ORIGINAL ARTICLE

Evaluation of the relative effectiveness of the 2017 updated Manchester scoring system for predicting *BRCA1/2* mutations in a Southeast Asian country

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ABSTRACT

Background Germline mutations in the *BRCA*1 and *BRCA*2 genes have significant clinical implications for both risk-reducing and early surveillance management. The third and most recent revision of the Manchester scoring system (MSS3) used to distinguish patients indicated for germline *BRCA1/2* testing included further adjustments for triple negative breast cancer, high-grade serous ovarian cancer and human epidermal growth factor 2 (HER2) receptor status. This study aims to evaluate the relative effectiveness of MSS3 in a Southeast Asian population.

Methods All patients in our centre were tested using next-generation sequencing (NGS) panels that included full gene sequencing as well as coverage for large deletions/duplications in *BRCA1/2*. We calculated MSS1-3 scores for index patients between 2014 and 2017 who had undergone *BRCA1/2* genetic testing and recorded their genetic test results. MSS1-3 outcomes were compared using receiver operating characteristic analysis, while associations with predictors were investigated using Fisher's exact test and logistics regression. Calculations *were* performed using Medcalc17.

Results Of the 330 included patients, 47 (14.2%) were found to have a germline mutation in *BRCA1* or *BRCA2*. A positive HER2 receptor was associated with a lower likelihood of a *BRCA1/2*mutation (OR=0.125, 95% CI 0.016 to 0.955; P=0.007), while high-grade serous ovarian cancer was conversely associated with an increased likelihood of a *BRCA1/2* mutation (OR=5.128, 95% CI 1.431 to 18.370; P=0.012). At the 10% threshold, 43.0% (142/330) of patients were indicated for testing under MSS3, compared with 35.8% (118/330) for MSS1% and 36.4% (120/330) for MSS2. At the 10% threshold, MSS3 sensitivity was 91.5% and specificity 65.0%, significantly better than the previous MSS1 (P=0.037) and MSS2 (P=0.032) models.

Conclusion Our results indicate that the updated MSS3 outperforms previous iterations and relative to the Manchester population, is just as effective in identifying patients with *BRCA1/2* mutations in a Southeast Asian *population.*

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INTRODUCTION AND AIMS

Germline mutations in the *BRCA1* and *BRCA2* genes, (abbreviated together as *BRCA1/2)* are associated with increased lifetime risks of breast and ovarian cancer and are the most common cause of hereditary breast and ovarian cancer.[1 2] Hereditary cancers make up a relatively small fraction of all cancers, with 5%–10% of breast cancers being attributed to a hereditary cause. Patients with *BRCA1* and *BRCA2* mutations have cumulative risks of breast and ovarian cancer, respectively, of 69%–72% and 17%–40% by age 80.[3] Breast cancer is the most common malignancy among Singaporean females, accounting for 29.7% of all female malignancies.[4] Identifying patients with *BRCA1/2* mutations provides additional clinically relevant information to clinicians to guide patient management and treatment, and for risk-reduction purposes. In the context of surgical management during malignancy, knowledge of mutation status helps inform surgical decisions,[5] as *BRCA1/2* mutation patients who undergo breast conservative surgeries—rather than mastectomies—have elevated risk of a second ipsilateral breast cancer. Additionally, mutation status can guide personalised clinical treatment such as the use of platinum-based neoadjuvant chemotherapy for breast cancers in *BRCA1/2* mutations carriers.[6] Additionally, poly (ADP-ribose) polymerase inhibitors have been increasingly reported to be an effective means of treating *BRCA1/2* mutation tumours through synthetic lethality, thereby exemplifying the concept of precision medicine.[7] In terms of preventative care, patients who have a *BRCA1/2* mutation are advised to consider risk-reducing mastectomy and bilateral salpingo-oophorectomy once they have completed childbearing—typically around age 40.[8]

Regardless of the significant reductions in the cost of testing and the lowering of testing thresholds, prediction models are still useful tools within cancer genetics clinics; both to inform patients as to the likelihood of a *BRCA1/2* mutation and to *appropriately* allocate available resources.

While models such as *BRCA*PRO and BOADICEA are available to predict a patient's likelihood of carrying a *BRCA1/2* mutation, they require specialised software and a relatively lengthy time period to input the patient's family history data in order to accurately calculate the likelihood of a mutation.[9] The Manchester scoring system (MSS) is a validated model developed by Evans *et al*. as a convenient and practical method for identifying patients with ≥10% (or ≥20%) likelihood of

being a *BRCA1/2* mutation carrier. Initially, the original model (MSS1) calculated the likelihood of a *BRCA1/2* mutation via scores derived using age of diagnosis for each breast, ovarian, pancreatic and prostate cancer for each member of the proband's family.[10] MSS1 has since been updated twice in view of certain histological subtypes reportedly exhibiting greater associations with *BRCA1/2* mutations. The first iteration in 2009 (MSS2), added scores based on breast histopathological markers, such as grade, morphology and receptor status of the index patient.[11] This accounted for the increased prevalence of *BRCA1/2* mutations carriers noted in patients with triple negative breast cancers (TNBC) and lower incidence in human epidermal growth factor 2 (HER2) positive breast cancers.[12] The recent iteration in 2017 (MSS3), involved four major updates. The changes included adding scores for adopted patients (ie, family history unknown), increasing the downward adjustment for HER2 receptor status and increasing the weightage for TNBC and high-grade serous ovarian cancer (HGSOC).[13] These updates addressed patients with HGSOC, reporting 17.1% incidence of *BRCA1/2* mutations.[14] The weightage increase ensures that patients with apparently sporadic HGSOC diagnosed below age 60 will be indicated for testing when they will have otherwise been missed by MSS2.[15]

While there was a previous study examining the predictive performance of MSS1 in a Southeast Asian population (Malaysia), there have been no further investigations using MSS2 and MSS3. Hence, this study aims to evaluate the effectiveness of MSS3 in a Southeast Asian population, relative to the previous versions (MSS1-2) and the Manchester population.

METHODS

Patients

All patients seen at the Cancer Genetics Service at National Cancer Centre Singapore, between January 2014 and June 2017 were followed up in a prospective clinical registry. Consent was taken at the point of genetic consultation, and all data collection and usage were approved by the SingHealth Institutional Review Board, IRB 2011/826/B. We included consecutive index patients from unrelated families, who had undergone clinical primary germline mutation testing for *BRCA1/2* mutations. All patients in our centre were tested using next-generation sequencing (NGS) panels that included full gene sequencing as well as coverage for large deletion/duplications in *BRCA1/2*. The NGS panels used were either organ specific (eg, breast cancer panel) or pan-cancer panel, determined by a combination of family history factors and/or patient preferences. Patients from families with a known *BRCA1/2* mutation prior to genetic testing were excluded.

Data collected prospectively included a detailed three-generation pedigree that covers family history of cancer in first-degree, second-degree and third-degree relatives. *BRCA1/2* mutation status and clinicopathological features (eg, age of diagnosis, tumour histology, grade and tumour receptor status) were also collected. Patients with no positive family history of cancer and only a single primary malignancy were regarded as apparently sporadic ('sporadic'). Cancer family history was patient reported and not verified by medical records or death certificates. We omitted reported cancers where patients were unsure of the diagnosis. Histopathological data collected included breast and ovarian morphology and tumour grade. Breast cancers were tested for oestrogen, progesterone and human epidermal growth factor 2 receptor status. Oestrogen and progesterone receptors were considered positive when more than 1% of cells stained positive. Additionally, human epidermal growth factor 2 was designated positive when stained positive 2/3+ on immunohistochemistry in combination with amplification being detected by fluorescence in situ hybridisation.

Statistical analysis

Discrete data were presented as counts (per cent) and outcomes were analysed using Fisher's exact test. Differences in continuous variables such as median age of diagnosis for breast cancer were assessed with the Mann-Whitney U test, while Fisher's exact test was performed on categorical variables. For each index patient, the Manchester scores and the corresponding predicted *BRCA1/2* mutation probabilities were calculated for the MSS1, MSS2 and MSS3 models according to the published scoring rules and tabulations.[10 11 13] If index patient eligibility was not met for MSS3, the MSS2 score was substituted for MSS3 in the analysis. Similarly, if the patient was not eligible for MSS2, the MSS1 score was substituted for MSS2. Receiver operating characteristic (ROC) curves and area under the ROC curves (AUC) were used to quantify the performance of MSS1, MSS2 and MSS3 as univariate predictors. Multivariable logistic regression analysis was used to statistically assess MSS2 in combination with HGSOC, TNBC and HER2 as predictors of presence/absence of *BRCA1/2*. Differences in AUCs among the various predictive models were compared for statistical significance. $P \leq 0.05$ were considered to be statistically significant. Statistical analyses were performed using MedCalc V.17.0 and SAS V.9.4 for windows.

RESULTS

Overall clinical characteristics

A total of 330 index patients from unrelated families were included in this study. Clinicopathological characteristics are presented in table 1 comparing patients with and without *BRCA1/2* mutations. Effects of gender and adoptive families were not evaluated as there was only one male breast cancer index patient and two patients from adoptive families. Among all the patients, 48.8% (161/330) were 'sporadic' patients. Chinese patients made up 69.7% (230/330) of the cohort with 9.4% (31/330) Malay, 5.5% (18/330) Indian patients and 15.5% (51/330) of other ethnicities. Among the patients with breast or ovarian cancer seen in the genetic clinic, 14.2% (47/330) were reported to be a *BRCA1/2* mutation carrier, of which 53.2% (25/47) were *BRCA1%* and 46.8% (22/47) were *BRCA2* mutations. Additionally, 4.5% (15/330) of patients had non-*BRCA* mutations in the following genes: *ATM, FANCI, MLH1, MUTYH, NBN, PALB2, PTEN, RAD51D, SDHA* and *TP53*. Median age of diagnosis for the breast and ovarian cancer was 40.9 and 53.0 years, respectively. Among these patients, 66.1% (218/330) had breast cancer only, 26.7% (88/330) had ovarian cancer only and 7.3% (24/330) had a history of both breast and ovarian cancer. Most breasts cancers were invasive ductal carcinomas (75.6%) that were typically grade 2 (32.2%) or 3 (35.5%). The frequency of *BRCA1/2* mutations was seen in 20.0% (9/45) and 34.7% (26/75) of patients with TNBC and HGSOC, respectively. Histopathological data pertinent to tumour type necessary in evaluating MSS2 and MSS3 were available in 81.2% (268/330) and 80.0% (264/330) of patients, respectively.

Manchester score as a whole

Figure 1A, B and supplementary figure 1sC–1sF display histograms of the distribution of the MSS1-3 scores in *BRCA1/2* mutation carriers and non-carriers, respectively. Combined Manchester scores of 15–19 and 20+ points were equivalent to the 10% and 20% likelihood thresholds respectively. At the 10% threshold, 43% (142/330) of patients were indicated for

Table 1 Clinicopathological characteristics

Variable	Total populations (%)	Patients with *BRCA1* (%)	Patients with *BRCA2* (%)	Patients with either *BRCA1/2* (%)	No *BRCA1/2* mutation (%)	P value*
Index patients†	330	25	22	47	283	–
Median age of breast cancer diagnosis	40.9	42.6	44.6	43.2	40.5	0.060
Median age of ovarian cancer diagnosis	53.0	53.4	52.2	53.0	52.6	0.760
Ethnicity						
Chinese	230 (69.7)	15/230 (6.5)	16/230 (7.0)	31/230 (13.5)	199/230 (86.5)	0.567
Indian	18 (5.5)	1/18 (5.6)	1/18 (5.6)	2/18 (11.1)	16/18 (88.9)	
Malay	31 (9.4)	4/31 (12.9)	3/31 (9.7)	7/31 (22.6)	24/31 (77.4)	
Others	51 (15.5)	5/51 (9.8)	2/51 (3.9)	7/51 (13.7)	44/51 (86.3)	
'Sporadic' patients	161 (48.8)	5 (20.0)	4 (18.2)	9 (19.1)	152 (53.7)	0.001
Breast and ovarian cancer						
Breast cancer only	218 (66.1)	8 (32.0)	11 (50.0)	19 (40.4)	199 (70.3)	0.001
Ovarian cancer only	88 (26.7)	13 (52.0)	5 (22.7)	16 (34.0)	72 (25.4)	
Breast and ovarian cancer	24 (7.3)	4 (16.0)	8 (36.4)	12 (25.5)	12 (4.2)	
Breast histopathology						
Estrogen receptor status	242	12	19	31	211	
Positive	152 (62.8)	3 (25.0)	15 (78.9)	18 (58.1)	134 (63.5)	0.75
Negative	72 (29.8)	8 (66.7)	3 (15.8)	11 (35.5)	61 (28.9)	
Unknown	18 (7.4)	1 (8.3)	1 (5.3)	2 (6.5)	16 (7.6)	
Progesterone receptor status	242	12	19	31	211	
Positive	123 (50.8)	1 (8.3)	15 (78.9)	16 (51.6)	110 (52.1)	0.538
Negative	98 (40.5)	9 (75.0)	3 (15.8)	12 (38.7)	84 (39.8)	
Unknown	21 (8.7)	2 (16.7)	1 (5.3)	4 (9.7)	17 (8.1)	
HER2 status	242	12	19	31	211	
Positive	40 (16.5)	0 (0.0)	1 (5.3)	1 (3.2)	39 (18.5)	0.037
Negative	141 (58.3)	8 (66.7)	16 (84.2)	24 (77.4)	117 (55.5)	
Unknown	61 (25.2)	4 (33.3)	2 (10.5)	6 (19.4)	55 (26.1)	
Triple negative breast cancer	242	12	19	31	211	
Yes	45 (18.6)	6 (50.0)	3 (15.8)	9 (29.0)	36 (17.1)	0.278
No	135 (55.8)	2 (16.7)	13 (68.4)	15 (48.4)	120 (56.9)	
Unknown	62 (25.6)	4 (33.3)	3 (15.8)	7 (22.6)	55 (26.1)	
Morphology	242	12	19	31	211	
Invasive ductal carcinoma	183 (75.6)	10 (83.3)	14 (73.7)	24 (77.4)	159 (75.4)	0.927
Ductual carcinoma in situ	24 (9.9)	1 (8.3)	2 (10.5)	3 (9.7)	21 (10.0)	
Invasive Lobular carcinoma	14 (5.8)	0 (0.0)	1 (5.3)	1 (3.2)	13 (6.2)	
Unknown	21 (8.7)	1 (8.3)	2 (10.5)	3 (9.7)	18 (8.5)	
Grade	242	12	19	31	211	
1	22 (9.1)	0 (0.0)	1 (5.3)	1 (3.2)	21 (10.0)	0.546
2	78 (32.2)	3 (25.0)	9 (47.4)	12 (38.7)	66 (31.3)	
3	86 (35.5)	7 (58.3)	5 (26.3)	12 (38.7)	74 (35.1)	
Unknown	56 (23.1)	2 (16.7)	4 (21.1)	6 (19.4)	50 (23.7)	
Ovarian histopathology						
HGSOC	112	17	11	28	84	
Yes	75 (67.0)	17 (100.0)	9 (81.8)	26 (92.9)	49 (58.3)	0.001
No	30 (12.4)	0 (0.0)	1 (9.1)	1 (3.6)	30 (35.7)	
Unknown	7 (2.9)	0 (0.0)	1 (9.1)	1 (3.6)	5 (6.0)	

*P value from comparison of patients with and without pathogenic *BRCA1/2* mutations.
†Index patients from unrelated families receiving clinical primary germline mutation testing for *BRCA*1/2 mutations.
HER2, human epidermal growth factor 2; HGSOC, high-grade serous ovarian cancer.

testing under MSS3, this was an absolute 7% higher compared with MSS1 (118/330) and MSS2 (120/330). With regard to MSS3, sensitivity of the Manchester scoring system for identifying patients who were *BRCA1/2* mutation carriers was 91.5% (43/47) at the 10% threshold; specificity for correctly identifying patients without *BRCA1/2* mutations was 65.0% (184/283). Using ROC analysis and Youden's rule, a Manchester score of 15 was determined to be optimal for maximising the correct classification rate relative to a 50:50 guess. When limiting the evaluation to patients who had the required histopathological data to accurately calculate their MSS3 score, sensitivity was 90% and specificity 64.5%, which did not significantly differ from results based on all 330 patients. Additionally, of the 15 patients with mutations in genes other than *BRCA1/2*, 2 patients had scores above 20 points, 4 patients had 15–19 points and the remaining 9 (60%) patients had 14 or less points on MSS3.

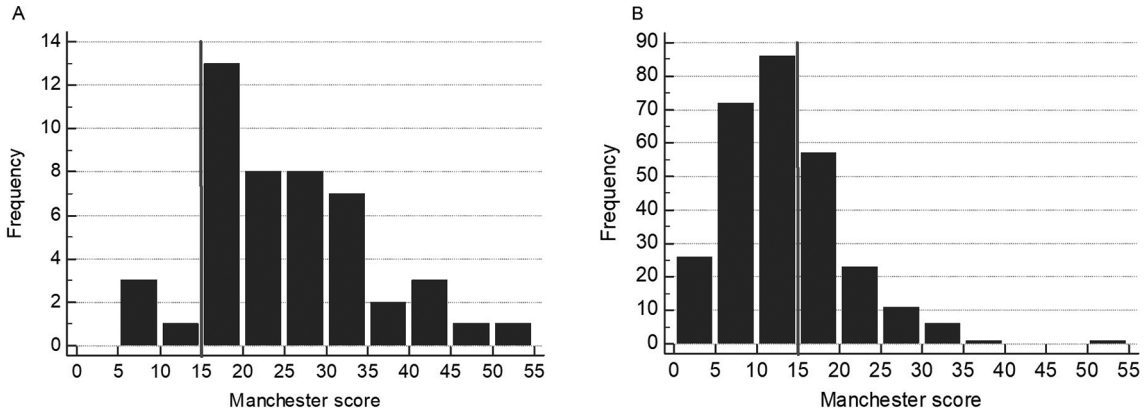

Figure 1A,B Histogram charting out frequency of patients based on Manchester scoring system 3 scores; horizontal line denotes cut-off score for patients indicated for *BRCA1/2* mutation testing.

Impact of 'sporadic' patients

Among all the patients who were *BRCA1/2* mutation carriers, 19.1% (9/47) were determined to be 'sporadic' patients. The distribution of 'sporadic' cancers is given in table 2. Only patients with 'sporadic' HGSOC cancer diagnosed below the age of 60 had consistent *BRCA1/2* mutations with an incidence of more than 10%. The pathological update to MSS3 meant that patients with 'sporadic' HGSOC diagnosed below the age of 60 were now indicated for genetic testing, thereby increasing the specificity of the scoring system from 83.0% to 91.5%. As a result, an additional five cases with *BRCA1/2* mutations were identified at the cost of testing 24 additional cases.

All four *BRCA1/2* mutation carriers not indicated for genetic testing based on MSS3 were patients with 'sporadic' cancers. These included one patient with a *BRCA1* mutation with TNBC and three *BRCA2* mutation carriers with ER+ HER2 breasts cancers diagnosed between 30 and 50 years. Overall, the prevalence of *BRCA1/2* mutations in 'sporadic' breast cancers and 'sporadic' TNBCs were low, at 3.4% and 3.8%, respectively.

Differences in performance between MSS3, MSS2 and MSS1

ROC analysis was performed to compare and quantify predictive performance of MSS3 versus MSS2 and MSS1. ROC curves for all three scores are compared in the plots of figure 2 and AUC was computed for each. MSS3 demonstrated the best overall accuracy as a classifier of *BRCA1/2* versus non-*BRCA1/2* patients with AUC (95% CI) equal to 0.845 (0.801, 0.882), which was significantly greater than AUCs for MSS1 (P=0.037) and MSS2 (P=0.032). Sensitivities for MSS1, MSS2 and MSS3 at the 10% likelihood threshold were 78.7%, 80.9% and 91.5% respectively, with associated specificities of 71.4%, 70.7% and 65.0%, respectively.

As previously mentioned, the update from MSS2 to MSS3 involved four major iterations. Excluding the update regarding adoptive families, the remaining three changes were investigated further to determine which change had the most impact on explaining the improvement in MSS3 by comparing AUCs of the associated ROC curves. We compared MSS2 scores against a MSS2 score modified to include one of the three updates to MSS3 (table 3). We found that increasing the score for HGSOC cancer contributed the largest single improvement to the MSS model (AUC: 0.842 vs 0.832; P=0.053). However, MSS3 which incorporates all three changes exhibited the largest improvement over MSS2 incorporating any single modification.

When assessing MSS as a predictor of *BRCA1* using the 10% likelihood threshold, MSS3 was the most accurate predictive score overall with an AUC of 0.880 versus 0.844 and 0.865 for MSS1 and MSS2, respectively. Finally, all three MSS iterations performed better as a predictor for *BRCA1* than for *BRCA2* (AUC: 0.761).

Table 2 'Sporadic' cancers: mutations/number (%) tested

Age of diagnosis (years)	Ovarian	HGSOC	Breast	TNBC
<30	0/1	0/0*	0/11	0/3*
30–39	0/2	1/2* (50.0)	2/48 (4.2)	0/12
40–49	0/3	1/9* (11.1)	1/21 (4.8)	1/10 (10.0)
50–59	0/4	3/13* (23.1)	0/4	0/1
60+	0/3	0/10	0/3	0/0
Total	0/14	5/34 (14.7)	3/87 (3.4)	1/26 (3.8)

*These patients are indicated for genetic testing under MSS3.
HGSOC , high-grade serous ovarian cancer; MSS, Manchester scoring system; TNBC, triple negative breast cancer.

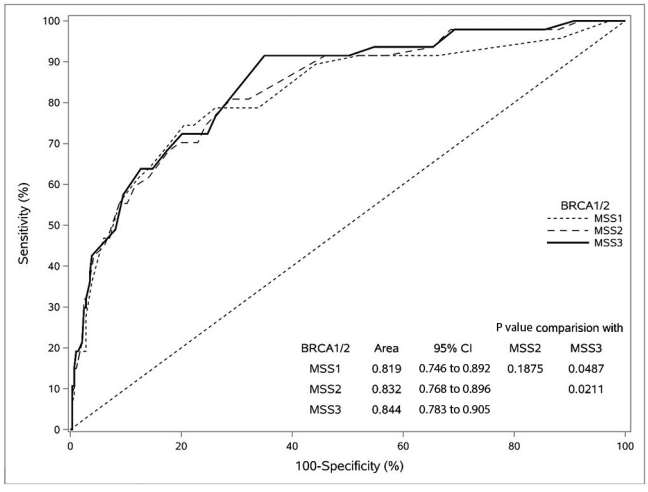

Figure 2 Receiver operating characteristic comparison between Manchester scoring system (MSS)1, MSS2 and MSS3.

Table 3 Receiver operating characteristic AUC comparison: MSS2 vs MSS3 updates

Comparison	AUC	95% CI	P value
MSS2 score	0.832	0.787 to 0.871	–
MSS2 score+HGSOC	0.842	0.798 to 0.879	0.053
MSS2 score+TNBC	0.829	0.784 to 0.868	0.330
MSS2 score+HER2	0.831	0.786 to 0.870	0.778
MSS3 score	0.844	0.800 to 0.882	0.021

HER2, human epidermal growth factor 2; HGSOC, high-grade serous ovarian cancer; MSS, Manchester scoring system; TNBC, triple negative breast cancer.

Performance comparison between Southeast Asian and Manchester populations

Table 4 juxtaposes the composition of patients seen in our cohort against the cohort by Evans *et al*.[13] Overall, there were no statistically significant differences in the incidence of *BRCA1/2* mutations seen in our clinic against those from Evans *et al*. MSS3 also achieved similar sensitivities in both studies (91.5% vs 92.8%, P=1.000). However, a much larger proportion of patients were indicated for genetic testing in the cohort by Evans *et al*. The root cause of the higher proportion of patients indicated for genetic testing was mainly in the patients with breast cancer only (55.7% vs 21.0%, P<0.001). Additionally, there were smaller proportions of *BRCA1/2* mutations in patients with breast cancers only (6.5% vs 12.4%, P=0.013) and among those with TNBCs diagnosed at or below age 40 (4.5% vs 32.9%, P=0.003) in our cohort compared with the cohort by Evans *et al*.

With regard to performance, MSS3 exhibited high sensitivity at identifying patients with *BRCA1/2* mutations in both cohorts. However, MSS3 was also statistically more specific in excluding patients without *BRCA1/2* mutations in our cohort (65.0% vs 42.8%, P<0.001). These results remained consistent even when evaluating patients with a full histopathological report.

Discussion

Overall, the distribution of histopathological features of patients with breast and/or ovarian cancer seen at a cancer genetic clinic in Singapore is largely similar to reports from previous western countries. The MSS3 is an improvement from previous MSS models and is highly sensitive in identifying patients with *BRCA1/2* mutations.

Breast cancer is the most common malignancy in women in both Singapore and the UK; moreover, ovarian cancer is the fifth most common malignancy in Singapore and sixth in the UK. However, the similarities end there as the UK has a higher incidence rate for both cancers. Based on the latest registry reports for 2015, the age-standardised incidence rates for breast (172.1 vs 65.3) and ovarian (23.5 vs 13.0) cancer in the UK were from twofold to threefold higher than in Singapore. Additionally, the median age of diagnosis for breast cancer (60–64 vs 45–54) was higher in the UK.[16 17] Previous *BRCA1/2* prevalence studies reported *BRCA2* mutation carriers were more common compared with *BRCA1* mutation carriers in the UK, while the converse was true in Singapore.[18 19] While these epidemiological differences pose potential difficulties for comparison, this is unlikely to be the case given the limited discrepancy in the efficacy of MSS3.

The ethnicity distribution of patients and the proportion of *BRCA1/2* mutation carriers seen in our genetic clinic were consistent with those reported by Thirthagiri *et al* in a previous Malaysian study.[20] This establishes the representativeness of our cohort for the region. However, while Thirthagiri *et al* reported that Malay patients had a higher proportion of *BRCA2* mutations over *BRCA1* mutations, this was not observed in our population. Interestingly, the Malay patients in our cohort had a higher proportion of *BRCA1/2* mutations that approached statistically significant. Hence, a further investigation to explore the possibility of *BRCA2* founder mutations in the Malay population may be warranted in view of these findings.

Positive HER2 receptor status and HGSOC were the only two markers that were statistically significant in predicting the likelihood of a *BRCA1/2* mutation in a patient. Additionally, patients who were *BRCA1* mutations carriers were more likely to have oestrogen receptor negative breast cancers or TNBC. The correlations between histopathological markers and *BRCA1/2* mutation status are consistent with previously reported studies[21] and in line with the iterations made to MSS2 to improve the model.

At the 10% threshold, MSS3 was an extremely effective model at identifying the majority of *BRCA1/2* mutation carriers and reducing the number of patients that were indicated for genetic testing. However, the high level of sensitivity dropped substantially at the 20% likelihood threshold where more than one-third of *BRCA1/2* mutation carriers would have been missed. It is not in the scope of this paper to determine the ideal cost-effective threshold

Table 4 Number (%) of patients tested: Evan *et al* vs our cohort

	Evans *et al*			Our study		
Manchester score	<15 *BRCA1/2* mutation	≥15 *BRCA1/2* mutation	Total *BRCA1/2*	<15 *BRCA1/2* mutation	≥15 *RCA1/2* mutation	Total *BRCA1/2*
All cases MSS3	52/1506 (3.5)	666/2610 (25.5)	718/4116 (17.4)	4/188 (2.1)	43/142 (30.3)	47/330 (14.2)
ER– HER2+ BC MSS3	0/26 (0.0)	6/22 (27.2)	6/48 (12.5)	0/14 (0.0)	0/1 (0.0)	0/15 (0.0)
ER+ HER2+ BC MSS3	2/80 (2.5)	4/50 (8.0)	6/130 (4.6)	0/20 (0.0)	1/3 (33.3)	1/23 (4.3)
TNBC (all ages) MSS3	7/112 (6.3)	152/371 (41.0)	159/483 (32.9)	1/24 (20.2)	8/21 (38.1)	9/45 (20.0)
TNBC <30	0/0 (0.0)	22/50 (44.0)	22/50 (44.0)	0/0 (0.0)	0/5 (0.0)	0/5 (0.0)
TNBC 30–39	3/75 (4.0)	57/129 (44.2)	60/199 (30.2)	0/12 (0.0)	1/5 (20.0)	1/17 (5.9)
TNBC 40–49	3/28 (10.7)	51/116 (44.0)	54/144 (37.5)	1/10 (10.0)	4/6 (66.7)	5/16 (31.3)
TNBC 50+	1/9 (11.1)	22/81 (27.2)	23/90 (25.6)	0/2 (0.0)	2/5 (40.0)	3/7 (42.9)
HGSOC MSS3	0/24 (0.0)	38/196 (19.4)	38/220 (17.3)	0/11 (0.0)	16/48 (33.3)	16/59 (27.1)
All ovarian MSS3	3/134 (2.2)	362/1133 (32.0)	365/1267 (28.8)	0/30 (30.0)	32/100 (32.0)	32/130 (24.6)
Breast-only MSS3	43/1292 (3.3)	310/1557 (19.9)	353/2849 (12.4)	4/158 (2.5)	9/42 (21.4)	13/200 (6.5)
Breast/ovarian path available MSS3	35/995 (3.5)	566/2090 (27.1)	601/3085 (19.5)	4/149 (2.7)	34/115 (29.6)	38/264 (14.4)

ER, Estrogen Receptor; BC, Breast cancer; TNBC, Triple negative breast cancer; HER2, human epidermal growth factor 2; HGSOC, high-grade serous ovarian cancer; MSS, Manchester scoring system.

for genetic testing. However, on a superficial analysis, taking the conservative approach of using the 20% threshold would render the model ineffective at ensuring patients with *BRCA1/2* mutations are correctly identified. The 10% likelihood threshold ensures that a substantial proportion of *BRCA1/2* mutation carriers will benefit from cost-effective risk-reduction strategies while reducing the cost from unnecessary genetic testing.

While MSS3 was sensitive in identifying patients with *BRCA1/2* mutations, MSS3 was seemingly unreliable in excluding patients with mutations in genes other than *BRCA1/2*. It is not within the scope of this study to carry out an evaluation of MSS for non-*BRCA1/2* mutations nor do our data allow us to reliably draw a conclusion as not all patients were tested with the same NGS panel. However, it would be interesting for future studies to investigate how the MSS3 model specificity can be improved to exclude patients for non-*BRCA1/2* mutations.

Patients with 'sporadic' breast or ovarian cancers constitute almost half of all patients with breast and ovarian cancer, yet approximately only a fifth of patients with *BRCA1/2* mutations are attributed to this population. Hence, this requires the need to be more selective in discerning which 'sporadic' patients are indicated for genetic testing. Patients with HGSOCs diagnosed below the age of 60 years consistently had an incidence of *BRCA1/2* mutations that was >10%. This supports the adjustment made to MSS3 to include patients with 'sporadic' HGSOC for genetic testing.

Overall, in terms of predictive performance, MSS3 was demonstrated to be an improvement compared with MSS2 and MSS1. All the updates to MSS3 have been shown to be contributory in improving the model. However, quantitatively, the largest gains in performance were mainly due to the inclusion of patients with 'sporadic' HGSOC for genetic testing.

Ensuring that patients with 'sporadic' HGSOC are identified is clinically significant. A randomised phase II trial by Ledermann *et al* reported that maintenance monotherapy with olaparib has the most benefit in prolonging progression-free survival in *BRCA1/2* mutation carriers with HGSOC.[22 23]

In our cohort, MSS1-3 were all consistently better at accurately predicting *BRCA1* mutations over *BRCA2* mutations. Of the four patients who had *BRCA1/2* mutations that were not identified by MSS3, three of them were *BRCA2* mutation carriers. This is in line with previously reported studies.[24–26] The discrepancy in the MSS predictive performance between *BRCA1* and *BRCA2* is quite possibly due to the heterogeneity in breast cancer tumour histology seen in *BRCA2* mutation carriers. Until a more distinctive feature is discovered to distinguish tumours in patients with *BRCA2* mutations, predictive models will continue to struggle at identifying patients with *BRCA2* mutations.

In our study, MSS3 at the 10% threshold achieved a similar level of sensitivity seen in the cohort by Evans *et al*.[13] This proves that MSS3 is a reliable model for ensuring that nearly all patients with *BRCA1/2* mutations will be indicated for genetic testing.

In terms of specificity, MSS3 performed significantly better in our cohort. The possible reason for the improvement is likely due to a combination of different complex factors. One of the more likely causes is the higher proportion of 'sporadic' patients in our cohort which was more than twice that of the cohort by Evans *et al*. Another possibility might be the different healthcare settings in the UK and Singapore. Breast and ovarian cancer rates are lower in Singapore and the participation rates for mammography for women aged between 50 and 69 years are much higher in the UK than in Singapore (70%–75% vs 55%–60%).[16 17] Ultimately, as the incidence of breast cancer increases, the effectiveness of using family history to distinguish *BRCA1/2* mutation carriers may diminish.

Another major difference observed was the smaller proportion of patients with TNBC diagnosed below the age of 40 who were *BRCA1/2* mutation carriers. This conflicts with previous reports of patients with early onset TNBC having higher incidence *of BRCA1/2* mutations, although the numbers from these studies were limited.[27] Moving forward, a reassessment on the prevalence of *BRCA1/2* mutations in Asian patients with TNBC may improve the MSS specificity in the Singaporean context.

Nearly all patients with non-HGSOC lacked a *BRCA1/2* mutation, echoing previous reports of lower prevalence of *BRCA1/2* mutations.[28] Hence, further examination between ovarian histopathological subtypes and the likelihood of *BRCA1/2* mutations may further improve the model's specificity. This is clinically relevant in the Asian population as some ovarian histological subtypes such as clear cell ovarian cancer have more than twice the incidence rate compared with a Caucasian population.[29]

Conclusion

In conclusion, our study has shown that MSS3 is consistent in being an improvement over its previous iterations and that it performed better in our Singapore cohort. This strongly supports MSS3 as an effective tool in directing genetic testing towards those more likely to carry *BRCA1/2* mutations among Chinese, Malay and Indian populations. As MSS was previously validated in a largely Caucasian population, this finding is highly significant as it suggest MSS3 is applicable to patients of these ethnic groups around the world. Wide usage of NGS panel testing has revealed new disease associations for known cancer predisposition genes as well as identified novel cancer predisposition genes. Future work will be needed to help distinguish patients with breast and/or ovarian cancer with *BRCA1/2* mutations from those with mutations in other cancer predisposition genes.

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REFERENCES

1 Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 1994;266:66–71.

2 Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G. Identification of the breast cancer susceptibility gene BRCA2. *Nature* 1995;378:789–92.
3 Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, Jervis S, van Leeuwen FE, Milne RL, Andrieu N, Goldgar DE, Terry MB, Rookus MA, Easton DF, Antoniou AC, McGuffog L, Evans DG, Barrowdale D, Frost D, Adlard J, Ong KR, Izatt L, Tischkowitz M, Eeles R, Davidson R, Hodgson S, Ellis S, Nogues C, Lasset C, Stoppa-Lyonnet D, Fricker JP, Faivre L, Berthet P, Hooning MJ, van der Kolk LE, Kets CM, Adank MA, John EM, Chung WK, Andrulis IL, Southey M, Daly MB, Buys SS, Osorio A, Engel C, Kast K, Schmutzler RK, Caldes T, Jakubowska A, Simard J, Friedlander ML, McLachlan SA, Machackova E, Foretova L, Tan YY, Singer CF, Olah E, Gerdes AM, Arver B, Olsson H, BRCA1 and BRCA2 Cohort Consortium. Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. *JAMA* 2017;317:2402–16.
4 Jara-Lazaro AR, Thilagaratnam S, Tan PH. Breast cancer in Singapore: some perspectives. *Breast Cancer* 2010;17:23–8.
5 Pierce LJ, Phillips KA, Griffith KA, Buys S, Gaffney DK, Moran MS, Haffty BG, Ben-David M, Kaufman B, Garber JE, Merajver SD, Balmaña J, Meirovitz A, Domchek SM. Local therapy in BRCA1 and BRCA2 mutation carriers with operable breast cancer: comparison of breast conservation and mastectomy. *Breast Cancer Res Treat* 2010;121:389–98.
6 Telli ML, Jensen KC, Vinayak S, Kurian AW, Lipson JA, Flaherty PJ, Timms K, Abkevich V, Schackmann EA, Wapnir IL, Carlson RW, Chang PJ, Sparano JA, Head B, Goldstein LJ, Haley B, Dakhil SR, Reid JE, Hartman AR, Manola J, Ford JM. Phase II Study of Gemcitabine, Carboplatin, and Iniparib As Neoadjuvant Therapy for Triple-Negative and BRCA1/2 Mutation-Associated Breast Cancer With Assessment of a Tumor-Based Measure of Genomic Instability: PrECOG 0105. *J Clin Oncol* 2015;33:1895–901.
7 Lim D, Ngeow J. Evaluation of the methods to identify patients who may benefit from PARP inhibitor use. *Endocr Relat Cancer* 2016;23:R267–R285.
8 Domchek SM, Friebel TM, Singer CF, Evans DG, Lynch HT, Isaacs C, Garber JE, Neuhausen SL, Matloff E, Eeles R, Pichert G, Van t'veer L, Tung N, Weitzel JN, Couch FJ, Rubinstein WS, Ganz PA, Daly MB, Olopade OI, Tomlinson G, Schildkraut J, Blum JL, Rebbeck TR. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. *JAMA* 2010;304:967–75.
9 Evans DG, Howell A. Breast cancer risk-assessment models. *Breast Cancer Res* 2007;9:213.
10 Evans DG, Eccles DM, Rahman N, Young K, Bulman M, Amir E, Shenton A, Howell A, Lalloo F. A new scoring system for the chances of identifying a BRCA1/2 mutation outperforms existing models including BRCAPRO. *J Med Genet* 2004;41:474–80.
11 Evans DG, Lalloo F, Cramer A, Jones EA, Knox F, Amir E, Howell A. Addition of pathology and biomarker information significantly improves the performance of the Manchester scoring system for BRCA1 and BRCA2 testing. *J Med Genet* 2009;46:811–7.
12 Brenton JD, Carey LA, Ahmed AA, Caldas C. Molecular classification and molecular forecasting of breast cancer: ready for clinical application? *J Clin Oncol* 2005;23:7350–60.
13 Evans DG, Harkness EF, Plaskocinska I, Wallace AJ, Clancy T, Woodward ER, Howell TA, Tischkowitz M, Lalloo F. Pathology update to the Manchester Scoring System based on testing in over 4000 families. *J Med Genet* 2017;54:674–81.
14 Alsop K, Fereday S, Meldrum C, deFazio A, Emmanuel C, George J, Dobrovic A, Birrer MJ, Webb PM, Stewart C, Friedlander M, Fox S, Bowtell D, Mitchell G. BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. *J Clin Oncol* 2012;30:2654–63.
15 Vang R, Shih I, Kurman RJ. Ovarian low-grade and high-grade serous carcinoma: pathogenesis, clinicopathologic and molecular biologic features, and diagnostic problems. *Adv Anat Pathol* 2009;16:267–82.
16 Lee HP, Annie L, Foo LL, Kuo SM, Lee E, Lim GH. Singapore Cancer Registry Annual Registry Report 2015. 2017 https://www.nrdo.gov.sg/docs/librariesprovider3/Publications-Cancer/cancer-registry-annual-report-2015_web.pdf?sfvrsn=10 (accessed 2 Nov 2017).
17 Kaur J, Poole J. Cancer Registration Statistics, England: 2015. 2017 https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/bulletins/cancerregistrationstatisticsengland/2015#background-notes (Accessed 2 Nov 2017).
18 Df E. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Anglian Breast Cancer Study Group. *Br J Cancer* 2000;83:1301–8.
19 Wong ESY, Shekar S, Met-Domestici M, Chan C, Sze M, Yap YS, Rozen SG, Tan M-H, Ang P, Ngeow J, Lee ASG. Inherited breast cancer predisposition in Asians: multigene panel testing outcomes from Singapore. *NPJ Genom Med* 2016;1:15003.
20 Thirthagiri E, Lee SY, Kang P, Lee DS, Toh GT, Selamat S, Yoon SY, Taib NA, Thong MK, Yip CH, Teo SH. Evaluation of BRCA1 and BRCA2 mutations and risk-prediction models in a typical Asian country (Malaysia) with a relatively low incidence of breast cancer. *Breast Cancer Res* 2008;10:R59.
21 Lakhani SR, Van De Vijver MJ, Jacquemier J, Anderson TJ, Osin PP, McGuffog L, Easton DF. The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. *J Clin Oncol* 2002;20:2310–8.
22 Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, Scott CL, Meier W, Shapira-Frommer R, Safra T, Matei D, Fielding A, Spencer S, Dougherty B, Orr M, Hodgson D, Barrett JC, Matulonis U. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncol* 2014;15:852–61.
23 Ledermann JA. PARP inhibitors in ovarian cancer. *Ann Oncol* 2016;27(Suppl 1):i40–i44.
24 Antoniou AC, Hardy R, Walker L, Evans DG, Shenton A, Eeles R, Shanley S, Pichert G, Izatt L, Rose S, Douglas F, Eccles D, Morrison PJ, Scott J, Zimmern RL, Easton DF, Pharoah PD. Predicting the likelihood of carrying a BRCA1 or BRCA2 mutation: validation of BOADICEA, BRCAPRO, IBIS, Myriad and the Manchester scoring system using data from UK genetics clinics. *J Med Genet* 2008;45:425–31.
25 Simard J, Dumont M, Moisan AM, Gaborieau V, Malouin H, Durocher F, Chiquette J, Plante M, Avard D, Bessette P, Brousseau C, Dorval M, Godard B, Houde L, Joly Y, Lajoie MA, Leblanc G, Lépine J, Lespérance B, Vézina H, Parboosingh J, Pichette R, Provencher L, Rhéaume J, Sinnett D, Samson C, Simard JC, Tranchant M, Voyer P, Easton D, Tavtigian SV, Knoppers BM, Laframboise R, Bridge P, Goldgar D. INHERIT BRCAs. Evaluation of BRCA1 and BRCA2 mutation prevalence, risk prediction models and a multistep testing approach in French-Canadian families with high risk of breast and ovarian cancer. *J Med Genet* 2007;44:107–21.
26 Kast K, Schmutzler RK, Rhiem K, Kiechle M, Fischer C, Niederacher D, Arnold N, Grimm T, Speiser D, Schlegelberger B, Varga D, Horvath J, Beer M, Briest S, Meindl A, Engel C. Validation of the Manchester scoring system for predicting BRCA1/2 mutations in 9,390 families suspected of having hereditary breast and ovarian cancer. *Int J Cancer* 2014;135:2352–61.
27 Young SR, Pilarski RT, Donenberg T, Shapiro C, Hammond LS, Miller J, Brooks KA, Cohen S, Tenenholz B, Desai D, Zandvakili I, Royer R, Li S, Narod SA. The prevalence of BRCA1 mutations among young women with triple-negative breast cancer. *BMC Cancer* 2009;9:86.
28 Herzog T, Xiu J, Bender R, Gatalica Z, Reddy S. 2771 BRCA1 and BRCA2 mutations in 1691 epithelial ovarian tumors identify subgroups with distinct molecular characteristics. *Eur J Cancer* 2017;09:S554–5.
29 Fuh KC, Java J, Kapp DS, Burger RA, Young RC, Alberts DS. Comparison of clear cell ovarian cancer in Asian versus Caucasians: A NRG/GOG study. *JCO* 2015;33:e16572–16572.