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Histopathological risk factors for ipsilateral breast events after breast conserving treatment for ductal carcinoma *in situ* of the breast – Results from the Swedish randomised trial

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ABSTRACT

Aim: The primary aims were to study risk factors for an ipsilateral breast event (IBE) after sector resection for ductal carcinoma *in situ* of the breast (DCIS) in a trial comparing adjuvant radiotherapy to no therapy and to assess predictive factors for response to radiotherapy. Secondary aims were to analyse reproducibility of the histopathological evaluation and to estimate correctness of diagnosis in the trial.

Setting: A randomised trial in Sweden (the SweDCIS trial), including 1046 women with a median of 5.2 years of follow-up in a population, offered routine mammographic screening.

Methods: A case-cohort design with a total of 161 cases of IBE (42 of those being members of the subcohort) and 284 sampled for the sub-cohort. Ninety five percent of the participants' slides could be retrieved and were re-evaluated by three experienced pathologists.

Results: Low nuclear grade (NG 1–2) and absence of necrosis halves the risk of IBE in both irradiated and non-irradiated patients. Lesion size, margins of excision and age at diagnosis did not modify these associations. The presence of necrosis modified the effect of radiotherapy: relative risk was 0.40 with necrosis present and 0.07 with necrosis absent (p-value for interaction 0.068). In all subsets of prognostic factors, radiotherapy conferred a substantial benefit. The risk factors for *in situ* and invasive IBE were similar. The agreement between pathologists was moderate ($\kappa = 0.486$). Correctness of diagnosis in the subcohort of SweDCIS was 84.8%.

Conclusion: Although nuclear grade and necrosis carry prognostic information, we could not define a group with very low risk after sector resection alone. Radiotherapy has a

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protective effect in all substrata of risk factors studied. The interaction between the presence of necrosis and radiotherapy is a clinically and biologically relevant research area.

1. Introduction

The national Swedish randomised DCIS trial (SweDCIS) was set up to study the effect of radiotherapy after breast conserving surgery for ductal carcinoma *in situ* (DCIS). Between 1987 and 1999, 1046 women were randomised to radiotherapy or no radiotherapy following a sector resection for DCIS. The recruitment basis for the study was a population offered mammography screening. After 5.2 years of follow-up, the cumulative incidence (and 95% confidence interval) for an ipsilateral breast recurrence (*in situ* or invasive) was 0.07 (0.05–0.10) in the radiotherapy arm and 0.22 (0.18–0.26) in the arm randomised to surgery only. There was no evidence for a differential effect of radiotherapy on *in situ* or invasive recurrences. Further details of the study and the results have been published.[1] To study histopathological risk factors for ipsilateral breast events, *in situ* or invasive, and to search for predictive factors for response to radiotherapy we undertook a histopathological re-evaluation using a case cohort design.[2] The study design also permitted a study of the reproducibility of diagnosis and of the histopathological evaluation and allowed us to estimate the correctness of diagnosis in the whole SweDCIS trial.

2. Patients and methods

2.1. Source population

The source population of this study is the SweDCIS Trial which accrued 1046 women from 1987 through 1999. The SweDCIS was a multicentre trial administered through the Regional Oncologic Centres in the six Swedish Health Care Regions. Inclusion criteria were a primary diagnosis of ductal carcinoma *in situ* of the breast occupying less than a quadrant of the breast, surgically treated with breast conservation, no prior history of cancer, no contraindication to radiotherapy and full informed consent. After a sector resection of the breast, women were randomised to postoperative radiotherapy of the breast or control only. A macroscopic surgical margin of 1 cm to the sides was aimed for. Scarpas'fascia and the pectoral fascia were the ventral and dorsal borders. Microscopically free margins were not requested but achieved in 80% of all participants (11% had positive margins and 8.5% had unknown margins). The specification dose of radiotherapy was 50 Gy given in 25 fractions over 5 weeks or 54 Gy given in two series with a gap of 2 weeks. No women were lost to follow-up. For further details, see Emdin et al.[1]

2.2. Study design

For the purpose of studying histopathological risk factors for ipsilateral breast events (IBE), a case cohort design was adopted. Eligible as cases were all women with an IBE of DCIS or invasive cancer that were identified through a full monitoring of all original medical records through 31st July 2001.

Eligible as a sample from the cohort – hereafter called subcohort – were all women included in the study. The subcohort was selected from the base line data at inclusion irrespective of if the members had a later event or not. The selection was done by day of birth in the month with different days randomly assigned for each of the participating six Health Care Regions. We aimed to sample at least 20% of the cohort. The study set-up is illustrated in the flowchart in Fig. 1.

2.3. Histopathological re-evaluation

The re-evaluation was done in two batches, hereafter called part A and part B (Fig. 1). To obtain a group where reproducibility could be studied and which at the same time made up the major part of the subcohort for the case-cohort study, 212 patients were selected by six birthdays as described above (part A). The sampling was done after the SweDCIS Trial was closed in December 1999. Slides were retrieved for all but 14 patients, for whom the slides were not available. The slides of the remaining 198 patients were sent to each of the three participating experienced breast pathologists. Diagnosis of DCIS, nuclear grade, presence of necrosis, inking of margins and use of large sections were evaluated by each pathologist independently and unknowingly of if the woman subsequently had developed an IBE or not. After the pathologists had submitted their results, a joint evaluation was undertaken for consensus concerning correctness of diagnosis (classified as DCIS, benign lesion, ADH, LCIS, Paget's disease of the nipple, microinvasive cancer and invasive cancer).

For part B all cases subsequently identified in the follow-up until 31st July 2001 were selected for study. To blind the pathologist to whether a re-evaluated slide belonged to a case or a representative of the subcohort, further women eligible as members of the subcohort were selected for study in part B. They were similar to the women in part A randomly selected from the SweDCIS trial at baseline, allocated by two more birthdays, different for each Health Care Region and these women together with those sampled in part A constitute the complete subcohort in the further analyses. The proportion in part B of cases and women without events was not revealed to the pathologists. In part B slides from 187 patients from a total of 194 eligible patients were evaluated for the same parameters as in part A at a consensus meeting between the three pathologists. At this time, no reproducibility study was attempted.

2.4. Histopathological definitions and clinical covariates

Before the evaluation, it was agreed that the definition of DCIS was to follow the Consensus Conference on Classification of DCIS[3] and the updated definitions of AFIP[4] and Ellis et al.[5]

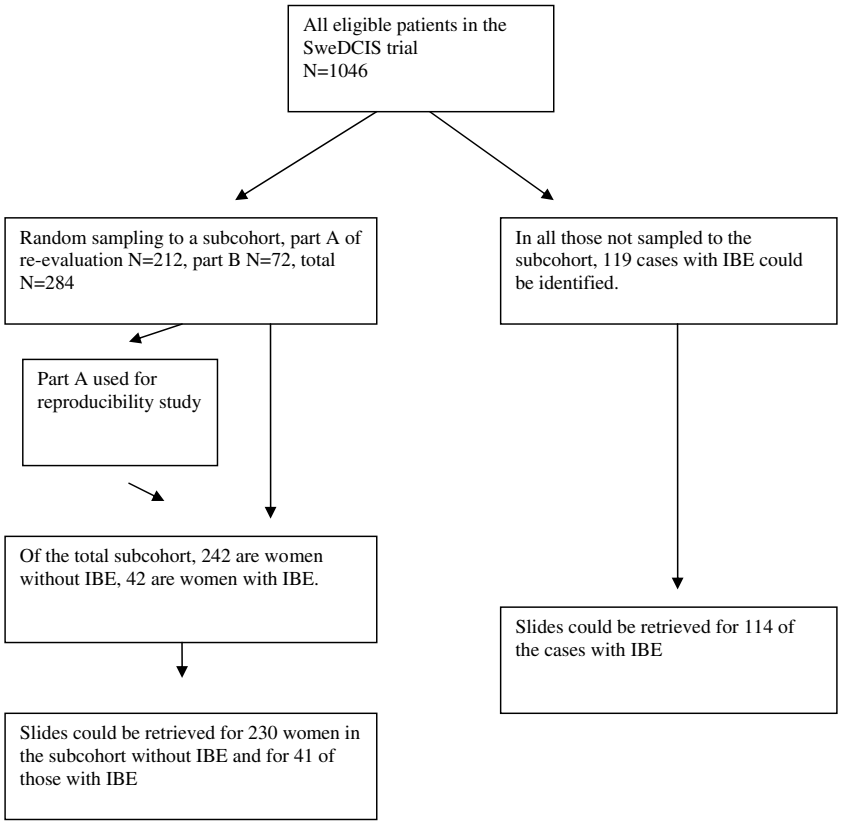

Fig. 1 – Flow chart of the selection of women to the subcohort and the case-series with ipsilateral breast events (IBE). The main analysis of the histopathological characteristics as prognostic and predictive factors was done according to Self and Prentice,[8] where three groups are considered: all 155 cases with IBE, subcohort members with IBE and subcohort women without IBE.

Thus, DCIS lesions of nuclear grade (NG) 1 or 2 up to 2 mm size and without necrosis were considered to be ADH. Definition of NG 1 was monomorphic nuclei with a size 1.5–2 times of normal red blood cells. NG 3: markedly pleomorphic nuclei, size larger than 2.5 times red blood cells. NG 2: what is not NG 1 or 3. Definition of microinvasion was the presence of a focus of invasion up to 1 mm where growth had to be seen outside of the specialised stroma of the duct. Definition of invasion was invasive foci larger than 1 mm.[5] Necrosis was defined as five or more pycnotic nuclei in eosinophilic intraductal debris.[6] In accordance with the eight-year update of the NSABP B-17,[7], we also applied a subdivision between women with NG 1–2 and no necrosis *versus* those with NG 1–2 with necrosis and including all those with NG 3. The baseline data in the SweDCIS trial also contain data on lesion size, margins (clear, involved or missing information) and age. These variables were used as co-variates in multivariate analyses of the association between the histopathological factors and risk of IBE.

2.5. *Statistical methods*

The subcohort was obtained by random sampling from the whole study base and this made it possible to straightforwardly estimate histopathological characteristics by study arm as well as the correctness of the DCIS diagnosis. The agreement of the DCIS diagnosis between the three pathologists was analysed by means of Cohen's kappa.

Cox proportional hazards models were assumed for DCIS and invasive ipsilateral events, and hazard ratios and variance estimates for the case-cohort design were determined according to Self and Prentice.[8] Technically, this was done by using the statistical program package *R* [9], and following Therneau and Li.[10] The analyses of prognostic factors were stratified on treatment arm to allow for different baseline hazards in the control and RT groups. The treatment effect was estimated in subgroups defined by the prognostic factors, and to compare two subgroups the log hazard ratios of RT *versus* control were compared by a simple *z*-test.

In the case-cohort design, the distribution of prognostic factors is only known for a subset of the non-cases. Thus, absolute differences between the groups cannot be directly observed as in a traditional cohort study. However, since information about the absolute risk reduction following radiotherapy is clinically important especially in subgroups of apparently low risk, we determined the cumulative incidence according to Kalbfleisch and Prentice[11] adopted to the case-cohort design by estimating the numbers at risk in subgroups of low risk.

In all our time-dependent analyses, follow-up was censored at the end of study or death if no recurrence had occurred previously. When analysing DCIS events only, follow-up was also censored if a woman experienced an

invasive recurrence. In analyses of invasive events only, follow-up was censored if a woman had a DCIS recurrence. In estimations of cumulative incidence, death without recurrence was considered as a competing event to IBE.

3. Results

3.1. Cases and subcohort

Fig. 1 and Table 1 show the study design and inclusion of patients. A subcohort of 284 women was selected by random sampling from the whole study cohort. Of these, 42 had experienced an IBE at the completion of follow-up (31st December, 2001), while 242 were free of IBE at that time. In the non-sampled part of the study cohort, 119 women experienced an IBE, making the total number of cases 161.

For the cases and subcohort non-cases, slides for 155 (96%) and 230 (95%) women, respectively, could be retrieved for evaluation.

3.2. Reproducibility of diagnosis

Reproducibility of diagnosis was studied in the part A evaluation of the subcohort. In the re-evaluation, the diagnosis was identical for all three pathologists already in their individual, separate evaluation in 157 of the 198 women (79%). A Cohen's Kappa of 0.486 for three raters was reached. At the joint evaluation consensus was reached in further 32 women, so that the diagnosis was agreed upon in 95.5% (189/198) of all evaluated. For the purpose of the case cohort study of risk factors, the majority rule was applied for determining the characteristics of the remaining nine histopathological specimens.

3.3. Correctness of diagnosis

Correctness of diagnosis was studied among the 271 women in the entire subcohort for which slides could be retrieved. The three pathologists judged 230 of these (84.8%) to be correctly diagnosed as DCIS. Around 9% of the women were deemed to have benign lesions and 4% of the women as having microinvasive or invasive tumours (Table 1).

Looking at the cases, there was an overrepresentation of specimens judged to be microinvasive or invasive (10%) as compared to the sub-cohort non-cases (3%) (Table 1). Among all the histopathologically reevaluated patients in the subcohort, 15.1% (41/271) had IBE. Among the 230 women who were judged as correctly diagnosed with DCIS, 15.6% (36/230) had IBE. The proportion of events in the whole SweDCIS trial was 15.4% (161/1046).

3.4. Histopathological characteristics by study arm

Table 2 shows estimates of the distribution of the histopathological characteristics in the entire SweDCIS trial. Both the characteristics of the tumours finally classified as DCIS and the misclassification with regard to the main diagnosis are similar between the radiotherapy and control arms. If anything, there was a somewhat higher proportion of benign lesions in the control group than in the radiotherapy group.

3.5. Risk factors for new ipsilateral breast events

Table 3 displays the results of the analysis of potential risk factors for IBE for the entire case-cohort study. The presence of NG 1–2 *versus* NG 3 halves the risk of an IBE. Similar estimates (relative risks 0.49–0.72) were seen for the absence of necrosis. When women were subdivided according to the low risk concept suggested in NSABP B-17[7] with the exception that we used the absence or the presence of necrosis instead of their comedonecrosis as criterion, 50–60% relative reductions in the risk of IBE were noted for those with NG 1–2 without necrosis. Large sections were only used in 20% of the subcohort, but a statistically non-significant reduction of

Table 1 – Number of women with re-evaluated slides in the subcohort (non-cases and cases) and number of women with re-evaluated slides among all the remaining cases in the SweDCIS Trial, not identified by the subcohort sampling

	Subcohort		Non-subcohort cases	Total
	Subcohort non-cases	Subcohort cases		
Diagnosis at re-evaluation	RT/no RT	RT/no RT	RT/no RT	RT/no RT
DCIS	194	36	96	326
	112/82	11/25	26/70	149/177
Benign/ADH/LCIS[a]	23	1	3	27
	8/15	1/0	0/3	9/18
Paget/microinvasive/invasive	7	4	12	23
	5/2	1/3	2/10	8 / 15
Not possible to evaluate	6	0	3	9
	1/5	0/0	2/1	3/6
Total[b]	230	41	114	385
	126/104	13/28	30/84	169/216

The distribution by randomisation arm (RT = surgery + postoperative irradiation; No RT = surgery alone) is also shown.
a ADH = atypical ductal hyperplasia; LCIS = lobular carcinoma *in situ*.
b 15 patients among the subcohort non-cases, 1 patient among the subcohort cases and 5 patients among the non-subcohort cases excluded due to missing slides.

Table 2 – Results of re-evaluation of the slides for the 271 women in the subcohort shown by randomisation arms (RT = surgery + radiotherapy; No RT = surgery alone)

Diagnosis	Subcohort (n = 271) (subcohort non-cases + subcohort cases)[a]	
	RT No. (percent of subcohort)	No RT No. (percent of subcohort)
DCIS	107 (81.1)	123 (88.5)
NG = 1	7 (5.3)	13 (9.4)
NG = 2	46 (34.8)	49 (35.3)
NG = 3	54 (40.9)	61 (43.9)
Necrosis present	81 (61.4)	89 (64)
Necrosis absent	26 (19.7)	34 (24.5)
Holland = 1	7 (5.3)	12 (8.6)
Holland = 2	48 (36.4)	50 (36)
Holland = 3	52 (39.4)	61 (43.9)
Large section	8 (13.6)	28 (20.1)
No large section	89 (67.4)	95 (68.3)
Inking	38 (28.8)	46 (33.1)
No inking	69 (52.3)	77 (55.4)
Benign/ADH/LCIS	9 (6.5)	15 (11.4)
Paget/microinvasive/ invasive	6 (4.3)	5 (3.8)
Inadequate	1 (0.7)	5 (3.8)
Total	139 (100)	132 (100)

(NG, nuclear grade)
a 21 cases (No RT 12/RT 9) not reported since glasses were not retrieved

the risk of IBE was seen. No such trend was seen when inking of margins had been applied.

All analyses were also performed by treatment arm (data not shown), and the risk estimates were similar in the radiotherapy and control arm with one exception: for all types of ipsilateral events, when necrosis was not present *versus* present. For the absence of necrosis, the hazard ratio was 0.14 in the radiotherapy arm, while being 0.83 in the control group.

Since lesion size, margins of excision and age at diagnosis possibly could have confounded the analyses of nuclear grade and necrosis, multivariate analyses adjusting for these factors were undertaken repeating the basic comparisons (the adjusted analyses in Table 3). The results were without any exception very similar to the univariate results.

3.6. *Predictive factors for response to radiotherapy*

Table 4 shows the results of an analysis to study the relative risk reduction of radiotherapy in the different prognostic subsets. The risk reduction from radiotherapy was similar in most subsets and although the confidence intervals in some subgroups were wide, the overall pattern is a substantial relative risk reduction following radiotherapy. However, parallel to the findings of the analyses by treatment arm mentioned above, there were indications of an effect modification of absence *versus* presence of necrosis on the radiotherapy effect for all types of events (p-value for interaction = 0.068). The findings indicate a very strong risk reduction for all types of events by radiotherapy when necrosis is absent, and this is further emphasised when ng and absence of necrosis is com-

Table 3 – Hazard ratios with 95% confidence intervals for risk of IBE, unadjusted models and models adjusted for lesion size, margins (free, involved or unknown) and patient age at diagnosis

Comparison	Type of comparison	All ipsilateral events	Ipsilateral DCIS	Ipsilateral invasive
		Hazard ratio (95% confidence limits)	Hazard ratio (95% confidence limits)	Hazard ratio (95% confidence limits)
NG 1–2 *versus* NG 3	Not adjusted	0.46 (0.28, 0.75)	0.39 (0.22, 0.71)	0.58 (0.29, 1.14)
	Adjusted[a]	0.56 (0.33, 0.93)	0.50 (0.27, 0.94)	0.63 (0.31, 1.28)
No necrosis *versus* Necrosis	Not adjusted	0.57 (0.31, 1.06)	0.49 (0.22, 1.08)	0.72 (0.31, 1.65)
	Adjusted[a]	0.55 (0.29, 1.04)	0.46 (0.20, 1.06)	0.66 (0.28, 1.57)
NG 1–2 without necrosis *versus* NG 3 or NG 1–2 with necrosis	Not adjusted	0.41 (0.21, 0.82)	0.37 (0.15, 0.88)	0.49 (0.19, 1.26)
	Adjusted[a]	0.43 (0.21, 0.88)	0.40 (0.16, 0.99)	0.47 (0.18, 1.26)
Large section Yes *versus* No	Not adjusted	0.64 (0.33, 1.25)	0.63 (0.28, 1.44)	0.65 (0.25, 1.7)
	Adjusted[a]	0.62 (0.30, 1.26)	0.61 (0.25, 1.47)	0.63 (0.23, 1.7)
Inking Yes *versus* No	Not adjusted	1.08 (0.66, 1.77)	1.05 (0.59, 1.89)	1.13 (0.56, 2.28)
	Adjusted[a]	1.37 (0.80, 2.35)	1.37 (0.72, 2.62)	1.36 (0.63, 2.91)

a Adjusted for age, tumour size and histopathological margins.

Table 4 – Hazard ratios with 95% confidence intervals for IBE comparing women randomised to radiotherapy versus no radiotherapy, stratified for each subset of nuclear grade (NG), absence or presence of necrosis, large section or inking used

	All ipsilateral events		Ipsilateral DCIS		Ipsilateral invasive	
	Hazard ratio, RT *versus* control (95% confidence interval) *p*-value for test of interaction		Hazard ratio, RT *versus* control (95% confidence interval) *p*-value for test of interaction		Hazard ratio, RT *versus* control (95% confidence interval) *p*-value for test of interaction	
Group						
NG 1–2	0.30 (0.13, 0.67)	0.98	0.21 (0.07, 0.68)	0.61	0.42 (0.15, 1.21)	0.57
NG 3	0.30 (0.16, 0.56)		0.31 (0.15, 0.64)		0.28 (0.11, 0.70)	
Necrosis						
No	0.07 (0.01, 0.42)	0.068	0.07 (0.01, 0.58)	0.15	0.07 (0.00, 1.13)	0.20
Yes	0.40 (0.23, 0.67)		0.36 (0.19, 0.68)		0.46 (0.22, 0.98)	
NG 1–2 without necrosis	0.05 (0.01, 0.38)	0.055	0.10 (0.01, 0.86)	0.28	NA NA	NA
NG 3 or NG 1–2 with necrosis	0.38 (0.23, 0.64)		0.34 (0.18, 0.64)		0.46 (0.22, 0.96)	
Large section						
Yes	0.21 (0.05, 0.89)	0.55	0.28 (0.05, 1.63)	0.95	0.12 (0.01, 1.10)	0.31
No	0.33 (0.20, 0.56)		0.30 (0.16, 0.57)		0.39 (0.19, 0.82)	
Inking						
Yes	0.20 (0.08, 0.50)	0.24	0.20 (0.06, 0.63)	0.41	0.21 (0.06, 0.74)	0.34
No	0.38 (0.21, 0.69)		0.35 (0.17, 0.72)		0.43 (0.19, 0.99)	

bined similar to the B-17 analysis (Table 4) (*p*-value for interaction = 0.055).

Neither the findings in Table 3 nor those in Table 4 showed any convincing pattern of different risk factors for IBE or predictive factors of response to radiotherapy by type of recurrence (a new DCIS or an invasive cancer).

3.7. *Cumulative incidence of IBE in low risk groups*

To further explore if we could find a group with a low risk of IBE also when no radiotherapy was given and to estimate the absolute risk reduction of radiotherapy in low risk groups, we estimated the cumulative incidence of IBE in the subsets of patients defined by a combination of NG and necrosis (Fig. 2). Even the group with NG 1–2 without necrosis reached 20% recurrence rate at 7 years of follow-up without radiotherapy and also in this subset radiotherapy conferred a large benefit, 19.2% in absolute risk reduction since there were very few events in the radiotherapy arm. When we combined NG 1–2, the absence of necrosis with free margins or with lesion size less than 20 mm, we found cumulative incidences of 17% and 13%, respectively, at 7 years and in both subsets a sub-

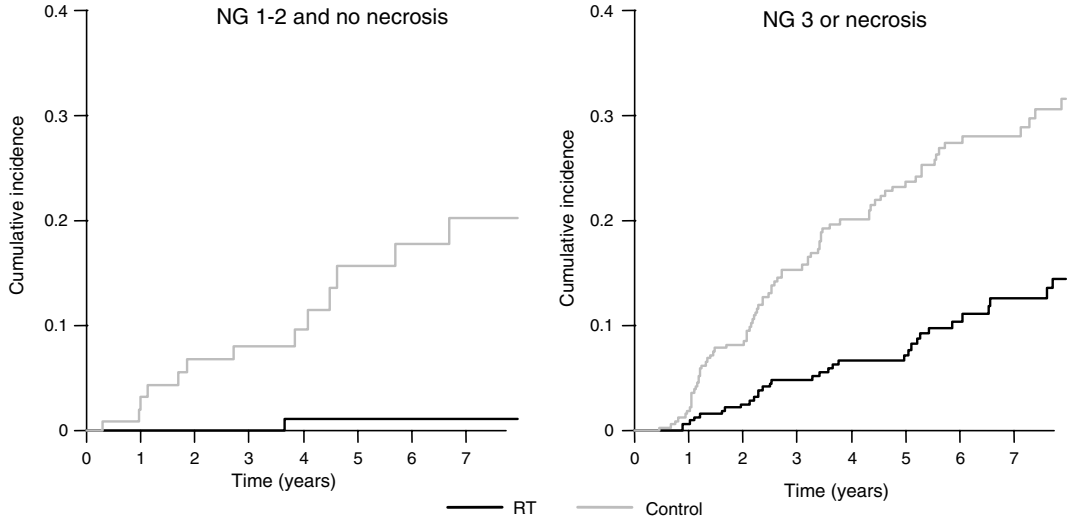

Fig. 2 – Cumulative incidence of IBE in women randomised to radiotherapy (RT) *versus* control stratified by NG 1–2, absence of necrosis and presence of necrosis and/or NG 3. The decrease in estimated numbers at risk over the first 7 years ranges from 117 to 29 among patients stratified for NG 1–2 and absence of necrosis treated with RT and from 87 to 26 among the similarly stratified controls. Numbers at risk among patients with NG 3 or necrosis decrease from 332 to 87 (RT) and from 303 to 77 (ctrl).

stantial reduction of risk following radiotherapy. When lesion size in this analysis according to the Van Nuys'classification,[12] was lowered to 15 mm, the cumulative incidence was 11% at 7 years without radiotherapy, but the subset was small with few events and the estimate thus unreliable.

4. Discussion

DCIS was correctly diagnosed in 85% in the routine histopathological examination judging from a histopathological re-evaluation of a random sample of 26% of the SweDCIS Trial. In about 4%, invasion or microinvasion was missed in the initial routine histopathological evaluation at inclusion into the study. Nine percent were judged as benign, ADH or LCIS. Low nuclear grade (NG 1–2) and absence of necrosis were associated with a lowered risk of IBE. We could not with certainty delineate a group not given radiotherapy that would have a yearly risk of two percent or lower. Radiotherapy confers reduction of IBE in all subsets of patients, with possibly even stronger effect in the absence of necrosis.

The analyses are based on a large randomised clinical trial, and the great majority of the slides from the random sample and from the cases could be retrieved and re-evaluated. The re-evaluation was done by three experienced breast pathologists who reached moderate agreement. The re-evaluation was done without any knowledge about the individual patients' follow-up status. The follow-up in the study was complete and classification of events was done by monitoring of individual patient records. The case-cohort design enables us to draw conclusions about the distribution of characteristics in the whole main trial. Furthermore, the design also allowed us to estimate the cumulative incidences of IBE to make inferences about absolute risks and not only relative risks.

A drawback of the study is that some subsets are small rendering a low statistical precision and the risk to miss important differences. Another risk with analyzing many subsets is spurious findings. However, we have not relied in our interpretation on multiple significance tests, but looked at overall patterns and studied interaction rather than small differences in subsets. Further, we have used risk factors as defined by others before us and the results are stable over subgroups and in line with what would be expected from the main analyses. Still, we underscore that as in all subgroup analyses, the results should be interpreted cautiously.

A further drawback is that in retrospective evaluation of DCIS specimens, margins and lesions size are difficult to assess with the same validity as the other re-evaluated characteristics.[7,13] For this reason, we refrained from classifying the lesions according to the Van Nuys'classification, size and margin status being central to that classification. However, the multivariate analyses in Table 3 show that the clinical data on margins and lesion size from the initial case record forms did not increase the association between NG or necrosis and prognosis. Our intention was to include the Holland classification for DCIS[14] in the analyses. However, in part A of the evaluation, we found that NG and Holland corresponded nearly completely and it was decided to only use NG.

Our finding of a possible interaction between the absence or presence of necrosis and radiotherapy is clinically and biologically interesting. The NSABP B-17 eight-year update[7] and the results of a meta-analysis of risk factors for IBE after DCIS[15] also point towards an effect modification of radiotherapy by the presence of necrosis, but go in the opposite direction from what we found. There are two differences between our and the other publications that may account for the discrepancies: First, the NSABP B-17 used 'comedonecrosis' and not just 'necrosis' as the classifier. 'Comedonecrosis' was graded as 'moderate/marked' when it occurred in more than one-third of ducts exhibiting DCIS; fewer or none were graded as 'absent/slight'. Our definition required as little as five pycnotic nuclei or more in eosinophilic ductal debris to be noted as 'necrosis'. Second, the meta-analysis relied mainly on observational studies with probably a range of definitions of necrosis used. In the observational studies there may also be a confounding by indication, since the presence of necrosis may have been associated with other treatment decisions that influence the risk of IBE and modify the effect of irradiation (*e.g.* re-excision, use of tamoxifen).[2]

In a recent follow-up of the EORTC trial, the investigators concluded that the effect of radiotherapy was similar over different strata of differentiation and architectural characteristics.[16] This coincides with our data over NG. Looking at interaction by necrosis, their classification and analysis are not parallel to ours and cannot directly support or contradict our findings. The interaction noted in the present study could be a chance finding. Nevertheless, it is biologically plausible that DCIS with and without necrosis may respond differently to different treatment modalities. The presence of necrosis is highly correlated to hypoxia[17] and a number of changes in the expression of transcription factors,[18] reduced ER-alpha expression,[19,20] a different pattern of induction of carbonic anhydrases[21] and a different pattern of gene expression[22,23] as compared to DCIS without necrosis.

Large sections were not associated with a statistically significant reduction of risk of IBE and inking did not seem to influence the risk of IBE in this study. According to others,[12] the use of large sections and inking of the margins together with serial sectioning should increase the sensitivity to detect involved margins and multifocal lesions , thus lowering the risk of ipsilateral breast events. In this study, large sections and inking were not used systematically and were used only in a subset of patients. Thus, this study can not exclude that these techniques are beneficial and if anything there is a tendency in our data for the use of large sections to be protective for an IBE.

Our estimates of the correctness of diagnosis in the main trial are similar to the EORTC 10853 Trial.[24] In that study, about 5% of the patients were classified as missed invasive diagnoses. We deem that the results from the SweDCIS trial similarly to the results of the EORTC trial are largely generalisable to a broad spectrum of patients with DCIS today. Notable is the fact that the Swedish trial was conducted in a population offered routine mammography screening. The histopathological re-evaluation also points to the fact that randomisation resulted in a similar distribution of tumour characteristics in the radiotherapy and control arm, thus supporting a high validity of the findings of the effect of radiotherapy in the main SweDCIS trial.

In line with other researchers, we found that the presence of necrosis is an important prognostic factor in DCIS, that we still cannot reliably define a group with very low risk without radiotherapy, and that radiotherapy has a protective effect – both in relative and absolute measures – for IBE in all substrata defined by hitherto known risk factors. These findings imply that cohorts of women not recommended radiotherapy should be followed long term for quality control and eventual revision of guidelines. Our findings of a probable modification by the presence of necrosis on the effect of radiotherapy underline that the biological differences between DCIS with and without necrosis is a highly relevant field of study with possible implications also for other forms of cancer.

Conflict of interest statement

None of the authors have any conflicts of interest with regard to the content of this article.

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