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Addition of pathology and biomarker information significantly improves the performance of the Manchester scoring system for *BRCA1* and *BRCA2* testing

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SUMMARY

Background: Selection for genetic testing of *BRCA1/BRCA2* is an important area of healthcare. Whilst testing costs for mutational analysis are falling, costs in North America remains in excess of US \$3,000 (UK price can be £690). Guidelines in most countries use a 10-20% threshold of detecting a mutation in *BRCA1/2* combined within a family before mutational analysis is considered. A number of computer based models have been developed. However, use of these models can be time consuming and difficult to use. The Manchester scoring system was developed in 2003 to simplify the selection process without losing accuracy.

Methods: In order to increase accuracy of prediction we have incorporated breast pathology of the index case into the Manchester scoring system based on 2156 samples from unrelated non-Jewish patients fully tested for *BRCA1/2*, and adapted the scores accordingly.

Results/Discussion: Data from breast pathology allowed adjustment of *BRCA1* and combined *BRCA1/2* scores alone. There was a lack of pathological homogeneity for *BRCA2*, therefore specific pathological correlates could not be identified. Upward adjustments in *BRCA1* mutation prediction scores were made for grade 3 ductal cancers, ER and triple negative tumours. Downward adjustments in the score were made for Grade 1 tumours, lobular cancer, DCIS and ER/HER2 positivity. Application of the updated scoring system led to 4/9 more mutations in *BRCA1* being identified at the 10/20% threshold respectively. Furthermore, there were 65/58 fewer cases meeting the 10/20% threshold for testing. Moreover, the adjusted score significantly improved the trade-off between sensitivity and specificity for *BRCA1/2* prediction.

Key words *BRCA1*, *BRCA2*, Manchester Score, BOADICEA, BRCAPRO, Myriad,
sensitivity, threshold, breast cancer, ovarian cancer

INTRODUCTION

Mutations in *BRCA1* and *BRCA2* confer a marked increased lifetime risk of developing breast and ovarian cancers and have a propensity to give rise to cancer at a young age. Consequently, despite contributing to less than 5% of new breast and 15% of new ovarian cancer diagnoses, it has been shown to be beneficial for healthcare systems to screen for these patients prior to providing surveillance, prevention or therapeutic strategies. The challenge for the clinician is to identify the subset of patients who are most likely to benefit from costly genetic testing. Even at extremes of age the presence of a *BRCA1/2* mutation will confer higher risks [1].

A number of models and scoring systems have been derived to assess the probability of a *BRCA1* or *BRCA2* mutation in a given individual dependant on their family history. Some earlier probability models such as the Couch [3] and Shattuck-Eidens models [4] were derived before widespread genetic testing had been introduced. Two tabular scoring systems have also been derived from the Myriad laboratories genetic testing programme [5,6]. However, the most widely used and validated model is BRCAPRO [7,8], which requires computer entry of the family history information. In 2003 we developed the Manchester scoring system to facilitate a more simple and accurate selection of families for *BRCA1/2* testing (Table 1) [9]. This development was undertaken primarily because existing systems were either laborious or had little validation. Since that time, the Manchester Scoring system has compared well with existing models such as BRCAPRO and Myriad as well as the newer BOADICEA model [9-13].

Recent evidence has shown that the type of breast pathology, including hormonal receptor status can influence the chance of detecting a *BRCA1* but not so much a *BRCA2* mutation and these factors have been incorporated into the BRCAPRO model and are in the process of

being incorporated into BOADICEA. Therefore, it appears timely to assess incorporation of breast pathology into the Manchester Scoring system and to determine what effect this has on the model's performance as well as its continued ease of use in the clinic setting.

Patients and Methods

The Manchester Scoring system was initially developed using parameters outlined in table 1. The score was derived from two datasets and validated in a third [9,14]. Although initially used to discriminate for each *BRCA* gene in isolation at the 10% detection threshold, a combined score for mutation prediction for both genes has now also been validated [12-14]. In our clinics we use a combined score of 16 points for the 10% threshold and 20 points for the 20% threshold of mutation probability. We have already assessed the effect of pathology in ovarian cancer on the scoring system [15], but now determine the additional effect of the proband's breast pathology in addition to ovarian pathology. We reviewed the data available to us from hospital records, pathology reports and cancer registries on 1918 breast cancer index cases out of 2156 non-Jewish index cases (238 cases had ovarian cancer only) who were fully tested for *BRCA1/2* including Multiple Ligation dependant Probe Amplification (MLPA) which detects large single or multi-exonic deletions and duplications. Oestrogen receptor (ER) status was taken as negative if the quickscore was 0/8 or if < 5% cells were positive by immunohistochemistry. Her2 was regarded as positive if the tumour showed gene amplification by fluorescent in-situ hybridization (FISH) or, in the absence of FISH, if the immunohistochemical 'Hercept test' was 3+ (scale of 0-3) Adjustments were made to the Manchester Score of the index case based on the following parameters: tumour grade, lobular pathology, DCIS, ER and Her2 receptor status. In addition a detailed pathology and receptor review was carried out in 113 breast cancer cases from a population based study of women diagnosed aged <31 years [16]. In all other instances only available pathology from cancer

registries and hospital records were used. Multiple analyses using additions or subtractions were performed until the best discriminating set was identified. Modifications to the Manchester Score were made for parameters that differed substantially between the *BRCA1* and *BRCA2* datasets and the non *BRCA1/2* datasets particularly samples testing negative for both *BRCA1* and *BRCA2*. The accuracy of the adjusted scoring system was assessed by the overall sensitivity and specificity of the model as well as its positive and negative predictive value. In addition to the accuracy, the discriminatory precision for individual cases was evaluated using receiver operating characteristic (ROC) curves. These are plots of the true positive rate against the false positive rate for different possible cut off points and hence show the trade off between sensitivity and specificity. For binary outcomes, the concordance statistic (C-statistic) is equivalent to the area under the ROC curve. Therefore, the Wilcoxon estimate of the area under the ROC curve was calculated for the original and adjusted models.

Results

Although breast cancer was confirmed in all cases, the reporting system for pathology in the UK meant that reliable data on tumour grade was not available on most cases until 1990 and on ER until 1996. Furthermore, Her2 was only widely reported after 2001. Consequently, as a large number of index cases were diagnosed with breast cancer between 1960-1990, pathology data (that included at least grade of tumour) was obtained on 1116 cases (57%) only (Table 2). ER receptor status was available on 707 (37%) of these patients and Her2 on 286 (15%). *BRCA1* tumour pathology was available in 126/203(62%) breast cancer index cases and ER was available in 43%. Table 2 shows the distribution of pathological subtypes in the sporadic, familial and *BRCA1/2* patients. As expected, *BRCA1* tumours were typically grade 3 invasive ductal cancers (IDC), which, where results were available, were mainly ER and Her2 receptor negative. Fifty one of 87 *BRCA1* cancers (63%) fulfilled the “triple

negative” criteria. *BRCA1* cancers were 4-5 fold less likely than non-*BRCA1* tumours to be lobular or grade 1. No *BRCA1* tumours over expressed Her2. In contrast, the pathological heterogeneity between *BRCA1* and non-*BRCA1/2* cases was not demonstrated with *BRCA2*. No striking differences were seen in the distribution of histology and receptor status in tumours from *BRCA2* and non *BRCA1/2* familial breast cancer patients. Consequently, the Manchester Scoring system was modified for *BRCA1* probability alone. Adjustments of 0 to 4 points (upwards or downwards) were considered for all histological and receptor parameters. Features that were less prevalent in *BRCA1* associated tumours were given a negative score and those more prevalent in *BRCA1* tumours were given a positive score. Scores above four were not investigated since our data showed that even for a triple negative breast cancer patient under 31 years of age with no family history, the frequency of constitutional *BRCA1* mutation was 2/16 (12.5%) and therefore only just reached the 10% likelihood for *BRCA1*. In other words four additional points added to the usual six for a breast cancer <30 years (Table 1) adds up to ten points-the *BRCA1* 10% threshold. Of interest, the frequency of *BRCA1* mutations found in a similar woman with sporadic, pathological grade 3 and ER negative (rather than triple negative) breast cancer was just 9% (2/22). In such cases, the 6 points attained for a breast cancer in the twenties plus 4 points for triple negative tumour would also just reach the 10 point 10% threshold for *BRCA1* while if only pathological grade 3 and ER negative, would score 9 points and not reach the testing threshold. The maximum for downward adjustment was based on the premise that even if a case at 50% risk of a known *BRCA1* mutation developed an atypical tumour for *BRCA1* they would still have at least a 50% risk of a family mutation. As such a score of 20 points (equivalent to a 20% risk) should not be reduced below 16 points (equivalent to a 10% risk of either *BRCA1* or *BRCA2*).

After considering pathology score multiple analyses were carried out to determine adjustments, which best discriminated between the probability or not of *BRCA1* mutations in an individual. The adjustments based on breast pathological correlates are shown in Table 3. Adjusting the scores to provide the best adjustments for sensitivity and, specificity improved the overall discrimination. For example, at the 10 point *BRCA1* total score, 23 *BRCA1* mutations were missed with the unadjusted score but only 11 with the adjusted score and 15 fewer cases required genetic testing. Using the combined scoring thresholds of 16 points and 20 points for the 10 and 20% mutation probability thresholds, 65 and 58 fewer samples respectively qualified for testing yet four and ten more *BRCA1* mutations respectively were identified (Table 4) with the non-identification of only one *BRCA2* mutation carrier.

Table 5 shows the overall adjustments to scores produced for *BRCA1*, *BRCA2* and non *BRCA1/2* breast cancers. It confirms how index *BRCA1/2* cases were more likely to have upward adjustment while index *BRCA1/2* negative cases were more likely to have a downward adjustment.

Table 6 shows the sensitivity, specificity, positive predictive value and negative predictive value for the unadjusted and adjusted model. Figures 1 and 2 show the ROC curves estimates for *BRCA1* and combined *BRCA1/2* scores and C-statistic and 95% confidence intervals for these curves at the 10% threshold respectively. Figure 3 shows the ROC curves for the unadjusted and adjusted Manchester scoring system estimates for the combined *BRCA1/2* score at the 20% threshold as well as the C-statistic and 95% confidence intervals for these curves. Overall, these results show that the adjustments we used significantly improved the discriminatory accuracy of the scoring system for both *BRCA1* alone (at the 10% threshold) and for the combined *BRCA1/2* score at both the 10% and 20% thresholds. This overall

improvement was evident despite specific breast pathology being available on only 43% of cases tested.

We estimate that incorporation of the breast pathology adjustment adds no more than 20 seconds to the time to calculate the Manchester score.

Discussion

Accurate, individualised assessment for the risk of *BRCA1* or *BRCA2* gene mutation is essential to provide clinicians with information concerning which patients to select for expensive genetic testing. A number of models have been developed to aid clinicians in this process, but many of these are time consuming and hence are difficult to use in busy clinical practices. Our group developed and validated the Manchester score [9] as a quick scoring system to predict BRCA mutation carriers. The model has compared well with existing as well as newer models [10-13]. More recently, many of these models, which usually derive their risk from age and family history alone, have been improved by including pathological features of the proband's breast or ovarian cancer. In this article, we report the result of a validation of the Manchester scoring system adjustment for pathological correlates.

We undertook a pragmatic study using available data from pathology reports and hospital records of women with breast cancer who had *BRCA1/2* testing. Available breast pathology information on 1116 breast cancers from patients tested for *BRCA1* and *BRCA2* were used to develop a modification to the Manchester score. It was only possible to derive a modification for *BRCA1* as *BRCA2* tumours were not sufficiently different to sporadic or familial non *BRCA1/2* tumours. Upwards modifications of between 1 and 4 points were made for a number of pathological features including ER negativity (+1), pathological grade 3 (+2) and grade 3, triple negativity (+4). Downward modification of -1 to -4 were made for ER Positivity (-1), DCIS (-1), pathological grade 1 (-2), lobular cancer (-2), LCIS (-3) and Her2 positivity (-4). These results, based on a large series of familial breast cancer cases, show that pathology in the proband can be used effectively to significantly improve the discrimination of the Manchester Scoring system. This improvement was seen not only in overall accuracy

as evidenced by improved sensitivity and specificity, but also for individualised risk assessment as evidenced by significant improvement in concordance statistics derived from ROC curves.

As has been shown in previous studies [17-20], our data has confirmed that *BRCA1* related breast cancers were more likely to be pathological grade 3 IDC and to be ER and Her2 negative. Tumours were also less likely to be pathological grade 1 or lobular. The need to discriminate is greatest for familial rather than sporadic breast cancers. Nonetheless the question remains as to which sporadic cases should be offered formal BRCA gene testing. Population based studies of early onset breast cancer in outbred non-founder populations have reported a low (<10%) rate of *BRCA1/2* mutations in diagnosed women with no family history [16,21,22]. Lakhani et al [17] provided data on the likelihood of breast cancer being caused by a *BRCA1* mutation by pathological grade and ER status at various ages. This data was for all women not those without a family history. Women aged between 20-29 years with grade 3 ER negative breast cancers had a 35% chance of a *BRCA1* mutation, with a similarly high risk for women aged 30-34 years at 26.5%. Only after the age of 34 years did the risks fall below 10% [17]. Unfortunately, it appears that some clinicians have misinterpreted these data and called for all women under 35 years of age with pathological grade 3 and ER negative breast cancer to be tested or offered testing based on a 10-20% testing threshold. The population-based data presented in this article confirm a similar 29% risk for young women (age less than 31 years). However, these data were derived from all women, not those without a family history. For sporadic breast cancer the mutation rate was only 9%, although this rose to 12.5% for triple negative breast cancer. This suggests that only triple negative, pathological grade 3 breast cancers in women less than 31 years qualify for testing irrespective of family history based on guidelines using a 10% threshold in outbred populations. Clearly the confidence intervals are wide on these numbers and further studies to

assess the rates of mutations in sporadic triple negative patients will help inform models and scoring systems further. Thresholds may also change if *BRCA1/2* status becomes more important in determining choice of treatment regimen such as with the validation of use of poly ADP ribose polymerase (PARP) inhibitors. These have been shown to have preferential activity in *BRCA* mutated individuals [23,24]. When such treatment becomes validated testing thresholds will need to be reassessed.

The methodology used in this article may be criticized for the incompleteness of pathological data and for its retrospective analysis. However, this largely pragmatic approach, although a potential failing of the analysis, is similar to the approach that clinicians are faced with, when deciding to test a family in the clinic. Whilst UK guidelines for testing employ a 20% threshold [25], these are not universal. However, they are especially important in nationally funded healthcare systems. Even outside these systems insurance companies are likely to insist on some sort of a threshold before funding a test. Pathology characteristics are now being incorporated with some success into computer models such as BRCAPRO [26] and BOADICEA [27]. Adjustments in the total score can be made to account for lower breast cancer incidence such as a 15 point, 10% threshold score in Malaysia, where the Manchester score was as effective or better than BOADICEA [28]. The Manchester score has been independently validated as taking 40 seconds per family tree [29]. We estimate that addition of the pathology will add no more than 20 seconds to the time required. Clinicians and genetic counsellors wishing to use the simpler Manchester scoring system can now improve the prediction in families by incorporating a pathology score for the breast cancer in the index case at the cost of minimal extra time.

Conclusion

The Adjusted Manchester Scoring system enables a more accurate prediction to be made of the likelihood of a given individual having a mutation in *BRCA1* or *BRCA2*. We would suggest using the basic scoring system and then adjusting scores using pathology information to enable accurate prediction and best use of limited financial resources.

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Table 1: Manchester Scoring system. Scores are added for each cancer in a direct blood lineage (cancers on the same side of the family)

	<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 (if <i>BRCA2</i> tested) For combined score =5 without prior testing	8
MBC >59	5 (if <i>BRCA2</i> tested) For combined score =5 without prior testing	5
Ovarian Cancer <60	8	5 (if <i>BRCA1</i> tested) For combined score =5 without prior testing
Ovarian Cancer >59	5	5 (if <i>BRCA1</i> tested) For combined score =5

		without prior testing
Pancreatic cancer	0	1
Prostate cancer <60	0	2
Prostate cancer >59	0	1

FBC –female breast cancer – MBC -male breast cancer

The combined score is determined by adding both the *BRCA1* and *BRCA2* scores without consideration for prior testing, thus MBC scores 5 points for *BRCA1* and ovarian cancer 5 for *BRCA2*. A combined score of 16 points can be used as a 10% threshold and 20 points as a 20% threshold in non founder Western populations. In families with no unaffected females a lower threshold could be used. Other tumour types such as Cholangiocarcinoma and ocular melanoma can contribute to the *BRCA2* score but the numbers of these tumours is too low to validate a precise score.

Table 2: Available breast pathology and receptor status in 1116 index cases tested for *BRCA1* and *BRCA2* mutations

	Sporadic <i>BRCA1/2</i> Negative	Familial <i>BRCA1/2</i> Negative	<i>BRCA1</i> index	<i>BRCA1</i> negative phenocopies*	<i>BRCA2</i> index
Number	113	756	126	15	106
Median age	32	46	40	56	43
Lobular	7/113 (4%)	80/756 (11%)	2/126 (2%)	3/15 (20%)	3/106 (3%)
LCIS	1/113 (1%)	11/756 (1.5%)	0/126	0/15	0/106
DCIS	9/113 (8%)	65/756 (9%)	5/126 (4%)	1/15 (7%)	6/106 (6%)
Mucinous	1/113 (1%)	2/756	1/126	1/15(7%)	0/106
Grade 1 IDC	5/95 (6%)	111/598 (18%)	3/117 (3%)	1/10 (10%)	3/97 (3%)
Grade 2 IDC	20/95 (21%)	249/598 (43%)	19/117 (16%)	5/10 (50%)	42/97 (44%)
Grade 3 IDC	70/95 (73%)	238/598 (39%)	96/117 (82%)	4/10 (40%)	52/97 (53%)
Her2+	7/40 (17%)	52/169 (31%)	0/51 (0%)	1/5 (20%)	2/22 (9%)
ER pos	30/66 (45%)	373/463 (81%)	22/87 (24%)	8/11 (73%)	65/80 (78%)
ER neg	36/66 (55%)	90/463 (19%)	65/87 (76%)	3/11 (27%)	15/80 (22%)
Grade 3 triple neg	20/66 (30%)	34/463 (7%)	51/87 (63%)	1/11 (9%)	9/80 (11%)
Grade 3 ER neg no her2 status	9/66 (13%)	19/463 (4%)	7/87 (9%)	3/11 (25%)	2/80 (3%)
Grade 3 ER neg her2+	4/26 (15%)	12/43 (28%)	0/51 (0%)	1/12 (8%)	1/9 (11%)
Assumed* grade 3 triple negative	28/95 (30%)	48/463 (10%)	58/87 (67%)	2/10 (20%)	11/80 (14%)

*This is assumed on the basis that untested grade 3 ER negative tumours have the same ratio as those tested for Her2.

Table 3: Calculated adjustments to the Manchester Score for predicting *BRCA1* and *BRCA2* mutations according to pathology and receptor status of breast cancer in the index case and the presence of ovarian cancer in family.

Pathology	BRCA1 adjustment	BRCA2 adjustment	Notes
Breast			
Her2+	-4*	0	No other alteration to score on basis of pathology needed
Lobular	-2	0	Add or subtract ER status
DCIS only (no invasive cancer)	-1	0	Add or subtract ER status
LCIS only (no invasive cancer)	-4*	0	No other adjustment
Grade 1 IDC	-2	0	Add or subtract ER status
Grade 2 IDC	0	0	Add or subtract ER status
Grade 3 IDC	+2	0	Add or subtract ER status
ER pos	-1	0	Add or subtract grade
ER neg	+1	0	Add or subtract grade
Grade 3 triple neg	+4*	0	No other alteration to score on basis of pathology needed
Ovary			
Epithelial (endometrioid, serous, clear cell, NOS) Granulosa cell	0 no change to MS	0	No adjustment to ovarian score ie 5 points for cancers >59 years for each gene.
Mucinous	No score given for index case or other relative	No score given for index case or other relative	Do not include in scoring at all
Borderline	No score given for index case or other relative	No score given for index case or other relative	Do not include in scoring at all
Germ cell tumours except granulosa cell	No score given for index case or other relative	No score given for index case or other relative	Do not include in scoring at all

* These adjustments are final and no further adjustment based on other pathological features is necessary,

Notes: neg-negative; pos-positive, ER –Oestrogen receptor, IDC invasive ductal carcinoma; NOS-not otherwise specified

Examples

1. Grade 3 ER pos would score +1 (+2 for grade 3, and -1 for ER pos)

2. Grade 3 ER neg would score +3 (+2, +1) unless triple negative (+4) 4 points added to total score
3. Grade 1 ER Pos scores -3 (-2, -1) 3 points deducted from total score
4. Grade 2 but no receptor status known (0) no adjustment
5. Lobular carcinoma ER Pos would score -3 (-2, -1)

Table 4: Potential gains in the need to test fewer samples and detect more mutations using the adjusted pathology score

	Fewer samples requiring testing~	Saving*	BRCA mutations gained	BRCA mutations lost	Net gain in mutations identified
10% combined	65	\$195,000	4 <i>BRCA1</i>	0	4
20% combined	58	\$174,000	10 <i>BRCA1</i>	1 <i>BRCA2</i>	9
10% BRCA1	22	\$66,000	12 <i>BRCA1</i>	0	12

~Using the adjusted 16 or 20 point score fewer samples meet the threshold and would not need to be tested (All samples were tested to derive these figures)

* Assuming cost of mutation analysis is \$3000

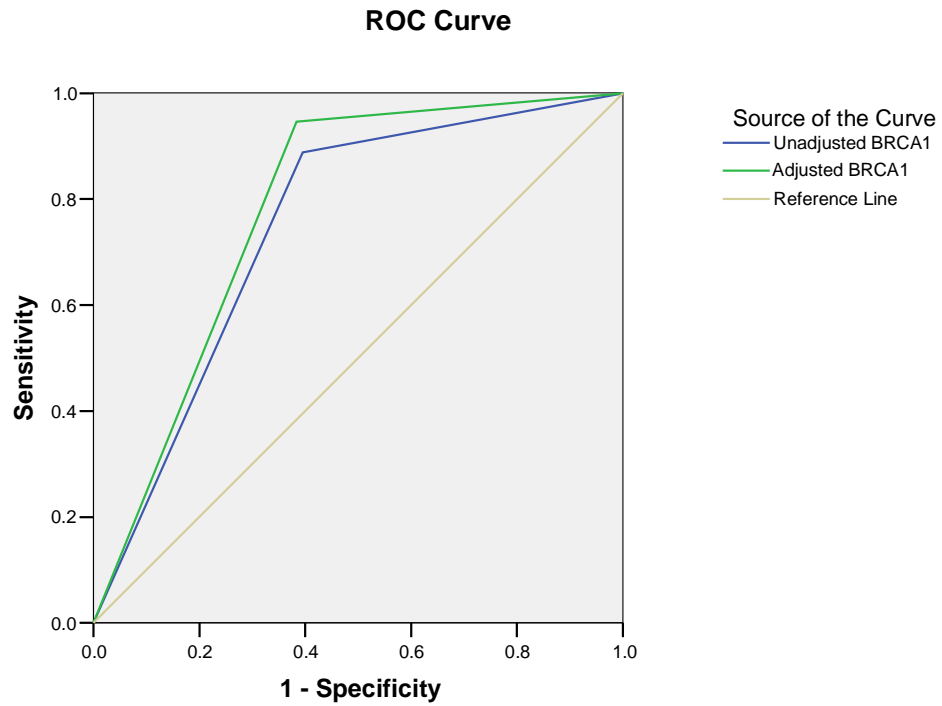
Table 5: Adjustments performed in the scores of index cases using the Manchester scoring system including pathology and receptor status

	index BRCA1/2 negative	<i>BRCA1</i> index	<i>BRCA2</i> index
Number with upward adjustment	288 (33%)	99 (80%)	52 (49%)
Number with downward adjustment	492 (57%)	13 (10%)	42 (40%)
Number with no change in score	89 (10%)	12 (10%)	12 (11%)
Total cases	869 (100%)	124 (100%)	106 (100%)

Table 6: Sensitivity, specificity and positive predictive value of Manchester Scores at 10% and 20% threshold adjusted and not adjusted for pathology and receptor status

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
BRCA1 10 points Unadjusted	180/203 (89%)	1184/1953 (61%)	180/947 (19%)	1184/1207 (98%)
BRCA1 10 points adjusted	192/203 (95%)	1211/1953 (62%)	192/934 (21%)	1211/1222 (99%)
Combined 16+ points unadjusted BRCA1	192/203 (95%)	860/1953 (44%)	192/1283 (15%)	860/871 (98.7%)
Combined 16+ points adjusted BRCA1	196/203 (97%)	899/1953 (46%)	196/1250 (16%)	899/906 (99.2%)
Combined 16+ points unadjusted BRCA1/2	361/389 (93%)	843/1767 (48%)	361/1283 (28%)	843/871 (97%)
Combined 16+ points adjusted BRCA1/2	365/389 (94%)	914/1767 (52%)	365/1218 (30%)	890/914 (97.5%)
Combined 20+ points unadjusted BRCA1/2	319/389 (82%)	1211/1767 (70%)	319/873 (36.5%)	1211/1281 (94.5%)
Combined 20+ points adjusted BRCA1/2	328/389 (84%)	1280/1767 (74%)	328/815 (40%)	1280/1341 (95.5%)

Figure 1: ROC Curves for BRCA1 at the 10% threshold

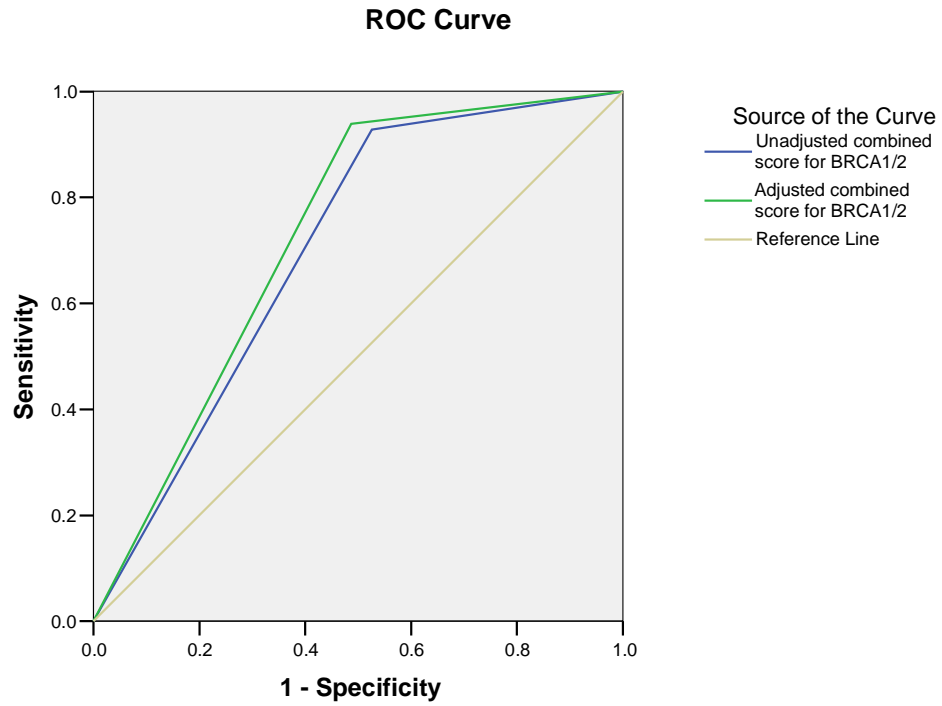


Diagonal segments are produced by ties.

C Statistic

Test Result Variable(s)	Area	Asymptotic 95% Confidence Interval		p
		Lower Bound	Upper Bound	
Unadjusted BRCA1	0.746	0.716	0.776	<0.001
Adjusted BRCA1	0.782	0.756	0.807	<0.001

Figure 2: ROC Curves for combined BRCA1/2 using the 10% threshold

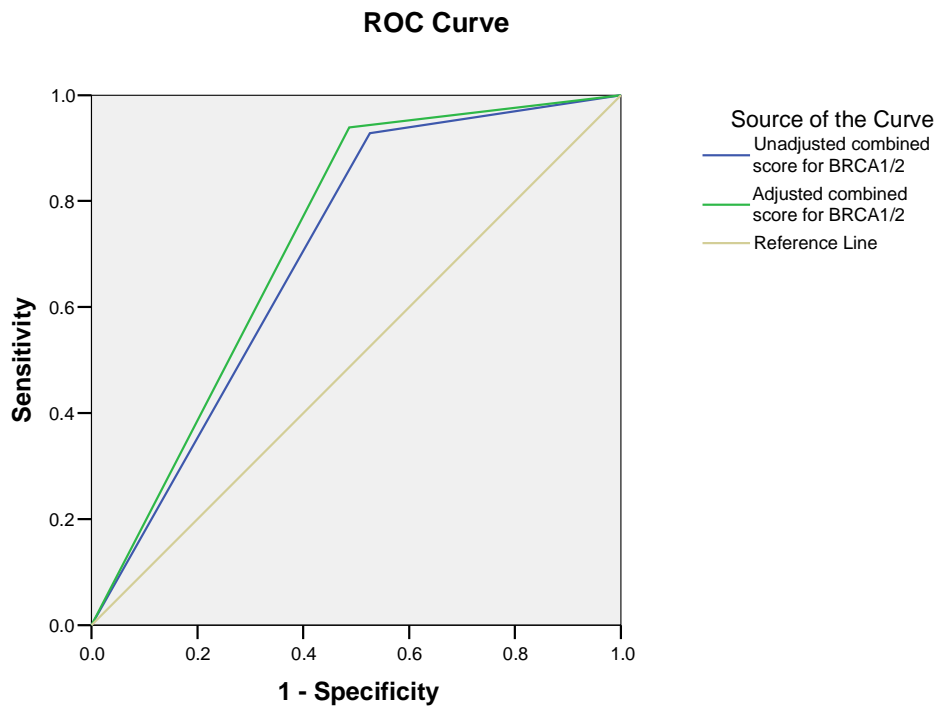


Diagonal segments are produced by ties.

C Statistic

Test Result Variable(s)	Area	Asymptotic 95% Confidence Interval		p
Unadjusted combined score for BRCA1/2	0.701	0.677	0.726	<0.001
Adjusted combined score for BRCA1/2	0.726	0.702	0.749	<0.001

Figure 3: ROC Curves for combined BRCA1/2 using the 20% threshold



Diagonal segments are produced by ties.

C Statistic

Test Result Variable(s)	Area	Asymptotic 95% Confidence Interval		p
		Lower Bound	Upper Bound	
Unadjusted combined score for BRCA1/2	0.752	0.727	0.778	<0.001
Adjusted combined score for BRCA1/2	0.783	0.758	0.807	<0.001