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Basal phenotype identifies a poor prognostic subgroup of breast cancer of clinical importance

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ABSTRACT

Background: Breast cancer is recognised to be a heterogeneous disease with a range of morphological appearances and behaviours. The recently recognised basal phenotype (BP) is associated with poor survival, but the clinical implications of this class of breast cancers remain to be adequately defined.

Methods: We have examined a well-characterised series of 1872 invasive breast carcinomas with a long term follow-up to assess the clinical significance of BP.

Results: A pragmatic definition of the BP as immunophenotypic evidence of basal cytokeratins CK5/6 and/or CK14 expression was used. These tumours were associated with shorter overall survival and disease-free interval in our series as a whole and in both the lymph node (LN) negative and LN positive subgroups. When stratified by histological grade, BP was of highly significant prognostic value in grade 3 but not in grades 1 or 2 tumours. Similarly, it was associated with poor survival in the moderate group of the Nottingham prognostic Index but not in the other groups. In a subgroup comprising LN negative grade 3 tumours, BP was the most powerful prognostic marker followed only by tumour size, while the other variables were non-significant. Patients with BP were more likely to respond to chemotherapy than those with non-basal tumours.

Conclusions: Our results provide robust evidence that BP is an important class of breast cancers with a particularly aggressive behaviour in patients with LN negative grade 3 disease. We recommend routine identification of BP in breast cancer and the development of effective adjuvant treatment strategies. These are important observations as these tumours typically lack hormone receptor and HER-2 overexpression limiting the range of relevant adjuvant therapies.

1. Introduction

Although the concept of the basal-like or basal phenotype (BP) of breast cancer has been known for some time, it has recently become highly topical following the recognition of this class in the high profile cDNA expression analysis[1,2] and the high frequency of these tumours developing in BRCA1 gene mutation carriers.[3,4] However, these studies have not focused on routine identification of the BP nor been of sufficient size to examine its clinical relevance in terms of behaviour and association with other prognostic factors in routine clinical usage.

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The potential poor survival associated with the expression of basal cell-type cytokeratins (CKs) in tumour cells was first reported by Dairkee et al. in 1987[5] and followed by other studies.[2,6–8] However, the association between BP and patients' outcome showed dissimilar results in the different subgroups of breast cancer. Previous studies have demonstrated that the expression of basal CKs (BP) is an independent marker of poor prognosis in breast cancers as a whole.[1,9–11] In a previous study, van de Rijn[7] examined the expression of CK5/6 and/or CK17 in 474 unselected breast carcinomas and found that basal CKs were associated with shorter survival in the lymph node (LN) negative tumours but not in the LN positive group. Similar results were reported by Potemski et al.[11] Contrasting this, Nielsen et al.[12] and Malzahn et al.[13] showed an association between basal CK expression and survival in the LN positive group, but not in the LN negative group.

In this study, we have examined the clinical significance of the BP, defined in a pragmatic fashion applicable to routine clinical practice, in a much larger and well-characterised series of breast cancers with a long term follow-up. We aim to identify the prognostic value of BP in the different prognostic categories using current clinical practice principally based on stage and grade, to determine which group(s) could benefit the most from BP characterisation in routine practice. Furthermore, we aim to explore the reasons behind the inconsistency of the previous results regarding association between BP and outcome in different cohorts of patients.

2. Materials and methods

2.1. Patients and tumours

This study was based on a well-characterised series of 1944 primary operable invasive breast carcinoma cases entered into the Nottingham Tenovus Primary Breast Carcinoma Series between 1986 and 1998. Patients were under the age of 70 and treated in a conventional uniform manner. The series has been used previously to study the expression of a wide range of biomarkers.[9,14] Patient's clinical history and tumour characteristics including age, menopausal status, tumour type,[15] histological grade,[16] tumour size, lymph node status, vascular invasion (VI),[17] and Nottingham Prognostic Index (NPI)[18] were assessed in a uniform fashion. Information on local, regional and distant recurrence and survival was maintained on a prospective basis. The patients were followed up at 3 month intervals initially, followed by 6 monthly and annually. The median overall survival was 73 months and the median time of event-free survival was 66 months (range 1–206 months). Median follow-up was defined as median follow-up for those patients still alive and disease-free at the latest hospital visit. The disease-free interval was defined as the interval (in months) from the date of the primary surgery to the first loco-regional recurrence or distant metastasis. The overall survival was the time, in months, from the date of the primary surgery to the time of breast cancer related death. Hormonal therapy was given to 536 patients (35.8%), chemotherapy to 261 (17.4%) and radiotherapy to 334 patients (22.3%).

Table 1 – Source, dilution and pretreatment of antibodies used

Antibody, clone	Dilution	Source	Pretreatment
Basal cell markers			
CK5/6 [cloneD5/16134]	1:100	Boehringer Biochemica	Microwave
CK 14 [clone LL002]	1:100		
Hormone receptors			
ER [clone 1D5]	1:80	DakoCytomation	Microwave
EGFR family members			
EGFR [clone EGFR.113]	1:10	Novocastra	Microwave
HER-2 (cerbB-2)	1:250	DakoCytomation	No
Tumour suppressor genes			
p53 [clone DO7]	1/50	Novocstra	Microwave
BRCA1 Ab-1 [clone MS110]	1:150	Oncogene Research Products	
Anti-FHIT [clone ZR44]	1/600	Zymed Laboratories	
Cell adhesion molecules			
Anti E-cadherin [clone HECD-1]	1:100	Zymed Laboratories	Microwave
Anti P-cadherin [clone 56]	1/200	BD Biosciences	
Mucins			
NCL-MUC-1 [clone Ma695]	1/300	Novocastra	Microwave
BCl2 (Clone 124)	1/400	Dako	Microwave
Neuroendocrine differentiation			
Chromogranin A [clone DAK-A3]	1/100	DakoCytomation	Microwave
Synaptophysin [clone SY38]	1/30	DakoCytomation	

Breast cancer tissue microarrays (TMA) were prepared and immunohistochemically stained for basal cytokeratins (CK5/6 and CK14) and other markers (Table 1) as previously described.[9,10,19–21] Positive and negative controls for each marker were used according to the supplier's data sheet. Two cores were evaluated from each tumour and only staining of the invasive malignant cells was considered. Each core was scored individually and the mean of the two readings was calculated. Immunohistochemical scoring was performed in a blind fashion. For the purposes of this study, basal positivity was defined as detection of expression in 10% or more of invasive malignant cells for CK5/6 and/or CK14.

This research was approved by Nottingham Research Ethics Committee 2 under the title of 'Development of a molecular genetic classification of breast cancer'. None of the authors has any competing interests.

2.2. Statistical analysis

Statistical analysis was performed using SPSS 10.0 statistical software. Cutoff values for the different biomarkers included in this study were chosen before statistical analysis. Standard cutoffs were used for established prognostic factors and were the same as for previously published patient series.[10] All factors were used as dichotomous covariates in the statistical analysis with the exception of age, grade and NPI which were analysed as 4, 3 and 3 groups, respectively. Univariate and multivariate analyses were performed by χ^2-test, Long rank and Cox regression analysis, respectively. Survival curves were analysed by the method of Kaplan–Meier.[22] A p-value <0.05 (two sided) was considered significant.

3. Results

After excluding the uninformative TMA cores, 1841 and 1821 tumours were available for CK5/6 and CK14 immunohistochemical analyses, respectively. Of the informative cases (1872), 347 cases (18.6%) showed BP. Of all cases, 348 cases were grade 1, 626 cases were grade 2 and 898 were grade 3. At the time of the primary diagnosis, 1186 (63.4%) patients had LN negative disease. Recurrence occurred in 367 cases (25.1%), distant metastases in 348 cases (18.7%) and 190 (10.2%) patients died from breast cancer.

3.1. Relationship to other prognostic factors and outcome

In this study, we have focused on the importance of BP in the whole series as well as key clinical categories of breast cancer in order to identify the group(s) in which BP can provide the relevant prognostic information.

3.2. BP in the whole series

In the whole series, BP was associated with larger primary tumour size, recurrence, development of distant metastasis (with high frequency of liver, lung and brain metastasis [>3-fold] and low bone metastasis) and shorter survival. In multivariate analysis with adjustment for other prognostic factors including tumour size, grade, lymph node status and vascular invasion, basal phenotype was an independent predictor of DFI and OS. Similar results were found in LN positive and LN negative groups (Tables 2 and 4, Figs. 1 and 2); vascular invasion (VI) positive and VI negative groups and in pre-menopausal and post-menopausal women. However, when stratified according to different NPI groups (good, $\geqslant$3.4; moderate 1, 3.41–4.4; moderate 2, 4.41–5.4; and poor, >5.4), we found that BP was associated with poor outcome in the moderate 1 group (LR = 5.9, p = 0.01 and LR = 21.0, p<0.001 in case of OS and DFI, respectively) but not in the other groups.

3.3. BP in the different histologic grades

When we stratified the cases according to histological grades, we found that in grade 3 tumours, which form a large part of the series (898 cases, 48% of the whole series), the BP was associated with most of the established poor prognostic factors included in this study and with poorer OS and DFI (Tables 3 and 4). No such associations were seen in grade 1 or 2 tumours (LR = 0.5, p = 0.32 and LR = 0.97, p = 0.5 in case of OS and DFI, respectively). We have further examined grade 3 tumours stratified according to LN positivity (Table 5). Univariate analyses showed that BP was associated with other poorer prognostic factors and with shorter survival in both LN negative and LN positive subgroups (Table 4). In the LN negative subgroup of grade 3 tumours (27.4% of the whole series), the BP showed the strongest prognostic value (LR = 8.8, p = 0.003 and LR = 15.73, p<0.001 in case of OS and DFI, respectively) followed only by tumour size (LR = 9.8, p = 0.002 and

Table 2 – Association between BP and clinicopathological variables in the whole series and in different LN groups (number of positive cases)

Variables	Tumours		χ^2	p-value
	Non-basal	Basal		
Size (>2 cm)				
Whole series	538/1525	158/347	12.2	<0.0001
LN negative	249/952	89/234	12.9	<0.001
LN positive	287/573	67/113	3.1	0.08
Distant metastasis				
Whole series	145	66	26.2	<0.0001
LN negative	57	34	18.9	<0.0001
LN positive	87	32	11.1	0.001
Recurrence				
Whole series	247	102	32.1	<0.0001
Regional recurrence	75	40	20.9	<0.0001
Local recurrence	98	36	6.3	0.012
LN negative	131	62	21.9	<0.0001
Regional recurrence	42	26	15.5	<0.0001
Local recurrence	61	21	1.9	0.17
LN positive	115	40	12.3	<0.001
Regional recurrence	32	14	6.8	0.009
Local recurrence	37	15	6.1	0.014
Patients died from BC				
Whole series	123	61	28.1	<0.0001
LN negative	48	32	21.9	<0.0001
LN positive	74	29	11.7	0.001

BP, basal phenotype; LN, lymph node; BC, breast cancer.

Table 3 – Association between BP and clinicopathological variables in the different tumours' grades (number of positive cases)

Variables	Tumours		χ^2	*p*-value
	Non-basal	Basal		
Size (>2 cm)				
Grade I	41/304	16/44	14.9	<0.001
Grade II	176/570	15/56	0.4	0.52
Grade III	493/651	206/247	5.3	0.02
LN disease				
Grade I	50	11	2.2	0.14
Grade II	212	19	0.25	0.66
Grade III	302	82	13.3	<0.0001
Distant metastasis				
Grade I	6	2	1.1	0.3
Grade II	42	5	0.2	0.6
Grade III	97	59	9.1	0.003
Recurrence				
Grade I	32	9	3.6	0.08
Grade II	78	10	6.9	0.4
Grade III	137	83	14.4	<0.0001
Patients died from BC				
Grade I	6	0	0.9	0.99
Grade II	32	4	0.2	0.6
Grade III	85	57	12.8	0.0001

LN, lymph node; BC, breast cancer.

Table 4 – Association between BP and outcome in different patients' groups

	Number	Overall survival		Disease-free interval	
		LR	*p*-value	LR	*p*-value
Whole series	1872	22.5	<0.0001	30.0	<0.0001
LN					
LN negative	1186	18.5	<0.0001	21.6	<0.0001
LN positive	686	9.2	0.002	12.2	0.0005
Grade					
Grade I	348	1.01	0.32	1.8	0.175
Grade II	626	0.01	0.932	0.1	0.76
Grade III	898	8.8	0.003	14.4	0.0001
LN negative group					
Grade I	287	0.7	0.4	0.2	0.7
Grade II	389	0.1	0.7	0.01	0.9
Grade III	510	8.8	0.003	15.7	0.0001
LN positive group					
Grade I	61	0.5	0.5	3.9	0.048
Grade II	237	0.2	0.6	0.5	0.5
Grade III	388	5.6	0.018	4.4	0.036

LN, lymph node.

LR = 5.57, $p = 0.02$ in case of OS and DFI, respectively). Interestingly, in this subgroup, the following was observed: (A) The majority were ER negative (54% of the cases), HER-2 negative (63%) and EGFR negative (76%). (B) Other known prognostic biomarkers such as patients' age, VI, menopausal,

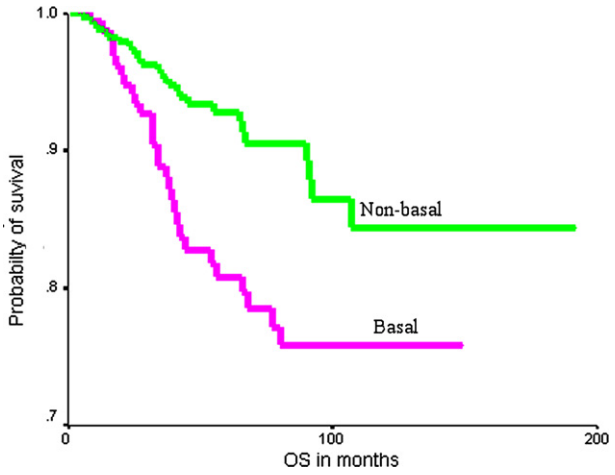

Fig. 1 – Correlation between BP and overall survival (OS) in the lymph node negative group of the whole series.

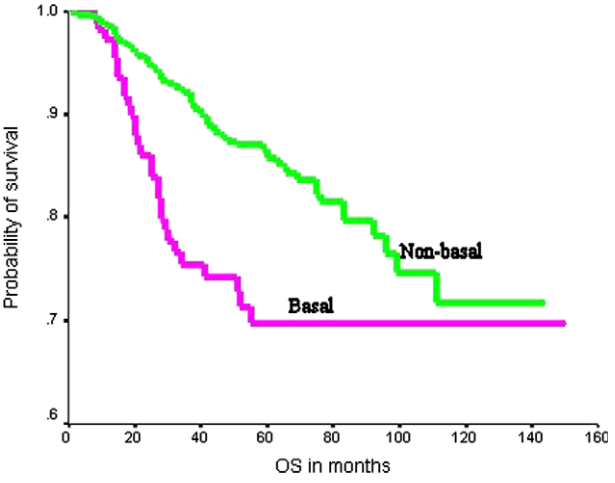

Fig. 2 – Correlation between BP and OS in the lymph node positive group of the whole series.

status, ER, PgR, HER-2, EGFR, p53, E-cadherin, P-cadherin, BRCA1, FHIT protein, BCl-2, MUC-1 and neuroendocrine markers were not associated with OS or DFI (the association between combined BP and ER expression and patient outcome in the whole series compared to the LN negative subgroup of grade 3 tumours is shown in Figs. 3 and 4). However, in the LN positive subgroup, although BP was associated with other established poor prognostic factors and patients outcome (Table 4), other variables including size, ER, p53 and P-cadherin showed stronger association with survival.

In addition, we examined low grade tumours that showed BP (43 cases; 12% of BP tumours) and compared them with grade 1 tumours that did not show BP (295 cases) and we noticed the following findings: higher percentage of BP tumours were of metaplastic type (12% compared to 0.3% of non-BP grade 1 tumours) and mucinous carcinomas (14% compared to 5% in non-BP), while lower percentage were of pure tubular carcinomas (7% compared to 22%). The majority of grade 1 BP tumours showed moderate degree of tubule formation (82%

Table 5 – Patients and tumour characteristics in grades 1, 2 and 3 tumours

Variables	Total cases	Grade 1 (348 cases)	Grade 2 (626 cases)	Grade 3 (898 cases)	
				LN negative (510)	LN positive (388)
Size (>2 cm)	696	57	191	213	235
Positive VI	772	74	246	182	270
NPI					
Good	634	321	313	0	0
Moderate	949	23	295	510	123
Poor	289	0	18	0	271
Distant metastasis	348	23	91	95	139
Recurrence	367	50	123	138	156
Patients died from BC	190	6	37	57	90
ER negative	545	30 (6%)	58 (11%)	274 (50%)	183 (33%)
HER-2 negative	1238	241 (19%)	429 (35%)	321 (26%)	247 (20%)
EGFR positive	291	26 (10%)	52 (18%)	124 (43%)	89 (29%)

LN, lymph node; VI, vascular invasion; BC, breast cancer.

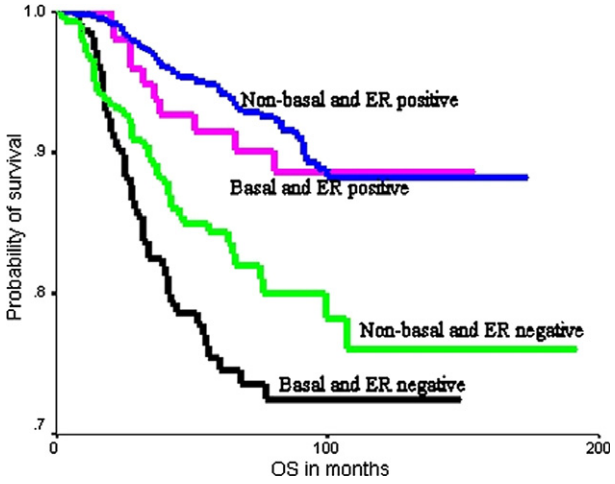

Fig. 3 – Correlation between combined BP and ER expression and OS in the whole series.

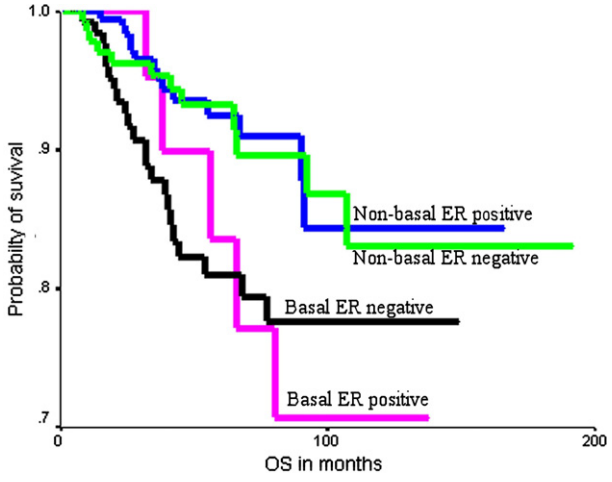

Fig. 4 – Correlation between combined BP and ER expression and OS in the LN negative grade 3 tumours.

compared to 64% in non-BP) and mitotic activity (10% compared to 2.2%). Higher percentage of BP tumours were ER and PR negative (27% and 33% compared to 7% and 18% in non-BP grade 1 tumours in case of ER and PR, respectively).

Regarding adjuvant therapy (chemotherapy, hormone therapy or radiotherapy), when we stratified our series into treatment groups, we found that basal tumours responded better to chemotherapy (less patients have relapsed and less have died during the 206 months of follow-up) than the LN, grade and size matched non-basal tumours in certain groups but not in others. For example, in LN positive grade 3 tumours with primary size <2 cm (120 patients), there was no statistical difference between patients who received chemotherapy or not in the non-basal group (LR = 0.11, $p = 0.74$ and LR = 1.21, $p = 0.271$ in case of OS and DFI, respectively), while in the basal group, patients who received chemotherapy showed better outcome (LR = 7.49, $p = 0.0062$ and LR = 13.65, $p = 0.0002$ in case of OS and DFI, respectively).

Multivariate analyses (MVA) including other established prognostic factors (histologic grade, LN status, primary tumour size, menopausal status, patients' age, ER, HER2, chemotherapy, radiotherapy and hormonal therapy) showed that BP is an independent predictor of survival in the whole series. Similar results were obtained in LN negative group (after excluding LN as a covariate in the analysis). In the LN positive subgroup, MVA including grade, size age and HER2, showed that BP was an independent predictor of survival; however, in this subgroup, when ER or menopausal status was included, BP became non-significant. Regarding tumour grade, BP is an independent predictor of survival in grade 3 tumours but not in grade 1 or 2. MVA with adjustment for prognostic factors including LN status, size, VI, menopausal

Table 6 – Multivariate Cox regression analysis of factors associated with overall survival and disease-free interval in grade 3 tumours

Predictor	Overall survival		Disease-free interval	
	Hazards ratio (95% confidence interval (CI))	*p*-value	Hazards ratio (95% CI)	*p*-value
LN negative group				
BP	1.96 (1.06–3.62)	0.031	2.1 (1.34–3.17)	<0.001
Size ⩽2 cm *versus* >2 cm	2.11 (1.15–3.9)	0.017	1.48 (0.96–2.28)	0.08
Vascular invasion	1.2 (0.64–2.25)	0.57	1.2 (0.77–1.88)	0.41
Menopausal status pre *versus* post-menopausal	1.45 (0.82–2.58)	0.21	0.88 (0.6–1.3)	0.52
LN positive group				
BP	1.9 (1.09–3.17)	0.02	1.33 (0.86–2.07)	0.19
Size ⩽2 cm *versus* >2 cm	2.02 (1.17–3.5)	0.01	1.4 (0.93–2.14)	0.1
Vascular invasion	0.69 (0.42–1.14)	0.15	1.03 (0.67–1.6)	0.09
Menopausal status pre *versus* post-menopausal	0.76 (0.3–1.9)	0.56	1.37 (0.99–1.88)	0.06

LN, lymph node; BP, basal phenotype.

status, age, ER, HER-2, EGFR and p53 showed that BP was the only significant variable in predicting regional recurrence in grade 3 tumours. Furthermore, in LN negative grade 3 tumours, BP was an independent predictor of survival and followed only by tumour size while other factors were not (Table 6). These results demonstrate that BP is the most significant prognostic factor in this important group of breast cancers.

4. Discussion

Breast cancer is a heterogeneous disease with a range of histological types and grades exhibiting different behavioural outcomes and different patterns of genetic derangement. Although its incidence is still high, the overall mortality due to breast cancer has decreased, attributed in part to the early application of various treatment modalities.[23] In order to reduce the mortality from breast cancer further, there is a desire to examine and characterise tumours, which are of poor prognosis, to predict their biology, ensure adequate therapy and improve patients' outcome. There is also a desire to identify those classes of breast cancer which benefit from targeted biological therapies such as hormone or trastuzumab (Herceptin) therapy. There is also a need to develop additional forms of systemic treatment effective in those tumours failing to express known targets such as ER or HER-2.

Currently, routine clinical management of breast cancer relies on traditional prognostic factors including nodal status, tumour histological grade, primary tumour size that can be combined into the NPI, in addition to VI, ER and HER-2.[24,25] Although these prognostic factors are useful, the clinical course of a patient with breast carcinoma remains difficult to predict as tumours of apparently homogeneous morphological characters still vary in response to therapy and have divergent outcomes.[26] Therefore, additional molecular markers are being sought to further refine classification, especially in patient subgroups whose outcome cannot be predicted accurately using conventional parameters.

Although previous studies have demonstrated several proteins which are useful in predicting outcome and the tumours which respond to specific therapies, in multivariate analyses, most of these markers co-vary and are therefore not independently informative. Moreover, they can only provide an additional prognostic value in certain subgroups of breast cancer.[27–31] For these reasons, the prognostic value of many biomarkers in breast cancer shows a wide range of variations depending on patient cohorts included in each study.

In the current study, we have investigated the clinical relevance of the BP in breast cancer. Our results demonstrated that the BP shows an association with the other established markers of poor prognosis and with poor patient outcome in our whole series. However, when we stratified the tumours into different grades, an association with poor outcome was seen in grade 3 tumours only. This can be explained by the following factors: (1) the majority of BP tumours are grade 3 (248 cases; 72% of BP) that have a worse prognosis compared with lower frequency in grades 1 and 2 that generally have a better outcome. BP is also associated with specific types of tumours such as salivary type tumours with known indolent behaviour.[32] and (2) multivariate analyses showed that the prognostic effect of BP is independent of grade. In addition, the presence of this inverse association with survival in grade 3 tumours, but not in grade 1 or 2, might also be related to the difference in the genetic abnormalities that characterise different grades of breast cancers.[33,34] Thus, BP is likely to be the outcome of molecular genetic alterations arising during the development of a more aggressive subgroup of grade 3 tumours. Specifically, BP defines a distinct class of grade 3 tumours which originate either from cells with a stem/progenitor cell features, which are known to express these basal markers,[35,36] or through acquisition of these characteristics during early neoplastic transformation, or less likely that these basal markers may be expressed later during the evolution of a subgroup of grade 3 that acquire aggressive, dedifferentiated features (reviewed in [37,38]). This latter explanation may be supported by observations that grade 3 tumours are characterised by a high degree of genetic instability and a wide range of genetic alterations which may virtually affect any regions of the genome.[34] Also of importance is the fact that BP morphology and phenotype are found at significantly higher frequency in tumours developing in patients with BRCA1 germline mutations.[3,4,39]

Our results are consistent with previous studies that reported an association between the expression of basal cell markers and shorter OS and DFS in the whole series[7,11] and in LN negative[7,11,13] or positive tumours.[12,13] Our results are also in agreement with Malzahn et al.[13] who examined basal CKs profile and demonstrated an association with shorter survival in a subgroup of grade 3 tumours but not in other groups. We also found that in a large subgroup that comprised LN negative, grade 3 tumours (27% of the whole series), BP was significantly associated with poor patients' outcome while other known prognostic markers including NPI, VI, tumour type, menopausal status and ER expression did not. Importantly, the majority of tumours in this subgroup was characterised by ER, HER-2 and EGFR negativity and therefore, lacks the benefit of specific therapy that targets these biomarkers. In addition, they were highly proliferative and may benefit from conventional chemotherapy. It is also known that grade 3 tumours are more responsive to chemotherapy than other grades.[40] These results may have a major impact on the management of a certain group of patients with breast cancer. Further study and characterisation of these tumours may have a potential to identify new targets for new agent treatments in this group.

In the current study, we also found that there is a trend for tumours with BP to have a more favourable outcome following the use of adjuvant; chemotherapy, however, because of the limited number of patients located within each comparable group and lack of randomisation to treatment this observation needs further study in a more appropriate or clinical trial setting to confirm these preliminary results.

In conclusion, our study provides robust data that BP can provide additional prognostic information on breast cancer and particularly in a selected subgroup that comprises LN negative, grade 3 tumours in which it provides prognostic information stronger than any other well established marker and can identify a specific subgroup of patients that may benefit from a more aggressive approach to adjuvant therapy. We therefore recommend routine staining of breast cancer with basal CKs, particularly in grade 3 tumours.

Conflict of interest statement

None declared.

REFERENCES

1. Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 2001;**98**:10869–74.
2. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature* 2000;**406**:747–52.
3. Jacquemier J, Padovani L, Rabayrol L, et al. Typical medullary breast carcinomas have a basal/myoepithelial phenotype. *J Pathol* 2005;**207**:260–8.
4. Lakhani SR, Reis-Filho JS, Fulford L, et al. Prediction of BRCA1 status in patients with breast cancer using estrogen receptor and basal phenotype. *Clin Cancer Res* 2005;**11**:5175–80.
5. Dairkee SH, Mayall BH, Smith H, Hackett A. Monoclonal marker that predicts early recurrence of breast cancer. *Lancet* 1987;**1**:514.
6. Jones C, Ford E, Gillett C, et al. Molecular cytogenetic identification of subgroups of grade III invasive ductal breast carcinomas with different clinical outcomes. *Clin Cancer Res* 2004;**10**:5988–97.
7. van de Rijn M, Perou CM, Tibshirani R, et al. Expression of cytokeratins 17 and 5 identifies a group of breast carcinomas with poor clinical outcome. *Am J Pathol* 2002;**161**:1991–6.
8. Sorlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci USA* 2003;**100**:8418–23.
9. Abd El-Rehim DM, Pinder SE, Paish CE, et al. Expression of luminal and basal cytokeratins in human breast carcinoma. *J Pathol* 2004;**203**:661–71.
10. Abd El-Rehim DM, Ball G, Pinder SE, et al. High-throughput protein expression analysis using tissue microarray technology of a large well-characterised series identifies biologically distinct classes of breast cancer confirming recent cDNA expression analyses. *Int J Cancer* 2005;**116**:340–50.
11. Potemski P, Kusinska R, Watala C, et al. Prognostic relevance of basal cytokeratin expression in operable breast cancer. *Oncology* 2005;**69**:478–85.
12. Nielsen TO, Hsu FD, Jensen K, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin Cancer Res* 2004;**10**:5367–74.
13. Malzahn K, Mitze M, Thoenes M, Moll R. Biological and prognostic significance of stratified epithelial cytokeratins in infiltrating ductal breast carcinomas. *Virchows Arch* 1998;**433**:119–29.
14. Rakha EA, Putti TC, Abd El-Rehim DM, et al. Morphological and immunophenotypic analysis of breast carcinomas with basal and myoepithelial differentiation. *J Pathol* 2006;**208**:495–506.
15. Ellis IO, Galea M, Broughton N, et al. Pathological prognostic factors in breast cancer. II. Histological type. Relationship with survival in a large study with long-term follow-up. *Histopathology* 1992;**20**:479–89.
16. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* 1991;**19**:403–10.
17. Pinder SE, Ellis IO, Galea M, et al. Pathological prognostic factors in breast cancer. III. Vascular invasion: relationship with recurrence and survival in a large study with long-term follow-up. *Histopathology* 1994;**24**:41–7.
18. Galea MH, Blamey RW, Elston CE, Ellis IO. The Nottingham Prognostic Index in primary breast cancer. *Breast Cancer Res Treat* 1992;**22**:207–19.
19. Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med* 1998;**4**:844–7.
20. Camp RL, Charette LA, Rimm DL. Validation of tissue microarray technology in breast carcinoma. *Lab Invest* 2000;**80**:1943–9.
21. Abd El-Rehim DM, Pinder SE, Paish CE, et al. Expression and co-expression of the members of the epidermal growth factor receptor (EGFR) family in invasive breast carcinoma. *Br J Cancer* 2004;**91**:1532–42.
22. Kaplan EL, Meier P. Non-parametric estimation from incomplete observations. *J Am Stat Assoc* 1958;**53**:457–81.
23. Mincey BA, Palmieri FM, Perez EA. Adjuvant therapy for breast cancer: recommendations for management based on consensus review and recent clinical trials. *Oncologist* 2002;**7**:246–50.
24. Mori I, Yang Q, Kakudo K. Predictive and prognostic markers for invasive breast cancer. *Pathol Int* 2002;**52**:186–94.

25. Hayes DF, Isaacs C, Stearns V. Prognostic factors in breast cancer: current and new predictors of metastasis. *J Mammary Gland Biol Neoplasia* 2001;**6**:375–92.
26. Alizadeh AA, Ross DT, Perou CM, van de Rijn M. Towards a novel classification of human malignancies based on gene expression patterns. *J Pathol* 2001;**195**:41–52.
27. Korkolis DP, Tsoli E, Fouskakis D, et al. Tumor histology and stage but not p53, Her2-neu or cathepsin-D expression are independent prognostic factors in breast cancer patients. *Anticancer Res* 2004;**24**:2061–8.
28. Seshadri R, Lee CS, Hui R, et al. Cyclin DI amplification is not associated with reduced overall survival in primary breast cancer but may predict early relapse in patients with features of good prognosis. *Clin Cancer Res* 1996;**2**:1177–84.
29. Linderholm B, Andersson J, Lindh B, et al. Overexpression of c-erbB-2 is related to a higher expression of vascular endothelial growth factor (VEGF) and constitutes an independent prognostic factor in primary node-positive breast cancer after adjuvant systemic treatment. *Eur J Cancer* 2004;**40**:33–42.
30. Hlupic L, Jakic-Razumovic J, Bozikov J, et al. Prognostic value of different factors in breast carcinoma. *Tumori* 2004;**90**:112–9.
31. Cornfield DB, Palazzo JP, Schwartz GF, et al. The prognostic significance of multiple morphologic features and biologic markers in ductal carcinoma in situ of the breast: a study of a large cohort of patients treated with surgery alone. *Cancer* 2004;**100**:2317–27.
32. Pia-Foschini M, Reis-Filho JS, Eusebi V, Lakhani SR. Salivary gland-like tumours of the breast: surgical and molecular pathology. *J Clin Pathol* 2003;**56**:497–506.
33. Buerger H, Otterbach F, Simon R, et al. Different genetic pathways in the evolution of invasive breast cancer are associated with distinct morphological subtypes. *J Pathol* 1999;**189**:521–6.
34. Roylance R, Gorman P, Harris W, et al. Comparative genomic hybridization of breast tumors stratified by histological grade reveals new insights into the biological progression of breast cancer. *Cancer Res* 1999;**59**:1433–6.
35. Boecker W, Buerger H. Evidence of progenitor cells of glandular and myoepithelial cell lineages in the human adult female breast epithelium: a new progenitor (adult stem) cell concept. *Cell Prolif* 2003;**36**:73–84.
36. Stingl J, Eaves CJ, Zandieh I, Emerman JT. Characterization of bipotent mammary epithelial progenitor cells in normal adult human breast tissue. *Breast Cancer Res Treat* 2001;**67**:93–109.
37. Li Y, Rosen JM. Stem/progenitor cells in mouse mammary gland development and breast cancer. *J Mammary Gland Biol Neoplasia* 2005;**10**:17–24.
38. Gusterson BA, Ross DT, Heath VJ, Stein T. Basal cytokeratins and their relationship to the cellular origin and functional classification of breast cancer. *Breast Cancer Res* 2005;**7**:143–8.
39. Lakhani SR, Van De Vijver MJ, Jacquemier J, et al. The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. *J Clin Oncol* 2002;**20**:2310–8.
40. Pinder SE, Murray S, Ellis IO, et al. The importance of the histologic grade of invasive breast carcinoma and response to chemotherapy. *Cancer* 1998;**83**:1529–39.