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Biology of primary breast cancer in older women treated by surgery: with correlation with long-term clinical outcome and comparison with their younger counterparts

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Background: As age advances breast cancer appears to change its biological characteristics, however, very limited data are available to define the precise differences between older and younger patients.

Methods: Over 36 years (1973–2009), 1758 older (⩾70 years) women with early operable primary breast cancer were managed in a dedicated clinic. In all, 813 underwent primary surgery and 575 good quality tumour samples were available for biological analysis. The pattern of biomarkers was analysed using indirect immunohistochemistry on tissue microarrays. Comparison was made with a previously characterised series of younger (<70 years) patients.

Results: There was high expression of oestrogen receptor (ER), PgR, Bcl2, Muc1, BRCA1 and 2, E-cadherin, luminal cytokeratins, HER3, HER4, MDM2 and 4 and low expression of human epidermal growth factor receptor (HER)-2, Ki67, p53, EGFR and CK17. Oestrogen receptor and axillary stage appeared as independent prognostic factors. Unsupervised partitional clustering showed six biological clusters in older patients, five of which were common in the younger patients, whereas the low ER luminal cluster was distinct in the older series. The luminal phenotype showed better breast cancer-specific survival, whereas basal and HER2-overexpressing tumours were associated with poor outcome.

Conclusion: Early operable primary breast cancer in older women appears as a distinct biological entity, with existence of a novel cluster. Overall older women showed less aggressive tumour biology and ER appeared as an independent prognostic factor alongside the time-dependent axillary stage. These biological characteristics may explain the differences in clinical outcome and should be considered in making therapeutic decisions.

Age is an important risk factor of breast cancer and one-third of cases occur in women aged >70 years (Office for National Statistics, 2006; Jemal *et al*, 2009). Older patients tend to present with comorbidities and a considerable proportion eventually die from non-breast cancer causes (Fleming *et al*, 1999; Yancik *et al*, 2001). Available data suggest changing biology with advancing age, for example, increased oestrogen receptor (ER) positivity and decreased human epidermal growth factor receptor (HER)-2 expression (Diab *et al*, 2000). However, there is limited literature precisely delineating any biological differences because of a lack of, or under-representation of, older women in related studies. Most studies are based on registry databases with non-standardised laboratory protocols with a lack of long-term clinical data.

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We have recently reported on the conventional pathological features and their correlation with long-term clinical outcome in older women with early operable primary breast cancer, regardless of primary therapy (Syed *et al*, 2011a).

This study aimed to analyse tumour biology in the same population treated by primary surgery and to correlate it with long-term clinical outcome. Comparison was also made with their younger counterparts (Abd El-Rehim *et al*, 2005; Aleskandarany *et al*, 2011). The focus of this study was on biomarkers analysed using tissue microarrays (TMAs) and partitional clustering analysis, rather than conventional pathological features as reported (Syed *et al*, 2011a).

PATIENTS AND METHODS

Study patients. Over 36 years (1973–2009), 1758 older ($\geqslant$70 years) women with early operable primary breast cancer (T0-2, N0-1, M0) were managed in a dedicated clinic with clinical information available from diagnosis till death/last follow-up. In all, 813 patients underwent primary surgery (with optimal adjuvant therapy as per unit policy at the time (Syed *et al*, 2011a). After excluding 238 tumour samples that were checked and confirmed to have insufficient materials, a total of 575 good quality formalin-fixed paraffin-embedded surgical specimens were available for TMA construction. All patients were managed following the same management guidelines (which obviously evolved with time because of the long period that the study covered), 267 (46.6%) patients received adjuvant endocrine therapy and 105 (18.3%) received postoperative radiotherapy. None of these patients received chemotherapy.

For comparison, a previously characterised disease stage-matched series of younger (<70 years) patients ($N=1809$) was retrieved from the unit's database (Abd El-Rehim *et al*, 2005; Rakha *et al*, 2008; Aleskandarany *et al*, 2011). Conventional pathological parameters, as part of standard reporting for surgical specimens, included size, grade (Elston and Ellis, 1991) and axillary stage (according to number of positive nodes, 1 = 0 positive, 2 = 1–3 positive and 3 = $\geqslant$4 positive). The prognostic significance of biomarkers was analysed for disease-free survival, calculated from diagnosis to first recurrence (including any recurrence and/or cancer in the contralateral breast) and breast cancer-specific survival, calculated from diagnosis to death from breast cancer. Owing to the 'bias' in terms of selection for adjuvant systemic therapy across both age groups (e.g., chemotherapy was not standard in the older series as opposed to the younger series in cases of poor prognostic tumours), thus subgroup analysis based on adjuvant systemic therapy was not carried out.

TMA construction. Tissue microarrays of formalin-fixed paraffin-embedded tumour sections were constructed as described (Camp *et al*, 2000). Briefly, 0.6 mm diameter cores of the representative part of the tumour blocks were implanted in the TMA blocks using Beecher's manual tissue microarrayer (MP06 Beecher Instruments Inc., Sun Prairie, WI, USA).

Immunohistochemistry (IHC). Twenty-five biomarkers (ER, PgR, HER2, HER3, HER4, EGFR, BRCA1 and 2, p53, Ki67, Bcl2, Muc1, E-cadherin, basal and luminal cytokeratins, including CK5, CK5/6, CK7/8, CK14, CK17, CK18 and CK19, MDM2 and 4, VEGF, CD44 and LKB1) were analysed using indirect IHC by StreptAvidin Biotin Complex and EnVision methods as described (Abd El-Rehim *et al*, 2005; Aleskandarany *et al*, 2011).

Scoring. Immunohistochemistry staining of biomarkers was assessed by the percentage of cells stained as well as McCarty's immunohistochemical scoring (H-score; range 0–300; Howell *et al*, 1984). The cutoffs of the percentage of cells were used to define positivity/negativity (Table 1). Uncategorical H-scores were used for cluster analysis for all biomarkers except HER2, where Herceptest scoring was used.

Cluster analysis. The biological pattern was characterised by partitional clustering method as described (Soria *et al*, 2010), using R (R 2.11.1, a free software environment) clustering software.

Statistical analysis. The X-tile Bio-informatics software was used to define cutoffs (Camp *et al*, 2004). The Statistical Package for Social Sciences (SPSS, version 16.0, Chicago, IL, USA) was used for data collection and analysis. Chi-squared test was used for comparisons of biomarker expression between groups. Survival analysis was performed using Kaplan–Meier methods with application of log-rank and generalised Wilcoxon tests as appropriate. The Cox-regression model was used for multivariate analysis. A P-value of <0.05 was considered significant.

Results were reported as per Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK) criteria (McShane *et al*, 2005).

Ethical consideration. The study was approved by the local research ethics committee.

RESULTS

Biology in older women

Overall pattern. There were high expression of ER, PgR, BRCA1/2, HER3/4, VEGF, Bcl2, E-cadherin, Muc1, luminal cytokeratins, MDM2, MDM4 and LKB1 and low expression of HER2, p53, EGFR, Ki67, CD44 and basal cytokeratins (Table 1).

Prognostic significance. The median follow-up for all patients was 60 months (longest = 160) and for surviving patients it was 63 months (longest = 160). The prognostic significance of the biomarkers is described below according to disease-free and breast cancer-specific survival.

Disease-free survival. Expression of ER, PgR, Muc1 and Bcl2 was associated with better disease-free survival, whereas p53 was associated with poor outcome. Patients having tumours with lower grade and axillary stage, and smaller size had better disease-free survival (Table 1, Figure 1).

Multivariate analysis including ER, PgR, Muc1, Bcl2, p53, grade, axillary stage and pathological size showed ER (HR = 2.95, 95% CI = 1.30–6.71, $P=0.01$) and axillary stage (HR = 5.57, 95% CI = 2.86–10.8, $P<0.001$) as independent predictors.

Breast cancer-specific survival. Positive expression of ER, PgR, Bcl2, CK19 and Muc1 was associated with significantly better survival, and so was the absence of HER2, EGFR, Ki67, p53 and CK17. Among the conventional markers, lower grade and axillary stage, and smaller pathological size were associated with better survival on univariate analysis (Table 1, Figure 2).

On multivariate analysis, only ER (HR = 5.76, 95% CI = 1.35–24.6, $P=0.01$) and axillary stage (HR = 10.1, 95% CI = 3.67–27.73, $P<0.001$) showed independent prognostic significance.

Biological characterisation. Including 19 biomarkers cluster indices suggested six clusters, with bi-plots of the pattern shown in Figure 3. The minimum number of biomarkers and maximum sample size to reproduce the same clusters were determined using the unsupervised cluster tree. Seven biomarkers (Figure 3C) appeared as key drivers. The remaining biomarkers were included one by one to reproduce the same clusters as had been produced by the 19 biomarkers. Eventually, the same clusters were produced by 12 biomarkers: ER, PgR, HER2, Bcl2, CK5, CK7/8, CK18, CK5/6,

Table 1. Methodology, expression and the prognostic significance of biomarkers in older women with early operable primary breast cancer

		Results							
		Expression pattern, *N*(%)		5-Year breast cancer-specific survival (%)			5-Year disease-free survival (%)		
Biomarker (*N* available)	**Cutoff (%)**	**Positive**	**Negative**	**Positive**	**Negative**	***P*-value**	**Positive**	**Negative**	***P*-value**
ER (***N***=518)	0	365 (70.5)	153 (29.5)	93	76	0.002	80	63	<0.001
PgR (***N***=517)	0	293 (56.7)	224 (43.3)	92	82	0.001	80	69	0.01
HER2 (***N***=537)	3+ (Hercept score)	41 (7.6)	496 (92.4)	75	90	0.01	70	76	0.74
Ki67 (***N***=575)[a]	10	194 (33.7)	381 (66.3)	84	90	0.01	75	73	0.95
p53 (***N***=479)	5	188 (39.2)	291 (60.8)	85	91	0.04	68	80	0.001
BRCA1 (***N***=465)	30	442 (95.1)	23 (4.9)	88	92	0.40	82	88	0.45
BRCA2 (***N***=404)	0	317 (78.5)	87 (21.5)	90	81	0.05	75	72	0.28
CK5 (***N***=519)	0	155 (29.9)	364 (70.1)	83	90	0.09	72	75	0.68
CK5/6 (***N***=477)	0	223 (46.8)	254 (53.2)	90	85	0.85	77	70	0.16
CK7/8 (***N***=515)	0	500 (97.1)	15 (2.9)	2 Events in negative group			75	87	0.26
CK14 (***N***=471)	0	113 (24.0)	358 (76.0)	86	89	0.90	76	75	0.51
CK17 (***N***=506)	0	101 (20.0)	405 (80.0)	82	90	0.04	73	77	0.92
CK18 (***N***=498)	0	479 (96.2)	19 (3.8)	88	75	0.08	75	62	0.31
CK19 (***N***=511)	0	488 (95.5)	23 (4.5)	88	74	0.006	74	77	0.90
EGFR (***N***=470)	0	93 (19.8)	377 (80.2)	81	90	0.02	70	77	0.31
HER3 (***N***=482)	0	474 (98.3)	8 (1.7)	No events in negative group			1 Event in negative group		
HER4 (***N***=481)	0	444 (92.3)	37 (7.7)	88	88	0.43	82	93	0.11
Bcl2 (***N***=501)	0	422 (84.2)	79 (15.8)	90	74	<0.001	78	59	0.001
E-cadherin (***N***=504)	30	311 (61.7)	193 (38.3)	85	91	0.48	73	77	0.64
Muc1 (***N***=515)	10	445 (86.4)	70 (13.6)	90	80	0.005	76	68	0.01
VEGF (***N***=427)	0	354 (82.9)	73 (17.1)	88	95	0.89	75	80	0.51
CD44 (***N***=514)	0	112 (21.8)	402 (78.2)	75	79	0.37	80	74	0.29
LKB1 (***N***=407)	30	318 (78.1)	89 (21.9)	88	85	0.74	75	79	0.15
MDM2 (***N***=447)	0	447 (100)	0	88	92	0.59	76	76	0.31
MDM4 (***N***=401)	0	399 (99.5)	2 (0.5)	1 Event in negative group			2 Events in negative group		

Abbreviations: Bcl2=B-cell lymphoma 2; BRCA1=Breast Cancer gene 1; EGFR=Epidermal Growth Factor Receptor; ER=oestrogen receptor; HER2=human epidermal growth factor receptor 2; Ki67=KI67 antigen; LKB1=Liver kinase B1; MDM=Mouse double minute; Muc1=Mucin 1; PgR=Progesterone receptor.
[a]Whole tumour sections were used.

CK19, p53, Muc1 and E-cadherin. Box plots in Figure 4 present their expression pattern. High ER expression was seen in clusters 1 and 2 and the rest showed low/negative ER expression:

Cluster 1 (luminal A) showed high hormone receptors, luminal cytokeratins, Bcl2, Muc1 and HER3, and low expression of HER2, p53, EGFR, CD44, E-cadherin and basal cytokeratins.

Cluster 2 (luminal B) differed from cluster 1 only in PgR expression (low instead of high).

Cluster 3 (normal-like) showed low expression of all biomarkers.

Cluster 4 (low ER luminal) had high expression of luminal cytokeratins, Muc1 and HER3 and low expression of the remaining biomarkers.

Cluster 5 (basal-like) had high expression of basal cytokeratins and HER3, and low expression of luminal cytokeratins.

Cluster 6 (HER2-overexpressing) showed also high luminal cytokeratins, Muc1 and HER3 and negative hormone receptor expression.

Long-term clinical outcome. The ER and luminal cytokeratins expressing clusters (1 and 2) were associated with the longest breast cancer-specific survival, followed by the low ER luminal and all low expressions/normal-like clusters (3 and 4; Figure 4). The basal-like (cluster 5) and HER2-overexpressing (cluster 6) tumours were associated with the worst prognosis.

Comparison with younger patients

Expression pattern of biomarkers. Expression of the 12 key biomarkers (ER, PgR, Her2, Bcl2, CK5, CK7/8, CK18, CK5/6, CK19, p53, Muc1 and E-cadherin) in older women were compared with a younger cohort (Table 2). Breast carcinomas in older women showed significantly higher positivity for ER, CK5/6, CK5,CK18, p53 and Bcl2 and lower expression of CK7/8 and E-cadherin. There was no significant difference in the expression of PgR, HER2 and CK 19. The difference in Muc1 expression only reached borderline significance.

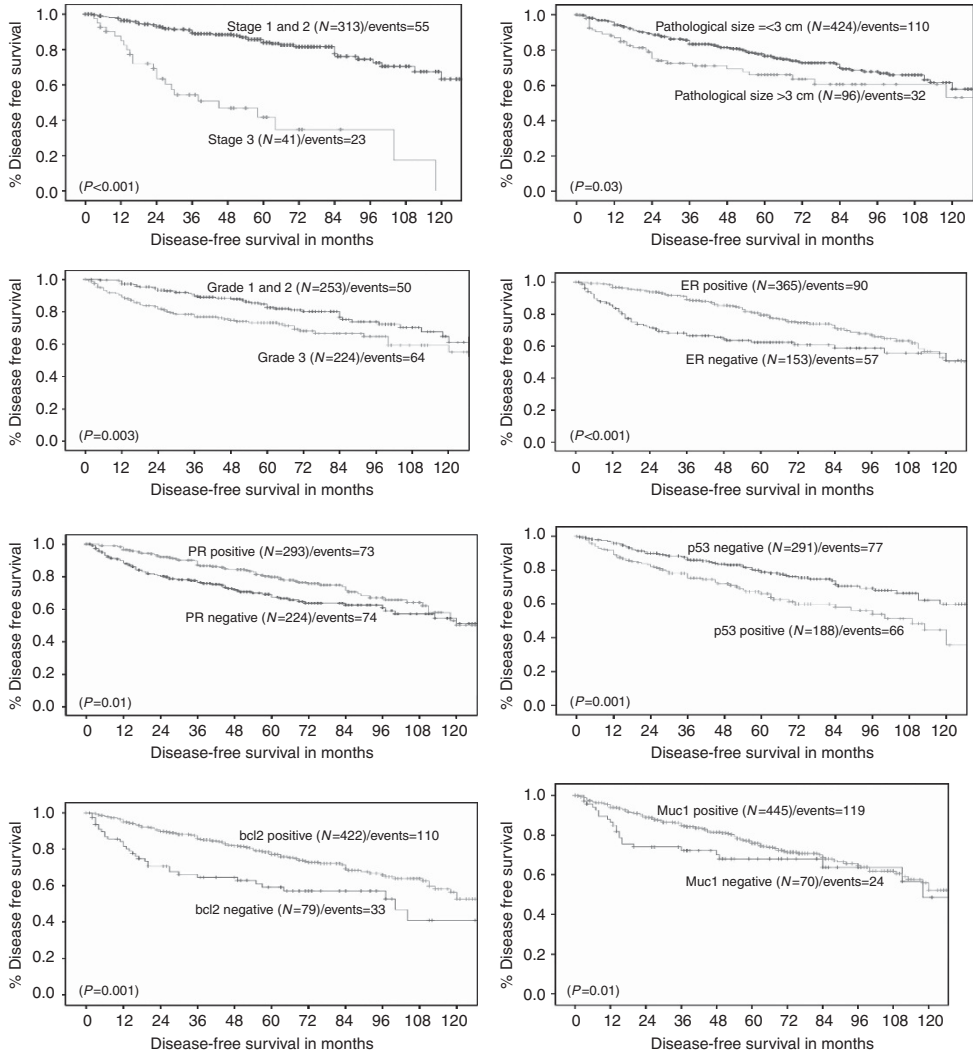

Figure 1. Kaplan–Meier plots of the biomarkers showing significant association with disease-free survival in older women with early operable primary breast cancer.

Biological clusters. The panel of 12 biomarkers was used to reproduce clusters in the younger series. Cluster indices and bi-plots indicated only five clusters as opposed to six observed in the older series (Figure 4). The pattern of these five clusters was essentially identical to clusters 1, 2, 3, 5 and 6 in the older series. Cluster 3 showed a pattern of ER, PgR and HER2 similar to that seen in the basal-like low-p53 cluster in younger patients but they were different mainly in the expression of basal cytokerations. However, the triple-negative pattern in this all low expression normal-like cluster suggests its linkage with the basal phenotype. The pattern noted in cluster 4, showing low ER and PgR with high luminal cytokeratin expression, was not seen in the younger series.

Both luminal types were characterised by high hormone receptor expression in both age groups, however, the median expression was considerably higher in older women (average H-score nearly 300 *vs* 150 in younger patients). In both age groups, PgR remained the differentiating point between luminal A and B clusters. High-p53 basal-like tumours in both age groups showed similar characteristics with low hormone receptor expression. CK19 expression, however, was higher in older women. Human epidermal growth factor receptor 2-overexpressing group was identical in both age groups except in the patterns of ER and PgR, where in older women, they were almost negative and in younger patients both showed low expression.

DISCUSSION

High expression of good prognostic biomarkers (ER, PgR, Bcl2 and Muc1) and low expression of poor prognostic biomarkers (HER2, Ki67, p53 and EGFR) were seen in the older series. There was also high expression of breast cancer-related genes BRCA1 and BRCA2, and luminal cytokeratins, E-cadherin, HER3, HER4, LKB1, MDM2 and MDM4, and low expression of stem cell marker and basal cytokeratins. Biological characterisation found six distinct patterns including a novel cluster, which was not observed in the younger patients. It possessed high luminal cytokeratins and low expression of hormone receptors and basal cytokeratins and has never been reported in the literature. The five clusters (luminal A, luminal B, basal, normal-like and HER2-overexpressing) were similar in both age groups. Oestrogen receptor, PgR, HER2, Bcl2, CK17, CK19, Muc1, p53, Ki67, EGFR, grade, pathological size and axillary stage were shown to be prognostic

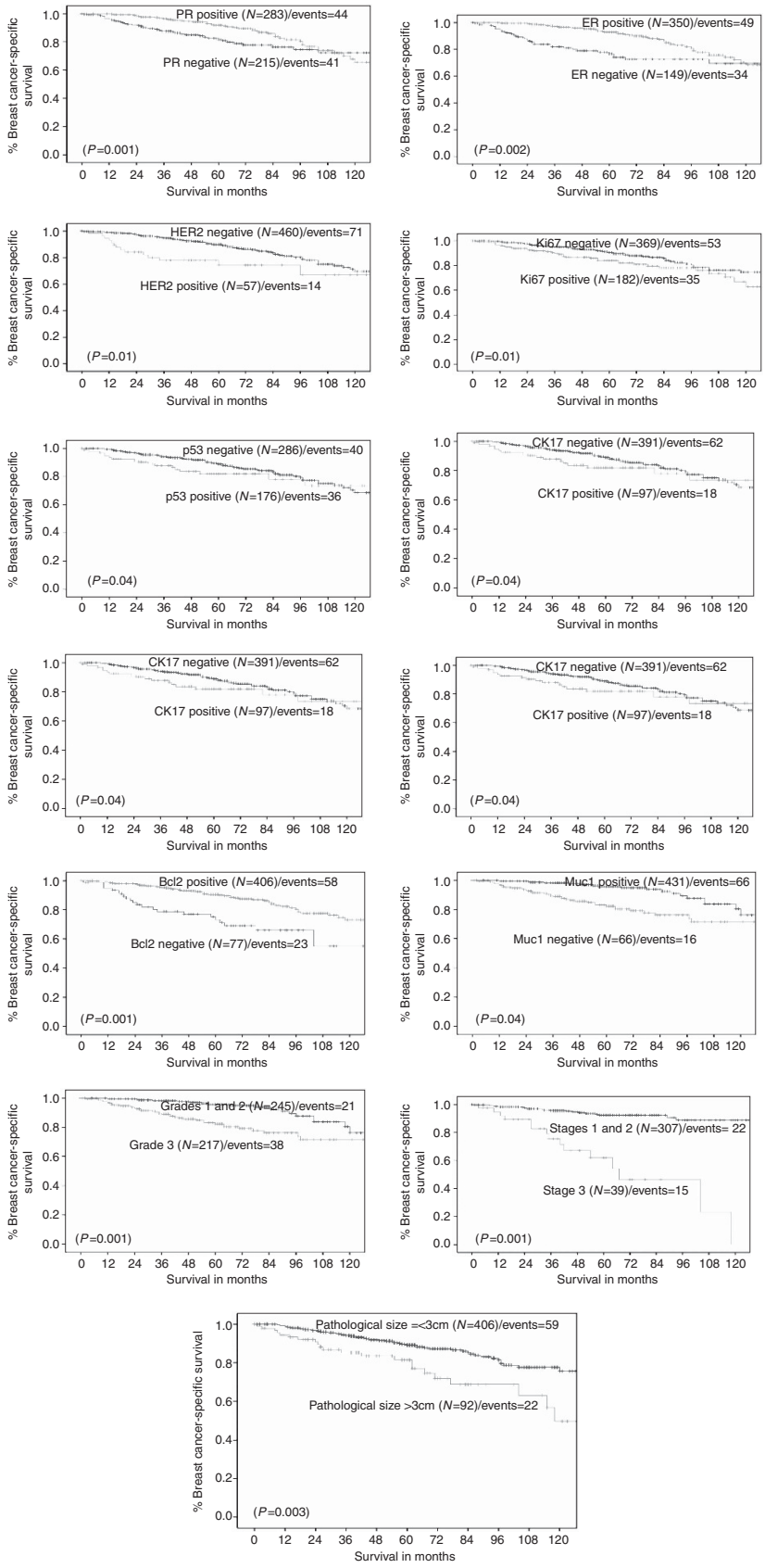

Figure 2. Kaplan–Meier plots of the biomarkers showing significant association with breast cancer-specific survival in older women with early operable primary breast cancer.

factors on univariate analysis, whereas only ER and axillary stage had independent prognostic significance. When combined, the three luminal phenotypes, regardless of ER, were associated with better breast cancer-specific survival as compared with other types, where basal and HER2-overexpressing tumours were associated with poor outcome.

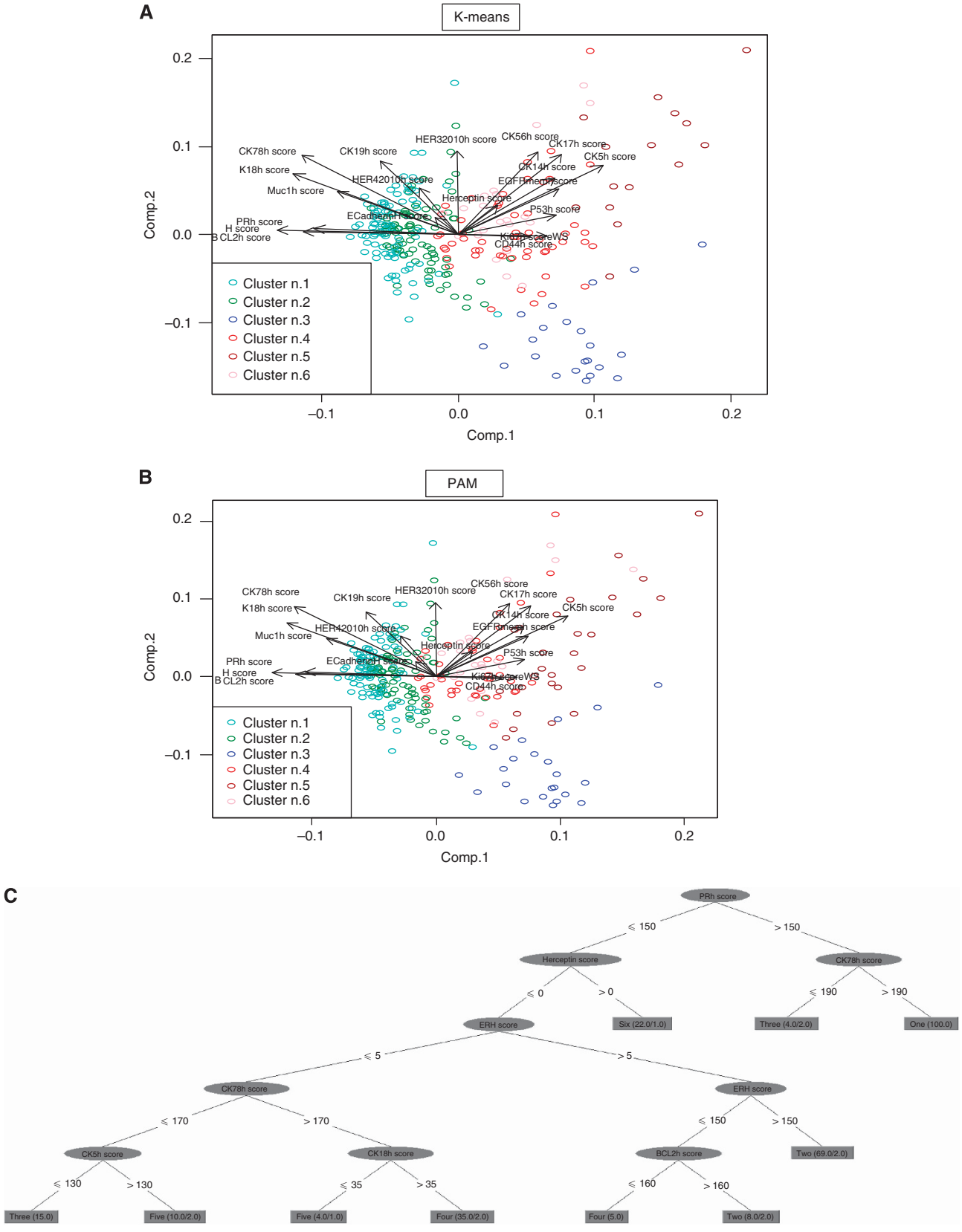

Figure 3. Bi-plots of clusters of early operable primary breast cancer in older women using (**A**) K-means and (**B**) partitioning around medoids (PAM) methods (each colour represent a cluster and the results of both methods suggest six clusters) and (**C**) inter-relationship tree of key biomarkers in clusters (showing H-score cutoffs).

This study, from a single centre with long-term clinical outcome with analysis of a large number of biomarkers, has the potential to provide a robust data set. The analysis was based on IHC analysis of surgical specimens. This, however, may be a caveat in terms of representation of the older population. Given that there is a higher proportion of patients with ER-positive tumours undergoing

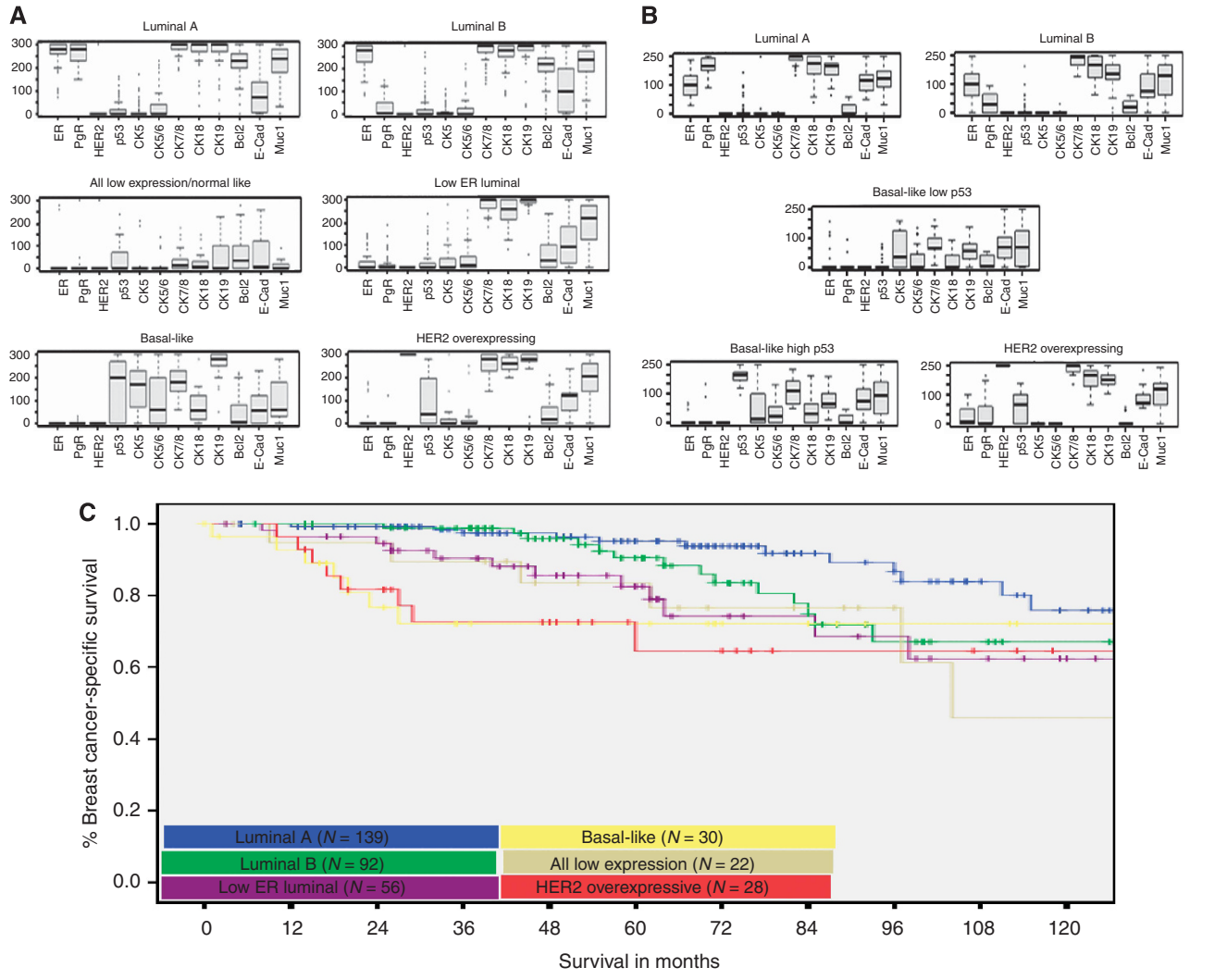

Figure 4. Biological classes of early operable primary breast cancer – (**A**) older (⩾70 years) ***vs*** (**B**) younger (<70 years) women. (**C**) Breast cancer-specific survival of older women with early operable primary breast cancer according to the biological clusters.

primary endocrine therapy instead of surgery, as opposed to those with ER-negative tumours where most of the time surgery would have been performed, the tumours in this cohort would be expected to have relatively more ER-negative disease when compared with the whole group of older women with primary breast cancer. This should be considered when projecting the conclusion to the whole elderly population.

The high expression of ER, PgR, Bcl2, CK7/8 and CK18 and low expression of HER2, Ki67, p53, CK5/6, CK14 and EGFR are in keeping with the literature (Diab *et al*, 2000; Eppenberger-Castori *et al*, 2002; Daidone *et al*, 2003; Pappo *et al*, 2007; Cheung *et al*, 2008; Durbecq *et al*, 2008; Ma *et al*, 2009; Tse *et al*, 2009) and supporting the theory of less aggressive tumour biology in older women. With the possible relative over-representation of ER-negative tumours as explained, it would be entirely conceivable that breast cancer in the overall older population is even less aggressive than shown here. The comprehensive analysis of further biomarkers including Muc1, cytokeratins, E-cadherin, breast cancer-related genes, stem cell markers, HER3 and HER4 is a new addition to the literature.

The prognostic significance of ER, regardless of age, has long been recognised (Allan *et al*, 1985; Akhtar *et al*, 1991; Ciatto *et al*, 1996). Bcl2 also appeared as an important prognostic factor in this study. Bcl2 expression has been reported to have strong prognostic significance in studies focusing on younger patients. A meta-analysis of 17 studies of breast cancer ($N = 5892$) has shown Bcl2 as an independent prognostic factor (Callagy *et al*, 2008). Studies have also shown its prognostic significance independent of adjuvant therapy and Nottingham Prognostic Index (NPI) (Callagy *et al*, 2006; Dawson *et al*, 2010). However, in our study Bcl2 was a prognostic factor on univariate analysis only, where ER and the time-dependent axillary stage seemed to produce more powerful impact. The prognostic significance of Bcl2 could be utilised in older women to predict response to primary endocrine therapy and anti-Bcl2 therapy (O'Brien *et al*, 2007) could potentially be used in high-risk/hormone-resistant tumours as adjuvant/primary therapy.

Another important biomarker observed in our cohort is Muc1. This has already been recognised as an immunogen and a vaccine has been produced (Gilewski *et al*, 2000). Its prognostic significance in our study is in keeping with the literature (Rakha *et al*, 2005). A small study from a single centre ($N = 243$); aged 27–89 years) with a median follow-up of 26 months has shown highly significant association of Muc1 with disease-free and overall survival (van der Vegt *et al*, 2007). Another study reported a significant reduction of Muc1 expression after 3 weeks of neoadjuvant tamoxifen (Hanson *et al*, 2001). It would therefore be interesting to evaluate its role in patients who receive primary endocrine therapy. Given the high expression of Muc1, the

Table 2. Expression pattern of biomarkers in early operable primary beast cancer – young (<70 years) *vs* older (⩾70 years) women

	Categorical variables <70 *vs* ⩾70 years		
Biomarker	**<70 *N* (%)**	**⩾70 *N* (%)**	***P*-value**
ER			0.04
Positive	953 (66.7)	401 (70.1)	
Negative	476 (33.3)	171 (29. 9)	
PgR			0.08
Positive	1005 (58.9)	317 (55.5)	
Negative	702 (41.1)	254 (44.5)	
HER2			0.40
Positive	140 (8.2)	45 (7.7)	
Negative	1574 (91.8)	538 (92.3)	
CK5			<0.001
Positive	204 (15.8)	155 (29.9)	
Negative	1086 (84.2)	364 (70.1)	
CK5/6			<0.001
Positive	288 (16.7)	194 (35.0)	
Negative	1432 (83.3)	360 (65.0)	
CK7/8			0.001
Positive	1717 (99.1)	548 (97.2)	
Negative	15 (0.9)	16 (2.8)	
CK18			<0.001
Positive	1453 (89.4)	527 (95.6)	
Negative	172 (10.6)	24 (4.4)	
CK19			0.46
Positive	1633 (94.4)	533 (94.7)	
Negative	96 (5.6)	30 (5.3)	
Muc1			0.05
Positive	1258 (89.4)	510 (91.9)	
Negative	149 (10.6)	45 (8.1)	
E-cadherin			<0.001
Positive	1458 (84.8)	421 (73.9)	
Negative	261 (15.2)	149 (26.1)	
p53			<0.001
Positive	468 (27.4)	199 (35.7)	
Negative	1241 (72.6)	359 (64.3)	
Bcl2			<0.001
Positive	301 (43.8)	441 (82.7)	
Negative	387 (56.3)	92 (17.3)	

utilisation of Muc1 vaccine could be explored as a potential adjuvant/primary therapy in older patients, especially in those who present with high-risk/ER-negative tumours.

Human epidermal growth factor receptor 2 showed its prognostic significance on univariate analysis only. Previous studies have shown an association with shorter disease-free and overall survival regardless of age (Knoop *et al*, 2001; Tsutsui *et al*, 2002; Kim *et al*, 2008; Linderholm *et al*, 2009). Trastuzumab has been in clinical practice for a few years, however, because of the lack of robust data focusing on older women it has not yet received wide acceptance in this population. Currently available studies on trastuzumab analysed its efficacy in combination with chemotherapy (Marty *et al*, 2005; Romond *et al*, 2005; Viani *et al*, 2007), which is again controversial from older patients' perspective. Robust data for its short-term use or as monotherapy are urgently required (Syed *et al*, 2011b). Other biomarkers including PgR, Ki67, p53 and EGFR also showed prognostic significance on univariate analysis, but were absorbed into ER in multivariate analysis. EGFR has recently received much attention as a therapeutic target in triple-negative disease, where it may have a role to play in older women (Tan *et al*, 2008; Kim *et al*, 2009; Lee *et al*, 2010). Further studies may give some insights into this. The conventional prognostic factors including grade, pathological size and axillary stage have been recognised as independent prognostic factors in younger patients and used to compute the NPI (Haybittle *et al*, 1982). Similar prognostic significance was seen in the older series in this study.

A limited number of studies characterised breast cancer using IHC, and most were restricted to younger (<70 years) patients. Studies that include older patients did not stratify them and also older women were under-represented (8–14%; Durbecq *et al*, 2008; Vallejos *et al*, 2010). Data are scarce in characterising breast cancer using IHC focusing on older women and correlating them with long-term clinical outcome. The number and panel of biomarkers used in studies tend to vary, however, the key drivers remain the same including ER, PgR, HER2, basal and luminal cytokeratins, EGFR and p53. Given these, our study is unique and important for being a large series from a single centre, characterising breast cancer specifically in older women, correlating the biology with long-term clinical outcome and also comparing them with the younger series from the same unit.

The five common clusters found in both age groups are in keeping with the literature (Sorlie *et al*, 2003). Both gene array and IHC studies have reported similar patterns (Sorlie *et al*, 2003; Abd El-Rehim *et al*, 2004). The low ER luminal cluster appears novel in the older cohort, with distinct biological pattern and associated breast cancer-specific survival. A third luminal cluster was previously reported on gene array and IHC studies (Sorlie *et al*, 2003; Sotiriou *et al*, 2003; Soria *et al*, 2010), but they classified luminal as ER-positive phenotype and that cluster was not further characterised and later on absorbed in the other two luminal types (A and B). The low ER luminal may be unique to older patients, and possibly has developed as a result of age-related changes in tumour biology. The reason that it was not noted in previous studies could be due to their small sample size with minimal representation of older patients.

Before we conclude that this cluster is novel and specific to older women, one consideration is the configuration of biomarkers, which may impact on the possibility of its detection in younger patients. Therefore, the clusters were reproduced in younger patients managed in the same unit, using the same methods and the same list of biomarkers, hence further supporting its novelty and specific existence in the older population. Having said that, it is still possible that this group might exist in younger patients but is probably too small to manifest itself as a cluster and as such may have merged into the luminal clusters.

This cluster, being ER, PgR and HER2-negative may be considered as basal-like by simple definition. However, as previously described, CK5/6 and EGFR expression is most prevalent in the basal phenotype (Nielson *et al*, 2004; Livasy *et al*, 2006). The low p53 expression in this cluster is atypical in the basal category and the very high expression of luminal cytokeratins justifies its grouping with the luminal class. Interestingly, the survival of the group was in between the classical luminal and basal phenotypes.

The basal clusters have recently been subgrouped according top 53 phenotypes by our group (Soria *et al*, 2010; Biganzoli *et al*, 2011). The characterisation of the younger series in this study showed the same classification, whereas the normal-like cluster in the older patients appeared different from the basal-like low p53 cluster seen in the younger patients in the expression of basal cytokeratins. It is possible that the overall low expression of basal biomarkers in older women has influenced this pattern. A review of the histological types of the normal-like cluster in the older series here showed typical basal type with apocrine and metaplastic varieties. Thus, it can be assumed that the normal-like phenotype in older women is a subgroup of basal-like tumours. This observation warrants further investigation.

Another interesting finding is that in older women there was high expression of hormone receptors and luminal cytokeratins as compared with their younger counterparts.

Each defined cluster showed different breast cancer-specific survival pattern where excellent outcome was observed with luminal A cluster and worst in HER2-overexpressing cluster. The pattern of clinical outcome in our series is consistent with the literature, where regardless of age luminal phenotype was reported to be associated with the best outcome and the HER2 and basal-like clusters with poor outcome (Sorlie *et al*, 2003; Calza *et al*, 2006; Vallejos *et al*, 2010).

CONCLUSION

Breast cancer in older women appears as a distinct biological entity with an additional novel cluster associated with distinct clinical outcome, when compared with their younger counterparts. Even within the apparently similar clusters the expression pattern differs in the two age groups where the older patients show higher expression of good prognostic factors further supporting the clinical observation that the majority of older women tend to get less aggressive tumours. Oestrogen receptor expression and the time-dependent axillary stage have been shown to be independent prognostic factors; ER status can now be easily obtained from needle core biopsies and provides a very reliable indication of prognosis allowing management decision be made.

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AUTHOR CONTRIBUTIONS

BMS manuscript is part of BMS's PhD project, she collected data, did all laboratory work, analysed data and written the manuscript. ECP, DS, JG, LM contributed in data collection and analysis and writing the manuscript. ARG, DALM, IOE, KLC conceived concept of study, provided overall supervision during conduct of the study, have written and approved final version of the manuscript for submission.

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