Prognostic Significance of HER-2/neu Expression in Patients with Ductal Carcinoma In Situ

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ABSTRACT: **Background:** The prognostic significance of biologic markers in women with ductal carcinoma in situ is not fully understood. HER2/neu is a marker of prognostic significance that is routinely assessed in invasive cancer but its correlation with clinical outcome in DCIS is still obscure.

Objectives: To evaluate the significance of HER-2/neu expression as a prognostic marker in DCIS.

Methods: Clinical and pathologic data from 84 patients treated for DCIS were analyzed. HER-2/neu expression was determined by immunohistochemical staining. Histopathologic parameters (nuclear grade, histologic subtype, necrosis, calcifications, margins) were reviewed by an experienced pathologist. Local recurrence and/or metastatic spread were used as endpoints to determine the prognostic significance of HER-2/neu expression.

Results: With a median follow-up of 94.8 months, nine recurrences were reported. Neither univariate nor multivariate analysis showed a significant correlation between HER-2/neu expression and disease recurrence or the time to disease recurrence. Although HER-2/neu expression demonstrated a significant association with high nuclear grade ($P < 0.0001$) and comedo subtype ($P < 0.0001$), there was no correlation between these histologic features and recurrence rate. The correlation between high nuclear grade and disease recurrence approached statistical significance ($P = 0.07$).

Conclusions: No significant association was found between HER-2/neu expression in DCIS and disease recurrence. However, HER-2/neu correlated with negative markers such as nuclear grading and comedo necrosis, and its role should therefore be investigated in larger studies.

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KEY WORDS: HER-2/neu, ductal carcinoma in situ, prognosis, markers, recurrence

In the last decade the incidence of ductal carcinoma in situ has increased, probably related to the introduction of mammography screening. In asymptomatic women, 15–25% of all screening-detected breast cancers are DCIS lesions [1].

DCIS = ductal carcinoma in situ

Treatment guidelines for DCIS have changed over the last two decades and most patients are now treated with breast-conserving therapy. Although these lesions do not metastasize, they carry a relatively high risk for local recurrence and of subsequently developing into invasive carcinoma, particularly when high nuclear grade or comedo-type necrosis is found in the primary lesion [2,3].

Although adjuvant breast irradiation and hormonal therapy reduce the number of ipsilateral breast recurrences by about half [4], local recurrence and the development of invasive cancer are still of concern. In an effort to optimize treatment outcome in these cases, multiple studies have examined the association of various clinical, pathologic, molecular and treatment-related factors with subsequent invasive breast carcinoma or local recurrence. To date, only histologic features like comedo-necrosis, nuclear grade and tumor size have been shown to correlate with local recurrence [4-8].

The last decade has witnessed an increasing number of biologic markers of breast neoplasms. One of the markers with probable prognostic significance is over-expression of the HER-2/neu oncogene within the tumor. Although HER-2/neu over-expression has been shown to correlate with a poor prognosis in invasive breast cancer [9,10], little information is available on the relationship between its over-expression and the outcome of patients with DCIS. Previous studies have indeed demonstrated that HER-2/neu expression correlates with aggressive histologic features in DCIS [11-20], but the prognostic significance of HER-2/neu expression in the clinical setting has yet to be determined.

The purpose of the present study was to assess the correlation of HER-2/neu over-expression and the long-term outcome in DCIS patients, and to evaluate its prognostic significance as well as its association with other histopathologic and biological characteristics.

PATIENTS AND METHODS

The medical records of all patients who underwent breast surgery in our hospital between 1990 and 1998 were reviewed. The study population included patients with the histopathologic finding of DCIS, without invasion. The data

were retrieved using the institution's computerized data bank. All cases diagnosed as DCIS were retrieved and the pathology was revised. Patients were excluded if any type of invasion was found or if the follow-up data were incomplete. Demographic parameters were retrieved from the patients' medical records. Using the outpatient clinic files, we evaluated the clinical course with regard to the development of local recurrence of DCIS or invasive cancer, metastatic spread and mortality. Further data were obtained by means of a telephone interview.

HISTOPATHOLOGIC REVIEW

Histopathologic assessment was performed on paraffin sections stained with hematoxylin-eosin. The following information and pathologic features were recorded for the initial and re-excision specimens:

- Predominant histologic subtype, categorized as comedo, cribriform, papillary, micro-papillary, or solid patterns
- Predominant and highest nuclear grade
- Presence or absence of necrosis
- Presence or absence of micro-calcifications
- Margin status, classified as positive, close, negative or uncertain (when the specimen was not inked or was fragmented such that the specimen margins could not be determined).

Nuclear grade was categorized into two groups: favorable (low or intermediate) and unfavorable (high). Histologic pattern was grouped as comedo and non-comedo subtypes.

CLINICAL OUTCOME

Local recurrence was defined as the reappearance of DCIS or invasive carcinoma in the ipsilateral breast. All recurrences were confirmed histologically. Distal metastases were diagnosed by computed tomography scan, bone scans or ultrasound. When the imaging finding was doubtful, fine needle or core biopsies were performed. All time intervals were calculated from the date of the first diagnosis.

HER-2/NEU EXPRESSION

HER-2/neu oncogene expression was determined by immunohistochemical staining of tissue sections with monoclonal antibodies. The monoclonal antibody used was at a dilution of 1:100 (monoclonal mouse anti-HER-2, Zymed Laboratories, San Francisco, USA). The primary antibody was visualized using biotinylated horse antibody to mouse immunoglobulin and streptavidin-horseradish peroxidase, followed by diminobenzidine solution. Breast carcinomas, positive or negative to HER-2/neu, were used as negative and positive controls, respectively. The specimens were classified as positive or negative solely on the basis of tumor cell membrane reactivity. The percentage of positive cells in the tumor population was determined by visual estimation, and a semi-quantitative analysis of HER-2/neu staining was performed. According to the literature and common practice, the staining pattern was scored as follows:

- 0 = no staining or membrane staining in less than 10% of the tumors cells
- 1+ = faint/barely perceptible membrane staining in more than 10% of the tumor cells with the cells stained only in part of their membrane
- 2+ = weak to moderate staining of the entire membrane in more than 10% of the cells
- 3+ = strong staining of the entire membrane in more than 10% of the cells.

A sample was considered positive for HER-2/neu over-expression if more than 10% of the epithelial cells demonstrated a moderate (2+/3+) to strong (3+) staining of the entire membrane.

STATISTICAL ANALYSIS

The association between over-expression of HER-2/neu and recurrence and all categorical variables (clinical, pathologic, and treatment-related variables) was analyzed using chi-square and Fisher's exact test. Using the *t*-test, mean age was compared between HER-2/neu positive and negative patients and between those with and without recurrence. Kaplan-Meier survival method was used to evaluate survival rates in the various patient subgroups. Log-rank test was performed to compare disease-free time between patient subgroups. Logistic regression and Cox proportional hazards model were applied to study simultaneously the effect of possible risk factors on recurrence and time to recurrence respectively. Statistical significance was accepted at $P < 0.05$. The data were analyzed using SAS software (version 9.1, Cary, North Carolina, USA)

RESULTS

A total of 112 women who underwent surgery between 1990 and 1998 with postoperative finding of DCIS were initially included in the study. Twenty-eight patients were excluded due to inadequacy of the pathologic material, death due to unrelated diseases soon after diagnosis of DCIS, or because of incomplete follow-up. The remaining 84 women were followed for a median period of 94.8 months (range 48–168 months). Mean age of the patients was 55.8 years (range 35–82 years).

In 80 of the 84 remaining patients (95%) the initial treatment was breast-conserving surgery by lumpectomy, and in 4 patients mastectomy was performed as the initial treatment. Twenty-seven patients (34%) from the group treated initially with lumpectomy underwent subsequent mastectomy due to multifocal disease, widespread DCIS within the breast, or due to massively involved margins in the first operation. Another 10 patients (12.5%) from the initial lumpectomy

group underwent further excision of the primary tumor site due to close, positive or uncertain margins. Of the 52 patients finally treated by breast-conserving surgery, 43 also underwent breast irradiation or a combination of breast irradiation and tamoxifen. Of the 31 women who finally underwent mastectomy, 15 received tamoxifen after the operation. All women treated postoperatively with tamoxifen had estrogen receptor-positive tumors.

Table 1. Comparison of recurrence rate by clinical parameters and histopathologic variables

Variable	No. of patients (%)	No. of patients with recurrence (%)	No. of patients without recurrence (%)	*P value*
Nuclear grade				
High	32 (38.1)	6 (18.75)	26 (81.25)	0.07
Intermediate and low	52 (61.9)	3 (5.77)	49 (94.23)	
Histologic pattern				
Comedo type	38 (45.78)	5 (13.16)	33 (86.84)	0.72
Non-comedo	45 (54.2)	4 (8.89)	41 (91.11)	
Margins				
Free	49 (94.23)	7 (14.9)	42 (85.71)	0.4
Not free	3 (5.77)	1 (55.33)	2 (66.67)	
Necrosis				
Present	73 (86.9)	7 (9.56)	66 (90.41)	0.33
Absent	11 (13.1)	2 (18.18)	9 (81.81)	
Calcifications				
Present	66 (78.57)	7 (10.61)	59 (89.29)	1.0
Absent	18 (21.43)	2 (11.11)	16 (88.89)	
Family history of breast cancer				
Present	16 (19.04)	3 (18.75)	13 (81.25)	0.37
Absent	64 (76.19)	6 (9.38)	58 (90.63)	
No data	4 (4.76)			
Menopausal state				
Before menopause	24 (28.57)	2 (8.33)	22 (91.67)	07
After menopause	57 (67.85)	7 (12.28)	50 (87.72)	
No data	3 (3.57)			

Table 2. Comparison of recurrence rate by HER-2/neu expression in surgical subgroups (lumpectomy vs. mastectomy respectively)

HER-2/neu over-expression	No of patients (%)	No. of patients (%) with recurrence	No. of patients (%) without recurrence	*P value*
Lumpectomy with and without adjuvant treatment				
Positive	22 (41.50)	5 (22.73)	17 (77.27)	0.2
Negative	30 (58.50)	3 (10)	27 (90)	
Mastectomy with and without adjuvant treatment				
Positive	15 (48.38)	0 (0)	15 (100)	1
Negative	16 (51.61)	1 (6.25)	15 (93.75)	
Lumpectomy without adjuvant treatment				
Positive	4 (57.14)	1 (25)	3 (75)	0.48
Negative	3 (42.86)	2 (66.6)	1 (33.3)	
Lumpectomy with adjuvant treatment (radiotherapy +/- tamoxifen)				
Positive	17 (37.77)	4 (23.53)	13 (76.47)	0.07
Negative	26 (57.77)	1 (3.85)	25 (96.15)	
Mastectomy without adjuvant treatment	8 (80)	0 (0)	8 (100)	
Positive	2 (20)	0 (0)	0 (0)	
Negative				
Mastectomy and tamoxifen treatment				
Positive	4 (19.06)			
Negative	10 (47.61)	0 (0)	4 (0)	1
Unknown	7 (33.33)	1 (10)	9 (90)	

During the follow-up period, recurrence of disease in the ipsilateral breast was diagnosed in 9 patients (10.7%). The primary surgical treatment was lumpectomy in eight patients and mastectomy in one. Five of these patients had been treated by adjuvant radiotherapy after the local excision and three patients also received tamoxifen. The woman who underwent mastectomy was also treated with tamoxifen.

In two patients the recurrent lesion was DCIS, discovered 22 and 82 months after primary surgery, respectively. These women were subsequently treated by mastectomy and were alive at the end of the study (4 months and 6 years respectively after the recurrence). In another six patients the recurrent tumor was invasive carcinoma, diagnosed between 13 and 142 months after the primary surgery. Four of them underwent subsequent mastectomy and were alive 24–72 months after the second surgery. In the remaining two, the breast tumors were diagnosed simultaneously with distant metastatic disease. One of them was treated with chemotherapy and died shortly after, and the other woman is still alive 8 months after the diagnosis of recurrence.

The woman with recurrent disease who was initially treated by mastectomy for DCIS presented 72 months later with local recurrence of invasive cancer in the mastectomy scar and metastatic spread. She received chemotherapy but died a few months later. Histopathologic revision of the resected breast did not show any focus of invasion.

Two patients died during the study period due to causes other than breast cancer and five patients developed other malignancies.

None of the following parameters – age, menopausal state, family history, resection margins, histologic pattern, and presence of necrosis or micro-calcifications – significantly influenced the rate of disease recurrence. However, a tendency to higher recurrence rates was observed in patients with high nuclear grade tumors ($P = 0.07$) [Table 1].

From among the 84 patients finally included in the study, in 37 (44.05%) the tumors were positive for HER-2/neu over-expression and in 47 patients (55.95%) they were negative. No correlation was found between the recurrence rates in patients positive for HER-2/neu as compared to patients without over-expression of HER-2/neu: 13.5% (5 of 37) versus 8.51% (4 of 47) ($P = 0.46$).

When the patients were grouped according to surgical treatment subgroups (patients who underwent lumpectomy versus patients who underwent initial mastectomy) and subgroups according to adjuvant treatment administration (patients given versus patients not given the treatment), the potential influence of the type of therapy on the outcome was eliminated. No significant correlation was found regarding

the relationship between HER-2/neu over-expression and disease recurrence for these subgroups as presented in Table 2, although patients initially treated by lumpectomy and adjuvant therapy showed an almost statistically significant association between expression of HER-2/neu and disease recurrence ($P = 0.07$).

The association between HER-2/neu and the different histologic features are summarized in Table 3. Most patients (61.9%) had DCIS with favorable nuclear grade. HER-2/neu over-expression was significantly higher in DCIS showing high nuclear grade compared to low nuclear grade (71.8% vs. 26.9, $P < 0.001$). HER-2/neu over-expression was also significantly higher in DCIS showing comedo compared to non-comedo pattern (68.4% vs. 24.4, $P < 0.001$). A tendency to HER-2/neu over-expression was also observed in DCIS lesions showing calcifications ($P = 0.05$).

Familial history of breast cancer did not influence the incidence of HER-2/neu over-expression and no correlation was found between HER-2/neu and the timing of recurrence ($P = 0.58$).

In multivariate analysis using a logistic regression model that included HER-2/neu, clinical variables (menopausal state, age, family history, type of surgery, tamoxifen treatment) and pathologic variables (histologic subtype, nuclear grade, margins), no HER-2/neu or any other marker was found to be of significance, either alone or together with other markers.

Table 3. Correlation between HER-2/neu expression and clinical and histopathologic variables

Variable	No. of patients (%)	No. of HER-2/neu negative patients (%)	No. of HER-2/neu positive patients (%)	*P value*
Nuclear grade				
High	32 (38.10)	9 (28.13)	23 (71.87)	‹ 0.0001
Non-high	52 (61.90)	38 (73.07)	14 (26.92)	
Histologic pattern				
Comedo type	38 (45.78)	12 (31.58)	26 (68.42)	‹ 0.0001
Non-comedo type	46 (54.22)	34 (75.56)	12 (24.44)	
Necrosis				
Present	73 (86.90)	38 (52.05)	35 (47.95)	0.1
Absent	11 (13.1)	9 (81.82)	2 (18.18)	
Calcifications				
Present	66 (78.57)	33 (50)	33 (50)	0.05
Absent	18 (21.43)	14 (77.78)	4 (22.22)	
Family history of breast cancer				
Present	16 (19.04)	10 (62.50)	6 (37.5)	0.5
Absent	64 (76.19)	33 (51.56)	31 (48.44)	
Unknown	4 (4.76)			

DISCUSSION

Since the incidence of non-invasive breast cancer has increased, and as conservative breast surgery became the new standard of care, local recurrence of either DCIS or invasive cancer has become the major concern when treating patients with this potentially curable disease. In the last two decades several studies assessed prognostic markers in a continuous quest for improving outcome. In the NSABP study B-17 moderate-to-marked comedo-necrosis was the single histologic feature found to predict recurrent disease [4]. Solin et al. [5] found that no single histopathologic parameter was associated with local recurrence. Ottesen and collaborators [6] reported that comedo-necrosis, large nuclei, and tumor size > 1 cm were significant predictors of local recurrence after excision alone, and Silverstein et al. and the Van Nuys group [7] created a DCIS pathologic classification based on nuclear grade and the presence or absence of comedo-necrosis. The Van Nuys Prognostic Index builds on this classification with the addition of two parameters with prognostic significance, as identified in multivariate analysis, namely tumor size and margin width [8].

The last decade has witnessed the emergence of increasing numbers of biologic markers to characterize breast neoplasms. These markers typically reflect alterations in genes that regulate cell growth, development and proliferation. The biologic marker profiles of patients with DCIS and their potential prognostic significance are currently being investigated [9,10]. One of the markers with probable prognostic significance is the HER-2/neu oncogene.

The human epidermal growth factor receptor 2 (HER-2)/neu (c-erbB-2) oncogene is localized on chromosome 17q, and encodes a trans-membrane tyrosine kinase receptor protein that is a member of the epidermal growth factor receptor (EGFR) or HER family [11,12]. Amplification of the HER-2/neu oncogene with the expression of its protein is found in 10–30% of invasive breast carcinomas [13,14] and 30–60% of DCIS cases [14-16]. In invasive breast cancer, HER-2/neu over-expression has been shown to correlate with poor prognosis and poor response to treatment [13,14]. However, the clinical significance of HER-2/neu over-expression in DCIS is presently unknown. In previous studies HER-2/neu expression in DCIS was shown to correlate with aggressive histologic features such as: high nuclear grade, comedo type, the presence of necrosis, and Ki-67 over-expression [10,14-20]. A significant inverse correlation has also been found between HER-2/neu staining and hormone receptor expression in patients diagnosed with DCIS [10,14,15,18]. All these studies showed that HER-2/neu over-expression correlates with various pathologic and molecular factors believed to be associated with a more aggressive behavior, leading to the assumption that DCIS that over-expresses HER-2/neu represents a biologically definable category with prognostic significance.

In the present study we assessed a rather large cohort of DCIS patients with a relatively long follow-up period, to investigate this correlation and to evaluate the prognostic impact of HER-2/neu in DCIS patients. During the study period 10.7% of patients initially operated on for confirmed

DCIS had recurrences. The median follow-up period was 94.8 months, which is longer than in most previous series [6,9,19,21]. The relatively lower recurrence rate in the present study could likely be attributed to the wide use of adjuvant irradiation in our patients.

No significant association between HER-2/neu expression and rate of disease recurrence was found, which concurs with the results of previous studies [14,17,18,22]. In the study reported by Ringberg and co-authors [14], three biological markers were associated with disease recurrence in a univariate analysis: Ki-67 levels, p53 and bcl-2 but not HER-2/neu. Reporting on their 49 patients who were treated with either mastectomy or conservative surgery with or without irradiation, Perin et al. [22] found no significant association between a wide variety of biologic markers including ER, PR, HER-2/neu, and p53, and the rate of disease recurrence. Cornfield and team [17], in a study of 151 patients with DCIS who underwent wide excision and observation alone, also reported no significant association between any of the biological markers evaluated including HER-2/neu and disease recurrence. Roka et al. [18] also found that high nuclear grade and negative estrogen receptor are risk factors for recurrence, whereas other factors like HER-2/neu and p53 did not have any prognostic significance.

Regarding the relationship between HER-2/neu over-expression and histopathologic parameters, the present study demonstrated a significant association between HER-2/neu over-expression and both the high grade and comedo subtypes of DCIS. Similar results were also reported in previous studies [10,15,16,18,19].

When the association between diverse variables and the rate of disease recurrence was analyzed, none of the following parameters – age, menopausal state, family history, resection margin, histologic pattern, presence of necrosis or microcalcifications, presence of microinvasion – significantly influenced the rate of local recurrence. However, as expected, a tendency to higher recurrence rates was observed in high grade DCIS ($P = 0.07$).

Although mastectomy is considered to be curative in the treatment of DCIS, with very low recurrence rates [23], most patients are currently treated by breast-conserving surgery with comparable results in terms of survival. Recurrence rates of 6% to 12% were reported in cases in which adjuvant treatment was administrater after breast-conserving surgery [4,24]. In the present study the outcomes were similar: 3.23% recurrence rate in patients treated by mastectomy, 11.63% in patients treated by breast-conserving surgery and subsequent adjuvant therapy (radiotherapy or/and tamoxifen), and 42.86% in patients treated by local excision alone – further highlighting the benefit of adjuvant therapy in patients treated by local excision.

ER = estrogen receptor
PR = progesterone receptor

In conclusion, although in the present study HER-2/neu was not an independent risk factor for recurrence, it was associated with other risk factors such as comedo subtype and high grade. This paradox was also noted in the previous studies. However, since most studies were relatively small, it is still possible that larger series will reveal the subgroups of DCIS patients in which HER-2/neu will be of value in therapeutic decisions.

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