LUND UNIVERSITY

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.

Ringberg, Anita; Nordgren, H; Thorstensson, S; Idvall, Ingrid; Garmo, H; Granstrand, B; Arnesson, L G; Sandelin, K; Wallgren, A; Anderson, Harald; Emdin, S; Holmberg, L

Published in:
European Journal of Cancer

DOI:
10.1016/j.ejca.2006.09.018

2007

Link to publication

Citation for published version (APA):
Ringberg, A., Nordgren, H., Thorstensson, S., Idvall, I., Garmo, H., Granstrand, B., Arnesson, L. G., Sandelin, K., Wallgren, A., Anderson, H., Emdin, S., & Holmberg, L. (2007). 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. *European Journal of Cancer*, *43*(2), 291-298. https://doi.org/10.1016/j.ejca.2006.09.018

Total number of authors:
12

General rights
Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.
• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Download date: 10. Oct. 2026

LUND UNIVERSITY
Faculty of Medicine

LU:*research*

Institutional Repository of Lund University

This is an author produced version of a paper published in European Journal of Cancer. This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.

Citation for the published paper:
Ringberg, A and Nordgren, H and Thorstensson, S and Idvall, I and Garmo, H and Granstrand, B and Arnesson, L G and Sandelin, K and Wallgren, A and Anderson, H and Emdin, S and Holmberg, L.
"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."
European Journal of Cancer, 2006, Issue: Nov 21.

http://dx.doi.org/10.1016/j.ejca.2006.09.018

Access to the published version may require journal subscription.
Published with permission from: Elsevier

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

Ringberg A[1], Nordgren H[2], Thorstensson S[3], Idvall I[4], Garmo H[5], Granstrand B[6], Arnesson, L G[7], Sandelin K[8], Wallgren A[9], Anderson H[10], Emdin S[6], Holmberg L[5]

(1)Department of Plastic Surgery, Malmö University Hospital, Malmö, Sweden; (2)Department of Pathology, University Hospital, Uppsala, Sweden; (3)Department of Pathology, Central Hospital, Kalmar, Sweden; (4)Department of Pathology, Malmö University Hospital, Malmö, Sweden; (5)Regional Oncologic Center, University Hospital, Uppsala, Sweden; (6)Department of Surgery, Norrland University Hospital, Umeå, Sweden; (7)Department of Surgery, University Hospital, Linköping, Sweden; (8)Department of Surgery, Karolinska Hospital, Stockholm, Sweden; (9)Department of Oncology, Sahlgenska University Hospital, Gothenburg, Sweden; (10)Regional Oncologic Center, University Hospital, Lund, Sweden.

Address for correspondence:

Lars Holmberg, Professor
Regional Oncologic Center
University Hospital
SE-751 85 Uppsala
SWEDEN

Tel: +46 18 151915
Fax: +46 18 711445
Email:lars.holmberg@akademiska.se

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 analyze reproducibility of the histopathological evaluation and to estimate correctness of diagnosis in the trial.

Setting: A randomized 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 in total 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 percent.

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

Introduction

The national Swedish randomized 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 to 0.10) in the radiotherapy arm and 0.22 (0.18 to 0.26) in 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.

Patients and methods

Source population

The source population of this study is the SweDCIS Trial which accrued 1046 women from 1987 through 1999. The SweDCIS was a multicenter 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 one centimetre 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% unknown margins) . The specification dose of radiotherapy was 50 Gy given in 25 fractions over five weeks or 54 Gy given in two series with a gap of two weeks. No women were lost to follow-up. For further details see Emdin et al 2006 (1).

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 July 31 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 figure 1.

Histopathological re-evaluation

The re-evaluation was done in two batches, hereafter called part A and part B (figure 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 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 July 31, 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 just as 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.

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). 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 presence of a focus of invasion up to 1 mm where growth had to be seen outside of the specialized stroma of the duct. Definition of invasion was invasive foci larger than 1 mm (5). Necrosis was defined as 5 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.

Statistical methods

The subcohort was obtained by random sampling from the whole study base and this made it possible to straightforwardly estimate histopathological characterisics 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 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.

Results

Cases and subcohort (Figure 1 and Table 1)

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 (December 31, 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.

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.

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 that 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).

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.

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. Presence of ng 1-2 vs ng 3 halves the risk of an IBE. Similar estimates (relative risks 0.49 to 0.72) were seen for 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 absence or presence of necrosis instead of their comedonecrosis as criterion, 50 to 60 per cent relative reductions in the risk of IBE were noted for those with ng 1-2 without necrosis. Large sections were only used in 20 per cent of the subcohort, but a statistically non-significant reduction of 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 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.

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 emphasized when ng and absence of necrosis is combined similar to the B-17 analysis (table 4) (p-value for interaction=0.055).

Neither the findings in Table 3 nor 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).

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 (Figure 2). Even the group with ng 1-2 without necrosis reached 20 per cent recurrence rate at seven years of follow up without radiotherapy and also in this subset radiotherapy conferred a large benefit, 19.2 per cent in absolute risk reduction since there were very few events in the radiotherapy arm. When we combined ng 1-2, absence of necrosis with free margins or with lesion size less than 20 mm we found cumulative incidences of 17 and 13 per cent respectively at seven years and in both subsets a substantial 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 per cent at seven years without radiotherapy, but the subset was small with few events and the estimate thus unreliable.

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 four percent 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 (ng1-2) 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 that 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 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 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 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 5 pycnotic nuclei or more in eosinophilic ductal debris to be noted as “necrosis”. Secondly, 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 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. Presence of necrosis is highly correlated to hypoxia (17) and a number of changes in expression of transcription factors (18), reduced ER-alpha expression (19, 20), a different pattern of induction of carbonic anhydrases (21) and 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 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 generalizable to a broad spectrum of patients with DCIS today. Notable is that the Swedish trial was conducted in a population offered routine mammography screening. The histopathological re-evaluation also points to that the 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 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 presence of necrosis on the effect of radiotherapy underlines 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.

Acknowledgement: The study was supported by the Swedish Cancer Society.

Conflict of interest: None of the authors have any conflicts of interest with regards to the content of this article.

References

1. Emdin SO**,** Granstrand B, Ringberg A et al. Radiotherapy after sector resection for ductal carcinoma in situ of the breast. Results of a randomised trial in a population offered mammography screening. Acta Oncologica 2006; xx: xx-xxx (accepted).

2. Prentice RL. A case-cohort design for epidemiologic cohort studies and disease prevention trials. Biometrika 1986; 73: 1-11.

3. Schwartz GF. Consensus Conference on the Classification of DCIS. Human Pathol 1997; 28,:1221-5.

4. Lininger RA, Tavassoli FA. Atypical intraductal hyperplasia of the breast. In: Ductal Carcinoma of the Breast, Ed Silverstein MJ, Williams and Wilkins 1997, Chapter 19, 195-222.

5. Ellis IO, Elston CW, Poller DN. Ductal carcinoma in situ. In Systemic Pathology, 3rd Ed/ vol 13, Em Ed W. St C. Symmers, The Breast, Churchill Livingston 1998, Chapter 14, 249-81.

6. Douglas-Jones AG, Gupta SK, Attanoos RL, Morgan JM, Mansel RE. A critical appraisal of six modern classifications of DCIS of the breast. Histopathology 1996; 29: 397-409.

7. Fisher ER, Dignam J, Tan-Chiu E, Costantino J, Fisher B, Paik S, Wolmark N. Pathologic findings from the National Surgical Adjuvant Breast Project (NSABP). Eight-year update of protocol B-17. Intraductal carcinoma. Cancer 1999; 86: 429-38.

8. Self SG, Prentice RL. Asymptotic distribution theory and efficiency results for case-cohort studies.Annals Statistics 1988; 16: 64-81.

9. R Development Core Team (2005). A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

10. Therneau TM, Li H. Computing the Cox Model for Case Cohort Designs. Technical Report Number 62, June 1998, Technical Report Series, Section of Biostatistics, Mayo Clinic, Rochester, Minnesota, http://mayoresearch.mayo.edu/mayo/research/biostat/upload/62.pdf

11. Kalbfleisch JD, Prentice RL. The statistical analysis of failure time data. Wiley&Sons Inc, Hoboken New Jersey 2002, 2nd edition, p. 255.

12. Silverstein MJ, Lagios MD, Craig PH et al. A prognostic index for ductal carcinoma in situ of the breast. Cancer 1996; 77: 2267-74.

13. Ringberg A, Idvall I, Fernö M et al. Ipsilateral local recurrence in relation to therapy and morphological characteristics in patients with ductal carcinoma in situ of the breast. Eur J Surg Oncol 2000; 26(5): 444-51.

14. Holland R, Peterse JL, Millis RR et al. Ductal carcinoma in situ: A proposal for a new classification. Sem Diagn Pathol 1994; 11: 167-80.

15. Boyages J, Delaney G, Taylor R. Predictors of local recurrence after treatment of ductal carcinoma in situ: a meta-analysis. Cancer 1999; 85: 616-28.

16. Bijker N, Meijnen P, Peterse J et al. Breast-conserving treatment with or without radiotherapy in breast-carcinoma-in-situ: Ten-year results of European Organisation for Research and Treatment of Cancer randomised phase III trial 10853-A study by the EORTC Breast Cancer Cooperative Group and EORTC Radiotherapy Group. J Clin Oncol 2006: 24: 3381-87.

17. Bussolati G, Bongiovanni M, Cassoni P, Sapino A. Assessment of necrosis and hypoxia in ductal carcinoma in situ of the breast: basis for a new classification. Virchows Arch 2000; 437: 360-4.

18. Okada K, Osaki M, Araki K, Ishiguro K, Ito H, Ohgi S. Expression of hypoxia-inducible factor (HIF-1alpha), VEGF-C and VEGF-D in non-invasive and invasive breast ductal carcinomas. Anticancer Res. 2005;25:3003-9.

19. Cooper C, Liu GY, Niu YL, Santos S, Murphy LC, Watson PH. Intermittent hypoxia induces proteasome-dependent down-regulation of estrogen receptor alpha in human breast carcinoma. Clin Cancer Res 2004; 10: 8720-7.

20. Helczinska K, Kronblad Å, Jögi A et al. Hypoxia promotes a dedifferentiated phenotype in ductal breast carcinoma in situ. Cancer Res 2003; 63: 1441-4.

21. Wykoff CC, Beasley N, Watson PH, Campo L, Chia SK, English R, Pastorek J, Sly WS, Ratcliffe P, Harris AL. Expression of the hypoxia-inducible and tumor-associated carbonic anhydrases in ductal carcinoma in situ of the breast. Am J Pathol. 2001;58:1011-9.

22. Sowter HM, Ferguson M, Pym C et al. Expression of the cell death genes BNip3 and NIX in ductal carcinoma in situ of the breast; correlation of BNip3 levels with necrosis and grade. J Pathol 2003; 201(4): 573-80.

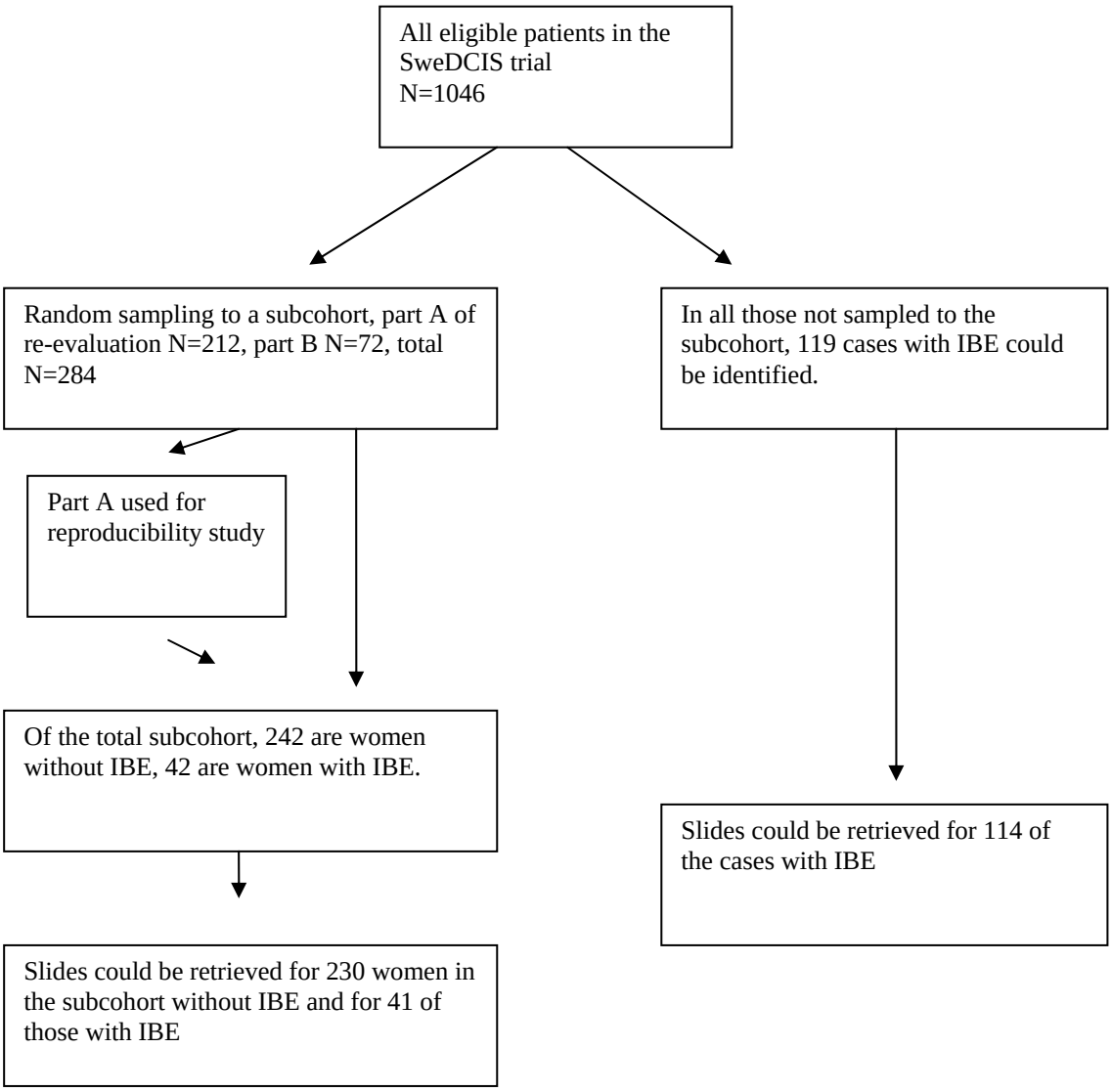
23. Adeyinka A, Emberley E, Niu Y et al. Analysis of gene expression in ductal carcinoma in situ of the breast. Clin Cancer Res 2002; 8(12): 3788-95.

24. Bijker N**,** Peterse L, Duchateau L et al. Risk factors for recurrence and metastasis after breast-conserving therapy for ductal carcinoma-in-situ: Analysis of European Organization for Research and Treatment of Cancer Trial 10853. J Clin Oncol 2001; 19: 2263-71.

Figure legends

Figure 1. Flowchart 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 Prentice et al (ref), where three groups are considered: All 155 cases with IBE, subcohort members with IBE and subcohort women without IBE.

Figure 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 seven 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 decreases from 332 to 87 (RT) and from 303 to 77 (ctrl).


All eligible patients in the SweDCIS trial N=1046
Random sampling to a subcohort, part A of re-evaluation N=212, part B N=72, total N=284
In all those not sampled to the subcohort, 119 cases with IBE could be identified.
Part A used for reproducibility study
Of the total subcohort, 242 are women without IBE, 42 are women with IBE.
Slides could be retrieved for 114 of the cases with IBE
Slides could be retrieved for 230 women in the subcohort without IBE and for 41 of those with IBE

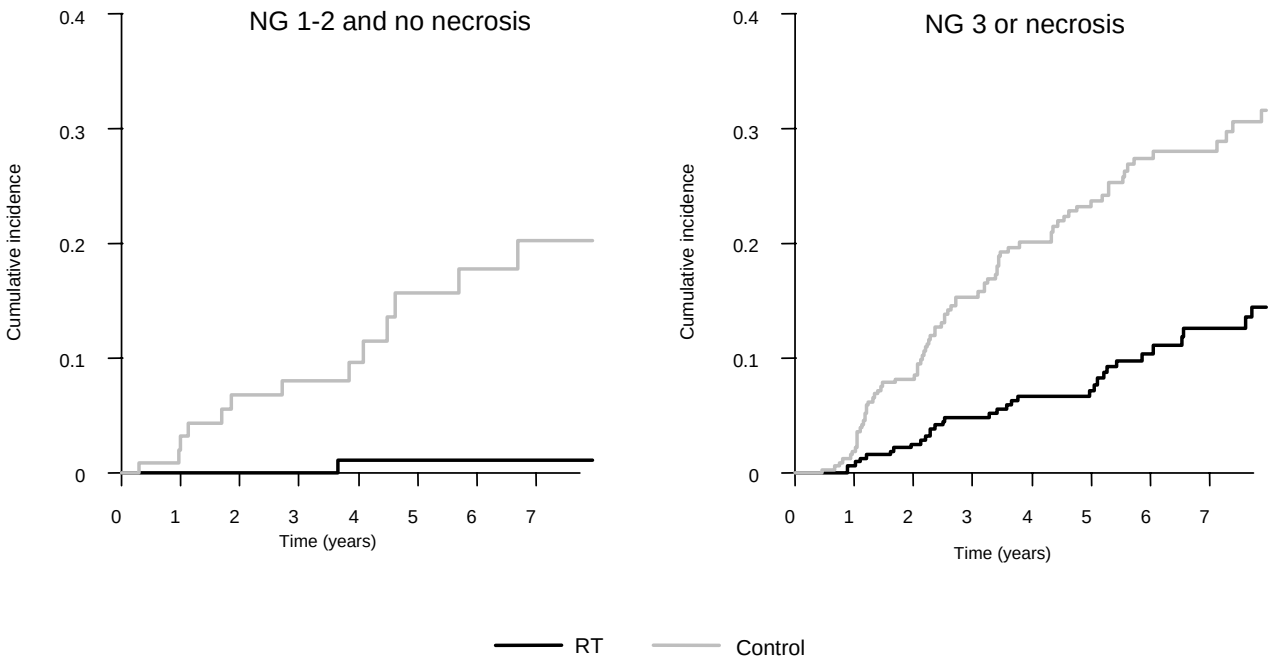

NG 1-2 and no necrosis
NG 3 or necrosis
Cumulative incidence
0.4
0.3
0.2
0.1
0
0 1 2 3 4 5 6 7
Time (years)
RT
Control

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. The distribution by randomisation arm (RT=surgery + postoperative irradiation; No RT = surgery alone) is also shown.

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

†ADH=atypical ductal hyperplasi; LCIS=lobular carcinoma in situ
[&]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).

	Subcohort (n=271)[*] (Subcohort non cases + Subcohort cases)	
Diagnosis	**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	18 (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 / microinv / invasive	6 (4.3)	5 (3.8)
Inadequate	1 (0.7)	5 (3.8)
Total	139 (100)	132 (100)

[*] 21 cases (No RT 12/RT 9) not reported since glasses were not retrieved

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

[&] Adjusted for age, tumour size and histopathological margins.