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Are cellular polarisation and mitotic frequency prognostic factors for local recurrence in patients with ductal carcinoma *in situ* of the breast?

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Abstract

There is still no generally accepted histopathological classification system for ductal carcinoma *in situ* (DCIS) of the breast. Nuclear grade, with or without other histopathological parameters (i.e. comedo-type necrosis and cellular polarisation), has been demonstrated to yield prognostic information. A detailed method for the evaluation of the mitotic frequency in DCIS, based on an approach by Contesso, was used in this study. We also investigated if cellular polarisation and mitotic frequency were important for the ipsilateral local recurrence-free interval (IL-RFI) in 121 DCIS patients who had been operated upon with breast-conserving treatment (BCT) without radiotherapy. Both cellular polarisation and the mitotic frequency were associated with histopathological and cellular biological factors (in previous evaluations), and were of borderline significance for IL-RFI in the univariate analyses. However, when nuclear grade was included in the multivariate analyses (with or without the growth pattern), neither cellular polarisation nor the mitotic frequency were of any independent prognostic value.

Keywords: Breast cancer; Ductal carcinoma *in situ*; Nuclear grade; Cellular polarisation; Mitotic frequency; Local recurrence

1. Introduction

There is still no generally accepted histopathological classification system for the clinical management of ductal carcinoma *in situ* (DCIS) of the breast. Nuclear grade and the presence of a comedo-type necrosis, originally described by Lagios in Ref. [1], have been shown to give prognostic information [2–4]. In the classification system proposed by Holland and co-workers [5], nuclear grade (cytonuclear differentiation) is combined with cellular polarisation (architectural differentiation). Silverstein and colleagues introduced the Van Nuys system and combined nuclear grade with comedo-type necrosis, into a high-grade with necrosis group, and a non-high-grade group with and without necrosis [6]. These last two systems have also been shown to give prognostic information for patients with DCIS [6,7].

Mitotic frequency is, together with tubular differentiation and nuclear grade, a parameter in the histological grading that is used for the prognostic evaluation of invasive breast cancers [8,9]. In general, mitotic frequency is assessed by counting the number of mitoses in ten high-power fields (HPFs) according to Elston [8]. Alternative methods to assess mitosis have been described. Contesso and colleagues registered the highest number of mitoses in a single HPF after examination of twenty successive HPFs [10]. Simpson and colleagues calculated the ratio between the total number of mitoses in ten HPFs and the total number of tumour cells in the same ten fields [11]. Mitotic frequency is mentioned by some authors in their description of the histological characteristics of DCIS [1,7]. The European Commission Working Group on Breast Screening Pathology (ECWGBSP) has

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stated that there is a need to find a usable method to estimate the mitotic frequency in DCIS [12].

In this study, we have chosen to use the approach of Contesso, although somewhat modified, in order to estimate the mitotic frequency in DCIS. Our aim was to study this parameter as well as cellular polarisation, and to assess their importance with regard to the ipsilateral local recurrence-free interval (IL-RFI), and their associations with the previously evaluated histopathological parameters and cellular biological factors [4,13].

2. Patients and methods

2.1. Patients

From 1987 to 1991, 327 cases of DCIS were registered in the population-based cancer registry of the Southern Swedish Health Care Region. Details about the patients have been previously described in Refs. [4,13]. After histopathological re-evaluation, 306 cases remained. Of these, 119 were treated with mastectomy and 66 with breast-conserving treatment (BCT) with postoperative radiotherapy (RT). In the present study, only the 121 patients who were treated with BCT without RT were included (median age 58 years, range 30–84 years). These cases were morphologically re-evaluated to determine their cellular polarisation and mitotic frequency.

2.2. Methods

All specimens were routinely prepared; fixed in 10% buffered formalin, embedded in paraffin, sliced into 5 μm thick sections and stained with haematoxylin-erythrosin. The cases were re-evaluated by one of the authors according to Holland's definition of cellular polarisation into three groups: (1) marked cellular polarisation with luminally oriented nuclei, (2) cellular polarisation present, but with solid areas without cellular polarisation, (3) absent or minimal cellular polarisation [5].

Evaluation of mitosis was performed by counting the mitotic figures when cells were in prophase, metaphase and anaphase. Hyperchromatic nuclei representing individual cell necrosis or cell apoptosis were excluded from the analysis. The strict criteria, originally described by Baak and presented again in detail in an editorial in 1990, were used [14,15]. These include absence of the nuclear membrane, presence of hairy projections of the mitotic figure, and basophilic rather than eosinophilic surrounding cytoplasm. Unlike Contesso, who calculated 20 HPFs within the tumour, we screened the whole lesion at a low magnification (×10), in the same manner as a cytological smear. This modification was necessary due to the distinct growth properties of DCIS, which mostly presents as a diffusely spread lesion with cancerous ducts that are surrounded by some mesenchymal tissue. This contrasts with invasive breast cancer tissue which presents as a distinct hearth. Suspected mitosis was noted, and then verified or rejected, using a high magnification (×40). Tumour cells with dense, eosinophilic cytoplasm, sometimes with a halo, and surrounding coarse, clumped nuclear material with rounded, broad-based triangular or spiky, rather than thin, hairy, projections, were judged to be apoptotic figures. The highest number of mitoses in any HPF within the tumour was registered. This HPF usually contained a whole or a part of a large cancerous duct, but could in individual cases contain several small cancerous ducts or a lobule with cancerisation. The DCIS lesions evaluated in this study were categorised into three subgroups: 0, 1 and $\geqslant 2$ mitoses. Contesso and co-workers used the same field area (0.159 mm^2), and a similar subgrouping: 0–1, 2 and 3 or more mitoses [10]. The evaluation of nuclear grade, comedo-type necrosis and growth pattern, as well as immunohistochemical and flow cytometric analyses of cellular biological factors, using this material, has been previously described in Refs. [4,13].

2.3. Statistics

The primary end-point of this study was the determination of the ipsilateral local recurrence-free interval (IL-RFI). This was defined as the time from diagnosis of DCIS to first local recurrence (DCIS or invasive cancer) in the breast. Thus, follow-up was censored at death or at the last clinical investigation of the patient. Associations between the two new factors, cellular polarisation and mitotic frequency, and other histopathological and biological factors were analysed by means of cross-tables and Chi-square tests. The prognostic effect of the two new factors on the IL-RFI was analysed in univariate Cox analyses and in multivariate analyses, also including nuclear grade and growth pattern, that were previously found to be of prognostic importance. The original grouping with three groups (coded 1, 2 and 3 for cellular polarisation and 0, 1 and 2 or more for mitotic frequency) was used both in analyses with separate categories and in the analyses for trend. Kaplan-Meier curves were used for the illustrations.

3. Results

3.1. The association between cellular polarisation/mitotic frequency and other histopathological parameters/cell biological factors

When the samples were assessed for cellular polarisation, most of the 121 DCIS cases were classified as either 1 or 2, 40% (49/121) and 48% (58/121), respectively, whereas only 12% (14/121) were classified as 3.

No mitosis was found in 17 of the 121 DCIS cases (14%). A mitotic classification of 1 was found in 45 cases (37%) and two or more was observed in 59 (49%).

A lower extent of cellular polarisation, as well as a higher mitotic frequency, were significantly associated with a higher nuclear grade, the presence of necrosis, ER-negativity, a high expression of c-erbB-2 and Ki67 and the accumulation of p53 (Table 1). A higher mitotic frequency, but not cellular polarisation, was significantly associated with PgR-negativity and a non-diploidy DNA status. Neither of these factors correlated with the growth pattern and expression of bcl-2. Mitotic frequency and cellular polarisation were also associated with one another (Table 1).

3.2. The association between cellular polarisation/mitotic frequency and the ipsilateral local recurrence-free interval (IL-RFI)

In a previous study of the same patients and over the same follow-up period [4], we showed that patients with nuclear grade 3 (high-grade) lesions have a significantly shorter IL-RFI compared with those with nuclear grade 1 + 2 (non-high-grade) lesions (Relative Risk (RR) = 2.5;

Table 1
Association between cellular polarisation and mitotic frequency and other histopathological and cell biological factors

Parameter	Number of patients	*P* value	
		Cell. polarisation (1 versus 2 versus 3)	Mitotic frequency (0 versus 1 versus ⩾2)
Nuclear grade			
1	12		
2	48		
3	61	0.014	<0.001
Necrosis			
Not present	49		
Present	72	0.004	<0.004
Growth pattern			
Non-diffuse	66		
Diffuse	55	0.30	0.89
ER status			
Positive	65		
Negative	39	0.002	0.016
PgR status			
Positive	45		
Negative	60	0.29	0.005
p53			
Normal	82		
Accumulated	25	<0.001	<0.001
c-erbB-2			
Normal	49		
Overexpressed	59	0.017	<0.001
Ki67			
Low	63		
High	45	<0.001	<0.001
bcl-2			
Low	43		
High	65	0.13	0.082
DNA ploidy status			
Diploid	74		
Non-diploid	39	0.17	0.010
Mitotic frequency			
0	17		
1	45		
⩾2	59	<0.001	–

ER, Oestrogen Receptor; PgR, Progesterone Receptor.

95% CI: 1.2–5.4). Subgroups based on the growth pattern (diffuse versus non-diffuse) showed a similar trend [RR = 2.0 (95% CI: 0.9–4.1)]. In the present study, cellular polarisation (3 versus 2 versus 1) and mitotic frequency (⩾2 versus 1 versus 0) were significant factors for IL-RFI in the univariate Cox regression analyses (Table 2) (test for trend: $P = 0.049$ and $P = 0.040$; see also Figs. 1 and 2 for illustrations). However, in two

Table 2
Univariate and multivariate Cox regression analyses of ipsilateral recurrence-free interval (IL-RFI) with nuclear grade (ng), growth pattern, and with (a) cellular polarisation or (b) mitotic frequency as predictors (RR = relative risk)

Factor	Univariate analyses		Multivariate analyses	
	RR (95% CI)	*P* value	RR (95% CI)	*P* value
(a)				
Cellular polarisation				
(2 versus 1)	1.6 (0.7–3.6)	0.27	1.2 (0.5–2.9)	0.61
(3 versus 1)	3.2 (1.1–9.9)	0.039	2.0 (0.6–6.5)	0.24
Trend (RR increase per step)	1.8 (1.0–3.1)	0.049		
Grade (ng 3 versus ng 1 and 2)	2.5 (1.2–5.4)	0.017	2.1 (1.0–4.7)	0.065
Growth pattern (diffuse versus non-diffuse)	2.0 (0.9–4.1)	0.070	1.7 (0.8–3.6)	0.15
(b)				
Mitotic frequency				
(1 versus 0)	2.4 (0.5–11)	0.26	2.1 (0.5–9.9)	0.33
(⩾2 versus 0)	3.9 (0.9–17)	0.072	2.5 (0.5–12)	0.26
Trend (RR increase per step)	1.8 (1.0–3.2)	0.040		
Grade (ng 3 versus ng 1 and 2)	2.5 (1.2–5.4)	0.017	1.9 (0.8–4.7)	0.15
Growth pattern (diffuse versus non-diffuse)	2.0 (0.9–4.1)	0.070	1.8 (0.9–3.8)	0.11

95% CI, 95% Confidence Interval.

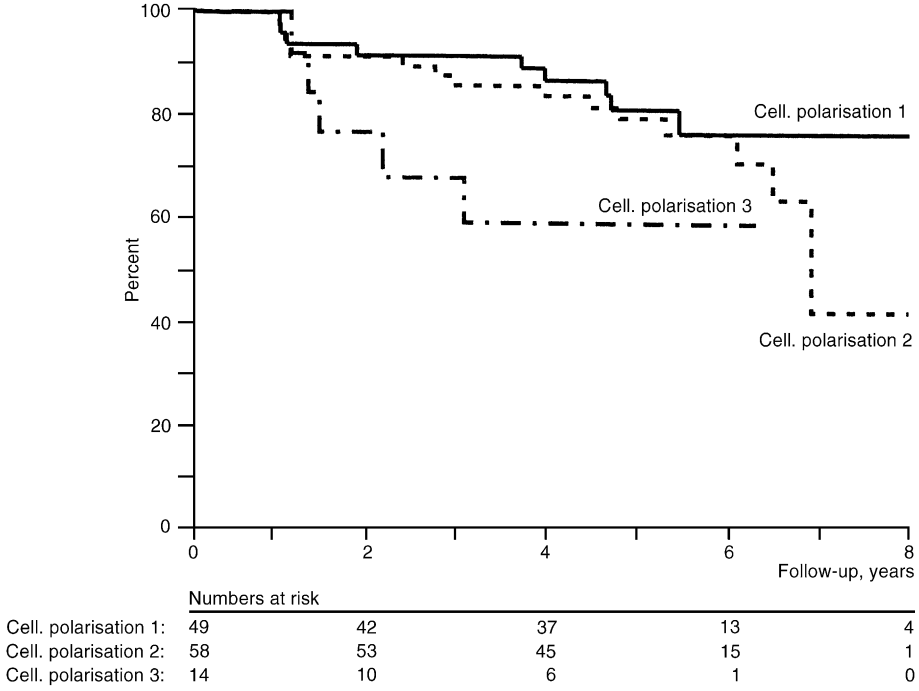

Fig. 1. Ipsilateral local breast recurrence rate according to cellular polarisation.

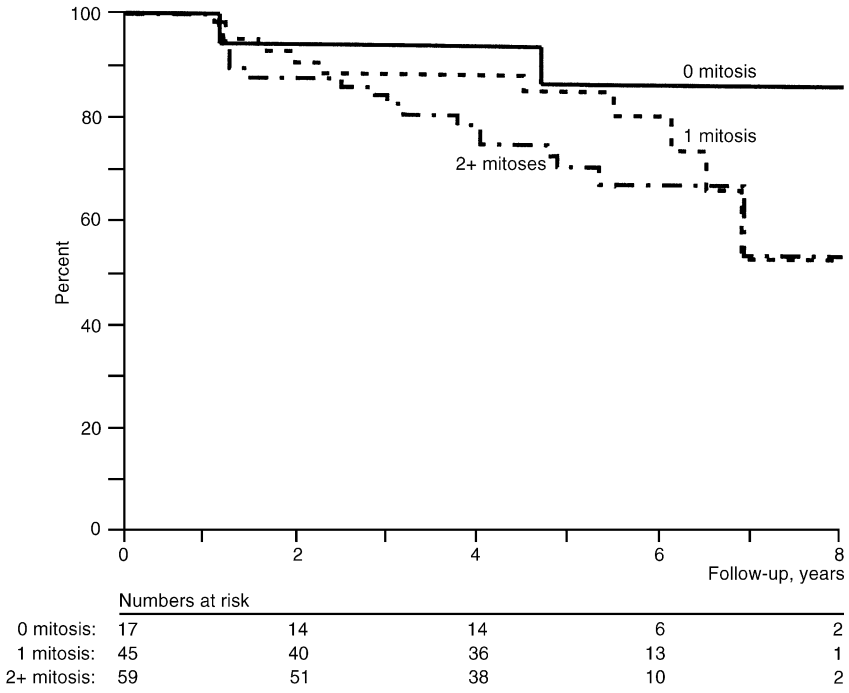

Fig. 2. Ipsilateral local breast recurrence rate according to the mitotic frequency.

separate multivariate analyses, neither cellular polarisation nor mitotic frequency were independent prognostic factors when analysed together with grade and the growth pattern (Table 2). Similarly, neither cellular polarisation nor mitotic frequency were independent prognostic factors when included with only nuclear grade in the bivariate analyses [test for trend: $P=0.28$ (cellular polarisation) and $P=0.30$ (mitotic frequency)].

The different combinations of cellular polarisation and nuclear grade, and also the frequency of ipsilateral local recurrences are illustrated in Table 3. In concordance with the results obtained in the multivariate analyses, differences in cellular polarisation in patients with the same nuclear grade did not provide any additional prognostic information. Similarly, when mitotic frequency and nuclear grade are combined, the mitotic frequency gave no additional information (Table 4).

Table 3
Percent of recurrences in patients grouped by nuclear grade and cellular polarisation. Number of recurrences/number of patients in brackets

Nuclear grade	Cellular polarisation		
	1	2	3
1	0 (0/7)	20 (1/5)	0 (0)
2	19 (5/26)	16 (3/19)	30 (1/3)
3	31 (5/16)	35 (12/34)	36 (4/11)

Table 4
Percent of recurrences in patients grouped by nuclear grade and number of mitoses. Number of recurrences/number of patients in brackets

Nuclear grade	Number of mitoses		
	0	1	⩾2
1	0 (0/2)	12 (1/8)	0 (0/2)
2	0 (0/11)	20 (5/25)	33 (4/12)
3	50 (2/4)	33 (4/12)	33 (15/45)

4. Discussion

Cellular polarisation and mitotic frequency were, in univariate analyses, factors of prognostic importance (IL-RFI) for our patients with DCIS ($P=0.049$ and $P=0.040$, respectively, test for trend). However, neither of them contributed significantly to prognosis in two separate multivariate analyses, including nuclear grade (with or without the growth pattern). These findings were further underlined when the frequency of recurrences was investigated for different combinations of nuclear grade and cellular polarisation/mitotic frequency (Tables 3 and 4). Both our study, as well as that of Wärnberg and colleagues [7], could separate a small group of highly differentiated DCIS (nuclear grade 1 and cellular polarisation 1) where no recurrences were found. However, a conclusion based on such a low number of patients [13 (8.7%) in the study by Wärnberg and colleagues and 7 (6%) in ours] is uncertain, and may be of limited clinical value. In another DCIS study, the impact of cellular polarisation is discussed, and the authors assert that the addition of cellular polarisation to nuclear grade is not of significant prognostic value

[16]. On the other hand, in a study of pathologists examining the reproducibility of different DCIS-classification schemes, the estimation of cellular polarisation together with nuclear grade according to Holland and colleagues [5] gave a better diagnostic consistency compared with the use of nuclear grade only [17].

The finding that the mitotic frequency was not an independent prognostic factor may be explained by the fact that the approach to assess mitoses is not optimal, and/or that the mitotic frequency is of limited prognostic value in DCIS. A further alternative explanation is that the results are due to chance; the present study is relatively small and thus the power to detect moderate independent prognostic effects is limited. 30th Elston's and Contessa's method of counting mitoses in DCIS, as used for invasive breast cancers [8,10], are problematic when examining the DCIS samples, due to their scattered growth, with the result that many HPFs contain a lot of normal tissue surrounding occasional cancerous ducts. This differs to observations using HPFs of invasive cancer samples where minimal normal tissue is seen. Mitoses in DCIS mostly occur in only a few ducts, even if the DCIS-lesion is widespread. This characteristic makes Contesso's method possible to use, but has to be modified so that mitotic frequency is counted not in 20 HPFs, but in all of the cancerous ducts of the lesion considering the highest number of mitoses in any of the examined HPFs. Due to the different sizes of the DCIS-lesion, the number of HPFs counted varied widely in our study, ranging from only a few to hundreds. Logically, the maximum number of mitoses was, on average, larger in cases where many HPFs were counted than in similar cases with only a few number being examined. However, this effect is reduced by including two or more mitoses in the same category. Nevertheless, the mitotic category also reflects the number of HPFs, at least to some extent. The mitotic frequency assessed according to the approach used in our study, was strongly associated with the proliferative marker Ki67, validating this assessment of mitosis in the DCIS samples.

In the present study, we speculated that it might be possible to construct a prognostic instrument for DCIS, corresponding to histological grade in invasive breast cancer cases [9]. But since we could not demonstrate that cellular polarisation and mitotic frequency were independent prognostic factors, this study does not support their use as a prognostic instrument for DCIS.

In conclusion, both cellular polarisation and mitotic frequency were associated with the ipsilateral recurrence-free interval. However, when including nuclear grade (with or without the growth pattern) in the multivariate analyses, neither of these factors were of any independent prognostic value. Nevertheless, this study is rather small and the power to detect the additional prognostic value of any new factors may be limited. Therefore, whether cellular polarisation and mitotic frequency give independent prognostic information in DCIS patients is still open to debate.

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