Original article

High Ki67 expression is a risk marker of invasive relapse for classical lobular carcinoma in situ patients

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ABSTRACT

Background: The clinical management of lobular carcinoma in situ lesions remains challenging. Our aim was to evaluate the risk of relapse for lobular carcinoma in situ (LCIS) patients, diagnosed on mammography performed for microcalcifications and according to proliferation assessed by Ki67 staining.

Methods: A series of 47 patient’s files with LCIS and followed in our institution were retrospectively selected. All patients underwent lumpectomy without radiation therapy. The expression of E-cadherin, estrogen receptor (ER), progesterone receptor (PR), EGFR and Ki67 were determined. Four different classes were then defined with the following criteria: ER+ and Ki67 ≤ 10%; ER+, Ki67 > 10%; ER−; ER−PR− and EGFR−.

Results: Patient’s mean age was 51.3 yrs. The majority of the lesions were classical LCIS (97%). All cases were E-cadherin either negative (71%) or weak and incomplete (29%). Among the 44 evaluable cases, 34 cases were ER or PR positive with Ki67 ≤ 10% (79%), 9 cases ER positive with Ki67 > 10% (21%), 1 case was ER and PR negative and expressed EGFR. At five years, all patients were alive, 1/34 ER positive and Ki67 low experienced a relapse contrasting with 3 out of 9 ER positive and Ki67 high (3 invasive carcinomas including 2 ductal and 1 lobular) (p = 0.0054).

Conclusion: In this retrospective study, we observed a higher risk of relapse associated with a high proliferative activity of classical LCIS. If confirmed in larger series, this observation suggests that radiation therapy or hormonotherapy could be discussed for patients with Ki67 high classical LCIS in order to decrease their risk of relapse.

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Introduction

LCIS is an uncommon lesion, usually found on biopsy for microcalcifications. Its incidence remains difficult to determine, with reported values of 0.5–3.6%. Histologically, these lesions are characterized by the proliferation of non cohesive cells developed in the ductulo-terminal unit. These cells harbor an E-cadherin inactivation due to gene mutation or promoter inhibition in most cases.1,2 Lobular neoplasia constitutes a spectrum of quantitatively different lesions encompassing atypical lobular hyperplasia, in which only some of the acini are involved and lobular carcinoma in situ, in which carcinoma cells distend the acini, that will be named for the purpose of this study as LCIS. Pleomorphic LCIS is also characterized by extensive pleomorphism of the nuclei and nucleoli. When associated with necrosis, these lesions were formerly recognized as ductal carcinoma in situ, before the discovery of associated changes in E-cadherin expression.3,4

Classical and pleomorphic LCIS is associated with a breast cancer risk seven to 10 times higher than that of women in the reference population. These lesions are generally seen as risk factors, but half of the cases of invasive carcinoma developing in patients with a previous diagnosis of LCIS are of lobular type and this incidence is higher than that in the general population, in which lobular invasive carcinomas account for 15% of breast carcinomas.5,6 The risk is even higher for pleomorphic LCIS. In addition, invasive lobular carcinomas harbor LCIS lesions in 80–90% of cases.7 These observations suggest that rather than being a simple risk factor, LCIS is
a precursor lesion of invasive lobular carcinomas. The association between the presence of LCIS at surgical margins after conservative breast surgery and the risk of relapse remains a matter of debate.8,9

In addition, the natural history of these lesions is poorly understood.

In cases in which LCIS is detected as the only lesion on surgical specimens removed from the breast due to microcalcifications, no marker of the risk of recurrence has yet been identified to guide the adaptation of clinical follow-up.

Molecular subtypes of invasive tumors have been identified on the basis of transcriptomic analysis and have been translated into clinical practice with a combination of immunohistochemical markers for ductal carcinomas in situ (DCIS).10,11 It has been suggested that the risk associated with relapse in DCIS may be higher for ERBB2 DCIS subtypes12 or for Ki67 high combined with p16 high DCIS,13 but further studies are required to confirm these observations. For LCIS, the link between proliferation activity and the risk of relapse has not been yet established.

Our aim was to evaluate the risk of LCIS relapse as a function of proliferation assessed by Ki67 staining. We selected a retrospective series of 47 LCIS cases from our files. These cases were chosen on the basis of the availability of long-term clinical follow-up data in our cancer center records.

Patients

We selected 58 LCIS patients retrospectively, according to the availability of both clinical follow-up and paraffin embedded blocks in our institution. The records of all these patients were reviewed and the clinical data were recorded. These patients did not have previous personal cancer history. For the majority of them, the patients were diagnosed on a screening mammography. Histological tissue sections (hematein-eosin-safran sections and E-cadherin staining) were retrospectively reviewed when available, by one junior (AR) and one senior (AVS) pathologist.

All patients have given their informed consent and the institutional review board approved this study. Data were analyzed anonymously.

Methods

Immunohistochemistry

Immunohistochemistry was performed on the most representative paraffin block. We cut 4-μm sections from formalin-fixed tissues embedded in paraffin blocks. The sections were dried, deparaffinized and rehydrated according to standard procedures. All sections were subjected to heat-induced antigen retrieval in citrate buffer (pH 6.1), by heating in a 850 W microwave oven. Tissue sections were first digested in 0.1% trypsin and 0.1% calcium chloride in triphosphate-buffered saline pH 7.6 for 5 min for Ki67 staining. They were then incubated for 1 h with all primary antibodies. The expression of E-cadherin (clone 4A2C7, Invitrogen, 1/50), Ki67 (Clone MIB1; 1/100, Dako A/S, Glostrup, Denmark), estrogen receptor (ER, clone 6F11; 1/200; Novocasta), progesterone receptor (PR, clone 1A6; 1/200; Novoceastra), EGFR (HER1, clone 31G7, Zymed, 1/40).

For each antibody, internal and external controls were included in the experiments. Staining was detected with the Vectastain Elite ABC peroxidase mouse IgG kit (Vector Burlingame, CA) and diaminobenzidine (Dako A/S, Glostrup, Denmark) as the chromogen. Cases were considered positive for ER, PR, EGFR and Ki67 when at least 10% of the epithelial cells were positive in ductulo-terminal units involved with LCIS lesions.

Cases were then classified to their phenotype and proliferation activity: ER+ve and Ki67 < 10%; ER+ve and Ki67 > 10%; ER–ve; ER–ve PR–ve and EGFR+ve.

Statistical methods

Data are expressed as means or absolute numbers with percentages in brackets. Time to relapse was defined as the time from breast cancer primary tumor diagnosis to occurrence of the event. Kaplan–Meier relapse-free survival curves were calculated for each molecular classification and were compared in log-rank tests. Significance was defined as p ≤ 0.05. Statistical analyses were performed with R2.12.1 software (http://www.R-project.org).

Results

Clinical and pathological data

The median age of the patients was 51.3 years (range: 31–72). The majority of the patients was pre-menopausal (69%; 40/58 cases). Among menopausal patients, 6 out of 12 received a hormonal replacement therapy. The median follow-up was 97.12 months (range: 70.47–141.54). Most lesions were diagnosed on surgical specimen for microcalcifications (48/58 cases; 83% of the cases) or for a mass (18/58 cases; 31% of the cases) discovered on mammography and treated by lumpectomy (57/58 cases; 98%) without radiotherapy. The lesions were pluri focal in the resected surgical specimen in 11 out of the 58 cases (19%).

The excision was considered complete in 74% of the cases (43 of 58 cases).

Tissue sections and high-quality representative paraffin blocks were available for review for 47 of the initial 58 patients with clinical follow-up data. After review, 44 of the 47 cases were considered to correspond to classical LCIS, based on both morphological criteria and E-cadherin expression profile. Most of the cases (31 of 44 cases, 71%) were E-cadherin-negative (Fig. 1a). Thirteen cases (29%) displayed partial, fragmented and incomplete E-cadherin staining.

Epithelial cell immunophenotypes and proliferation activity

Among cases, the Ki67 staining pattern was often heterogeneous distributed within a single lobule, with the positive cells being located at the periphery of the lobule (Fig. 1c). However, the Ki67 expression was homogeneous from one lobule to another within a single case. All carcinomatous cells from a single case were evaluated.

The results of this analysis are displayed in Table 1. Most cases were ER+ve (91%), PR+ve (66%), Ki67 low (80%) and EGFR–ve (89%).

In total, 30 cases were defined as ER+ve Ki67 low (70%), nine cases as ER+ve and Ki67 high (21%) (Fig. 1) and one case was ER–ve PR–ve and EGFR–ve (2%). The remaining cases were for three of them ER–ve and PR+ve (7%) and were pooled with the ER+ve cases for the statistical analysis and one un-assigned.

Association of LCIS proliferation assessed by Ki67 with prognosis

At five years, all but one of the patients were alive. Median survival was 263 months.

One of the 34 ER or PR+ve and Ki67 low tumors and four of the nine high Ki67 tumors (1 DCIS, 3 invasive carcinomas including 2 ductal and 1 lobular) relapsed (p = 0.0054).

The relative risk of relapse associated with the ER+ve and Ki67 high LCIS group was 10.42 (IC 95%: 0.67–8.08) (Fig. 2).
Discussion

The clinical management of patients with LCIS varies between institutions, but generally involves the close observation of patients undergoing lumpectomy. This pragmatic attitude is based on long-term clinical follow-up studies in LCIS patients, which have shown that these patients have a risk of breast cancer seven to 10 times higher than women of the same age from the reference population. Furthermore, there seems to be a higher risk of developing invasive lobular carcinoma in the population of patients previously treated for LCIS, this subtype accounting for 25–75% of the cancers observed. Adjunct treatments based on selective estrogen receptor modulators may be introduced in the near future, based on recent results clearly demonstrating a lower risk of subsequent carcinoma in patients treated with exemestane. Additional radiotherapy has also been proposed, particularly for patients with variants of LCIS, such as pleomorphic LCIS or LCIS associated with necrosis, or PLCIS in contact with surgical margins. However, there is currently no objective parameter for predicting the true risk of recurrence at initial diagnosis of LCIS.

We investigated the natural course of classical LCIS lesions diagnosed on surgical specimens performed in the majority of the cases for microcalcifications, a clinical situation reported to be more often

Table 1

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<tr>
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<td>KI67 ≤ 10% (low)</td>
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Fig. 1. Examples of ER and low and high KI67 stainings in E-cadherin-negative LCIS. a. LCIS with no E-cadherin expression. b. LCIS strong ER nuclear positivity. c. KI67 nuclear staining in less than 10% of carcinomatous cells; d. KI67 nuclear staining in more than 10% of carcinomatous cells.

Fig. 2. Disease free survival curves of LCIS groups according to proliferation activity. Disease free survival curves of the ER or PR+ve and KI67 low (in black and green) and the ER-ve KI67 high (in red) sub-groups of LCIS are compared. Time in abscissa is expressed in months from 0 to 300. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
encountered than previously reported and the risk of these lesions progressing to invasive carcinoma, by analyzing the impact on patient outcome of proliferation activity, assessed by immunohistochemistry in a series of LCIS patients from our institution for whom clinical outcome data and paraffin embedded blocks were available.

We found that the classical LCIS with a high proliferative activity was associated with a risk of relapse. Despite the modest size of this series, this observation shows that ERBB2 may be involved in the progression from in situ to invasive ductal carcinomas.

It has been suggested that estrogen-positive tumors associated with high levels of proliferation or ERBB2 overexpression are good surrogate markers for the definition of luminal B invasive carcinomas. For DCIS, we and others have previously demonstrated that several different molecular classes exist that can be defined by immunohistochemistry. However, the link between molecular DCIS subtypes and the risk of relapse is still unclear. LCIS lesions are subclinical in most cases and are almost always diagnosed on fixed, paraffin embedded specimens. Immunohistochemistry is therefore the only approach that can realistically be used in these cases. The threshold defining high levels of proliferation for LCIS lesions with Ki67 staining has not been clearly fixed, but Ki67 levels exceeding 10% in an estrogen-positive low-grade lesion, such as LCIS, would appear to be a reasonable definition for validation in an independent series.

Evaluation of the risk of LCIS progressing to invasive lobular carcinoma remains highly challenging. Recent reports have shown that microinvasive LCIS is a very rare disease (0.02%; 16 of 75,250 cases) but that this condition may occur in either classical or pleomorphic carcinoma, both estrogen-positive. The putative role of specific genomic/transcriptomic alterations that could drive tumor progression from in situ to invasive lobular carcinoma remains to be defined. For ductal carcinoma in situ, it has been suggested that ERBB2 may be involved in the progression from in situ to invasive ductal carcinomas in some, but not all cases. It has also recently been suggested that the combination of high levels of expression of Ki67, COX2 and p16 could be used to identify DCIS associated with a higher risk of recurrence as invasive cancer. No such biological marker has yet been proposed for identifying LCIS lesions associated with a higher risk of relapse. However, molecular heterogeneity of pleomorphic LCIS has been demonstrated in one multicenter series published by Chen, in which ERBB2 was amplified in 25% of cases and was, in some cases, associated with apocrine differentiation. However, no link has been found between molecular changes and the risk of relapse.

In conclusion, the classical ER+ve Ki67 high LCIS lesions are associated with a higher risk of relapse after surgical excision. These findings require confirmation in a larger series of cases, but they suggest that pathologists could determine LCIS phenotype in particular when diagnosed on surgical specimens performed for microcalcifications and radiotherapy or hormone therapy may be beneficial for patients with these ER+ve and Ki67 high LCIS lesions.

Conflict of interest statement
None declared.

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References