Histopathological and Cell Biological Factors of Ductal Carcinoma in Situ Before and After the Introduction of Mammographic Screening

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With the introduction of mammographic screening the incidence of ductal carcinoma in situ (DCIS) has increased to 10–15% of all breast cancers. The aim of this study was to investigate whether there were any morphological and cell biological differences between DCIS detected during the pre-screening (n = 39) as opposed to the screening period (n = 120). We could not demonstrate any statistically significant differences between the pre-screening and the screening period with regard to nuclear grade, presence of necrosis, the Van Nuys classification system, growth pattern, or cell biological factors (estrogen and progesterone receptors, c-erbB-2, p53, DNA ploidy status, Ki67, and Auer classes). These findings suggest that DCIS tumors detected during the two time periods have a similar malignant potential. DCIS detected during the screening period was further divided into the prevalence period versus the period thereafter, and symptomatic versus screening-detected asymptomatic cases. More cases with diffuse growth patterns were seen during the prevalence period than after the prevalence period, and screening-detected asymptomatic DCISs were more often 15 mm or smaller in diameter than DCISs detected symptomatically.

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With the introduction of mammographic screening, the incidence of ductal carcinoma in situ (DCIS) has increased from a few percent (1) to 10–15% of all breast carcinomas (2, 3). This development has initiated a discussion on whether these DCIS lesions have a lower aggressive potential than the DCIS diagnosed before the introduction of screening (4). To our knowledge, this question has only been investigated in one study, showing no major difference with regard to cellular size (5). Therefore, the aim of the present study was to make a more extensive comparison of DCIS lesions detected during the pre-screening period with those detected during the screening period with regard to both histopathological characteristics (i.e. nuclear grade, necrosis, the Van Nuys classification system, and growth pattern) and cell biological factors (i.e. estrogen and progesterone receptors, c-erbB-2, p53, DNA ploidy status, and Ki67). We also compared the distributions between the following subgroups of DCIS detected during the screening period: the prevalence period versus the period thereafter, and symptomatic versus screening-detected asymptomatic DCISs.

MATERIAL AND METHODS

Material

All cases diagnosed as carcinoma in situ (CIS) at the Department of Pathology/Cytology at Lund University Hospital during two periods, 1978–1982 (pre-screening period, i.e. the period before the mammographic health control was introduced) and 1989–1994 (screening period with mammographic health control) were studied. During these periods surgical specimens from three hospitals were sent for diagnosis. A total of 186 cases of CIS were diagnosed. A re-evaluation was made and some cases, not classified as DCIS according to the criteria of Azzopardi (6), were excluded from further analysis (see below).

The pre-screening period. We chose a time period (1978–1982) well ahead of the start of mammographic healthcare control (1989) in order to prevent interference in the successively increasing use of mammography. Fifty-three cases of CIS were found during this period. Six cases of lobular carcinoma in situ (LCIS) were excluded, 5 cases reclassified as atypical ductal hyperplasia (ADH), and 3

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cases of Paget's disease restricted to the nipple. The remaining 39 cases were classified as DCIS, 7 of them combined with Paget's disease of the nipple. Mean patient age was 59 years (38–86).

The screening period. The cases detected during this period were further divided into two groups: those detected during the first screening period after the introduction of mammographic screening (prevalence period, 1 March 1989 to 30 June 1991), and those detected thereafter, during the following screening rounds (1 July 1991 to 31 December 1994). During the prevalence period, 54 cases of CIS were found. Two cases of LCIS and 6 cases re-evaluated as ADH were excluded. Of the remaining 46 cases of DCIS, one was combined with Paget's disease. Twenty-two cases (48%) were asymptomatic detected by mammographic screening and the remaining 24 (52%) presented symptoms.

In the period after prevalence screening, 79 cases of CIS were diagnosed. Three were LCIS and two were Paget's disease restricted to the nipple and were excluded. Of the remaining 74 cases, classified as DCIS, 47 cases (64%) were asymptomatic detected by mammographic screening and 27 (36%) because of symptoms.

The mean patient age during the screening period was 56 years (27–87).

Histopathological classification

Macroscopy. The tissue sections from surgical specimens of pre-screening DCIS were taken as small pieces from areas suspected by the pathologist of having a discernible lesion. In screening-detected DCIS, areas that had been marked with an indicator wire preoperatively were sampled also as small pieces by the pathologist, as well as areas where there was a macroscopic suspicion of a discernible lesion. All cases were routinely prepared: fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin-erythrosin.

During 1992–1994, 27 cases were prepared as whole-specimen serial sections. With this technique, the surgical breast specimens were transported cold in ice-bags to the Department of Pathology where the margins were inked and the entire specimen was sliced into 3–4 mm-thick sections and all sections with any fibrous tissue were sampled for histopathological evaluation. After stabilization in a microwave oven for 30 min, the sections were routinely processed as described above for the small pieces.

Microscopy. All cases in the present study were re-examined by one of us (II) according to the standardized histopathological protocol used for CIS by the South Sweden Breast Cancer Group (7). In the standardized protocol, several parameters are included, among which the following factors are considered in the comparison of histopathological characteristics of pre-screening and screening DCIS:

- Nuclear grade (ng) was assessed in order of increasing pleomorphism, as described for invasive breast carcinomas (8). Thus, DCIS with little variation in nuclear grade was designated as ng 1, moderate variation, ng 2, and marked variation, ng 3.
- Comedo-type necrosis, henceforth merely necrosis, was registered and combined with nuclear grade resulting in three subgroups in the classification of the Van Nuys system (9): non-high-grade (ng 1 + ng 2) without necrosis, non-high-grade with necrosis, and high-grade independent on the presence of necrosis.
- Growth pattern was classified according to Andersen and co-workers as microfocal, tumor-forming, diffuse or mixed (microfocal and/or tumor-forming + a diffuse component) growth pattern (10), and reported as diffuse versus non-diffuse growth patterns. A microfocal DCIS is a lesion of DCIS up to 5 mm without surrounding stromal reaction. A tumor-forming DCIS is a macroscopically discernible lesion larger than 5 mm with a stromal reaction more or less evident microscopically. A diffuse growth pattern is a macroscopically as well as microscopically ill-defined lesion, larger than 5 mm. The growth pattern was determined by a combined evaluation of the original macroscopic histopathological description and the actual findings on the slides.
- Size was categorized into three groups $\leq$ 15 mm, $>$ 15 mm and not possible to evaluate.

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

Serial sections (4 μm) from formalin-fixed, paraffin-embedded blocks were cut. 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 (11). The slides were stained in an automatic immunostainer TechMate 500 (Ventana Biotek) with Dako ChemMate Detection Kit peroxidase/DAB. The dilution used for each antibody is detailed in Table 1. The incubation time was 45 min for the antibodies against ER and PgR, and 25 min for the remaining antibodies. As previously (12), we defined ER and PgR positivity, overexpression of c-erbB-2, accumulation of p53, high rate of

Table 1

Antibodies used in this investigation

Antigen	Clone	Dilution	Purchased from	Cat. 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
Ki67	MIB-1	1 : 100	Immunotech	0505

Table 2

Distribution (%) of histopathological characteristics of ductal carcinoma in situ (DCIS) detected during the pre-screening and screening periods. The screening period is further divided into the prevalence period and the post-prevalence period (in parentheses)

	Pre-screening (n = 39)	Screening n = 120 (46/74)	p-value
Size (mm)			
≤15	36	51 (46/54)	
>15	15	30 (35/27)	
Not evaluated	49	19 (20/19)	0.002 (0.62) [1]
Nuclear grade			
1	18	16 (17/15)	
2	31	40 (35/43)	
3	51	44 (48/42)	0.60 (0.65)
Necrosis			
No	44	32 (35/31)	
Yes	56	68 (65/69)	0.25 (0.69)
The Van Nuys classification			
Non-high-grade without necrosis	28	31(30/31)	
Non-high-grade with necrosis	21	25(22/27)	
High-grade	51	44(48/42)	0.75 (0.77)
Growth pattern			
Non-diffuse	38	39 (26/47)	
Diffuse	62	61 (74/53)	1.00 (0.023)

[1] The p-values outside the parentheses correspond to the comparison between the pre-screening and screening periods, whereas the p-values in parentheses correspond to the comparisons between the prevalence period and post-prevalence period.

Ki67 as more than 10% stained nuclei (ER, PgR, p53, Ki67), or cell membrane (c-erbB-2). In DCIS, 10% has also been applied as the cut-off by Quinn and co-workers for defining ER, c-erbB-2, and p53 positivity (13), and by Rudas and colleagues for ER, PgR, and p53 (14). For the analyses of cell biological factors, 27 cases were excluded because only large sections were available.

Flow cytometric DNA analysis. A 50 µm section was disintegrated and analyzed principally according to the method described by Schutte and co-workers (15) including treatment with trypsin and staining with propidium iodide (16). The DNA content in individual nuclei was analyzed in Ortho-Cytoron Absolute (Ortho Diagnostic Systems, Rariton, New Jersey, USA). In accordance with the Convention of Nomenclature for DNA Cytometry (17), ploidy status was defined as: diploid if one DNA cell population and non-diploid if two or more cell populations.

Image DNA analysis

A volume of 250–300 µl of the cell suspension after the incubation with trypsin (see above, Flowcytometric DNA analysis) was taken for cytospin (30 × g, 4 min). The cytospin was fixed in 4% neutral buffered formalin for at least 30 min and then Feulgen stained and assessed for the nuclear DNA content with an Image analysis system (Innovative Vision AB, Linköping Sweden, Lab-eye 3PC) as previously described (18). Interpretation of the image cytometric DNA histograms was performed according to the Auer classification (I–IV, (19)). Auer I corresponds to diploid/near diploid with a low rate of proliferation, Auer II to tetraploid with low proliferation, Auer III to either diploid with high proliferation or aneuploid with a low proliferation, and Auer IV to either highly proliferative aneuploid, between 2C and 4C, and tetraploid cases, or hypertetraploid cases above 4C (regardless of proliferation).

Statistics

The software package Stat 5.0 (Stata Corp) was used to perform all the statistical analyses. Subgroup differences in histopathological parameters between pre-screening and screening periods and the association between different factors were tested by means of Fisher's exact test or a χ^2 test for trend. Two-sided p-values below 0.05 were considered significant.

RESULTS

Histopathological characteristics

The pre-screening (1978–1982) vs. the screening period (1989–1994). When comparing the distribution of nuclear grade, necrosis, the Van Nuys classification system and growth pattern between the two periods, no statistically significant differences were found (Table 2). Fifty-one percent were ng 3 (high-grade) during the pre-screening pe-

Table 3

Distribution (%) of histopathological characteristics of ductal carcinoma in situ (DCIS) in relation to the presence of symptoms (symptomatic vs. asymptomatic) during the screening period, 1989–1994

Histopathologic parameter	Symptomatic n = 51	Asymptomatic n = 69	p-value
Size			
≤ 15 mm	39	59	
> 15 mm	41	22	0.047
Not evaluated	20	19	
Nuclear grade			
1	20	13	
2	31	46	
3	49	41	0.22
Necrosis			
No	35	30	
Yes	65	70	0.69
The Van Nuys classification			
Non-high-grade without necrosis	31	30	
Non-high-grade with necrosis	20	29	
High-grade	49	41	0.50
Growth pattern			
Non diffuse	37	41	
Diffuse	63	59	0.85

riod as compared with 44% during the screening period. The percentage of cases with a diffuse growth pattern was almost identical (62% vs. 61%). Size was more often evaluated during the screening period (81% vs. 51%).

During vs. after the prevalence period. In the screening group, the distributions of histopathological characteristics were also similar during and after the prevalence period, with the exception of growth pattern. Significantly more cases with a diffuse growth pattern were found during the prevalence period than after (74% vs. 53%) (Table 2).

Symptomatic vs. screening-detected asymptomatic DCIS during the screening period. The percentage of mammographically detected asymptomatic DCIS cases was 58 (69/120) during the screening period. Consequently, there were 42% (51/120) symptomatically detected DCISs during this time period. When comparing DCIS for distribution of histological parameters detected in these two ways, no significant differences were found, with the exception of size. The percentage of small lesions ($\leq$ 15 mm) was significantly greater for the screening-detected asymptomatic DCISs than for symptomatic DCISs (59% vs. 39%). The percentage of cases, where size was not evaluated, was similar in both groups (Table 3).

The association between growth pattern and other histopathological parameters. A diffuse growth pattern was significantly associated with higher nuclear grade ($p = 0.022$; test for trend), presence of necrosis ($p = 0.001$), and with larger tumors (more than 15 mm in diameter) or tumors where size was not evaluated ($p < 0.001$).

Cell biological factors

The pre-screening vs. the screening period. ER and PgR were present in 46% and 34%, respectively, of DCISs detected during the pre-screening period (Table 4). Similar values were obtained for DCISs detected during the screening period (42% and 29%, respectively). The percentage of cases showing overexpression of c-erbB-2 was also similar between the two time periods (54% vs. 63%). Accumulation of p53 tended to be more common during the pre-screening period than during the screening period (42% vs. 25%; $p = 0.078$). No significant differences were found in DNA ploidy status (DNA-non-diploidy: 47% vs. 44%), proliferation rate (high Ki67: 28% vs. 41%) and Auer classes between the two time periods (Table 4). During the screening period, the distributions of cell biological factors were compared in different subgroups: 1) during versus after the prevalence period (Table 4), and 2) symptomatic versus screening-detected asymptomatic (Table 5). No significant differences were found in these subgroups for any of the examined factors.

The association between cell biological factors and histopathological parameters. ER and PgR negativity, overexpression of c-erbB-2, accumulation of p53, non-diploidy, high Auer classes and high proliferation were positively associated with higher nuclear grade ($p = 0.001$ or less; test for trend), and presence of necrosis ($p = 0.015$ for p53, $p = 0.069$ for ploidy status, and $p < 0.001$ or less for the remaining factors). In contrast, only PgR and c-erbB-2 were significantly associated with growth pattern; DCISs with diffuse growth patterns were more PgR

negative and c-erbB-2 overexpressed than those with non-diffuse growth patterns ($p = 0.031$ and $p = 0.028$, respectively).

DISCUSSION

No significant differences in the distribution of the histopathological characteristics nuclear grade, necrosis, the Van Nuys classification system, and growth pattern were found between DCISs detected during the pre-screening and screening periods. These findings indicate that DCIS detected after the introduction of mammographic screening has a similar aggressive potential compared to that of DCIS detected before screening. Size was evaluated more often in the screening group than in the pre-screening group. This could be attributed to the examination of whole-specimen serial sections instead of the previously used routine method by which small tissue pieces were sampled from macroscopically suspected or wire-indicated areas. Another reason could be a heightened awareness when mammographic material was being examined.

When the prevalence period in the screening period was compared with the post-prevalence period, no differences were found in the distribution of nuclear grade, necrosis, the Van Nuys classification system and size. However, significantly more cases with diffuse growth patterns were found during the prevalence period. When size was related to the way of detection, a greater proportion of small lesions ($\leq$ 15 mm) were found in the screening-detected asymptomatic group than in the symptomatic group (59% vs. 39%). No significant differences in the distribution of the other histopathological parameters were found. It is thus indicated that growth pattern and size were related to when and how DCIS was detected. For the 97 cases with information on both size and growth pattern, during the screening period, the percentage of large lesions with a diffuse growth pattern was reduced from 43% during the prevalence period to 22% during the post-prevalence

Table 4

Distribution (%) of cell biological factors of ductal carcinoma in situ (DCIS) detected during the pre-screening and screening periods. The screening period is further divided into the prevalence period and the post-prevalence period (in parentheses)

Cell biological factor [1]	Pre-screening	Screening	p-value
ER			
Negative	54	58 (60/57)	
Positive	46	42 (40/43)	0.69(0.83) [2]
PgR			
Negative	66	71 (67/76)	
Positive	34	29 (33/24)	0.54 (0.36)
c-erbB-2			
Normal	46	37 (33/41)	
Overexpressed	54	63 (67/59)	0.42 (0.52)
p53			
Normal	58	75 (69/80)	
Accumulation	42	25 (31/20)	0.078 (0.24)
DNA ploidy status			
Diploid	53	56 (58/55)	
Non-diploid	47	44 (42/45)	0.85 (0.83)
Auer class			
I	44	35 (27/44)	
II	25	24 (30/19)	
III	8	11 (9/12)	
IV	22	30 (34/25)	0.75 (0.28)
Ki67			
Low	72	59 (60/58)	
High	28	41 (40/42)	0.22 (1.00)

[1] The number of analyzed cases during the pre-screening period varied between 33 and 38. The corresponding figures during the screening period were 88 and 91.
[2] The p-values outside the parentheses correspond to the comparison between the pre-screening and screening periods, whereas the p-values in parentheses correspond to the comparisons between the prevalence period and the post-prevalence period.

Table 5

Distribution (%) of histopathological characteristics of ductal carcinoma in situ (DCIS) in relation to the ways of detection during the screening period (symptomatic vs. asymptomatic)

Cell biological factor [1]	Symptomatic	Asymptomatic	p-value
ER			
Negative	57	59	
Positive	43	41	1.00
PgR			
Negative	69	73	
Positive	31	27	0.65
c-erbB-2			
Normal	45	31	
Overexpressed	55	69	0.19
p53			
Normal	71	78	
Accumulation	29	22	0.63
DNA ploidy status			
Diploid	57	55	
Non-diploid	43	45	1.00
Auer class			
I	42	29	
II	18	29	
III	12	10	
IV	28	31	0.47
Ki67			
Low	60	58	
High	40	42	1.00

[1] The number of asymptomatic cases varied between 40 and 42. The corresponding figures for symptomatic cases were 47 and 49.

period. The corresponding figures when comparing symptomatic and screening-detected asymptomatic lesions were 44% and 20%, respectively. These figures suggest that mammographic screening results in detection of DCIS at an early stage, and thereby in a reduction in large DCISs with diffuse growth patterns.

Comparisons with other DCIS studies are difficult to make as most are pre-screening studies and some include tumors with a minor invasive component in the definition of DCIS, or use a different type of classification, mainly an architectural pattern (20, 21). Only one study comparing clinically detected and screening-detected DCIS has to our knowledge been performed, and has shown, in agreement with our results, no major difference in cellular size (5).

In agreement with a previous study from our group (22), a diffuse growth pattern was strongly associated with large lesions and to cases where size was not evaluated. Our finding that growth pattern is strongly associated with size and margins (22) suggests that growth pattern may be useful as a complement for prognostic considerations, when the histopathological evaluation of these two parameters is difficult or not possible to do.

Likewise, we compared the distribution of cell biological factors between DCIS detected during the pre-screening and screening periods, without finding any statistically significant differences in the presence of ER and PgR, expression of c-erbB-2, accumulation of p53, DNA ploidy status, Auer classes and Ki67 level. The mode of detection during the screening period, screening-detected asymptomatic versus symptomatic, or detection of DCIS during as opposed to after the prevalence period, was not associated with any differences in the distribution of the cell biological factors. In a study by Poller et al., both symptomatic and screening-detected DCIS were included when investigating ER, c-erbB-2, and p53, but these researchers did not correlate their findings with the mode of detection (23).

In the present study ER and PgR positivity (43% and 34%, respectively) was in the range of those results obtained by other investigators (23–27), and also of those in our previous study [(12) ER +: 60%, and PgR +: 43%]. Furthermore, the percentages of c-erbB-2 overexpression (60%), p53 accumulation (30%), DNA non-diploidy (45%), and Ki67 (38%) were similar to those in our previous study [(12) 54% (c-erbB-2), 26% (p53), 60% (non-diploid), and 42% (Ki67)]. The small amount of material and/or different values for cut-off in the present study could be the reason for any differences compared to the work of other investigators.

The association between cell biological factors and the histopathological pattern is in concordance with other studies, showing an association between high-grade/high nuclear grade DCIS and ER and/or PgR negativity, DNA non-diploidy, overexpression of c-erbB-2, and accumulation of p53 (12, 21–26). The overall strong association between cell biological factors and nuclear grade and the prognostic classification system according to Silverstein and co-workers, and the comparable weak association between the cell biological factors and growth pattern are also a confirmation of the results from our group (12), and indicate that growth pattern and the other histopathological parameters reflect two different characteristics of DCIS. It therefore seems reasonable to include the growth pattern, together with the other histological classification systems, when evaluating the prognostic importance of histopathological parameters in DCIS. A recent investigation from our group has also demonstrated that both nuclear grade and growth pattern are independent prognostic factors for ipsilateral recurrence-free survival, and that a subgroup of DCIS with non-high-grade lesions and a non-diffuse growth pattern with a very low recurrence rate (2/34 (5.9%)) could be identified (22). The usefulness of cell biological factors as prognostic instruments has only been investigated in a few small studies, suggesting that c-erbB-2 and p53 may be useful for prognostic considerations (27–29). We have previously shown that bcl2, p53, and Ki67 are on the borderline of significance for ipsilateral recurrence-free survival in univariate analyses, whereas ER, PgR, c-erbB-2 and DNA ploidy status did not yield any prognostic information (12). When the cell biological factors were included one-by-one in multivariate analyses, together with grade and growth pattern (the two histopathological parameters showing the greatest prognostic impact), none of them reached significance. There was, however, a strong relationship between the cell biological factors and hence a summary cell biological index was introduced. This index was a prognostic factor in both uni- and multivariate analyses (12).

In summary, we found a higher number of cases with diffuse growth patterns during the prevalence period than thereafter, and more small lesions when the DCIS was screening detected in asymptomatic women with mammography screening than in symptomatic women. For all other histopathological characteristics and cell biological factors, no significant differences were found. Taken together, these findings suggest a similar malignant potential for DCIS during the pre-screening and screening periods.

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