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European Journal of Cancer 37 (2001) 1514–1522

European Journal of Cancer

www.ejconline.com

Cell biological factors in ductal carcinoma *in situ* (DCIS) of the breast-relationship to ipsilateral local recurrence and histopathological characteristics

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Received 27 September 2000; received in revised form 23 March 2001; accepted 23 March 2001

Abstract

All cases of ductal carcinoma *in situ* (DCIS) diagnosed from 1987 to 1991 in the Southern Health Care Region of Sweden, and operated upon with breast conserving treatment (BCT) with ($n=66$) or without ($n=121$) postoperative radiation (RT) were clinically followed, morphologically re-evaluated and analysed for cell biological factors (immunohistochemical assays or DNA flow cytometry). Median age at diagnosis was 58 years (range 29–83 years) and median follow-up was 62 months. Oestrogen (ER)- and progesterone receptor (PR)-negativity, c-erbB-2 overexpression, low bcl-2 expression, p53 accumulation, DNA non-diploidy and high Ki67, were strongly associated with high grade DCIS, and comedo-type necrosis. In contrast, significant associations to growth pattern (not diffuse versus diffuse) were seen only for c-erbB-2 and PgR. There was also a strong relationship between the cell biological factors, and a summary cell biological index based on principal component analysis was introduced (CBI-7). In the group that had not received postoperative RT, 31 ipsilateral local recurrences occurred (13 invasive, 18 DCIS). Ipsilateral recurrence-free interval (IL-RFI) was in univariate analyses significantly, or almost significantly, shorter for patients showing p53 accumulation, high Ki67 or low bcl-2, compared with patients with normal p53, low Ki67 and high bcl-2. The prognostic importance of the remaining cell biological factors was less pronounced. On the other hand, the index CBI-7, was a strong predictor for recurrence.

Keywords: Breast cancer; Ductal carcinoma *in situ*; DCIS; Nuclear grade; Growth pattern; Cell biological factors; Prognosis; Outcome; Recurrence

1. Introduction

Since the introduction of mammographic screening, the incidence of *in situ* carcinoma of the breast has increased in frequency from a few per cent up to 10–15% of all breast cancers [1–3]. Treatment with mastectomy (ME) has been reported to be curative in 95–100% of the cases [4–6]. Breast conserving treatment (BCT) and postoperative radiotherapy (RT) also had a good prognosis, similar to that after ME [5-year ipsilateral recurrence-free interval of 94% (BCT + RT) versus 96% (ME)], in a population-based consecutive series of patients with DCIS from our healthcare region [7]. In another study, a higher ipsilateral recurrence rate was, however, obtained after BCT + RT than after ME [8]. Patients operated upon with BCT without RT have, compared with those operated upon with BCT + RT, an increased frequency of ipsilateral breast tumour recurrences [7,9–12]. It would be desirable to identify a group of patients, with a very low risk of developing ipsilateral recurrences, that will not need RT after BCT. Several histopathological parameters have been shown to yield such prognostic information: e.g. nuclear grade, comedo-type necrosis, margins, size of the lesions, degree of differentiation and growth pattern [13–16]. Our group has recently demonstrated that nuclear grade was a prognostic factor for ipsilateral recurrence-free

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0959-8049/01/$ - see front matter
PII: S0959-8049(01)00165-4

interval (IL-RFI), and that both growth pattern and margins seem to give additional information [7].

DCIS can also be characterised on the basis of cell biological factors, e.g. steroid receptors, oncogenes, tumour suppressor genes, DNA ploidy status and proliferative markers. So far, analyses of these cell biological factors in DCIS have mainly provided information on their occurrence and relationship to histological characteristics [17–22]. Their prognostic importance has only been investigated in a few small studies, indicating that c-erbB-2, bcl-2, p53 and Ki67 might predict recurrence [23–25].

The aim of the present study was to perform a more extensive investigation concerning cell biological factors [oestrogen receptor (ER), progesterone receptor (PgR), c-erbB-2, bcl-2, p53, DNA ploidy status and Ki67] and their correlation to the development of ipsilateral recurrences. Furthermore, the associations between these factors and histopathological characteristics (nuclear grade, comedo-type necrosis and growth pattern) were examined.

2. Patients and methods

2.1. Patients

Between 1 September 1987 and 31 December 1991, 327 cases were registered as DCIS at the population-based Regional Tumour Registry in Lund. They were all subjected to histopathological re-evaluation, as previously described [7]. 21 cases were excluded [one invasive carcinoma and one lobular carcinoma *in situ* (LCIS) had been incorrectly coded (2/327 = 0.6%) and 15 microinvasive carcinomas, three ADH and one proliferating fibrocystic adenosis had initially been diagnosed as DCIS, but at re-evaluation the diagnosis was changed (19/327 = 5.8%)]. Of the remaining 306 cases, 119 (39%) were treated with ME and 187 (61%) with BCT. 67 patients (22%) had received postoperative RT, all but one after BCT. In the present investigation, only those 187 patients operated upon with BCT were included. 127 (68%) of these DCISs were detected by mammographic screening, 30 (16%) by clinical symptoms, and 30 (16%) as *en passant* findings. Median age was 58 years (range 29–83 years).

2.2. Histopathological re-evaluation

The 187 cases of DCIS, treated with BCT, have been retrospectively classified [7]. The following parameters were considered: Nuclear grade (1–3; [26]), comedo-type necrosis (not present versus present; [27]), growth pattern (not diffuse versus diffuse [7,28], margins ($\geqslant$1 mm versus involved versus not evaluated [7]), size ($\leqslant$15 mm versus $>$15 mm versus not recorded; [7]). Nuclear grade and necrosis were combined in the van Nuys classification [27], and thus grouped in non-high grade with or without necrosis, and high grade (independent of presence of necrosis). Growth pattern was classified according to Andersen and coworkers into microfocal, tumour forming, diffuse or mixed (microfocal and/or tumour forming + a diffuse component), and reported on as not diffuse versus diffuse growth pattern [7].

2.3. Immunohistochemical assay of ER, PgR, c-erbB-2, bcl-2, p53 and Ki67

For all cases, serial sections (4 μm) were cut from formalin-fixed, paraffin-embedded blocks. The sections were dried at 60°C for 1 h. After dewaxing and rehydration, the sections were treated with 10 mM citrate buffer (pH 6.0, 15 min) in a microwave oven [29]. The slides were stained in an automatic immunostainer TechMate 500 (Ventana Biotek) with Dako ChemMate Detection Kit peroxidase/DAB. The dilutions used for each antibody are detailed in Table 1. The incubation time was 45 min for the antibodies against ER and PgR and 25 min for the remaining antibodies. The blocks were sectioned and the slides were stained at one department. The evaluation of the staining was performed by two pathologists (one evaluated ER and PgR, whereas the other evaluated c-erbB-2, bcl-2, p53 and Ki67). ER and PgR positivity, overexpression of c-erbB-2, high bcl-2, accumulation of p53, high rate of Ki67 were defined as more than 10% stained nuclei (ER, PgR, p53, Ki67), cytoplasm (bcl-2), or cell membrane (c-erbB-2). In DCIS, 10% has previously been applied as a cut-off point by Quinn and coworkers for defining ER, c-erbB-2, bcl-2 and p53 positivity [22], and by Rudas and colleagues for ER, PgR and p53 [30].

2.4. Flow cytometric DNA analysis

A 50 μm section was disintegrated and analysed principally according to the method described by Schutte and coworkers [31] including treatment with trypsin and staining with propidium iodide. The DNA content in individual nuclei was analysed in an Ortho-Cytoron Absolute (Ortho Diagnostic System, Rariton, NJ, USA [32]). In accordance with the Convention of Nomenclature

Table 1
The description of antibodies used in this investigation

Antigen	Clone	Dilution	Purchased from	Catalogue no.
ER	1D5	1:100	Dako	M7047
PgR	Polyclonal	1:50	Dako	A0098
c-erbB-2	CB11	1:200	Novocastra	NCLCB11
p53	DO-7	1:200	Dako	M7001
bcl-2	124	1:50	Dako	M0887
Ki67	MIB-1	1:100	Immunotech	0505

ER, oestrogen receptor; PgR, progesterone receptor.

for DNA Cytometry [33], ploidy status was defined as follows: diploid if one DNA cell population and non-diploid if two or more cell populations.

2.5. Statistics

Associations within cell biological factors, and between cell biological factors and histopathological parameters were analysed using odds ratios and logistic regression. The interrelationship between the cell biological factors was further studied by means of a principal component analysis [34], and a simple cell biological index (CBI-7) was constructed based on the first principal component. Ipsilateral local recurrence-free interval (event: DCIS or invasive cancer) was analysed using the Cox proportional hazards model. Follow-up was censored at death or at the last clinical investigation of the patient. All statistical tests were two-sided.

3. Results

3.1. The distribution of histopathological and cell biological factors

Seventeen of the 187 DCIS were nuclear grade 1 (9%), 74 nuclear grade 2 (40%), and 96 nuclear grade 3 (51%). Presence of comedo-type necrosis was found in 118 (63%) of the lesions, and a diffuse or mixed growth pattern was found in 82 (44%). One hundred and eight (58%) of the tumours were operated with a margin $\geqslant 1$ mm, while in 8 cases (4%) the margins were involved, and in 71 cases (38%) not evaluated. The tumour size was 15 mm or below in 99 (53%), above 15 mm in 17 cases (9%), and not assessed in 71 cases (38%).

For 158 of the 187 samples (84%), all immunohistochemical analyses could be evaluated. The remaining 29 cases were completely or partially excluded for one of the following reasons: no cancer left in the sections ($n=11$), presence of cancer in only some of the sections ($n=15$), and no blocks available ($n=3$). Sections for flow cytometric DNA ploidy analysis were available in 175 cases, of which DNA ploidy status could be evaluated in 172 (98%), and for 152 cases there was information for all cell biological factors and ploidy status. ER and PgR positivity was found in 60% (97/163) and 43% (71/165) of the DCIS lesions, respectively. Fifty-four per cent (92/171) showed c-erbB-2 overexpression. The corresponding figures for high bcl-2 and p53 accumulation were 56% (96/170) and 26% (45/170), respectively. Non-diploidy was found in 60% (103/172) of the cases, whereas a high rate of proliferation (Ki67) was found in 42% (72/170). No association was found between age and the biological factors; the mean age varied between 57 and 59 years in the subgroups determined by the simple categorisations of the cell biological factors above.

3.2. The associations between the different cell biological factors, and between cell biological factors and histopathological characteristics

3.2.1. Between the cell biological factors

ER and PgR positivity were positively associated with high bcl-2 and negatively associated with overexpression of c-erbB-2, accumulation of p53 and high Ki67 (Table 2). For instance, the ratio of bcl-2 odds (high versus low) in the ER-positive compared with the ER-negative group was 5.8 (95% confidence interval (CI): 2.9–11.6). Overexpression of c-erbB-2 and accumulation of p53 were positively associated to each other and negatively to bcl-2. DNA non-diploidy was significantly associated only to the accumulation of p53. Since there was a strong interrelationship between the cell biological factors, it was further studied by means of principal component analysis (data not shown). The first principal component correlated very strongly with the sum of the number of factors indicating an aggressive tumour (ER and PgR negativity, overexpression of c-erbB-2, low bcl-2, accumulation of p53, non-diploidy, and high Ki67, correlation coefficient = 0.99). Hence this sum was used as a cell biological index, called CBI-7 in the further analyses.

3.2.2. Nuclear grade

ER and PgR negativity, overexpression of c-erbB-2, low bcl-2, accumulation of p53, non-diploidy and high proliferation were positively associated with high grade lesions in univariate analyses (Table 3; $P=0.003$ or less). In addition, the CBI-7 was strongly associated with grade (Table 3, Fig. 1). A multivariate logistic regression analysis yielded that PgR negativity, overexpression of c-erbB-2 and high Ki67 were strong independent determinants for high grade DCIS (Table 3).

3.2.3. Comedo-type necrosis

Similar to nuclear grade, comedo-type necrosis was also significantly associated with all the cell biological factors and CBI-7 in univariate analyses (Table 4, Fig. 1, $P=0.044$ or less) with Ki67, followed by DNA ploidy status, as the two strongest factors in a multivariate logistic regression analysis.

3.2.4. Growth pattern

PgR negativity and overexpression of c-erbB-2 were significantly associated with the growth pattern in both uni- and multivariate analyses (Table 5). No relationship was found between growth pattern and the other cell biological factors, nor the CBI-7 (Fig. 1).

3.2.5. Size

No significant association was found between size and the cell biological factors. The results were similar when excluding the cases of DCIS with no recorded size.

3.3. Ipsilateral local recurrence-free interval (IL-RFI) for patients treated with BCT without RT

Among the patients treated with BCT without RT ($n = 121$), 31 developed ipsilateral local recurrence (26%; 13 invasive and 18 DCIS) and 4 (3%) ended follow-up due to death from other causes than cancer. The median follow-up for the patients without recurrence was 62 months. In a previous study with the same patients and follow-up, analysing clinical and pathological risk factors [7], it was shown that patients with high grade lesions have a significantly shorter IL-RFI compared with those with non-high grade lesions [relative risk (RR) = 2.5 (95% CI: 1.2–5.4)]. Subgroups based on growth pattern (diffuse versus not diffuse) showed a similar trend [RR = 2.0 (95% CI: 0.9-4.1)].

Univariate Cox regression analyses of IL-RFI with the cell biological factors as predictors yielded that only

Table 2
Odds ratios (positive versus negative) and 95% confidence intervals (CIs) for all pairwise comparison between cell biological factors. A factor is positive if more than 10% of the cells are stained or if diploidy is present

	ER	PgR	c-erbB-2	bcl-2	p53	ploidy	Ki67
PgR	13.2*** (6.6–30.9)						
c-erbB-2	0.14*** (0.1–0.3)	0.32*** (0.1–0.6)	–				
bcl-2	5.8*** (2.9–11.6)	3.4*** (1.8–6.8)	0.3*** (0.2–0.6)	–			
p53	0.15*** (0.07–0.32)	0.35** (0.16–0.75)	4.3*** (2.0–9.5)	0.30*** (0.15–0.62)	–		
ploidy	1.4 (0.73–2.7)	1.0 (0.51–1.8)	0.62 (0.33–1.2)	1.2 (0.65–2.3)	0.43* (0.21–0.87)	–	
Ki67	0.53* (0.28–0.99)	0.47* (0.25–0.90)	2.6** (1.4–4.8)	0.41** (0.22–0.77)	4.9*** (2.3–10.3)	0.36** (0.19–0.69)	–

ER, oestrogen receptor; PgR, progesterone receptor.
*$0.01 < P \leqslant 0.05$; **$0.001 < P \leqslant 0.01$; ***$P \leqslant 0.001$.

Table 3
Distribution of cell biological factors by grade, (high grade versus non-high grade DCIS), and univariate and multivariate logistic regression analyses of grade with cell biological factors as predictors

Cell biological factor	Distribution		Logistic regression analysis	
	High grade (%)	Non-high grade (%)	Univariate OR (95% CI)	Multivariate OR (95% CI)
ER				
positive	48	72	0.4 (0.2–0.7)	1.6 (0.6–4.5)
negative	52	28		
PgR				
positive	27	60	0.2 (0.1–0.5)	0.3 (0.1–0.8)
negative	73	40		
c-erbB-2				
overexpressed	72	35	4.7 (2.5–9.0)	3.8 (1.6–8.7)
normal	28	65		
bcl-2				
high	43	71	0.3 (0.2–0.6)	0.6 (0.3–1.4)
low	57	29		
p53				
accumulated	39	13	4.1 (1.9–8.7)	1.8 (0.7–4.8)
normal	61	87		
DNA ploidy status				
non-diploid	51	29	2.6 (1.4–4.9)	2.4 (1.0–5.4)
diploid	49	71		
Ki67				
high	62	21	6.4 (3.2–12.7)	3.5 (1.5–8.0)
low	38	79		
CBI-7 (increase/unit)	–	–	2.0 (1.6–2.5)	–

OR, odds ratio; ER, oestrogen receptor; PgR, progesterone receptor; DCIS, ductal carcinoma *in situ*; CI, confidence interval; CBI-7, cell biological index.

bcl-2, p53 and Ki67 were significant, or almost significant (Table 6). To see if any of the cell biological factors might add prognostic information, when grade and growth pattern were accounted for, multivariate analyses were performed including these variables and one cell biological factor at a time. However, none of the individual cell biological factors reached significance (data not shown). In order to evaluate if the number of cell biological factors, indicating an aggressive tumour, was of prognostic importance, the CBI-7 was used for those 98 cases with data on all cell biological factors. CBI-7 was thereby found to be highly significant in a univariate analysis (RR = 1.3 for one unit increase of CBI-7; 95% CI: 1.1–1.6; Tables 6 and 7). The prognostic value of CBI-7 remained in a multivariate analysis also including grade and growth pattern RR = 1.3 (95% CI: 1.0–1.6). The latter two factors, however, were far from significance in this model [grade: RR = 1.4 (95% CI: 0.5–4.2], and growth pattern: RR = 1.5 (95% CI: 0.6–3.6].

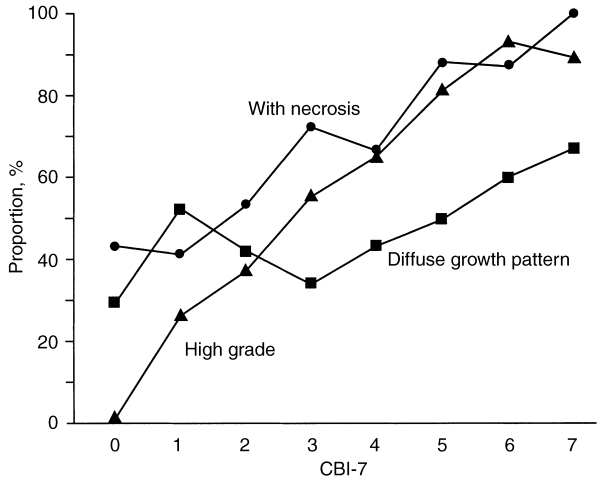

Fig. 1. Proportion of high grade tumours, tumours with comedo-type necrosis, and tumours with a diffuse growth pattern in relation to the sum of the number of cell biological factors (CBI-7 = cell biological index), indicating an aggressive tumour.

4. Discussion

All cell biological factors (ER, PgR, c-erbB-2, bcl-2, p53, DNA ploidy status and Ki67) were significantly associated with nuclear grade (non-high grade versus high grade) and comedo-type necrosis (not present versus present). In contrast, only c-erbB-2 and PgR were significantly associated with growth pattern. The cell biological factors were also strongly associated to one another, and the CBI-7 was therefore introduced, based on principal component analysis and equal to the sum of the number of factors indicating an aggressive

Table 4
Distribution of cell biological factors by comedo-type necrosis, (present versus not present), and univariate and multivariate logistic regression analyses of necrosis with cell biological factors as predictors

Cell biological factor	Distribution		Logistic regression analysis	
	With Necrosis (%)	Without Necrosis (%)	Univariate OR (95% CI)	Multivariate OR (95% CI)
ER				
positive	47	71	0.5 (0.2–0.9)	1.3 (0.5–3.5)
negative	53	29		
PgR				
positive	35	58	0.4 (0.2–0.8)	0.5 (0.2–1.3)
negative	65	42		
c-erbB-2				
overexpressed	65	35	3.4 (1.8–6.6)	1.9 (0.9–4.4)
normal	35	65		
bcl-2				
high	48	71	0.4 (0.2–0.7)	0.6 (0.2–1.3)
low	52	29		
p53				
accumulated	32	17	2.2 (1.0–4.7)	0.8 (0.3–2.2)
normal	68	83		
DNA ploidy status				
non-diploid	49	24	3.1 (1.6–6.3)	2.8 (1.2–6.2)
diploid	51	76		
Ki67				
high	55	21	4.5 (2.2–9.3)	3.2 (1.3–7.5)
low	45	79		
CBI-7 (increase/unit)	–	–	1.6 (1.3–1.9)	–

OR, odds ratio; ER, oestrogen receptor; PgR, progesterone receptor; DCIS, ductal carcinoma *in situ*; CI, confidence interval; CBI-7, cell biological index.

Table 5
Distribution of cell biological factors by growth pattern, (diffuse versus not diffuse), and univariate and multivariate logistic regression analyses of growth pattern with cell biological factors as predictors

Cell biological factor	Distribution		Logistic regression analysis	
	Diffuse (%)	Not diffuse (%)	Univariate OR (95% CI)	Multivariate OR (95% CI)
ER				
positive	57	62	0.8 (0.4–1.5)	2.4 (0.9–6.2)
negative	43	38		
PgR				
positive	33	52	0.5 (0.2–0.9)	0.3 (0.1–0.7)
negative	67	48		
c-erbB-2				
overexpressed	66	44	2.4 (1.3–4.5)	2.7 (1.2–6.0)
normal	34	56		
bcl-2				
high	54	59	0.8 (0.5–1.5)	0.9 (0.4–2.1)
low	46	41		
p53				
accumulated	32	22	1.7 (0.8–3.2)	1.8 (0.8–4.4)
normal	68	78		
DNA ploidy status				
non-diploid	43	38	1.2 (0.7–2.3)	1.3 (0.6–2.6)
diploid	57	62		
Ki67				
high	38	46	0.7 (0.4–1.4)	0.3 (0.1–0.8)
low	62	54		
CBI-7 (increase/unit)	–	–	1.1 (1.0–1.3)	— -

OR, odds ratio; ER, oestrogen receptor; PgR, progesterone receptor; DCIS, ductal carcinoma *in situ*; CI, confidence interval; CBI-7, cell biological index.

Table 6
Univariate Cox regression analyses of ipsilateral recurrence-free interval (IL-RFI) with cell biological factors and the cell biological index (CBI-7) as predictors (RR = relative risk). Only non-irradiated patients were included

Cell biological factor	Univariate analyses	
	RR (95% CI)	*P*-value
ER (positive versus negative)	0.5 (0.3–1.2)	0.12
PgR (positive versus negative)	0.6 (0.3–1.3)	0.18
c-erbB-2 (overexpressed versus normal)	1.7 (0.8–3.6)	0.20
bcl-2 (high versus low)	0.5 (0.2–1.0)	0.061
p53 (accumulated versus normal)	2.2 (1.0–4.7)	0.052
ploidy status (non-diploid versus diploid)	1.4 (0.6–3.0)	0.46
Ki67 (high versus low)	2.2 (1.0–4.7)	0.048
CBI-7 (increase/unit)	1.3 (1.1–1.6)	0.002

OR, odds ratio; ER, oestrogen receptor; PgR, progesterone receptor; DCIS, ductal carcinoma *in situ*; CI, confidence interval; CBI-7, cell biological index.

tumour. The CBI-7 was significantly associated with grade and necrosis, but not with growth pattern — a similar pattern as for the individual cell biological factors.

We have previously demonstrated that nuclear grade and growth pattern seem to provide independent prognostic information for IL-RFI in this series of patients with DCIS, treated with BCT without RT [7]. The results concerning the different association for nuclear grade and growth pattern to cell biological factors, obtained in the present study, further strengthened the suggestion that nuclear grade and growth pattern express different biological characteristics of DCIS. The close associations between nuclear grade and cell biological factors indicate that they all contain information on the inherent characteristics of the tumour cell. In contrast, the growth pattern may be more associated to the environmental condition of the surrounding stroma, or the inter-relationship between the tumour cell and the surrounding cells. Overexpression of c-erbB-2 was, together with high Ki67 and DNA ploidy status, the strongest determinant for high-grade lesions and presence of comedo-type necrosis, whereas c-erbB-2 and PgR were the only factors strongly associated with the growth pattern. The importance of c-erbB2 in DCIS is in line with a report showing that c-erbB-2, and its co-receptors c-erbB-3 and c-erbB4, are important for the effect of heregulin-α on cell migration of Paget's disease [35]. c-erbB-3 has previously been shown to have an inverse correlation to c-erbB-2, whereas no correlation was demonstrated between c-erbB-3 positivity and type of DCIS, p53 and PgR expression and proliferative activity [36].

No significant association between size and biological factors was found. However, for a large proportion of

Table 7
Univariate and multivariate Cox regression analyses of ipsilateral recurrence-free interval (IL-RFI) with the cell biological index (CBI-7), grade and growth pattern as predictors (RR = relative risk). Only non-irradiated patients included

Factor	Univariate analyses		Multivariate analyses	
	RR (95% CI)	*P*-value	RR (95% CI)	*P*-value
CBI-7 (increase/unit)	1.3 (1.1–1.6)	0.002	1.3 (1.0–1.6)	0.051
Grade (high grade versus non-high grade)	2.5 (1.2–5.4)	0.014	1.4 (0.5–4.2)	0.52
Growth pattern (diffuse versus not diffuse)	2.0 (0.9–4.1)	0.07	1.5 (0.6–3.6)	0.34

95% CI, 95% confidence interval; CBI-7, cell biological index.

cases (38%) size was not recorded, and this should be considered in the interpretation of the results.

In univariate analyses (median follow-up: 62 months) of predictors for IL-RFI, bcl-2, p53 and Ki67 were at the borderline of significance. The prognostic importance of these factors, and c-erbB-2, has previously been indicated in some small studies [23–25]. However, in multivariate analyses, including nuclear grade and growth pattern, all of the examined cell biological factors were far from significance. The very strong association between nuclear grade and the cell biological factors may explain this. Another approach to investigate their prognostic value was to combine them into the CBI-7. This prognostic index was strongly significant in univariate analysis ($P = 0.002$) and very close to significance in combination with grade and growth pattern ($P = 0.051$), the relative risk increased by 30% for one unit's increase in the CBI-7 in both analyses. The stronger prognostic effect of the CBI-7 compared with the individual cell biological factors may be due to measurement errors of the individual factors. Their relative importance is less when the factors are summed up into an index. One disadvantage with an index is the need for assaying several factors. Further investigations may, however, clarify if a smaller subset of factors will suffice. Other cell biological factors may also be of importance in DCIS (e.g. transforming growth factor beta, cyclins, pS2, cadherins, vascular endothelial growth factors [37–42]. In addition to their importance in prognosticating IL-RFI, the cell biological factors may also be useful for treatment prediction; e.g. ER and PgR may guide the use of adjuvant anti-oestrogen treatment. A beneficial effect of adjuvant tamoxifen for patients with DCIS operated upon with a lumpectomy and postoperative RT has been reported both with regard to ipsilateral and contralateral recurrences [43]. Therapies based on monoclonal antibodies directed against c-erbB-2 may also be a treatment alternative in the future, since a high proportion of DCISs overexpress c-erbB-2. It would also be desirable to find markers for sensitivity or resistance to RT. In invasive breast cancer, *p53* mutations have been suggested to predict sensitivity to radiotherapy [44].

The distribution of cell biological factors (ER-positivity 60%, PgR-positivity 43%, overexpression of c-erbB-2 54%, high bcl-2 56%, accumulation of p53 26%, non-diploidy 60%, and high Ki67 42%) are in line with those of other investigators [17–22]. A certain variation in the results may be explained by random variation due to small patient numbers and the use of different values for the cut-off point. In the publications, referred to above, several cut-off points have been used for defining positive staining: such as any positive staining, 5 and 10%. In comparison with an ongoing study in our region (using the same methodology), concerning the prognostic importance of cell biological factors in small ($\leqslant 10$ mm) invasive breast cancers, the distribution of cell biological factors was quite similar, but with one exception — a considerably smaller proportion of c-erbB-2-overexpressing cases was found (10% of 128 cases; Kaij, pers. commun.). Others have previously obtained similar results, and explained this discrepancy as possibly being due to a group of c-erbB-2-negative lesions with a very transient *in situ* stage [45].

In conclusion, a strong interrelationship was found between the cell biological factors, suggesting the use of a summary cell biological index. Moreover, the cell biological factors were strongly associated with nuclear grade, but not with growth pattern, emphasising that these pathological parameters express different characteristics of DCIS, and that they may be useful as independent prognostic factors. The cell biological index added prognostic information to predict ipsilateral local recurrence.

Acknowledgements

The following were sources of support: Swedish Cancer Society, the Gunnar Nilsson and Elisabeth Nilsson Foundation and the Malmöhus County Council. The excellent technical assistance of Carina Strand and Bo Baldetorp is gratefully acknowledged. We also thank Poul Boiesen, Lennart Bondeson, Erik Holm, Sven Johansson, Karin Lindholm, Otto Ljungberg and Görel Östberg for previously re-evaluating the histopathological slides. We are also indebted to the Regional Tumour Registry in Lund for support in identifying the patient material and Eva Henriksson for preparing the figure.

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