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Pleomorphic lobular carcinoma of the breast: is it a prognostically significant pathological subtype independent of histological grade?

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Pleomorphic lobular carcinoma is regarded as a biologically aggressive variant of invasive lobular carcinoma of the breast. However, there is no consensus on the definition and whether this subtype adds useful information to histological grade. Two-hundred and two grade 2 or grade 3 invasive lobular carcinomas were studied. Tumours were categorised according to the components of histological grade: tubules, pleomorphism and mitoses. Pleomorphic lobular carcinoma was defined as a carcinoma with a lobular growth pattern and marked nuclear pleomorphism (pleomorphism 3). Breast cancer-specific survival was used in analysis of prognosis. Grade 3 pleomorphic lobular carcinomas (tubules 3, pleomorphism 3, mitoses 2 and tubules 3, pleomorphism 3, mitoses 3) had a worse prognosis than grade 2 (tubules 3, pleomorphism 2, mitoses 1) carcinomas. Grade 2 lobular carcinomas with marked nuclear pleomorphism (tubules 3, pleomorphism 3, mitoses 1) had a similar prognosis to grade 2 carcinomas with moderate pleomorphism (tubules 3, pleomorphism 2, mitoses 1). Survival was associated with mitotic score, but not with nuclear pleomorphism on both univariate and multivariate analysis. A non-classical growth pattern was seen more frequently in all subgroups with marked nuclear pleomorphism and was associated with worse survival. Histological grade and nodal status were independent of prognostic factors. This study shows that histological grade (in particular the mitotic component) in invasive lobular carcinomas is of prognostic importance, but pleomorphic type does not provide useful additional prognostic information.
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Invasive lobular carcinoma is the commonest special type of breast cancer, representing about 10% of cases. It comprises several histological subtypes, all characterised by dyscohesive growth pattern and loss of function of the cell-adhesion protein E-cadherin.[1–5] Pleomorphic lobular carcinoma was first described in 1987 by Page[6] as a variant of classical invasive lobular carcinoma. It is characterised by a classical growth pattern, but has marked nuclear atypia and frequently shows a plasmacytoid, histiocytoid or apocrine morphological appearance. In contrast to the most common classical variant of invasive lobular carcinoma, which is typically strongly oestrogen receptor and progesterone receptor positive and *ERBB2* (*HER2*) negative, the pleomorphic variant may express oestrogen receptor and progesterone receptor at lower levels and occasionally shows amplification of oncogenes, including *ERBB2*.[7–9] Derksen *et al*[10] demonstrated that combined inactivation of *E-cadherin* and *TP53* in mice induces development of invasive and metastatic mammary carcinomas, which resemble human pleomorphic lobular carcinoma. Previous studies, although limited by small numbers of patients, concluded that this subtype shows an aggressive clinical behaviour compared with classical invasive lobular carcinoma.[9,11,12] One study reported a dismal outcome for pleomorphic lobular carcinoma with 9 out of 11 patients dying of their disease with a median survival of 2.1 years.[12] These authors concluded based on this small

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number of cases that the outcome of pleomorphic lobular carcinoma is significantly worse than either infiltrating ductal carcinoma or classical invasive lobular carcinoma, even when stratified by axillary lymph node status.[12] Pleomorphic lobular carcinomas have molecular genetic features characteristic of lobular carcinomas (loss of 16q and gain of 1q), but unlike the majority of classical invasive lobular carcinoma, they show additional genetic changes, such as *ERBB2* amplification, more analogous to high-grade ductal carcinoma, which may be responsible for the more aggressive biological behaviour of pleomorphic lobular carcinoma.[13,14]

However, pleomorphic lobular carcinoma remains a poorly defined entity and there is no consensus on whether it is a distinct entity independent of histological grade. Several authorities, including the WHO Classification of Breast Tumours, described it as a variant of invasive lobular carcinoma that retains the distinctive growth pattern, but exhibits a greater degree of cellular atypia and pleomorphism than the classical form.[1,8,11] However, some pathologists and oncologists regard pleomorphic lobular carcinoma and high-grade (grade 3) invasive lobular carcinoma as synonymous, regardless of the nuclear features, mitotic counts, growth pattern or presence of apocrine or histiocytoid features. Historically, the value of histological grading of invasive lobular carcinoma according to Bloom and Richardson has been questioned because the majority of tumours are characterised by lack of tubule formation, moderate nuclear pleomorphism and limited mitotic activity, and are therefore grade 2. Although some studies reported limited prognostic value for histological grading of invasive lobular carcinoma,[15] we and others have demonstrated that grading of these tumours, using the Nottingham grading system, is a strong and independent predictor of outcome, which supports the importance of routine assessment of histological grade in invasive lobular carcinoma.[16–21] Analysing the three components of grading separately revealed that mitotic count was of more prognostic power than the two other components.[16,21] This raises the question of whether pleomorphic lobular carcinoma as a distinct pathological subtype has any clinical value additional to grading, because conventional grading also includes cellular atypia and mitotic count as components. The aim of this study was therefore to evaluate the prognostic significance of pleomorphic lobular carcinoma and to provide recommendations regarding the relevance of using this separate subtype.

Materials and methods

Patients

A retrospective database of consecutive breast cancer patients was searched for patients with grade 2 or grade 3 invasive lobular carcinoma. These patients were diagnosed and treated at the Nottingham City Hospital from 1987–2010. Original slides of invasive lobular carcinoma classified as either mixed, solid, pleomorphic or grade 3 were retrieved and reviewed by specialized breast pathologists (CMHD, EAR and AHSL). Mixed lobular carcinoma was defined as a mixture of lobular subtypes with at least 10% of the second component. Carcinomas containing both lobular and ductal components were excluded; only pure lobular carcinomas were considered. Two-hundred and two grade 2 or grade 3 invasive lobular carcinomas were identified. Pleomorphic lobular carcinoma was defined as a carcinoma with a lobular growth pattern and marked nuclear pleomorphism (Figure 1). These carcinomas were compared with classical non-pleomorphic lobular carcinomas (tubules 3, pleomorphism 2, mitoses 1).

Clinicopathological Features

Clinicopathological features that were recorded included patient age, systemic treatment (endocrine and/or chemotherapy), tumour size, nodal status, grade (defined according to the modified Bloom-Richardson score based on tubule formation, nuclear pleomorphism and mitotic activity index),[22] oestrogen receptor and ERBB2 status and prognosis. Oestrogen receptor positivity was defined as at least 10% of invasive carcinoma nuclei, showing immunohistochemical staining. ERBB2 staining was routinely performed in cases diagnosed after 2004. Cases diagnosed before 2000 were stained on tissue microarray sections using immunohistochemistry and chromogenic *in situ* hybridisation in borderline cases. E-cadherin was defined as positive if 1% or more invasive carcinoma cells showed membranous staining. Some of the tumours were stained as part of a previous study.[2] Hormonal therapy was given to 107 patients and chemotherapy to 34 patients. None of the patients received adjuvant trastuzumab therapy.

Prognosis was expressed as breast cancer-specific survival and disease-free survival. Breast cancer-specific survival was defined as the interval from the operation to death from breast cancer. Disease-free survival was calculated from the date of operation to the first recurrence (local, regional or distant).

Statistical Analysis

The χ^2-test and the Student's *t*-test for trend were used to assess baseline differences between ordinal and continuous variables, respectively. The Kaplan–Meier method was used to estimate overall survival and disease-free survival. A Cox-proportional-hazards model was used to compare the cohorts and to adjust for known prognostic variables (tumour size,

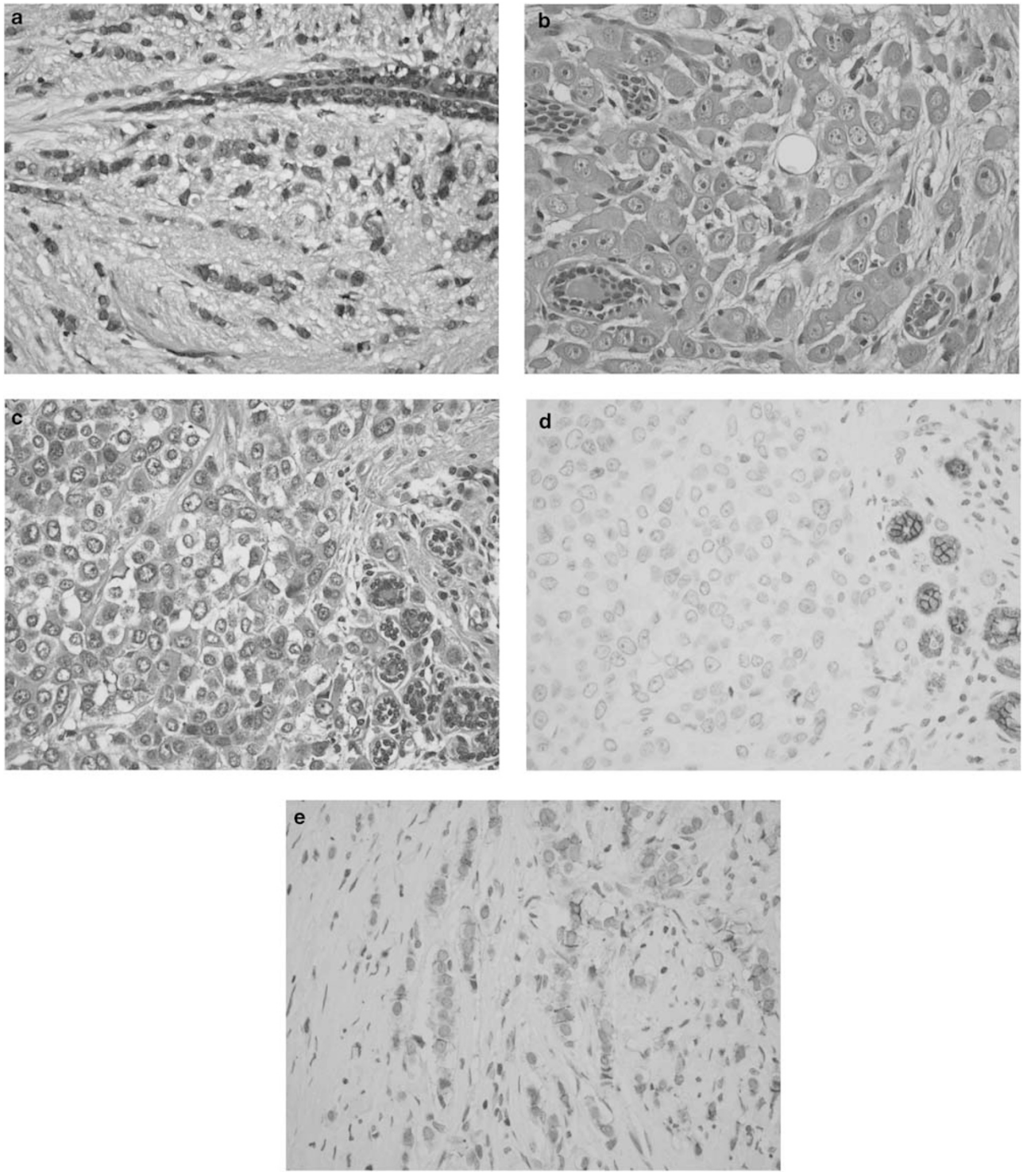

Figure 1 (**a**) Classical invasive lobular carcinoma with dyscohesive growth pattern and moderate nuclear pleomorphism. Pleomorphic lobular carcinomas (**b**) with classical growth pattern (**c**) with solid growth pattern (**d**) showing absence of E-cadherin expression and (**e**) showing patchy membranous E-cadherin. Note the membranous expression in normal breast epithelium in (**d**).

nodal status, vascular invasion, growth pattern and systemic therapy).

This study was approved by Nottingham Research Ethics Committee 2 under the title of 'Development of a molecular genetic classification of breast cancer'.

Results

The clinicopathological features of the invasive lobular carcinomas according to the subset of histological grade are summarised in Table 1. There were too few tubules 3, pleomorphism 2, mitoses 2

($n=7$) and tubules 3, pleomorphism 2, mitoses 3 ($n=9$) carcinomas for analysis. Compared with tubules 3, pleomorphism 2, mitoses 1 carcinomas, the non-classical growth patterns (mixed or solid) were more frequent in tubules 3, pleomorphism 3, mitoses 1 ($\chi^2=13$, $P=0.0003$), tubules 3, pleomorphism 3, mitoses 2 ($\chi^2=72$, $P<0.0001$) and tubules 3, pleomorphism 3, mitoses 3 carcinomas ($\chi^2=44$, $P<0.0001$). The mitotic score component of grade was higher in carcinomas with solid or mixed than classical growth patterns ($\chi^2=65$, $P<0.0001$ for both comparisons). Vascular invasion was more frequent in tubules 3, pleomorphism 3, mitoses 3 than tubules 3, pleomorphism 2, mitoses 1 carcinomas ($\chi^2=8$, $P=0.004$). Some pleomorphic lobular carcinomas had a central fibrotic focus, but we did not formally assess this feature. The majority (92%) of cases were oestrogen receptor positive and 2% were positive for ERBB2, and the expression of these markers was not significantly different among the subsets. A total of 89% of carcinomas showed no E-cadherin expression with similar proportions in all subsets.

Outcome

The survival curves of patients with the four-grade subsets are shown in Figure 2. The median follow-up was 7.6 years (range 0.8–15.7) with 29 deaths because of breast cancer. Patients with tubules 3, pleomorphism 3, mitoses 1 carcinomas had a similar survival to patients with tubules 3, pleomorphism 2, mitoses 1 carcinomas ($\chi^2=0.7$, $P=0.39$). Patients with tubules 3, pleomorphism 3, mitoses 3 ($\chi^2=4.9$, $P=0.027$) or tubules 3, pleomorphism 3, mitoses 2 carcinomas ($\chi^2=11$, $P=0.0008$) had a worse survival than those with tubules 3, pleomorphism 2, mitoses 1 carcinomas. There was no significant difference in survival between patients with tubules 3, pleomorphism 3, mitoses 3 and those with tubules 3, pleomorphism 3, mitoses 2 carcinomas ($\chi^2=0.26$, $P=0.61$). If the analysis is restricted to patients with E-cadherin-negative carcinomas, the results are similar: patients with grade 3 carcinomas have a worse prognosis than those with tubules 3, pleomorphism 2, mitoses 1 carcinomas ($\chi^2=6.7$, $P=0.01$) and the survival is similar for patients with tubules 3, pleomorphism 3, mitoses 1 and tubules 3, pleomorphism 2, mitoses 1 carcinomas ($\chi^2=0.7$, $P=0.39$).

On multivariate analysis of the pleomorphism and mitotic score components of histological grade as continuous variables, survival was associated with mitoses (relative risk 1.85 (95% confidence intervals 1.16–2.95, $P=0.01$), but not with pleomorphism (relative risk 1.16 (95% confidence intervals 0.52–2.59, $P=0.72$). Carcinomas with a non-classical growth pattern had a worse survival compared with carcinomas with a classical growth pattern ($\chi^2=4.2$, $P=0.04$). On multivariate survival analysis,

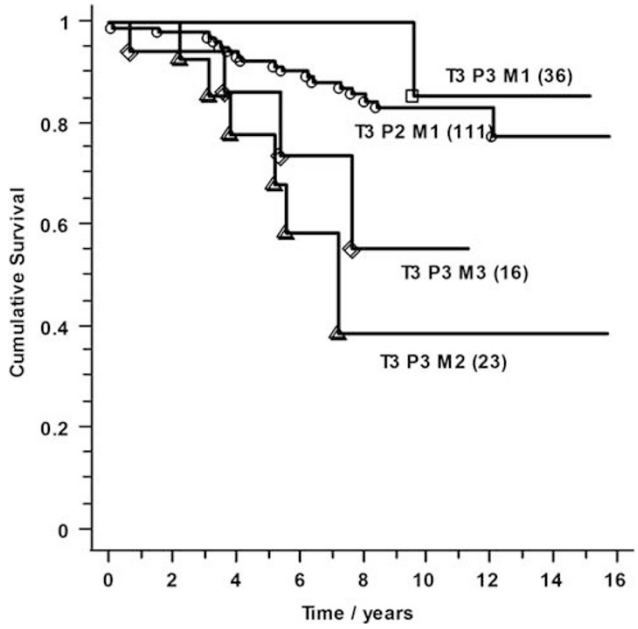

Figure 2 Breast cancer-specific survival in different grade subsets. The number of patients in each subset is indicated in brackets. (T = tubules, P = pleomorphism and M = mitoses).

Table 1 Subsets of histological grade and relation to other pathological variables

Grade subset	*ER positive*	*ERBB2 positive*	*Growth pattern*			*Median size (mm)*	*Lymph node positive*	*Vascular invasion present*	*E-cadherin negative*
			Classical	*Mixed*	*Solid*				
T3,P2,M1	99/106 (93%)	1/111 (1%)	107/111 (96%)	4/111 (4%)		20	45/110 (41%)	19/111 (17%)	99/111 (89%)
T3,P3,M1	26/30 (87%)	1/25 (4%)	27/36 (75%)	7/36 (19%)	2/36 (6%)	22.5	18/36 (50%)	6/34 (18%)	26/29 (90%)
T3,P3,M2	16/17 (94%)	1/14 (7%)	5/23 (22%)	12/23 (52%)	6/23 (26%)	27	9/22 (41%)	4/21 (19%)	18/20 (90%)
T3,P3,M3	12/14 (86%)	0/9	6/16 (37%)	4/16 (25%)	6/16 (37%)	26.5	9/14 (64%)	8/15 (53%)	9/11 (82%)

Abbreviation: ER = oestrogen receptor.

histological grade and nodal status were the only independent variables. Similar results were obtained for the above analyses with disease-free survival (data not shown).

Discussion

In the present study grade 3 invasive lobular carcinomas (tubules 3, pleomorphism 3, mitoses 3 and tubules 3, pleomorphism 3, mitoses 2) had a worse prognosis than grade 2 (tubules 3, pleomorphism 2, mitoses 1) carcinomas. Grade 2 carcinomas with marked nuclear pleomorphism (tubules 3, pleomorphism 3, mitoses 1) had a similar survival to grade 2 carcinomas with moderate nuclear pleomorphism (tubules 3, pleomorphism 2, mitoses 1). On univariate and multivariate analysis, the mitotic count was associated with survival, but nuclear pleomorphism was not associated. This shows that mitoses are of more prognostic importance than pleomorphism and is consistent with studies of breast cancer as a whole.[23] The number of patients in the present study is greater than previous studies of pleomorphic lobular carcinomas, but larger studies are needed to confirm or refute these observations and to examine the subsets with small numbers. Non-classical growth patterns were more frequent in carcinomas with marked nuclear pleomorphism. Talman *et al*[17] reported that the majority of grade 3 invasive lobular carcinomas have a non-classical growth pattern and have a worse outcome than grade 2 carcinomas, irrespective of histological type. Histological grade was an independent prognostic factor in keeping with previous studies.[18,21,24,25]

In our series, we observed heterogeneity of pleomorphism in a substantial proportion of pleomorphic lobular carcinomas in both the *in situ* and the invasive components. This is consistent with recently published genetic data, suggesting that pleomorphic lobular carcinomas may evolve through a genetic pathway similar to that of classical invasive lobular carcinomas, but with the acquisition of additional genetic events.[5,13,26]

In the present study, about 90% of carcinomas in all the grade subsets were oestrogen receptor positive. Some studies have found that over 90% of pleomorphic lobular carcinomas are oestrogen receptor positive,[7,9] but others have shown a lower proportion of about 80%.[13,27] The proportion of ERBB2-positive carcinomas was low in all subsets in the present study. Comparison with early studies is difficult as only immunohistochemistry was performed, but a recent study found ERBB2 amplification in 14% of pleomorphic lobular carcinomas.[9] Our results are similar to previous studies of unselected invasive lobular carcinomas, which show that the majority of invasive lobular carcinomas (80–100%) are oestrogen receptor positive and lack ERBB2 overexpression.[13,18,21,28–30]

We based the diagnosis of invasive lobular carcinoma on the typical dyscohesive growth pattern. We did not base it on E-cadherin expression as there is good evidence that about 10–15% of invasive lobular carcinomas show some membranous expression.[2,31] Indeed it is possible for an invasive lobular carcinoma with a typical morphology to have an E-cadherin mutation, but show E-cadherin expression.[31] Also, some non-lobular carcinomas lack E-cadherin expression.[32] In the present study, about 10% of all the grade subsets showed some staining for E-cadherin. Most E-cadherin-positive lobular carcinomas show marked reduction or absence of expression of at least one of the catenins,[2] consistent with the central role of loss of function of the the E-cadherin–catenin complex in this histological type. This absence of catenin expression is also seen in most E-cadherin-positive pleomorphic lobular carcinomas. If survival analysis is restricted to E-cadherin-negative carcinomas, the results are similar to the whole-patient group.

In conclusion, this study found that classification as the pleomorphic subtype added no useful additional prognostic information to histological grade in invasive lobular carcinomas. Histological grade should still be used for clinical decision making in invasive lobular carcinomas. Although the pleomorphic subtype is of biological interest, it does not appear to be of prognostic value.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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