Invasive lobular carcinoma: to grade or not to grade

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Grading of invasive ductal carcinoma of no special type using the Nottingham combined histologic grading system provides independent prognostic information. The prognostic utility of grading invasive lobular carcinomas, however, has not been fully elucidated. In addition, the relationship between grade in invasive lobular carcinomas and expression of predictive biomarkers is less certain. The purpose of this study was to correlate histologic grade in invasive lobular carcinoma with known prognostic and predictive markers. All primary resections for invasive mammary carcinomas diagnosed in Mount Sinai Hospital, Toronto, between the years 1996 and 2002 were reviewed ($n=1053$). Of these cases, 50 were pure invasive lobular carcinoma (incidence 4.7%). The median age at diagnosis was 64 years. These tumors were graded using the Nottingham combined histologic grading system and analyzed for estrogen receptor, progesterone receptor, HER2/neu and E-cadherin expression. Tumor grade was correlated with tumor size ($P=0.03$), and the American Joint Committee on Cancer nodal status ($P=0.05$). Assessment of the individual components of grade showed that the mitotic score was highly correlated with tumor size ($P=0.02$), lymph node positivity ($P=0.02$) and overall American Joint Committee on Cancer stage ($P=0.01$). Estrogen receptor and progesterone receptor were highly expressed irrespective of the grade of tumor. HER2/neu protein overexpression and E-cadherin protein expression was absent in all invasive lobular carcinomas studied. We conclude that pure invasive lobular carcinoma is uncommon and occurs predominantly in postmenopausal women. Increasing tumor grade is correlated with median tumor size and the American Joint Committee on Cancer nodal stage, but not correlated with the expression of estrogen receptor, progesterone receptor, E-cadherin or HER2/neu protein overexpression.

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Invasive mammary carcinomas are broadly classified into no special type (ductal) and special type cancers.¹ Invasive lobular carcinoma is a member of the latter category representing 0.6–20% of all invasive cancers.^{2–4}

Invasive ductal carcinomas of no special type constitutes the majority of invasive mammary cancers, and they are generally graded using the Nottingham combined histologic grading system, which is a modification of the Bloom and Richardson method.⁵ Histologic grade together with tumor size (measured pathologically) and histologic lymph node stage have been found to have a significant correlation with prognosis.⁶ In addition, the expression of prognostic markers in breast cancer, namely

estrogen receptor and progesterone receptor is grade dependent, as is the overexpression and amplification of HER2/neu. The application of the Nottingham combined histologic grading system to invasive lobular carcinoma is not routine in histopathology reporting. In addition, the relationship of biomarkers to grade in invasive lobular carcinoma is unknown. The objective of this study was to correlate histologic grade in invasive lobular carcinoma with tumor size, lymph node status and biomarker profile.

Materials and methods

A retrospective review of all primary resections for invasive mammary carcinomas conducted at Mount Sinai Hospital, Toronto, between the January 1996 and December 2002 was undertaken. A total of 1053 invasive mammary carcinomas were diagnosed in this period, 50 of which were pure invasive lobular carcinomas. Strict morphologic criteria were used to

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identify the cohort of invasive lobular carcinomas, namely a monotonous population of discohesive cells devoid of any tubule formation. Evidence of tubule formation in the representative sections of tumor archived made the tumor ineligible for the study and these tumors were classified as invasive ductal, no special type with lobular features.

Hematoxylin and eosin-stained sections of the formalin-fixed, paraffin-embedded tumors were reviewed. The 50 invasive lobular carcinomas were independently graded by two of the authors using the Nottingham combined histologic grading system.⁶ In accordance with this grading system, each tumor was assessed and scored numerically for the percentage of tubule formation, the degree of nuclear pleomorphism and the mitotic count in 10 high-power fields (field diameter 0.44 mm). The scores obtained for each morphological parameter were added to obtain an overall grade. In cases where assessment of grade differed, the disagreement was resolved by consensus after joint review using a multiheaded microscope. Tumor size, tumor grade, lymph node status and other prognostic parameters were recorded and tabulated (Table 1).

Representative sections of invasive tumor were selected. Sections of paraffin-embedded tumor, 4 μ m thick, were cut and stained for estrogen receptor (6F11, Novocastra, dilution 1/70), progesterone receptor (PgR, DAKO, dilution 1/1500), HER2/neu protein (CB11, Novocastra, dilution 1/200 & A0485, DAKO, dilution 1/750), and E-cadherin (HECD-1, Zymed, prediluted). Estrogen receptor and progesterone receptor were interpreted as positive if >1% of tumor cells demonstrated nuclear positivity.⁷ HER2/neu protein overexpression was recorded if >10% of tumor cells demonstrated complete, moderate to strong membrane staining. Complete, circumferential membrane staining with the E-cadherin antibody in any percentage of tumor cells was considered positive.

Differences between tumor grades were tested using the Jonckheere-Terpstra nonparametric test for both continuous (age, size, number of positive lymph nodes), binary (positivity of lymph nodes, estrogen receptor, progesterone receptor, Her2/neu, E-Cadherin) and categorical (American Joint

Committee on Cancer T and N, stage) variables. Categorical variables were treated as ordinal, using the natural ordering of these scoring systems. Exact (not asymptotic) *P*-values were calculated using the SAS software (version 8.2, SAS Institute, Cary NC, USA).

Results

Of 1053 primary resections for invasive mammary carcinoma conducted at Mount Sinai Hospital, during the study period, 50 cases of pure invasive lobular carcinoma were identified using the strict morphological criteria outlined (incidence 4.7%). Of the tumors, 10 (20%) were grade I (Figure 1), 33 (66%) were grade II (Figure 2) and seven (14%) were grade III (Figure 3), using the Nottingham combined histologic grading system.⁶

The median patient age at the time of diagnosis was 64 (range 37–85) years, and did not differ between tumor grades (*P*=0.63). The median tumor size, as determined by pathological examination, increased incrementally with increasing tumor grade. The median size was 1.2 cm for grade I tumors, 2.5 cm for grade II tumors and 4.4 cm for grade III tumors (*P*=0.03). The American Joint

Figure 1 Invasive lobular carcinoma, grade I/III, with cells exhibiting minimal nuclear pleomorphism ($\times 60$ magnification).

Table 1 Relationship of tumor grade to tumor size, lymph node status, and overall AJCC stage

ILC (n = 50)	Grade I	Grade II	Grade III	Statistical (P)
Number	10 (20%)	33 (66%)	7 (14%)	
Age (median) (years)	61	66	64	0.63
Median size (cm)	1.2	2.5	4.4	0.03
AJCC T (median)	T1c	T2	T2	0.13
LN positivity (%)	29	32	83	0.09
AJCC N (median)	N0	N0	N1a	0.05
Stage (median)	I	IIA	IIIA	0.09

ILC: invasive lobular carcinoma; AJCC T: American Joint Committee on Cancer Tumor Stage; LN: lymph node; AJCC N: American Joint Committee on Cancer Nodal Stage.

Committee on Cancer nodal stage was correlated with tumor grade ($P=0.05$) with a trend toward increasing tumor grade and both nodal metastases

Figure 2 Invasive lobular carcinoma, grade II/III, with cells exhibiting moderate nuclear pleomorphism but maintaining a single file growth pattern ($\times 60$ magnification).

Figure 3 Invasive lobular carcinoma, grade III/III, with cells exhibiting significant nuclear pleomorphism ($\times 60$ magnification).

($P=0.08$) and increasing median number of nodes involved ($P=0.06$). The overall stage of the tumors at diagnosis (a function of both tumor size and number of lymph nodes involved) increased progressively with tumor grade. The median stage of grade I tumors was stage I, for grade II tumors stage IIA and for grade III tumors stage IIIA ($P=0.08$). When tumor grade was divided into its constituent parts and re-examined, increasing nuclear grade was associated with increasing tumor size, lymph node positivity and tumor size. None of these parameters reached statistical significance (Table 2). Mitotic count, however, when analyzed in isolation was strongly associated with tumor size ($P=0.02$), lymph node positivity ($P=0.02$), and overall American Joint Committee on Cancer stage ($P=0.01$) (Table 3).

In our cohort, 100% of tumors expressed estrogen receptor, and progesterone receptor was expressed with high frequency ($>85\%$). Both were independent of tumor grade ($P\geq 0.95$). None of the tumors were found to overexpress HER2/neu protein. All of the tumors had HER2/neu protein expression assessed by two antibodies and in no case was there an equivocal result necessitating further investigation for gene amplification status using fluorescent *in situ* hybridization (FISH). E-cadherin was uniformly absent in all invasive lobular carcinomas studied (Table 4).

Discussion

Lobular carcinoma was first described by Foote and Stewart^{8,9} in 1941. They describe both the *in situ* form of the disease, confined to the lobule and terminal ducts and the invasive form. The morphologic features that distinguish this neoplasm from ductal carcinoma are two-fold.

- I. The cells are monotonous and discohesive with prominent intracytoplasmic lumina.
- II. The pattern of invasion is frequently in a single file arrangement with a targetoid growth pattern around terminal ducts.

Table 2 Tumor characteristics by nuclear grade ($n=50$)

ILC ($n=50$)	Nuclear grade I	Nuclear grade II	Nuclear grade III	Significance test
Number	10	34	6	
Age (median) (years)	61	65	64.5	0.99
Size (median) (cm)	1.15	2.55	2.60	0.12
AJCC T (median)	T1c	T2	T2	0.31
LN positivity (%)	29	38	60	0.37
Stage (median)	I	IIA	IIA	0.28
Number of positive lymph nodes (median)	0	0	3	0.35
AJCC N (median)	N0	N0	N0	0.25

ILC: invasive lobular carcinoma; AJCC T: American Joint Committee on Cancer Tumor Stage; LN: lymph node; AJCC N: American Joint Committee on Cancer Nodal Stage.

Table 3 Tumor characteristics by mitotic count per 10 high-power fields, field diameter 0.44 mm

ILC (n = 50)	MC 0–5	MC 6–10	MC > 11	Significance test
Number	40	7	3	
Age (median) (years)	65	64	48	0.22
Size (median) (cm)	1.7	3.0	5.0	0.02
AJCC T (median)	T1c	T2	T3	0.03
LN positivity (%)	30	60	100	0.02
Stage (median)	IIA	IIB	IIIC	0.01
Number of positive lymph nodes (median)	0	3	11	0.007
AJCC N (median)	N0	N0	N3	0.01

ILC: invasive lobular carcinoma; MC: mitotic count; AJCC T: American Joint Committee on Cancer Tumor Stage; LN: lymph node; AJCC N: American Joint Committee on Cancer Nodal Stage.

Table 4 Relationship of tumor grade with the expression of estrogen receptor, progesterone receptor, HER2/neu protein and E-cadherin protein

ILC (n = 50)	Grade I	Grade II	Grade III	Statistical (P)
Number	10 (20%)	33 (66%)	7 (14%)	
ER (%)	100	100	100	1.00
PR (%)	90	85	86	0.95
HER2/neu (%)	0	0	0	1.00
E-Cadherin (%)	0	0	0	1.00

ILC: invasive lobular carcinoma; ER: estrogen receptor; PR: progesterone receptor.

The reported incidence of invasive lobular carcinoma ranges from 0.6 to 20%.^{2–4} In the present series, the proportion of invasive lobular carcinoma was 4.7%. The variation in reported incidence is most probably due to different histopathological criteria used to define invasive lobular carcinoma rather than a real variation in incidence. We defined invasive lobular carcinoma on the basis of typical cytomorphologic characteristics, cited above and absent ductal formation.

Since the original description of invasive lobular carcinoma, variants other than the 'classical type' have been described. These include, the solid variant,¹⁰ the alveolar variant,³ the tubulo-lobular variant,¹¹ the signet ring variant,¹² the histiocytoid variant¹³ and a mixed subgroup.¹² The mixed subgroup, in Dixon *et al*'s¹² original paper, was recognized when an invasive lobular carcinoma was comprised of a mixture of variants or when the invasive lobular carcinoma had a classic growth pattern, but the degree of nuclear pleomorphism was greater than that seen in the classic subgroup. Eusebi *et al*¹⁴ described the clinical outcome in a group of cases he termed 'pleomorphic lobular carcinoma' which had the growth pattern of classic invasive lobular carcinoma but showed at least a moderate degree of nuclear pleomorphism. These tumors were associated with an aggressive behavior with six patients (60%) dying of disease within 42 months of diagnosis. Weidner and Semple¹⁵ divided invasive lobular carcinomas into 'pleomorphic' or 'classical' subtypes, based on nuclear characteristics, similar to the criteria described by Eusebi.

Their study demonstrated a trend toward a decrease in overall survival in patients with pleomorphic invasive lobular carcinoma compared with patients with classic invasive lobular carcinoma. Pleomorphic lobular carcinoma has subsequently been the subject of a number of papers^{16–19} and is described under the general rubric of invasive lobular carcinoma in the new World Health Organisation Classification of Tumours.⁴

Nuclear pleomorphism is only one of the features of the Nottingham combined grading system.⁶ This grading scheme involves semiquantitative evaluation of three morphological features—the percentage of tubule formation, the degree of nuclear pleomorphism and an accurate mitotic count using a defined field area. A numerical scoring system and the overall grade are derived from a summation of individual scores for the three variables: three grades of differentiation are used.⁶ Histologic grade is strongly correlated with prognosis, and is correlated with both disease-free and overall survival.^{20–23} The authors of the original study stressed that this grading system had clinical utility for both invasive lobular carcinoma and special type cancers. This was further validated by the work of Sastre-Garau *et al*,²⁴ who showed that histologic grade is as important for prognosis in invasive lobular carcinoma as it is in invasive ductal carcinomas. However, pathologists have been reluctant to utilize it in relation to invasive lobular carcinoma due primarily, it is thought, to the absence of any tubule formation in invasive lobular carcinomas, rendering that dimension of the three-tiered grading system redundant.

To investigate grading in invasive lobular carcinoma, du Toit *et al*²⁵ graded 171 such tumors in accordance with the Nottingham combined histologic grading system, and demonstrated that 20% were grade I, 64% grade II and 16% were grade III or high grade. These figures are similar to those in the current study.

In our cohort of pure invasive lobular carcinomas, we demonstrated that there was a positive correlation between increasing tumor grade and tumor size. The median size of tumors increased with tumor grade (Table 1). This result was statistically significant ($P=0.03$). Tumor size is a known independent prognostic factor,^{26–28} and the incidence of nodal metastases is known to increase with primary tumor size.²⁸

Axillary lymph node status is the single best predictor of patient survival.²⁹ The absolute number of involved axillary nodes at the time of diagnosis provides additional prognostic information, which is reflected in the revised American Joint Committee on Cancer nodal staging system.³⁰ The number of involved nodes also influences the choice of adjuvant therapy protocols.³¹ In our study of 50 invasive lobular carcinomas, there was a trend toward increasing lymph node involvement and increasing tumor grade ($P=0.08$); 29% of grade I tumors, 32% of grade II tumors and 83% of grade III tumors had axillary lymph node metastases at diagnosis. The median number of positive nodes was 0 for both grade I and grade II tumors, but patients with grade III tumors had a median number of 3.5 lymph nodes positive at diagnosis ($P=0.06$). There was a positive correlation between the median nodal stage, as defined by the new American Joint Committee on Cancer staging system and tumor grade. The median 'N' stage for grade I and II invasive lobular carcinomas was N0 but for grade III tumors it was 'N1a'. This result was statistically significant ($P=0.05$).

The Nottingham combined histologic grading system⁶ assesses three tumor parameters as discussed above. When we analyzed these parameters separately, tubule formation was noninformative as all invasive lobular carcinomas by definition score 3/3. Nuclear pleomorphism, despite being the initial morphologic factor that defined different grades of invasive lobular carcinoma, when analyzed alone showed only a weak correlation with tumor size, lymph node status or overall American Joint Committee on Cancer staging. However, the mitotic score when examined in isolation was highly correlated with all the prognostic parameters assessed (Table 3). These results were highly statistically significant. This fact suggests that the single most important component of the grading system in determining stage and biological aggressiveness in invasive lobular carcinomas is the mitotic index. This is not an unexpected finding as other investigators have assessed mitotic activity in invasive mammary carcinomas not classified by morphologic

subtypes and have also shown that it is the strongest predictor of decreased survival and it contributes predictive information beyond that obtained with the combined histologic grade.^{32–36} Indeed multivariate analysis of the Bloom Richardson grading system,⁵ of which the Nottingham combined histologic grading system is a modification, indicates that the mitotic index contributed much of the prognostic value.³³ Determination of estrogen receptor positivity is now routinely performed by immunohistochemistry on paraffin-embedded tumor sections. Estrogen receptor status carries both prognostic and predictive information. Estrogen receptor-positive tumors are known to have a better prognosis than estrogen receptor-negative tumors and estrogen receptor status is strongly correlated with tumor grade and differentiation, patient age and tumor size. In the clinical setting, the major importance of estrogen receptor lies in its predictive value. The response of both advanced and early breast cancer to endocrine therapy is proportional to estrogen receptor content.^{37,38} Our study illustrates that all invasive lobular carcinomas irrespective of patient age, tumor size or grade are positive for estrogen receptor and thus carry significant predictive value for the patient. However this data suggests that estrogen receptor status carries no discriminatory prognostic value in the setting of invasive lobular carcinoma. When Frolik *et al*¹⁹ graded their invasive lobular carcinomas, 93% of grade I and grade II and 100% of grade III invasive lobular carcinomas expressed estrogen receptor. Yet other studies that have addressed estrogen receptor status in pleomorphic lobular carcinomas not otherwise graded, have found varying degrees of receptor positivity, 9–81%.^{16–18}

The expression of progesterone receptor also carries both prognostic and predictive information. The prognostic utility of progesterone receptor has been validated in a number of studies^{39–45} and is thought to reflect the presence of a functional estrogen receptor pathway in the tumor and is used to predict the response of patients to adjuvant endocrine therapy.⁴¹ In our study cohort, there was a high incidence of progesterone receptor positivity (85–90%) that was not correlated with tumor grade ($P=0.95$). The literature shows that classic invasive lobular carcinomas, which correlate with grade I invasive lobular carcinomas are known to have a high incidence (>90%) of progesterone receptor expression.^{16–19} The reported incidence, however, of progesterone receptor positivity for 'pleomorphic' lobular carcinomas, corresponding to grade II and III in our study, is widely disparate, ranging from 9 to 97%.^{16,18,19}

HER2/neu is a proto-oncogene located on the long arm of chromosome 17.⁴⁵ Low levels of the protein expressed by this gene are normally present in many adult tissues including breast, endometrium, prostate and ovary. Amplification of this gene and increased levels of the protein product have been found in between 10 and 35% of invasive breast

cancers.⁴⁶ The presence of amplification and protein overexpression is associated with a number of adverse clinical outcomes, including decreased overall and disease-free survival, especially for patients with lymph node-positive disease.^{47–49} The presence of amplification carries predictive information with regard to responsiveness to anthracycline-based chemotherapy and is an absolute criterion for treatment with trastuzumab (Herceptin) therapy in the metastatic setting. *HER2/neu* gene amplification in invasive ductal carcinoma is correlated with grade. Tsuda *et al*⁵⁰ found *HER2/neu* amplification in 33% of grade III invasive ductal carcinomas, 10% of grade II carcinomas and 0% of grade I carcinomas. Similar results were obtained in our own institution.⁵¹ The amplification of this gene has been found in 0% of special type cancers and <1% of invasive lobular carcinomas.⁵² In the latter study, only one of 67 invasive lobular carcinomas was positive for *HER2/neu* gene amplification. This was designated as a 'pleomorphic' subtype of invasive lobular carcinoma with a nuclear grade of III/III. No reference to overall Nottingham combined histologic grade was given. In studies assessing *HER2/neu* overexpression by immunohistochemistry the results are highly variable. Middleton *et al*¹⁷ found *HER2/neu* overexpression in 81% of 'pleomorphic' invasive lobular carcinomas not further stratified by grade. Frolik *et al*¹⁹ found overexpression in 53% of grade III invasive lobular carcinomas and 6.66% of both grades II and I invasive lobular carcinomas, whereas none of the 15 cases of invasive lobular carcinomas studied by Porter *et al*⁵³ showed *HER2/neu* overexpression. Of the 50 invasive lobular carcinomas studied in our institution, none showed *HER2/neu* protein overexpression as assessed by immunohistochemical staining with two different antibodies. The lack of *HER2/neu* overexpression may be reflective of the small number of cases in this study. Indeed, in our consultation service, we have a small number of grade III invasive lobular carcinomas that are positive for *HER2/neu* protein overexpression and gene amplification (unpublished data). The discrepancy in the reported rates of *HER2/neu* overexpression in invasive lobular carcinoma may be accounted for by variation in histological classification or antibody sensitivity and specificity. Our results suggest that *HER2/neu* gene amplification is of minor importance in the development or progression of invasive lobular carcinoma regardless of grade, in contrast with invasive ductal carcinomas. Molecular characterization of invasive lobular carcinomas has shown that they have a higher incidence of loss of chromosome 17q than ductal carcinomas ($P=0.3$) and that ductal tumors have a higher incidence of 17q22–24 amplification than their lobular counterparts,⁵⁴ factors that may influence *HER2/neu* gene copy number and hence protein expression. A cDNA microarray study similarly showed that only two of 17 lobular carcinomas arrayed displayed *HER2/neu* gene overexpression.⁵⁵

E-cadherin is a calcium-dependent cell–cell adhesion molecule that is localized in lateral cell–cell contacts and enriched in the zonula adherens junction. Its function is to mediate intercellular adhesion via homophilic interactions.⁵⁶ It plays an essential role in the formation and maintenance of normal architecture and function of epithelial tissues.⁵⁷ It also shows an important invasion suppression activity.⁵⁸ The gene encoding the human E-cadherin, *CDH1* is localized on chromosome 16q22.1. Chromosome 16q is frequently lost in invasive lobular carcinomas and somatic mutations in this gene have been found in over 50% of invasive lobular carcinomas.⁵⁸ Somatic mutations are frequently accompanied by loss of the normal allele by loss of heterozygosity or alternatively by epigenetic silencing, signature features of a tumor suppressor gene. Loss of heterozygosity at the *E-cadherin* locus has been demonstrated in 27 of 27 invasive 'pleomorphic' lobular carcinomas in one study.⁵⁹ In most cases, the mutations are of the truncating type resulting in loss of protein expression,⁶⁰ which is compatible with the typical dispersed tumor growth with obvious loss of cell–cell adhesion. Lack of the protein expression can be assessed using immunohistochemistry. E-cadherin expression is normally present in the epithelial cell membranes in accordance with its function with cell adhesion. In our series, all tumors were negative for E-cadherin expression irrespective of grade, suggesting that loss of E-cadherin is a function of tumor morphology and not biological aggressiveness and is of no prognostic importance. Other authors have found E-cadherin expression in 0–45% of invasive lobular carcinomas.^{59–68} In these studies no correlation with grade was made. The discrepancy in the literature with regard to the percentage of invasive lobular carcinomas expressing E-cadherin may relate to differences between the populations studied, sensitivity and specificity of the antibodies used and diagnostic variation as applied to lobular carcinomas.

We have shown that invasive lobular carcinoma when characterized by strict morphological criteria is uncommon. Attempts have previously been made to subdivide invasive lobular carcinomas on the basis of morphological parameters and more recently by assessing 'nuclear pleomorphism'. We have applied the well-standardized and widely accepted Nottingham combined histologic grading system to a sequential series of invasive lobular carcinomas from one institution. We have correlated our results with tumor factors of known prognostic and predictive importance. We have shown that grade is correlated with overall tumor size and the American Joint Committee on Cancer nodal status. In contrast, estrogen receptor and progesterone receptor expression and *HER2/neu* overexpression are not correlated with grade. Expression of the tumor suppressor gene/cell adhesion factor, E-cadherin is universally lost in all invasive lobular

carcinomas irrespective of grade suggesting that its loss is a pre-requisite for the lobular phenotype but it does not dictate biological aggressiveness. It is clear from this study that invasive lobular carcinoma is both morphologically and biologically distinct from invasive ductal carcinomas and that grading is important. Further studies correlating the grade of invasive lobular carcinoma and response to adjuvant treatments and long-term survival are necessary to expand the body of knowledge concerning invasive lobular carcinomas.

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