The combined endocrine receptor (CER) in breast cancer, a novel approach to traditional hormone receptor interpretation and a better discriminator of outcome than ER and PR alone.

Esther J Campbell[1&2], Mathias Tesson[2], Flora Doogan[1], Zahra MA Mohammed[2&3], Elizabeth Mallon[4], Joanne Edwards[2].

[1] Department of Surgery, Queen Elizabeth University Hospital, Glasgow G51 4TF, UK

[2] Institute of Cancer Sciences, University of Glasgow, Wolfson Wohl Translationnal Cancer Research Centre, Garscube Estate, Glasgow G61 1HQ, UK

[3]Academic Unit of Colorectal Surgery, School of Medicine, University of Glasgow, Glasgow Royal Infirmary, Glasgow G4 0ET, UK

[4]Department of Pathology, Queen Elizabeth University Hospital, Glasgow G51 4TF, UK

Running title: The combined endocrine receptor

Corresponding Author:

Dr Esther Jennifer Campbell, Department of Surgery, Queen Elizabeth University Hospital, Glasgow, G51 4TF, UK. Telephone: 07792820049. Email:Esther.Campbell@ggc.scot.nhs.uk

Abstract

Background: The functional role of progesterone receptor (PR) signalling was previously unclear and PR testing in breast cancer is controversial. Recent defining work has highlighted the functional crosstalk that exists between the oestrogen receptor (ER) and PR. The purpose of this retrospective cohort study was to compare the prognostic value of the combined oestrogen receptor (ER) and progesterone receptor (PR) score with either ER or PR alone. Methods: Tumour Allred ER and PR scores were reclassified as negative, low and high. The combined endocrine receptor (CER) was calculated as the average of the reclassified ER and PR scores, resulting in 3 groups: CER negative, impaired and high. Cox proportional hazards models were used to estimate disease-free survival (DFS) and breast cancer-specific survival (BCSS). Results: The CER was a more powerful predictor of 5-year DFS and BCSS than either ER or PR alone. In multivariate analysis that included ER, PR and CER, only CER remained an independent prognostic variable for 5-years DFS (HR 0.393 CI 0.283-0.548, P=0.00001) and BCSS (HR 0.553 CI 0.423-0.722, P=2.506 x10^{-8}). In ER+ patients impaired CER was an independent marker of poor outcome for 5-years DFS (HR 2.469 CI 1.049-5.810, P=0.038) and BCSS (HR 1.946 CI 1.054-3.596 P=0.033) in multivariate analysis that included grade, LN, tumour size, HER 2 status and PR status. The results were validated in a separate cohort of patients. Conclusion: CER is a more powerful discriminator of patient outcome than either ER or PR alone. Economical and simple, it can identify risk in ER+ early breast cancer and potentially be utilised for adjuvant cytotoxic chemotherapy decision-making.

Keywords

Breast cancer, oestrogen receptor, progesterone receptor, endocrine therapy, combined endocrine receptor.

Introduction

Worldwide breast cancer is the most frequently diagnosed cancer in woman. The majority, approximately 70%, express the oestrogen receptor (ER). ER positive disease (ER+) has historically been perceived as the 'lesser of two evils', yet many women with ER+ breast cancer still succumb to their disease. Breast cancer is responsible for over 10,000 deaths each year in the UK [www.cancerresearchuk.org] and remains the leading cause of cancer deaths among females in less developed countries (Torre *et al*, 2012). The advent of gene expressing profiling and multi parametric assays has brought to the fore that ER+ breast cancer is a heterogeneous disease and highlights the importance of targeted individual treatment selection (Dowsett *et al*, 2010; Paik S *et al*, 2006). For most of the world, these validated methods to stratify risk and guide treatment decisions are are too expensive and subsequently not routinely available. As recognised by the St Gallen conference, surrogate markers or less expensive pathology tests may provide valuable information in such countries (Coates *et al*, 2015).

Semi-quantitative immunohistochemistry (IHC) is a near universal method of tumour hormone receptor (ER and progesterone receptor, PR) testing. Tumour ER expression is a powerful predictor of response to endocrine therapy and its value is undisputed. Until recently, the biological role of PR was less well defined and it was considered a biomarker of ER function (Horwitz and McGuire, 1975). ER+/PR+ tumours are associated with better clinical outcome (Blows *et al*, 2010; Purdie *et al*, 2014; Viale *et al*, 2007) however the underlying mechanism responsible for this was poorly understood. Recent, defining work has now elucidated that PR redirects where ER binds to chromatin and acts as a proliferative brake in ER+ breast cancer (Mohammed H *et al*, 2015). This highlights the role of functional crosstalk between both the ER and PR (Mohammed H *et al*, 2015) and underlines the value of both ER and PR testing in breast cancer.

In this study we hypothesised that semi-quantitative IHC ER and PR scores together may represent a surrogate 'snap shot' of functional hormone receptor crosstalk. We therefore analysed the ER and PR together as a combined endocrine receptor (CER) to test if this would be more informative of outcome than either factor independently. We report that the CER is a better predictor of outcome than either the ER or PR, and the CER is an independent significant prognostic factor. The results were validated in a separate cohort of breast cancer patients.

Patients and Methods

Derivation study patient population

1711 female patients were diagnosed with primary operable invasive breast cancer (symptomatic and screen detected) between October 1995 and September 1998 in Greater Glasgow NHS hospitals. The Greater Glasgow Breast Cancer (GGBC) database contains pathological, treatment and follow up details for these patients. Original pathology report included % tumour cells staining for ER. PR was not routinely tested during this period. Tumour samples were centrally re-analysed for 557 patients, randomly selected from the 1711 patients (33%) (supplementary figure 1A). All patients in this cohort received tamoxifen monotherapy for 5 years except for two whose prescribed endocrine agent was not documented as they were enrolled in the ATAC study. The Research Ethics committee of North Glasgow University Hospital approved the collection of patient data and use of human tissue in this study.

Tissue microarray (TMA) construction and Immunohistochemistry (IHC)

We have previously described the method for the TMA construction using formalin fixed paraffin embedded (FFPE) tissue, taken at time of surgical resection (Mohammed *et al*,

2012a; Mohammed *et al*, 2012b). Triplicate TMA were constructed to avoid heterogeneity of PR staining (Mohammed *et al*, 2012a). The IHC for ER, PR and HER2 was performed as we described previously (Mohammed *et al*, 2012a; Mohammed *et al*, 2012b) applying protocols established in the CPA accredited diagnostic pathology laboratory, Glasgow Royal Infirmary with appropriate positive and negative controls.

IHC scoring

Tumour Allred ER and PR scores were scored as we have previously reported (Mohammed *et al*, 2012a). A cut-off to define receptor positivity for ER and PR was an Allred score ≥3, the internationally accepted cut-off. High scores were defined as Allred 6-8, and low scores as Allred 3-5. Representative examples of ER and PR staining for each scoring category is shown in supplementary figure 2. HER2 membrane staining was scored as previously described (Mohammed *et al*, 2012b).

Combined Endocrine receptor (CER)

The Allred ER and PR scores were reclassified. A score of 0 was assigned to an Allred score of less than 3, 1 assigned to Allred scores 3-5 and 2 assigned to Allred scores 6-8. The CER was calculated as the average of the reclassified ER and PR scores. CER 0 represents negative endocrine receptor status, CER 0.5-1.5 represents impaired endocrine receptor status (CER impaired) and CER 2 represents high endocrine receptor status (CER high).

Validation study patient population

The validation cohort of patients consisted of a consecutive series of new diagnosed early invasive female breast cancer patients presenting at two Greater Glasgow Hospitals between January 2008 and January 2009 (supplementary figure 1B). The Caldicott Guardian granted

permission for the use of patient data. All patients underwent curative surgery and adjuvant treatment prescriptions as per national guidelines (SIGN, 2007) were discussed at a post-operative multidisciplinary meeting. ER and PR status for this cohort was obtained from routine pathology records.

Follow-up

Follow up data was confirmed with the registrar general and patient case records for the derivation study patient population included survival status (alive, death other cause and breast cancer specific death) and documentation of date and site of recurrence (none, local, regional, distant). For patients who died, the date of death was recorded; all deaths not attributable to breast cancer were censored at the date of death. The primary outcomes in this analysis were time from definitive surgery to breast cancer-specific death and time to recurrence. In addition, early 5-year disease free survival (DFS) was analysed by censoring events at 5-years. DFS was defined as alive and well with no documented local, regional or distant breast cancer recurrence or breast cancer specific death. Accordingly, the end points were breast cancer specific survival (BCSS) and DFS at 5-years.

The validation study patient population follow up was confirmed using electronic case records. For every patient, details of definitive surgery date and most recent clinical review date were collected to calculate time to outcome. Clinical review included either breast surgery follow-up clinic or oncology follow-up clinic. For patients who died, the date of death was recorded; all deaths not attributable to breast cancer were censored at the date of death. Patient status at most recent review date was recorded (alive and well, documented local, regional or distant breast cancer recurrence or breast cancer specific death). The end point was DFS.

Statistical analysis

Statistical analysis was carried out using SPSS version 22. Univariate survival analysis was performed using Kaplan Meier method analysed by the log-rank test. Calculation of hazard ratios (HR) for both univariate and multivariate analysis performed using Cox's proportional-hazards model; a stepwise backward procedure was used to derive a final model of variables that had a significant independent relationship with patient outcome.

Results

Derivation study population

A total of 1711 patients presented with operable invasive breast cancer from October 1995 to September 1998. 557 patient tumour samples were randomly selected for TMA construction and centrally tested for ER and PR. Male breast cancers were excluded due to their biological heterogeneity. Accurate follow up data and tumour Allred scores for ER and PR were available for 90% (n=503) patients. 63% (n=319) were ER+ and 42% (n=210) were ER+/PR+. Patient and tumour characteristics are detailed in Table 1. Median follow up was 12.7 years, 61% (n=305) patients were alive, 20% (n=102) had died as a result of breast cancer and 19% (n=96) had died from other causes. At 5-years, 16% (n=82) had a breast cancer specific event.

CER scores (0-2)

CER scores (0, 0.5, 1, 1.5, 2) survival analysis confirmed the selected cut-offs (figure 1) defining the classification of negative (CER 0), impaired (CER 0.5-1.5) and high (CER2). CER 0 (HR 6.915 CI 3.131-15.264, *P*=0.000002), CER 0.5 (HR 3.418 CI 1.085-10.771, *P*=0.036), CER 1 (HR 2.617 CI 1.044- 6.560, *P*=0.040) and CER 1.5 (HR 3.031 CI 1.099-8.360, *P*=0.032) with CER 2 as the indicator category.

Redistribution of endocrine response using the CER compared to ER

Of the 319 ER+ patients 263 patients had an Allred ER high (6-8), when the CER was applied 46% (n=121) of these patients were reclassified as impaired. In addition, 6% (n=12) of ER negative were reclassified as CER impaired (Table 1).

CER and patient outcome

The CER classification resulted in a statistically significant difference in both early 5-year DFS and BCSS between negative, impaired and high categories (figures 2A and 2D). No statistical difference was demonstrated between ER high and low (figures 2B and 2E) or PR negative and low (figures 2C and 2F).

Multivariate analysis

Survival analysis confirmed that tumour grade, tumour size and lymph node (LN) (0 nodes positive, 1-3 nodes positive and greater than 3 nodes positive) and HER2 positivity were all predictive of prognosis (data not shown).

The CER was a more powerful predictor of 5-year DFS and BCSS than either the ER or PR alone. In multivariate analysis that included ER, PR and CER, only the CER remained an independent prognostic variable for 5-years DFS (HR 0.393 CI 0.283-0.548, P=0.00001) and BCSS (HR 0.553 CI 0.423-0.722, P=2.506 x10^{-8}). In multivariate analysis that included grade, LN, tumour size category and HER2 status, CER impaired and negative were independent prognostic variables with CER high as the indicator category for 5-years DFS (Table 2). In terms of BCSS for the entire cohort, impaired CER was not statistically significant when analysed as a categorical variable (Table 2).

In contrast in subgroup analysis performed in ER+ patients (n=319), therefore excluding CER negative patients, impaired CER was an independent marker of poor outcome for 5-years DFS and BCSS (Table 2) in multivariate analysis that included grade, LN, tumour size, HER 2 status and PR status. Importantly for 5-years DFS, impaired CER was a better predictor of outcome than PR status, tumour size and tumour grade (Table 2).

Validation Study Population

Validation of the prognostic power of the CER was performed in 455 patients diagnosed with early invasive operable breast cancer between January 2008 and January 2009. Patient and tumour characteristics are detailed in Table 3.

There were notable differences between the study cohorts. The validation cohort had fewer patients with ER negative breast cancer, and PR negative breast cancer. As expected with a more recent cohort, the majority of HER 2+ patients received biological therapy and more patients underwent breast conservation surgery. More patients had LN negative disease and were over 50 years at age, presumably as a result of improved breast screening uptake. Almost all (98%) patients with ER+ breast cancer received endocrine treatment.

Median follow up was 68.25 months (5.7 years). 80% (n=364) patients were alive and well, 7% (n=31) had died as a result of breast cancer and 9% (n=42) had died from other causes. 4% (n=19) were alive with documented evidence of breast cancer recurrence, therefore 11 % (n=50) had a breast cancer specific event.

CER classification in this cohort was associated with highly significant differences in DFS between CER negative, CER impaired and CER high groups (figure 3A). There was no significant difference in outcome between ER low and negative (figure 3B) or PR low and negative (figure 3C). The CER was a more powerful predictor of DFS than either the ER or PR. In multivariate analysis comparing the three factors the CER classification was

independently significant, CER negative HR 6.416 (CI 3.129- 13.157, P=3.903 x10^{-7}) and CER impaired HR 2.627 (CI 1.327-5.202, P=0.006). In multivariate analysis that included grade, tumour size and LN (HER2 was not included as this was not significantly associated with poor outcome as most HER2+ patients received biological therapy) the CER was independently significant in the validation cohort, including ER+ subgroup (n=398) (Table 2). The CER was a more powerful predictor of DFS than grade and tumour size (Table 2). Tumour size, grade and LN were independently significant for DFS as expected when included in multivariate analysis without CER (data not shown).

Discussion

The combined endocrine receptor (CER) is economical and an easily reproducible algorithm using well validated routinely tested biomarkers. In the derivation study for patients with early breast cancer, the CER was observed to be a better predictor of DFS and BCSS than either ER or PR alone. In addition, the CER is independently significant in multivariate analysis when combined with grade, lymph node status and tumour size. These findings were validated in a separate, modern cohort of early breast cancer patients.

Semi quantitative IHC is the near universal choice of tumour hormone (ER and PR) receptor testing. Despite its widespread use there have been a number of controversies in recent years regarding hormone testing.

IHC is a semi quantitative technique and pre analytical, analytical and post analytical factors can influence the results and result in test variation (Allred *et al*, 2009). In the derivation study expression level of both receptors were centrally tested to avoid testing variation. The validation study utilised the Allred scores from the pathology reports. The receptor testing had been performed in CPA accredited laboratories and represent ‘real world’ data.

IHC assays of ER and PR are limited to determining whether the receptors are present in tumour cells and providing some information on the levels of ER and PR in the tumour. The primary purpose of evaluating the ER and/ or PR status for individual patients is to predict whether they will respond to endocrine therapy. For the purposes of selecting endocrine therapy it is the hormone receptor status that is primarily important. It is notable however, that 6% of patients of ER negative patients were reclassified as CER impaired (ER-/PR+) in the derivation cohort and 1% in the validation cohort, suggesting that the CER categorisation will ensure more patients with hormone receptor positive disease will be considered eligible for endocrine treatment.

The categorisation should be clinically useful in the context of guiding adjuvant chemotherapy. Importantly, in both cohorts a substantial number of patients with high Allred ER scores were reclassified as impaired using the CER. There is an open question regarding the importance of quantifying hormone receptor expression level by IHC. Fisher *et al* (2005) compared various methods of scoring ER and PR, involving percentage ranges, intensity, both summated and as a product and concluded that the 'any-or none' method was just as good at prediction and simpler. Certainly within our own study, the level of ER independently when analysed as negative, low and high did not have a linear relationship with outcome. However, when analysed as the combined endocrine receptor, a direct proportional benefit with outcome and level of receptors was identified. Higher amounts of hormone receptor levels as determined by IHC have been associated with improved patient outcomes (Barnes *et al*, 1996; Cowen *et al*, 1990; Dowsett *et al*, 2008; Elledge *et al*, 2000; Esteban *et al*, 1994; Lockwood *et al*, 1999; Stendahl *et al*, 2006; Yamashita *et al*, 2006). These studies suggest that patients with higher ER IHC levels will have a higher probability

of good outcome probably due to good response to endocrine therapy. Our study supports that the level of *both* hormone receptors is important for outcome.

While the predictive power of the ER is undisputed, the predictive power and clinical utility of PR is more controversial (Hefti *et al*, 2013; Olivotto *et al*, 2004). Since 2009 the UK National Institute of Clinical Excellence (NICE) no longer recommends PR measurement in routine pathological assessment of early breast cancer (National Institute for Health and, and Excellence, 2009). A number of studies have, however, reported the prognostic power of PR (Blows *et al*, 2010; Purdie *et al*, 2014; Viale *et al*, 2007; Mohammed *et al*, 2015). Our results are in keeping these studies demonstrating improved outcome in ER+/PR+ breast cancer and support the value of PR measurement in breast cancer patients.

The aim of this study was simple, combining the ER and PR will be more informative in terms of outcome than either independently. Our working hypothesis is that ER and PR should not be considered alone, both are required and semi-quantitative IHC ER and PR scores together may represent a surrogate 'snap shot' of functional hormone receptor crosstalk. The importance of ER and PR being functionally linked through complex crosstalk has recently been defined (Mohammed *et al*, 2015). To our knowledge we are the first study to report a combined ER and PR IHC. This was a retrospective study and relatively small in terms of patient numbers. We would urge for further testing and application in larger cohorts from different centres to validate this score. The cut-offs applied were based on consensus opinion of what is considered high and low receptor expression of ER and PR (Goldhirsch *et al*, 2009), and supported statistically to define the CER categories. Importantly, the cut-offs were robust in the validation cohort.

In conclusion, the CER is a more powerful predictor of patient outcome than either the ER or PR alone and is a simple and economical method to identify risk in ER+ early breast cancer.

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Conflict of interest

The authors disclose no potential conflict of interest.

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Title and legends to figures and tables

Table 1. Characteristics of the derivation study population.

For the derivation population study, patient and tumour characteristics in the column titled "total" are re-categorised according to the combined endocrine receptor (CER) classification. ER, oestrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; neg, negative; imp, impaired; hi, high.

Table 2. Multivariate cox analysis for 5-year DFS and BCSS in the derivation and validation cohorts.

CER, combined endocrine receptor; ER, oestrogen receptor; PR, progesterone receptor; HER2+, human epidermal growth factor receptor 2-positive; imp, impaired; neg, negative.

Table 3. Characteristics of the validation study population.

For the validation population study, patient and tumour characteristics in the column titled "total" are re-categorised according to the combined endocrine receptor (CER) classification. Patients received endocrine therapy in the form of tamoxifen monotherapy, aromatase inhibitor (AI) monotherapy, early switch within 5 years AI-tamoxifen or *vice versa* and extended switch, 5 years on AI switched to tamoxifen or vice *versa*. ER, oestrogen receptor; PR, progesterone receptor, HER2, human epidermal growth factor receptor 2; neg, negative; imp, impaired; hi, high.

Figure 1. Determination of the cut-offs for the combined endocrine receptor (CER).

Kaplan-Meier plots were constructed for all possible CER values 0, 0.5, 1, 1.5 and 2. The outcome is 5-year disease-free survival (DFS).

Figure 2. Kaplan-Meier plots in the derivation study.

5-year disease-free survival (DFS) was plotted according to the combined endocrine receptor (CER) scores (A), oestrogen receptor (ER) scores (B) or progesterone receptor (PR) scores (C). Breast cancer-specific survival (BCSS) at 15-years was plotted according to CER scores (D), ER scores (E) or PR scores (F). hi, high; imp, impaired; neg, negative.

Figure 3. Kaplan-Meier plots in the validation study.

5-year disease-free survival (DFS) was plotted according to the combined endocrine receptor (CER) scores (A), oestrogen receptor (ER) scores (B) or progesterone receptor (PR) scores (C). hi, high; imp, impaired; neg, negative.

Supplementary figure 1. CONSORT diagrams

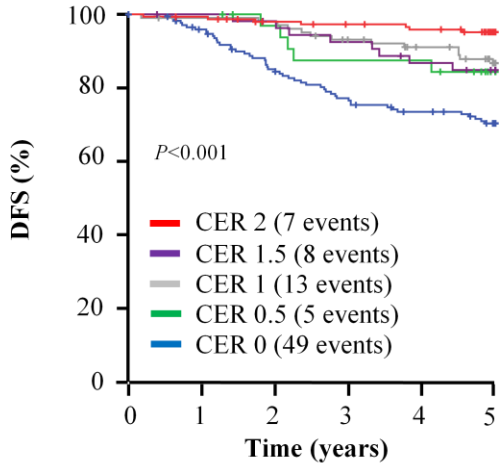
CONSORT diagrams for the derivation cohort (A) and the validation cohort (B). TMA, tissue microarray; ER, oestrogen receptor; PR, progesterone receptor; IHC, immunohistochemistry; HER2, human epidermal growth factor receptor 2.

Supplementary figure 2. Immunohistochemical staining of breast specimens for ER and PR.

Representative examples of negative, low (Allred score 3-5) and high (Allred score 6-8) ER and PR staining. The pictures show nuclear staining of tumour cells with intermittent stromal components. ER, oestrogen receptor; PR, progesterone receptor.

Figures

Figure 1



Number at risk						
CER 2	142	139	132	129	126	0
CER 1.5	55	54	51	48	44	0
CER 1	102	99	98	90	84	0
CER 0.5	32	31	28	28	25	0
CER 0	172	157	137	123	116	0

467 **Figure 2**

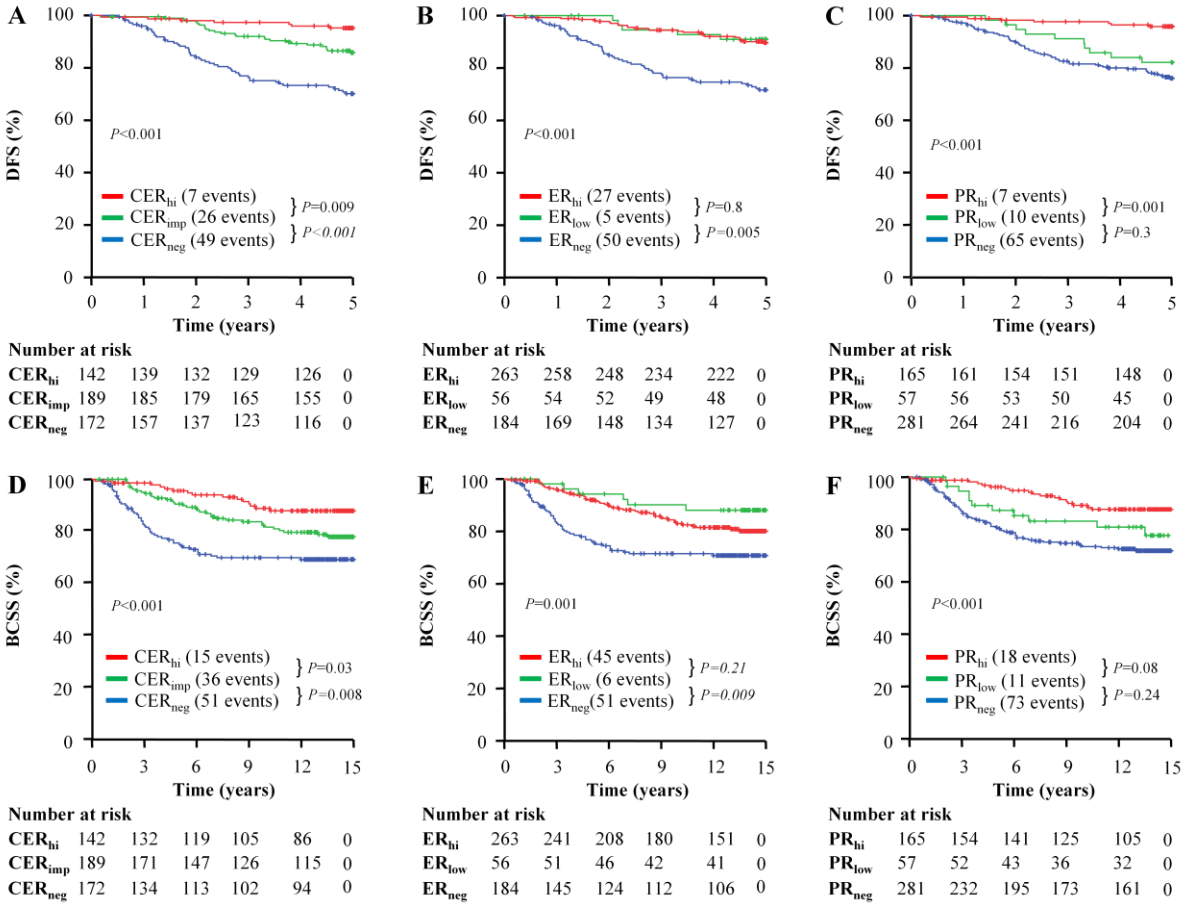


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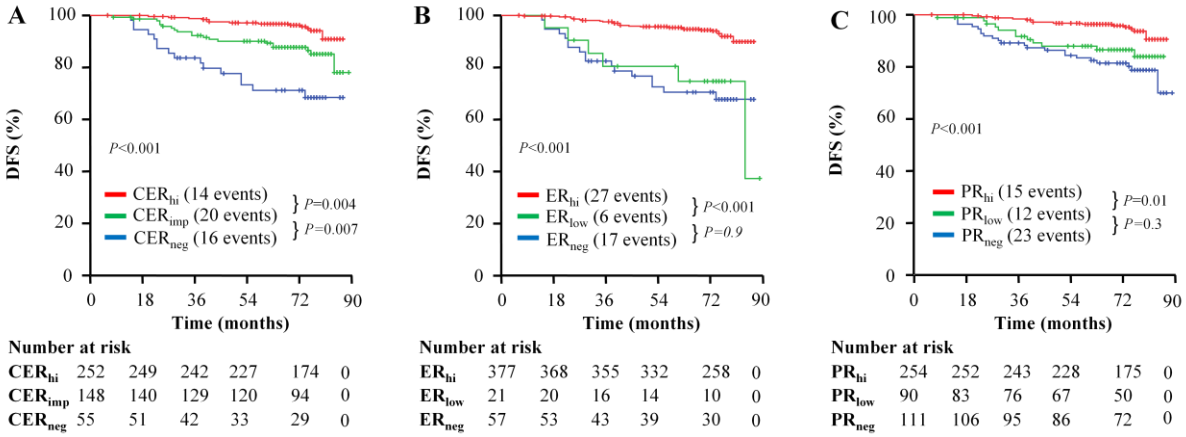
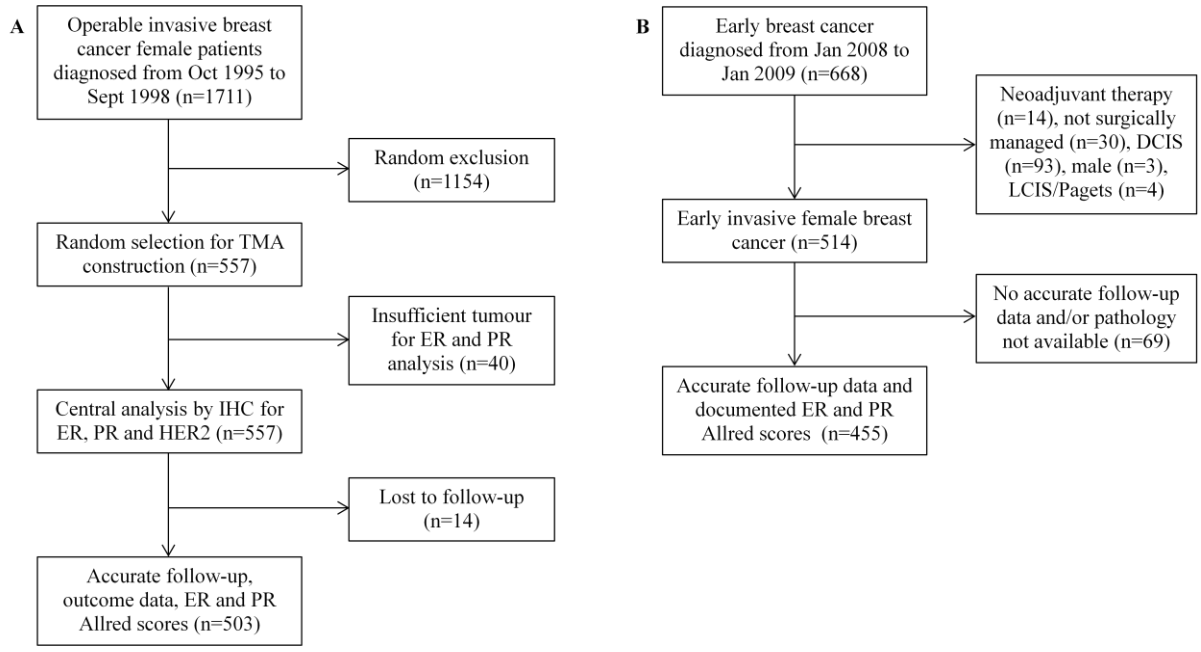
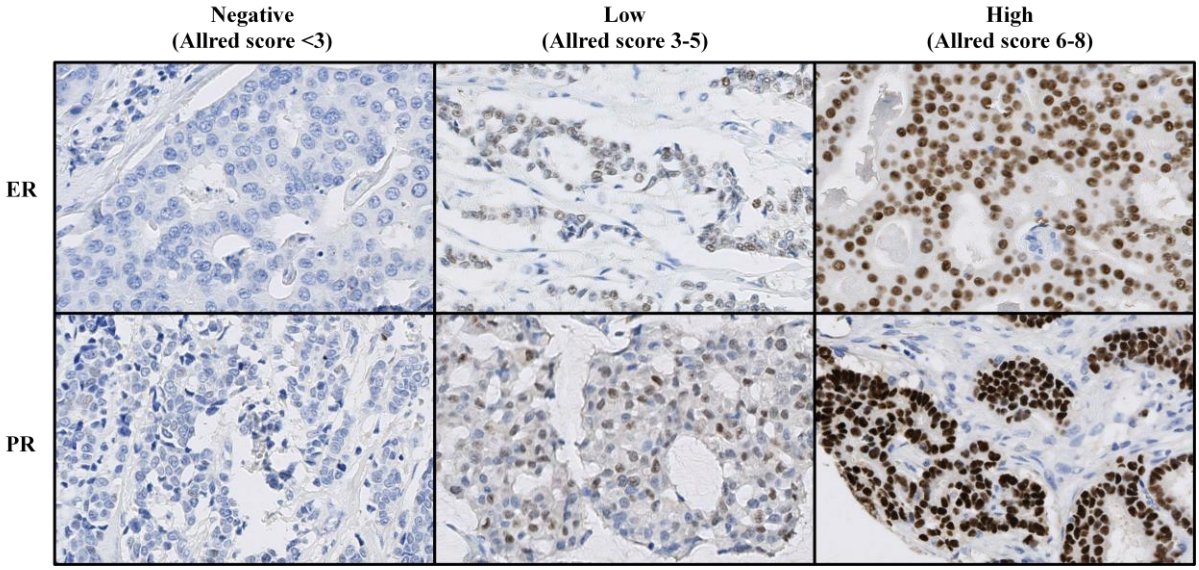
Figure 3


A
DFS (%)
P<0.001
CERhi (14 events)
CERimp (20 events)
CERneg (16 events)
P=0.004
P=0.007
Time (months)
Number at risk
CERhi 252 249 242 227 174 0
CERimp 148 140 129 120 94 0
CERneg 55 51 42 33 29 0
B
DFS (%)
P<0.001
ERhi (27 events)
ERlow (6 events)
ERneg (17 events)
P<0.001
P=0.9
Time (months)
Number at risk
ERhi 377 368 355 332 258 0
ERlow 21 20 16 14 10 0
ERneg 57 53 43 39 30 0
C
DFS (%)
P<0.001
PRhi (15 events)
PRlow (12 events)
PRneg (23 events)
P=0.01
P=0.3
Time (months)
Number at risk
PRhi 254 252 243 228 175 0
PRlow 90 83 76 67 50 0
PRneg 111 106 95 86 72 0

Supplementary figure 1



476 **Supplementary figure 2**



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Tables

Table 1: Characteristics of the derivation study population				
	Total N (%)	**CER_{neg} N (%)**	**CER_{imp} N (%)**	**CER_{hi} N (%)**
Age				
< 50	144 (29)	63 (37)	43 (23)	38 (27)
≥ 50	359 (71)	109 (63)	146 (77)	104 (73)
Grade				
1	93 (18)	3 (2)	51 (27)	39 (27)
2	217 (43)	33 (19)	108 (57)	76 (53)
3	191 (38)	134 (78)	30 (16)	27 (19)
unknown	2 (<1)	2 (1)		
Lymph node				
0	287 (57)	95 (55)	109 (58)	83 (58)
1-3	129 (26)	39 (23)	52 (28)	38 (27)
> 3	81 (16)	37 (21)	26 (14)	18 (13)
unknown	6 (1)	1 (<1)	2 (<1)	3 (2)
Size				
< 20mm	297 (59)	83 (49)	121 (64)	93 (66)
20-50 mm	189 (38)	81 (47)	62 (33)	46 (32)
> 50mm	16 (3)	7 (4)	6 (3)	3 (2)
Unknown	1 (<1)	1 (<1)		
ER Allred score				
< 3	184 (37)	172 (100)	12 (6)	
3-5	56 (11)		56 (30)	
6-8	263 (52)		121 (64)	142 (100)
PR Allred score				
< 3	281 (56)	172 (100)	109 (58)	
3-5	57 (11)		57 (30)	
6-8	165 (33)		23 (12)	142 (100)
HER2				
positive	76 (15)	51 (30)	16 (9)	9 (6)
negative	417 (83)	117 (68)	169 (89)	131 (92)
unknown	10 (2)	4 (2)	4 (2)	2 (2)
Surgical operation				
mastectomy	322 (64)	105 (61)	125 (66)	92 (65)
conservation	181 (36)	67 (39)	64 (34)	50 (35)
Endocrine therapy				
yes	368 (73)	69 (40)	170 (90)	129 (91)
no	127 (25)	100 (58)	16 (8)	11 (8)
unknown	8 (2)	3 (2)	3 (2)	2 (1)
Chemotherapy				
yes	208 (42)	116 (67)	49 (26)	43 (30)
no	292 (58)	55 (32)	138 (73)	99 (70)
unknown	3 (<1)	1 (<1)	2 (1)	

Table 2: Multivariate cox analysis for 5-year DFS and BCSS in the derivation and validation cohorts		
	Hazard ratio (CI)	**Significance**
Derivation cohort		
5-year DFS		
Lymph node	1.895 (1.453 - 2.472)	*P=0.00005*
Grade	1.560 (1.001- 2.431)	*P=0.050*
Size	1.380 (0.918 - 2.173)	*P=0.121*
CER_{neg}	4.441 (1.895 – 10.411)	*P=0.001*
CER_{imp}	2.869 (1.240-6.639)	*P=0.014*
HER2+	1.676 (1.004-2.798)	*P=0.048*
BCSS		
Lymph node	1.833 (1.428-2.353)	*P=0.000002*
Grade	1.504 (1.026-2.203)	*P=0.036*
Size	1.711 (1.196-2.448)	*P=0.003*
CER_{neg}	2.024 (1.065-3.848)	*P=0.031*
CER_{imp}	1.788 (0.974-3.283)	*P=0.061*
HER2+	1.182 (0.717-1.948)	*P=0.511*
5-year DFS in ER+ patients		
Lymph node	2.027 (1.281-3.209)	*P=0.003*
Grade	1.646 (0.899-3.012)	*P=0.106*
Size	1.208 (0.639-2.35)	*P=0.561*
CER_{imp}	2.469 (1.049-5.810)	*P=0.038*
PR_{neg}	0.956 (0.409-2.236)	*P=0.917*
HER2+	4.160 (1.803-9.603)	*P=0.001*
BCSS ER+ patients		
Lymph node	2.070 (1.406-3.049)	*P=0.0002*
Grade	1.825 (1.167-2.855)	*P=0.008*
Size	1.723 (1.167-2.806)	*P=0.029*
CER_{imp}	1.946 (1.054-3.596)	*P=0.033*
PgR_{neg}	0.928 (0.464-1.858)	*P=0.833*
HER2+	1.535 (0.644-3.629)	*P=0.329*
Validation cohort		
DFS		
Lymph node	1.818 (1.282-2.579)	*P=0.001*
Grade	1.266 (0.731-2.192)	*P=0.400*
Size	1.416 (0.825-2.428)	*P=0.207*
CER_{neg}	5.722 (2.727-12.003)	*P=0.000004*
CER_{imp}	2.431 (1.196-4.941)	*P=0.014*
DFS in ER+ patients		
Lymph node	2.388 (1.554-3.671)	*P=0.00007*
Grade	1.445 (0.805-2.594)	*P=0.218*
Size	1.299 (0.680-2.480)	*P=0.428*
CER_{imp}	2.096 (1.010-4.351)	*P=0.047*
PR_{neg}	0.763 (0.299-1.948)	*P=0.571*

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Table 3: Characteristics of the validation study population				
	Total N (%)	**CER_{neg} N (%)**	**CER_{imp} N (%)**	**CER_{hi} N (%)**
Age				
<50	68 (15)	15 (27)	18 (12)	35 (14)
≥50	387 (85)	40 (73)	130 (88)	217 (86)
Grade				
1	77 (17)		22 (15)	55 (22)
2	209 (46)	5 (9)	66 (45)	138 (55)
3	168 (37)	50 (91)	60 (40)	58 (23)
unknown	1 (<1)			1 (<1)
Lymph node				
0	311 (68)	36 (66)	94 (64)	181 (72)
1-3	97 (21)	11 (20)	31 (21)	55 (22)
>3	46 (10)	8 (14)	23 (15)	15 (6)
unknown	1 (<1)			1 (<1)
Size				
<20 mm	254 (56)	18 (33)	74 (51)	162 (64)
20-50 mm	176 (39)	35 (64)	61 (41)	80 (32)
>5 mm	13 (3)	2 (3)	8 (5)	3 (1)
unknown	12 (3)		5 (3)	7 (3)
ER Allred score				
<3	57 (12)	55 (100)	2 (1)	
3-5	21 (5)		21 (14)	
6-8	377 (83)		125 (85)	252 (100)
PR Allred score				
<3	111 (24)	55 (100)	56 (38)	
3-5	90 (20)		90 (61)	
6-8	254 (56)		2 (1)	252 (100)
HER2				
positive	70 (15)	18 (33)	35 (24)	17 (7)
negative	382 (84)	37 (67)	111 (75)	234 (93)
unknown	3 (<1)		2 (1)	1 (<1)
Surgical operation				
mastectomy	131 (29)	24 (44)	44 (30)	63 (25)
conservation	324 (72)	31 (56)	104 (70)	189 (75)
Endocrine therapy				
yes	392 (86)	2 (4)	140 (95)	250 (99)
tamoxifen	184 (40)		57 (39)	127 (50)
AI	138 (30)	2 (4)	57 (39)	79 (31)
early switch	46 (10)		14 (9)	32 (12)
late switch	24 (5)		12 (8)	12 (5)
no	63 (14)	53 (96)	8 (5)	2 (<1)
Chemotherapy				

yes	166 (37)	40 (73)	59 (40)	67 (27)
no	289 (63)	15 (27)	89 (60)	185 (73)
Biological therapy				
yes	50 (11)	14 (25)	24 (16)	12 (5)
no	405 (89)	41 (75)	124 (84)	240 (95)

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