Methodological quality assessment of studies investigating the Mammostrat test

Study feature	Qualities sought	Bartlett *et al.* (2010)[124]	Ring *et al.* (2009)[125]	Ross *et al.* (2008)[126]
Sample of patients	Inclusion criteria defined	Y	Y	Y
	Sample selection explained	Y	Y	Y
	Adequate description of diagnostic criteria	Y	Y	Y
	Clinical and demographic characteristics fully described	Y	N (NA for one of the cohorts used)	Y
	Representative (random or consecutive sample)	Y (consecutive)	U (unclear if either)	Y (from a RCT)
	Assembled at a common (usually early) point in the course of their disease	Y	U	U
	Complete (all eligible patients were included)	Y	U	U
Follow-up of patients	Sufficiently long	Y	U	Y
Outcome	Objective	Y	Y	Y
	Unbiased (e.g. assessment blinded to prognostic information)	Y	U	Y
	Fully defined	Y	Y	Y
	Appropriate	Y	Y	Y
	Known for all or a high proportion of patients	Y	U	U
Prognostic variable	Fully defined, including details of method of measurement if relevant	Y	Y	Y
	Precisely measured	Y (detail provided)	Y (detail provided)	Y (detail provided)
	Available for all or a high proportion of patients	Y	Y	U
	If relevant, cut-point(s) defined and justified	Y (reference provided)	Y (detail provided)	Y (detail provided)
Analysis	Continuous predictor variable analysed appropriately	Y	Y	Y
	Statistical adjustment for all important prognostic factors	U	Y	Y
Intervention subsequent to inclusion in cohort	Fully described	Y	N	Y (from prespecified treatment arms)
	Intervention standardised or randomised	N	N	Y

N, no; NA, not available; U, unclear/not reported; Y, yes.

Summary of results: Mammostrat test

Study	Outcomes/end points	Results	Authors' conclusions	Comments
Bartlett *et al.* (2010)[124]	DRFS RFS OS	**Assignment to risk groups**	Mammostrat can act as an independent prognostic tool for ER+, tamoxifen-treated breast cancer. This study revealed a possible association with outcome regardless of LN status and ER– tumours. These data provide further support for the use of this antibody panel as an aid to patient management in early breast cancer	Further data within the three groups were not extracted Data on small number of ER– and untreated cases not extracted (reported to show a similar pattern to other groups)

	All cases ($n=1540$), n (%)	**G1: all ER+ ($n=1189$), n (%)**	**G2: ER+, tamoxifen only ($n=831$), n (%)**	**G3: ER+, N0, tamoxifen only ($n=657$), n (%)**
Low risk	717 (46.6)	643 (54.1)	444 (53.4)	341 (51.9)
Moderate risk	305 (19.8)	244 (20.5)	175 (21.1)	139 (21.2)
High risk	278 (18.1)	168 (14.1)	112 (13.5)	88 (13.4)
Missing	240 (15.6)	134 (11.3)	100 (12.0)	89 (13.5)

Significantly more cases assigned to high-risk group in ER– vs. ER+ (45% vs. 16%, $p<0.001$). No differences between other groups

All cases ($n=1300$): Significant association between risk score and RFS, DRFS and OS (all $p<0.001$). Multivariate analysis: risk score independent predictor of RFS ($p<0.001$), DRFS ($p<0.001$) and OS ($p<0.01$) (along with clinicopathological predictors)

G1 ($n=1055$): Significant association between risk score and DRFS ($p<0.001$), RFS ($p<0.001$) and OS ($p<0.001$). Multivariate analysis: risk score independent predictor of RFS ($p<0.05$), DRFS ($p<0.01$) and OS ($p<0.01$) (along with clinicopathological predictors)

G2 ($n=731$): Significant association between risk score and DRFS ($p<0.001$), RFS ($p<0.01$) and OS ($p<0.01$). Multivariate analysis: risk score independent predictor of DRFS ($p<0.05$), OS ($p<0.05$), trend for RFS ($p=0.064$) (along with clinicopathological predictors)

G3 ($n=568$): Significant association between risk score and RFS ($p<0.05$), DRFS ($p<0.01$) and trend for OS. Multivariate analysis: trend towards Mammostrat score to predict RFS ($p=0.076$) and DRFS ($p=0.092$) (along with clinicopathological predictors)

Study	Outcomes/end points	Results	Authors' conclusions	Comments
Ring *et al.* (2006)[125]	DFS at 5 years	G1 (training/validation cohort, ER+): Cox model identified a group of patients as having either poor or moderate outcomes with a 5-year DFS of approximately 75% as opposed to patients classified as good with a 5-year DFS of approximately 95% ($p < 0.001$) G2 (ER+ patients): Cox model identified poor patients with a 5-year DFS of 50% compared with approximately 70% for patients classified as moderate and 87% for patients classified as good ($p = 0.008$) G3 (ER+ patients): Cox model identified ER+ patients classified as poor with OS of 55% compared with 75% for patients classified as moderate and 90% for patients classified as good ($p = 0.0039$) In both cohorts the Cox model was independent of stage, grade and LN status Combined G2 and G3: for patients with poor or good prognosis (82%), sensitivity for poor prognosis in predicting disease progression was 38% whereas specificity was 88%. The PPV of poor prognosis was 38% (95% CI 32% to 44%) whereas the NPV was 88% (95% CI 84% to 92%)	The test can significantly improve on traditional prognosticators in predicting outcome for ER+ breast cancer patients	Only data relating to validation were extracted (information about training results in paper) Only data relating to the Cox models (not tree models) were extracted
MS – reclassification data All CIC	[CIC information has been removed]	[CIC information has been removed]	[CIC information has been removed]	

Study	Outcomes/end points	Results	Authors' conclusions	Comments
Ross *et al.* (2008)[126]	RFI DRFI BCSD	**Association between clinical outcomes and stratification by test** *Tamoxifen treated (n = 711)* ~58% low risk, 21% moderate risk, 21% high risk Significant association between patients stratified by test and RFI (HR 1.3, 95% CI 1.1 to 1.6, $p = 0.006$). Low risk vs. moderate risk not significant (log-rank, $p = 0.05$); low risk vs. high risk significant (HR 1.8, 95% CI 1.2 to 2.6) Significant association between patients stratified by test and DRFI (HR 1.4, 95% CI 1.1 to 1.7, $p = 0.001$). Low risk vs. moderate risk not significant; high risk vs. low risk significant (HR 2.1, 95% CI 1.4 to 3.1, $p = 0.0004$) Significant association between patients stratified by test and BCSD (HR 1.5, 95% CI 1.2 to 1.9, $p = 0.0003$). Low risk vs. moderate risk not significant; high risk vs. low risk significant (HR 2.3, 95% CI 1.5 to 3.5, $p < 0.0001$) Kaplan–Meier estimate of proportion of patients recurrence free after 10 years Overall: 82% (95% CI 79% to 85%) Low risk: 85% (95% CI 81% to 88%) Moderate risk: 85% (95% CI 80% to 91%) High risk: 73% (95% CI, 65% to 80%) Multivariate Cox model – significant prognostic power independent of age and tumour size (HR 1.3, 95% CI 1.1 to 1.6, $p = 0.007$) **Chemotherapy responsiveness** *Tamoxifen and cytotoxic chemotherapy treated (n = 269) vs. NSABP B20 tamoxifen only (n = 161)* Kaplan–Meier estimate of RFI Low-risk: improved by 5% from 86% to 91% (HR 0.4, 95% CI 0.2 to 0.8, $p = 0.01$) High risk: improved by 21% from 64% to 85% (HR 0.4, 95% CI 0.2 to 0.9, $p = 0.02$) Moderate risk: not significant Interaction: not significant *Placebo treated (n = 287)* Non-significant association between RFI at 10 years and stratification by test	The risk index was significantly associated with clinical outcome among the ER-expressing, LN–, tamoxifen-treated patients. It seems that the test may be able to identify patients who have greater absolute benefit from adjuvant chemotherapy compared with unstratified patient populations	Data on subsets of patients (e.g. by age) within the treatment arms have not been extracted