1/2* variant. The prevalence of pathogenic *BRCA1/2* variants was >10% in women diagnosed with ovarian cancer earlier than 60 years old (21%) and those diagnosed later than 60 years old with a family history of breast and/or ovarian cancer (17%) or a past medical history of breast cancer (34%). The prevalence of pathogenic *BRCA1/2* variants was also >10% in women with a Manchester BRCA Score of ≥ 15 points (14%).

Discussion: Our study suggests that age at diagnosis, family history of breast and/or ovarian cancer, past medical history of breast cancer or a Manchester BRCA Score of ≥ 15 points are associated with a >10% prevalence of germline pathogenic *BRCA1/2* variants in epithelial ovarian cancer.

74 INTRODUCTION

75 Ovarian cancer is the eighth most common cancer occurring in women and the second
76 commonest cause of gynaecological-related cancer death worldwide [1]. Standard of care
77 treatments include cytoreductive surgery and platinum- and taxane-based chemotherapy [2,
78 3]. Molecularly targeted agents offer the promise of anti-cancer treatments that specifically
79 target biological vulnerabilities within tumour cells, thereby offering alternative therapies to
80 traditional cytotoxic agents. To date, pathogenic *BRCA1/2* variants are the only predictive
81 biomarkers validated in ovarian cancer [4]. Several phase 2/3 trials have shown that
82 poly(ADP-ribose) polymerase (PARP) inhibitors significantly improve progression-free
83 survival (PFS) in platinum-sensitive high-grade serous and endometrioid ovarian cancer, with
84 the greatest benefit achieved in women with a pathogenic *BRCA1/2* variant [5-10]. Indeed, a
85 recently reported randomised, double-blinded, placebo-controlled, phase 3 trial, SOLO1,
86 demonstrated that 24 months of olaparib maintenance therapy following a partial/complete
87 response to cytoreductive surgery and platinum-based chemotherapy in FIGO stage 3/4
88 *BRCA*-mutant high-grade serous or endometrioid ovarian carcinoma reduced the risk of
89 disease progression or death at 3 years with a hazard ratio 0.28 (95% confidence interval
90 0.20-0.39, $P < 0.001$) [9].

91 The prevalence of germline pathogenic *BRCA1/2* variants in ovarian cancer is estimated at
92 between 10 and 15%, with the majority of heterozygotes diagnosed with high-grade serous
93 ovarian carcinoma [11-15]. High-grade serous carcinoma is the commonest histological
94 subtype, accounting for approximately 70% of all cases of ovarian cancer [16, 17]. At
95 present, access to PARP inhibitors as maintenance therapy in Europe and North America is
96 restricted by morphological subtype (serous or endometrioid), *BRCA1/2* status (germline or
97 somatic) and/or platinum sensitivity (complete or partial response to the latest platinum-based

therapy). It is not surprising therefore that clinical demand for *BRCA1/2* testing has increased significantly as oncologists and patients seek to access these drugs [18-23]. As a result, germline *BRCA1/2* testing is increasingly prevalent in unselected populations of women with ovarian cancer, resembling routine tumour testing for somatic mutations in other tumour types e.g. *BRAF* (melanoma), *RAS* (colorectal cancer), *EGFR* (lung cancer) and *PDGFRA* and *KIT* (gastrointestinal tumours). Unlike routine tumour testing for somatic variants, testing for germline *BRCA1/2* variants could be stratified according to risk factors associated with hereditary breast and ovarian cancer syndrome [24].

In this study, we report the prevalence of germline pathogenic *BRCA1/2* variants in a large cohort of women diagnosed with epithelial ovarian cancer in the North West of England, correlating the prevalence of germline pathogenic *BRCA1/2* variants with risk factors associated with hereditary breast and ovarian cancer syndrome. Our aim is to inform risk stratification for germline *BRCA1/2* testing in epithelial ovarian cancer when conducted in an oncology clinic rather than a specialised genetics department.

METHODS

Patient selection

Women diagnosed with epithelial cancer of the ovary, fallopian tube or peritoneum (FIGO stage 1 to 4 [25]) who underwent germline *BRCA1* and *BRCA2* testing between 1st June 2013 and 1st September 2018 were included. Germline *BRCA1/2* testing took place in the oncology clinics at the Christie NHS Foundation Trust, Manchester or the genetics clinics at St Mary's Hospital, Manchester. Only women treated for ovarian cancer at The Christie Hospital or St Mary's Hospital were included in the study. Pathogenic (class 5) or likely pathogenic (class

4) *BRCA1* and *BRCA2* variants were included and will be referred to collectively as “pathogenic *BRCA1/2* variants” throughout this manuscript, whilst variants of unknown clinical significance (class 3) were excluded [26]. Cases of non-epithelial ovarian cancer were excluded. Women from a Jewish ancestry were excluded because across the North West of England this group undergo founder mutation testing first, and the Manchester BRCA Scoring System is not designed to assess risk in this population.

A family history was defined as any index case of epithelial ovarian cancer and a first-degree or second-degree relative with breast and/or ovarian cancer. An index case was diagnosed with sporadic ovarian cancer if she had no first-degree or second-degree relative with breast and/or ovarian cancer. All demographic data were extracted from case notes and/or electronic patient records.

Survival bias

In order to account for survival bias we performed a subgroup analysis according to the year the index case was diagnosed with ovarian cancer (pre versus post 2012). This strategy was adopted because the prevalence of pathogenic *BRCA1/2* variants detected in women diagnosed with ovarian cancer before 2012 may have been biased by long-term survivors [27, 28]. In women diagnosed with ovarian cancer before 2012, the minimum time from the diagnosis of ovarian cancer to subsequent germline *BRCA1/2* testing was 18 months (January 2012 to June 2013); an interval that approximates to half the median overall survival for ovarian cancer [17].

Germline *BRCA1/2* testing

Germline *BRCA1* and *BRCA2* variants were detected by testing DNA extracted from peripheral circulating lymphocytes. Next generation sequencing (NGS) was used to detect

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3 144 variants throughout the whole coding sequence of *BRCA1* and *BRCA2*, including at least 15
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5 145 base pairs beyond each exon-intron junction. Enrichment occurred using a custom designed
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7 146 long range PCR based approach followed by a normalisation step using SequelPrep
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9 147 normalisation plates and library preparation using the Illumina Nextera DNA Library
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11 148 Preparation Kit. NGS analysis was on an Illumina MiSeq using v2 2×150 base pair
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13 149 sequencing chemistry. Single nucleotide variants and small deletions, duplications, insertions
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15 150 and insertion/deletions (<40 base pairs) were called using a bioinformatic pipeline validated
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17 151 to detect heterozygous and mosaic variants in NGS data to an allele fraction of ≥4%. The
18
19 152 bioinformatic pipeline was developed for use across a broad range of inherited cancer
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21 153 syndromes, some of which have a high frequency of somatic mosaicism e.g.
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23 154 neurofibromatosis type 2. An allele fraction cut off of ≥4% for variant detection was
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25 155 determined following clinical validation, as this was the lowest allele fraction limit of
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27 156 detection where both sensitivity and specificity remained high.
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33 157 Testing for large genomic rearrangements/copy number variation (e.g. whole exon or whole
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35 158 gene deletions/duplications) in *BRCA1* and *BRCA2* was performed by multiplex ligation-
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37 159 dependent probe amplification (MLPA) [29]. The MLPA MRC Holland probe kits P002-D1
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39 160 (BRCA1) and P045-C1 (BRCA2) were used to analyse germline DNA. Amplified ligation
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41 161 products were subject to fragment analysis using an ABI 3130xl Genetic Analyser and size
42
43 162 called using GeneMapper v2.0 (Applied Biosystems). Copy number status calling was
44
45 163 performed using data exported from GeneMapper using custom developed MLPA
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47 164 spreadsheets that report relative dosage quotient for each probe compared to five reference
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49 165 control samples. All MLPA analysis assays were performed in duplicate for confirmation of
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51 166 results.
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57 167 **Manchester BRCA Scoring System**
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The Manchester Scoring System is a simple-to-use, paper-based model that can be used to determine the combined *BRCA1* and *BRCA2* carrier probability of an index case with a relevant cancer (Table 1) [30]. The development of the Manchester Scoring System was based on empirical data gathered from the Manchester mutation-screening programme [31]. Each individual, from one side of the family, is scored for each gene separately, *BRCA1* and *BRCA2* (Table 1). For index cases of breast cancer or any index case or unaffected relative of an index case of ovarian cancer (<60 years) the *BRCA1* and *BRCA2* scores are adjusted according to pathology [30]. The pathology adjustment takes into account the higher prevalence of germline pathogenic *BRCA1/2* variants in triple-negative breast cancer and high-grade serous ovarian carcinoma [32]. A Manchester Score of 15-19 points equates to a combined *BRCA1* and *BRCA2* probability of 10%, and 20 points to a 20% probability [30].

179

180 RESULTS

181 Five hundred and fifty-seven women of non-Jewish ancestry underwent germline *BRCA1* and
182 *BRCA2* testing following a diagnosis of epithelial ovarian cancer (Table 2). A total of 103
183 women (18%) had a pathogenic *BRCA1/2* variant (68 *BRCA1*, 35 *BRCA2*) (Table 2). The
184 mean age at which ovarian cancer was diagnosed differed in patients with pathogenic *BRCA1*
185 (51.9 years [range 36-76]) and *BRCA2* (59.4 years [range 33-86]) variants. The types of
186 pathogenic *BRCA1/2* variants detected are reported in Table 3. Twenty-three *BRCA1/2*
187 variants of unknown clinical significance (class 3) were detected.

188 Pathogenic *BRCA1/2* variants were most commonly detected in women diagnosed with high-
189 grade serous ovarian cancer, although women diagnosed with this histological subtype were
190 most frequently screened (Table 2). All women diagnosed with germline *BRCA*-mutant

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3 191 endometrioid ovarian cancer had poorly differentiated (high-grade) tumours. No *BRCA1/2*
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5 192 heterozygotes had low-grade serous, low-grade endometrioid, undifferentiated or mucinous
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7 193 ovarian cancer (Table 2) [16]. One woman diagnosed with FIGO stage 3C carcinosarcoma of
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9 194 the ovary had inherited a germline *BRCA1* variant, although the epithelial histological
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11 195 component of her invasive tumour was high-grade serous. Eighty-four women (15%) had
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13 196 been diagnosed with breast cancer and 268 (48%) had a first-degree or second-degree relative
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15 197 with breast and/or ovarian cancer (Table 2).
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20 198 The prevalence of pathogenic *BRCA1/2* variants was >10% in women diagnosed with ovarian
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22 199 cancer under the age of 60 years (21%) (Table 4). Also, the prevalence of pathogenic
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24 200 *BRCA1/2* variants was >10% in women diagnosed at 60 years or older with a family history
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26 201 of breast and/or ovarian cancer (17%) or a past medical history of breast cancer (34%) (Table
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28 202 4). In women diagnosed with sporadic ovarian cancer at 60 years or older the prevalence of
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30 203 pathogenic *BRCA1/2* variants almost reached 10% (7/76) (Table 4). However, in women
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32 204 diagnosed with sporadic ovarian cancer at 60 years or older without a past medical history of
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34 205 breast cancer, the prevalence of pathogenic *BRCA1/2* variants fell below 5% (2/46).
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39 206 Survival bias may have affected the prevalence of pathogenic *BRCA1/2* variants detected in
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41 207 the subgroup of women diagnosed with ovarian cancer at 60 years or older with a family
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43 208 history of breast and/or ovarian cancer, prior 2012, although the difference was not
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45 209 statistically significant (24% versus 15%; Fisher's exact test $P=0.21$) (Table 5).
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49 210 The prevalence of pathogenic *BRCA1/2* variants was >10% (101/463) in all women with a
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51 211 Manchester BRCA Score of ≥ 15 points, and there was a stepwise increase in prevalence as
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53 212 the Manchester Score increased (Table 6). In contrast, in women with a Manchester Score of
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55 213 <15 points the prevalence of pathogenic *BRCA1/2* variants was substantially <10% (2/94)
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57 214 (Table 6).
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215 Risk stratification by age alone confirmed women diagnosed with epithelial ovarian cancer
216 under the age of 30 years were unlikely to have a germline pathogenic *BRCA1/2* variant
217 (Table 7).

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219 DISCUSSION

220 By testing germline DNA in women diagnosed with epithelial ovarian cancer across North
221 West England we found the overall prevalence of pathogenic *BRCA1/2* variants exceeded
222 10% (103/557) (Table 2). Furthermore, by separating groups according to established risk
223 factors for hereditary breast and/or ovarian cancer syndrome we found the prevalence of
224 pathogenic *BRCA1/2* variants was consistently >10% in those women diagnosed with ovarian
225 cancer under the age of 60 years and in those diagnosed over 60 years old with either a family
226 history of breast and/or ovarian cancer or a past medical history of breast cancer (Table 2).

227 A number of studies have also assessed the prevalence of germline pathogenic *BRCA1/2*
228 variants in ovarian cancer. In an East of England series (GTEOC study), the prevalence of
229 germline pathogenic *BRCA1/2* variants amongst all high-grade serous and endometrioid
230 ovarian cancer cases was 8% (18/232) and increased to 12% (17/146) in women diagnosed
231 <70 years, but fell to 1% (1/86) in those aged ≥ 70 years [19]. Similarly, in a Scottish series
232 the prevalence of pathogenic *BRCA1/2* variants amongst unselected non-mucinous epithelial
233 ovarian cancer was 13.1% (31/236), but fell to 8.2% (13/159) in women diagnosed >70 years
234 old [20]. In an unselected series from Europe (AGO-TR-1 trial), the prevalence of pathogenic
235 *BRCA1/2* variants in epithelial ovarian cancer was 20.8% (109/523) and fell to 10.6% in
236 women diagnosed ≥ 60 years old, but increased to 31.9% (71/109) in women with a family
237 history of breast or ovarian cancer [33]. Moreover, in a large Australian study, the prevalence

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3 238 of pathogenic *BRCA1/2* variants in non-mucinous ovarian cancer was 14.1% (141/1,001), but
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5 239 fell to 8.3% (38/457) in women diagnosed ≥ 61 years old, 11.2% (103/738) in women without
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7 240 a personal history of breast and 8.3% (62/749) in women without a family history of breast
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9 241 and/or ovarian cancer [11]. The data from these series and our study therefore suggests that,
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11 242 three clinical features could be used to risk stratify for testing for germline *BRCA1/2* variants
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13 243 in women diagnosed with ovarian cancer, including age at diagnosis, family history of breast
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15 244 and/or ovarian cancer and past medical history of breast cancer. This is important if criteria
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17 245 for selecting which patients to tests are used by funding bodies.
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22 246 In our study, across the North West of England, selection criteria for germline *BRCA1/2*
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24 247 testing was mostly based upon an individual's pathology adjusted Manchester Score of ≥ 15
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26 248 points, with 17% (94/557) falling below the 15-point threshold [30]. This scoring system
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28 249 provides an alternative method for determining whether an individual's combined *BRCA1*
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30 250 and *BRCA2* carrier probability is $\geq 10\%$ (Table 1). In our series, a Manchester Score of ≥ 15
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32 251 points was associated with a $>10\%$ prevalence of pathogenic *BRCA1/2* variants, whereas a
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34 252 Manchester Score of <15 points was associated with a prevalence substantially $<10\%$.
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36 253 Furthermore, one of the *BRCA2* heterozygotes with a Manchester Score <15 had a strong
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38 254 family history of prostate cancer with two first-degree relatives diagnosed at <60 years old,
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40 255 giving Manchester Score of 14 (ovarian cancer <60 [5+5], 2 x prostate cancer <60 [+2, +2]).
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42 256 Overall therefore, the Manchester Score provides a better trade off of sensitivity and
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44 257 specificity than simply excluding women with sporadic ovarian cancer diagnosed after the
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53 259 Although this study is unlikely to unduly influence the debate regarding universal germline
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55 260 *BRCA1/2* testing in unselected populations of women diagnosed with ovarian cancer versus
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57 261 those at higher-risk of inheriting a variant, we consider a number of potential problems with
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unselected screening beyond the obvious financial burden. Firstly, pathogenic *BRCA1/2* variants occur much less frequently in non-high-grade non-serous ovarian carcinoma [11, 19, 20]. Indeed, somatic mutations in other genes are more commonly found in non-high-grade non-serous epithelial subtypes, including *PIK3CA*, *PTEN*, *KRAS*, *BRAF*, *ERBB2* and *ARID1A* [34-38]. Moreover, at present PARP inhibitors are only licensed in high-grade serous and endometrioid subtypes. Therefore, there does not seem to be a biological rationale or therapeutic incentive for unselected germline *BRCA1/2* testing in non-high-grade non-serous/endometrioid subtypes.

Secondly, if unselected germline *BRCA1/2* testing becomes the prerogative of oncologists, the additional clinical expertise provided by geneticists may be lost [39-42]. No *BRCA1/2* test is 100% accurate for all variants, and therefore accepting a diagnosis of *BRCA1/2* wild-type or variant of unknown clinical significance in an index case with a strong family history of cancer may be naive. Many NGS-based assays in use will identify variants in the coding regions of *BRCA1/2* +/- 5-10 base pairs either side of the intron-exon junction, but these assays would not detect rarer pathogenic variants such as deep intronic variants or those located in 5'-untranslated regions [43-45]. Furthermore, initially reported variants of unknown significance can be reclassified following further investigations such as segregation analysis, RNA sequencing or additional data from case-control analyses [39]. This level of genetic scrutiny only occurs in specialist genetics departments. There is therefore some concern that women diagnosed with epithelial ovarian cancer whom have a strong family history of cancer, may evade further necessary diagnostic investigations that would be performed by geneticists, if they are labelled as *BRCA1/2* wild-type or variant of unknown clinical significance by oncologists alone.

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Finally, by only screening for germline *BRCA1/2* variants there is a risk of missing other moderate-to-low penetrance actionable cancer-predisposition genes, such as *RAD51C/D*, *BRIP-1*, *MLH1*, *MSH2/6* and *PMS2* [24]. The prevalence of each individual cancer-predisposition gene is too low in ovarian cancer to warrant screening in an unselected population [46-48], however there is a risk that by focusing testing solely on *BRCA1* and *BRCA2*, other cancer-predisposition genes will remain undetected. In the North West of England, if a woman diagnosed with *BRCA1/2* wild-type ovarian cancer has a Manchester Score of ≥ 20 points, she is offered extended panel testing for alternative germline variants. We would therefore recommend that any patient diagnosed with ovarian cancer and a family history of cancer should be referred to the local genetic department irrespective of their *BRCA1/2* status.

There are some limitations with the study. Our study was biased by including mostly women with high-grade serous ovarian cancer and established risk factors for hereditary breast and/or ovarian cancer syndrome. Although we are confident that our series represents an almost comprehensive investigation of patients with high-grade serous ovarian cancer diagnosed under the age of 60 years, we acknowledge that a comparably smaller number of women diagnosed with ovarian cancer later than 60 years old were tested, especially those without risk factors for hereditary breast and ovarian syndrome. Consequently, the overall prevalence of germline pathogenic *BRCA1/2* variants reported in our study should be interpreted in the context of a selected population of women diagnosed with epithelial ovarian cancer.

In conclusion, the findings from our study suggest that if a 10% pre-test probability threshold is required prior to germline *BRCA1/2* testing in ovarian cancer then using age at diagnosis, a family history of breast and/or ovarian cancer, a past medical history of breast cancer or a Manchester Score of ≥ 15 should provide appropriate risk prediction.

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Cancer, age at diagnosis	<i>BRCA1</i>	<i>BRCA2</i>
FBC, <30	6	5
FBC, 30-39	4	4
FBC, 40-49	3	3
FBC, 50-59	2	2
FBC, >59	1	1
MBC, <60	5	8
MBC, >59	5	5
Ovarian cancer, <60	8	5
Ovarian cancer, >59	5	5
Pancreatic cancer	0	1
Prostate cancer, <60	0	2
Prostate cancer, >59	0	1
Pathology adjustment		
<i>Breast cancer (index case only)</i>		
Grade 3	+2	0
Grade 2	0	0
Grade 1	-2	0
ER positive	-1	0
ER negative	+1	0
Triple-negative*	+4	0
HER2 amplified†	-6	0
Ductal carcinoma <i>in situ</i>	-2	0
Lobular	-2	0
<i>Ovarian cancer (any case in family)†</i>		
Mucinous, germ cell or borderline tumours	0	0
High-grade serous, <60	+2	0
Adopted (no known status in blood relatives)	+2	+2

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Table 1. The Manchester Scoring System with pathology adjustment. Each individual and family characteristic (from one side of the family only) is given a numerical weight and these are added to give a score for each of the two genes, *BRCA1* and *BRCA2* [30]. Score “Cancer, age at diagnosis” first and then adjust score based on “Pathology adjustment”. Key: * Also score grade in addition to triple-negative; † Also score grade and ER status in addition to HER2 status; ‡ Only if the relative is not related to index case through more than one unaffected woman aged >60 years; FBC, female breast cancer; MBC, male breast cancer; ER, oestrogen receptor. As an example, a 34 year-old woman diagnosed with ER- HER2 amplified grade 3 invasive ductal carcinoma and a first-degree relative with high-grade endometrioid ovarian cancer diagnosed at 63 years old would score 4+4+2+1-6+5+5=15 points.

Demographics	Tested (n=557)	Combined <i>BRCA1/2</i> (n=103)
<i>Histology</i>		
Adenocarcinoma, NOS	13	5 (38)
Carcinosarcoma	6	1 (17)
Clear cell	18	2 (11)
Endometrioid	29	5* (17)
Low-grade serous	10	0
High-grade serous	475	90 (19)
Mucinous	4	0
Undifferentiated	2	0
<i>FH_x of BC/OC</i>	268	68 (25)
<i>PMH_x of BC</i>	84	28 (33)

Table 2. Demographic data. Data are reported as number (percentage; the denominator is column 2 “Tested”). Key: BC, breast cancer; FH_x, family history; NOS, not otherwise specified; OC, ovarian cancer; PMH_x, past medical history; *All *BRCA1/2* heterozygotes had poorly differentiated (high-grade) endometrioid ovarian cancer.

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<i>BRCA1/2</i> variant type	Number (%)
SNV	22 (21)
Insertion	1 (1)
Deletion	55 (53)
Duplication	11 (11)
Indel	1 (1)
Mosaic	1 (1)
LGR	12 (12)
- Deletion	5
- Duplication	7

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333 **Table 3. Germline *BRCA1/2* variant types.** Key: SNV, single nucleotide variant; indel,
 334 insertion/deletion; LGR, large genomic rearrangement.

Risk factors	Tested	<i>BRCA1</i>	<i>BRCA2</i>	Combined <i>BRCA1/2</i>
<60 y/o	352	56	18	74 (21)
<60 y/o sporadic OC	213	19	9	28 (13)
≥60 y/o + FH _x BC/OC	129	9	13	22 (17)
≥60 y/o + PMH _x BC	59	10	10	20 (34)
≥60 y/o + sporadic OC	76*	3	4	7‡ (9)
≥60 y/o + sporadic OC + no PMH _x BC	46	0	2†	2 (4)

Table 4. Risk factors for pathogenic germline *BRCA1/2* variants in epithelial ovarian cancer cohort. Data are reported as number (percentage; the denominator is column 2 “Tested”). Key: BC, breast cancer; FH_x, family history; OC, ovarian cancer; PMH_x, past medical history; y/o, years old; *30/76 (39%) had a PMH_x of breast cancer; † One patient had a Manchester Score of 14 and one patient had a Manchester Score of 10; ‡ 5/7 (71%) had a PMH_x of breast cancer.

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Date of screening	Tested	<i>BRCA1</i>	<i>BRCA2</i>	Combined <i>BRCA1/2</i>
<i>Pre-January 2012</i>	145			29 (20)
<60 y/o	96	16	4	20 (21)
<60 y/o + sporadic OC	47	4	1	5 (11)
≥60 y/o + FH _x BC/OC	33	3	5	8 (24)
≥60 y/o + PMH _x BC	15	2	2	4 (27)
≥60 y/o + sporadic OC	16*	0	1†	1 (6)
≥60 y/o + sporadic OC + no PMH _x BC	8	0	1†	1 (13)
<i>Post-January 2012</i>	442			74 (18)
<60 y/o	256	40	14	54 (21)
<60 y/o + sporadic OC	166	15	8	23 (14)
≥60 y/o + FH _x BC/OC	96	6	8	14 (15)
≥60 y/o + PMH _x BC	44	8	8	16 (36)
≥60 y/o + sporadic OC	60‡	3	3	6 (10)†
≥60 y/o + sporadic OC + no PMH _x BC	38	0	1^	1 (3)

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Table 5. Evaluation of survival bias according to date of ovarian cancer diagnosis. Data are reported as number (percentage; the denominator is column 2 “Tested”). Key: BC, breast cancer; FH_x, family history; OC, ovarian cancer; PMH_x, past medical history; y/o, years old; * 8/16 (50%) had a PMH_x of BC; † the same patient (Manchester Score 14); ‡ 22/60 (37%) had a PMH_x of BC; † 5/6 (83%) had a PMH_x of BC; † 1/6 (17%) had a Manchester Score <15 and 5/6 (83%) had a Manchester Score ≥15; ^ Patient had a Manchester Score of 10. All germline *BRCA1/2* testing took place after 1st June 2013. There was no evidence of survival bias between cohorts (pre- and post-January 2012).

Manchester Score	Tested	<i>BRCA1</i>	<i>BRCA2</i>	Combined <i>BRCA1/2</i>
<15	94	0	2	2 (2)
15-19	298	27	16	43 (14)
20-29	133	25	11	36 (27)
30-40	20	7	5	12 (60)
40+	12	9	1	10 (83)

Table 6. Manchester BRCA Score. The Manchester Score is reported in points. Data are reported as number (percentage; the denominator is column 2 “Tested”).

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Age	Tested	<i>BRCA1</i>	<i>BRCA2</i>	Combined <i>BRCA1/2</i>
<30	6	0	0	0 (0)
30-39	20	4	1	5 (25)
40-49	113	22	5	27 (24)
50-59	213	30	12	42 (20)
60-69	140*	9	12	21 (15)
≥70	65 [†]	3	5	8 (12)

362

363 **Table 7. Prevalence of pathogenic germline *BRCA1/2* variants according to age at**
 364 **diagnosis.** Data is reported as number (percentage; the denominator is column 2 “Tested”).
 365 Age is reported in years. Key: *84/140 (60%) had a family history of breast or ovarian cancer
 366 and 37/140 (26%) had a past medical history of breast cancer; [†] 45/65 (69%) had a family
 367 history of breast or ovarian cancer and 22/65 (34%) had a past medical history of breast
 368 cancer.

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