Prognosis in carcinoma in situ of the breast

BY

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ABSTRACT
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The incidence of breast cancer is rising steadily in Sweden and the proportion of carcinoma in situ (CIS) has increased appreciably, most likely due to mammography screening. The aim of this study was twofold: (1) to examine risk factors for subsequent invasive breast carcinoma and breast cancer death after primary ductal carcinoma in situ (DCIS) and (2) to study the biology in the progress between in situ and invasive carcinoma.

In a cohort-study based on 3,398 women with a primary CIS reported to the Swedish Cancer Registry (SCR) 1980-1992, women diagnosed in 1989-1992 ran a relative risk of 0.1 (CI 95%, 0.0-0.9) from dying of breast cancer as compared with women diagnosed in 1980-1982. Women in counties with mammography screening ran a relative risk of 0.2 (CI 95%, 0.0-2.1) for breast cancer death in comparison with women in non-screening counties.

In a case-control study derived from all 4,661 women with primary CIS reported to the SCR 1960-1992, we investigated risk factors for subsequent invasive breast carcinoma (n=118) and breast cancer death (n=39). Large size and multifocality were found to increase the risk for breast cancer death. Postoperative radiotherapy and mastectomy lowered the risk for ipsilateral invasive cancer.

The standardised incidence rates (SIR) for invasive breast cancer were estimated in the cohort from 1980-1992. The SIR after primary DCIS and primary lobular carcinoma in situ (LCIS) was 4.5 (CI 95%, 3.7-5.5) and 4.0 (CI 95%, 2.1-7.5), respectively.

New histopathological classification systems for DCIS were evaluated in 195 women consecutively diagnosed with primary DCIS between 1986-1994. One group with highly differentiated lesions was defined with the EORTC classification system and had an excellent prognosis.

Histopathological grade and expression of p53, c-erbB-2, Ki 67, hormone receptors, Bcl-2 and angiogenesis were compared in 626 women with either a pure DCIS, a small invasive carcinoma or a lesion with both an invasive and in situ component. When grade was taken into account, no change in tumour markers could be detected that signalled the progression from an in situ stage to invasiveness. All tumour markers correlated to grade and their distribution was very similar in the two components of mixed lesions.

Key words: Breast carcinoma, ductal carcinoma in situ, lobular carcinoma in situ, prognosis, histopathological grade, p53, c-erbB-2, Ki 67, Bcl-2, hormone receptors, angiogenesis.

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To my family: Anneli, Erik and Joel

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals (I-V)

I.   Wärnberg F, Bergh J, Holmberg L. Prognosis in women with a carcinoma in situ of the breast. A population based study in Sweden. Cancer Epidemiology, Biomarkers and Prevention 1999; Vol 8: 769-774.

II.   Wärnberg F, Bergh J, Zack M, Holmberg L. Risk factors for breast cancer death and subsequent invasive breast cancer after Ductal Carcinoma In Situ. A population-based case-control study in Sweden. *(Submitted)*

III.   Wärnberg F, Yuen J, Holmberg L. The risk of subsequent invasive breast cancer after breast carcinoma in situ. Lancet, 2000; Vol 355: 724-725.

IV.   Wärnberg F, Nordgren H, Bergh J, Holmberg L. Ductal carcinoma in situ of the breast from a population defined cohort: an evaluation of new histopathological classification systems. European Journal of Cancer, 1999; Vol 35: 5: 714-720.

V.   Wärnberg F, Nordgren H, Bergkvist L, Holmberg L. A comparison of tumour biology in small invasive and ductal carcinoma in situ of the breast. *(Submitted)*

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Abbreviations

BCS	Breast Conserving Surgery
CDR	Causes of Death Registry
CI	Confidence Interval
CIS	Carcinoma In Situ of the Breast
DCIS	Ductal Carcinoma In Situ of the Breast
EORTC	European Organisation for Research and Treatment of Cancer
ER	Oestrogen Receptors
IH	Immunohistochemistry
LCIS	Lobular Carcinoma In Situ of the Breast
NSABP	National Surgical Adjuvant Breast and Bowel Project
OR	Odds Ratio
PR	Progesterone Receptors
RFS	Relapse Free Survival
RH	Relative Hazard
ROC	Regional Oncological Center, Uppsala-Örebro
SCR	Swedish Cancer Registry
SIR	Standardised Incidence Ratio
XRT	Postoperative Radiation Therapy

Introduction

The incidence of breast cancer is steadily rising in Sweden and today almost 6,000 new breast cancers are diagnosed each year. During the last few decades the proportion of carcinoma in situ (CIS) among all detected breast cancers has increased and this is probably due to mammography screening programs, now used nation-wide in Sweden. During the 1960s and 1970s, CIS constituted about 1-2% of all breast cancers reported to the Swedish Cancer Registry (SCR). Today, the proportion of CIS is about 10% of all diagnosed breast cancers (ROC, 2000). In studies of screening detected lesions the proportion of CIS is even higher, reaching proportions as high as 35% (Lagios, 1996). Ductal carcinoma in situ (DCIS) has increased more than lobular carcinoma in situ (LCIS) (Lemanne et al., 1991; Ernster et al., 1996) which is largely because DCIS is often detected by mammography (Stomper et al., 1989) while LCIS is not.

Because of recent advances in breast cancer research, the current standard surgical therapy for small invasive breast cancer is breast-conserving surgery (BCS). Early detection, often as a result of mammography screening, has improved the survival as well as the local control of the disease (Liljegren et al., 1994; Tabar et al., 1995). Moreover, the addition of radiotherapy to the remaining breast parenchyma has significantly improved local control after surgery, both in invasive and in situ carcinoma of the breast (Fisher et al., 1989; Fisher et al., 1993; Fisher et al., 1999). More extensive surgery (i.e., mastectomy) is only necessary in cases with a large or locally advanced tumour. However, there is still considerable controversy regarding the therapy for patients with CIS (Silverstein et al., 1996). Our knowledge about CIS is not as exhaustive as it is for invasive breast cancer and, even if CIS *per se* has a better prognosis than invasive breast cancer, less extensive surgery is still not fully accepted as standard treatment.

One major task for further research is to identify those patients with CIS, who run a high risk to develop subsequent invasive breast cancer or to die from breast cancer. To distinguish such a group of patients (or - on the contrary - a group of patients with an excellent prognosis), would help in making therapy decisions and planning follow-up.

Background

The Swedish Cancer Registry

The SCR was established in 1958. The law requires that all malignant tumours, including carcinoma in situ of the breast, has to be notified and entered into the registry. Some benign lesions have to be reported as well. The registry has a high degree of completeness and includes 90-99% of all cases diagnosed with invasive breast cancer (Mattsson et al., 1984; Rutqvist et al., 1983; Garne et al., 1995). In 1997, approximately 43,000 cancers were registered of which approximately 5,800 were breast cancers. The total population of Sweden at that time was 8.8 million. In 1978, about 80% of cancers of all types were histologically confirmed (Mattsson et al., 1984) and in 1993 and 1997 the frequency was 97% (The National Swedish Board of Health and Welfare [Socialstyrelsen], 1996 and 1999).

Before and until 1980, DCIS was reported as invasive ductal cancer; however, from 1980 onwards, it was classified as in situ, as was also LCIS. In a validity control of the registry in the southern part of Sweden, the correctness of the registration of CIS was found to be 93.8% while the completeness was 78.0% during the period 1982 to 1988. Correctness and completeness during 1989 to 1991 rose to 95.9% and 94.6%, respectively (Garne et al., 1995). The SCR also includes data from the national Causes of Death Registry (CDR). The CDR has recently been validated concerning breast cancer deaths in more than 2,000 women. In 4.6%, the recorded cause of death disagreed with that in the official autopsy reports and medical records (Garne et al., 1996). In the CDR, both the direct and the indirect causes of death are entered.

Histopathological classification

Ductal carcinoma in situ of the breast

DCIS is a proliferation of malignant cells within ducts and acini of the breast parenchyma. It is distinguished from invasive breast carcinoma by the absence of evidence of invasion outside the basement membrane (Van Dongen et al., 1992). DCIS, first described in the early part of the 20th century (Bloodgood, 1931; Bloodgood, 1934), is a heterogeneous disease. Despite this early description, there is still no classification system that is unanimously accepted being clinically useful (Van Dongen et al., 1992; Consensus Conference Committee, 1997). The most frequently used

classification system is based on the architectural growth pattern (Harris et al., 1992). However, important disadvantages with this classification system exists. The major limitations include its lack of clear criteria for the different subgroups and that within the same lesion there may be a mixture of different growth patterns. These restrictions result in low reproducibility (Patchefsky et al., 1989; Silverstein et al., 1990; Rosai, 1991; Van Dongen et al., 1992; Lennington et al., 1994).

Several new proposals for classification systems of DCIS have recently been introduced (Holland et al., 1994; Silverstein et al., 1995; European Community, 1996). These systems are based principally on nuclear grade. Nonetheless, they also include such different histopathological features as architectural differentiation, with special reference to cellular polarisation (Holland et al., 1994) and the presence or absence of comedo-type necrosis (Silverstein et al., 1995).

Lobular carcinoma in situ of the breast

LCIS is a lesion characterised by a solid proliferation of small cells within breast acini, with small, uniform, round-to-oval nuclei and variably distinct cell borders (Harris et al., 1992). The distinction between LCIS and atypical lobular hyperplasia is not always distinct, and sometimes these two lesions are grouped together as lobular neoplasia.

Invasive Breast Carcinoma

Invasive breast carcinoma is also a heterogeneous group of lesions. They are almost exclusively adeno-carcinomas. Histologically, they are classified as ductal or lobular invasive breast carcinoma; these two entities accounts for approximately 75% and 5-10%, respectively, of all invasive breast carcinomas. Other special types of invasive breast carcinoma are tubular carcinoma, medullary carcinoma and mucinous carcinoma and constitute, in the order given, about 2%, 5-7% and 3% of all breast cancer. Additional to the carcinoma described here, there are some other, rarer, types (Harris et al., 1992).

Histological grading based on the degree of differentiation has been shown to correlate with prognosis and in the early 1990s a new classification system modified from the earlier Bloom & Richardson method (Bloom et al., 1957) was introduced (Elston et al., 1991). The grade is derived from three morphological features: tubule formation, nuclear pleomorphism and

mitotic counts, each of which is scored on a scale from 1-3, giving a possible total of 3-9 points. Tumour grade is then allocated as follows:

Scale points	Grade	Degree of differentiation
3-5	grade I	well differentiated
6-7	grade II	moderately differentiated
8-9	grade III	poorly differentiated

Prognostic Factors

The most important factors indicating a higher risk to succumb to breast cancer are tumour size and lymph nodal status (Carter et al., 1989). These determinants are used in clinical practice to predict the benefit of adjuvant therapy in addition to traditional surgery. Numerous other prognostic factors have been reported (Mansour et al., 1994). Some of these factors are used in the clinic, including hormone receptors. Still much uncertainty persists on how to practically use these factors individually or interactively. We have used some of these factors in one study, not as prognostic factors, but as indicators of different biological events in breast carcinomas. These indicators are briefly presented below.

P53

The p53 is a tumour suppressor gene encoding a protein predominantly localised in the cell nucleus. The role of the p53 protein is not clearly defined but it is known to take an active role in the regulation of transcription (Levine et al., 1991; Lane, 1992; Elledge et al., 1993; Marks et al., 1994; Fung et al., 1995). Mutations of the p53 gene are a common genetic alteration in various human cancers, including breast cancer. Most of the mutations result in a stable protein that can easily be detected by immunohistochemistry. Although not absolute, overexpression of the p53 protein is an indicator of p53 gene mutations (Sjögren et al., 1996). The frequency of p53 mutations ranges from 15-50% in human breast cancer. Several studies have demonstrated that tumours that overexpress the p53 protein have a poorer prognosis with more relapses and a shorter survival time (Harris et al., 1993; Bergh et al., 1995).

C-erbB-2

The c-erbB-2 or HER2/neu is an oncogene carried on chromosome 17q. Amplification of the gene is seen in 10-34% of breast carcinomas, as well as in various other human tumours (Allred et al., 1992a; Porter-Jordan et al., 1994; De Potter et al., 1995). The gene codes for a protein similar to the epidermal growth factor receptor. Overexpression of the c-erbB-2 protein, as well as the amplification of the gene, has been shown to be a prognostic factor for poor outcome in node-positive breast cancer. The results concerning node-negative tumours are less convincing, however. The evidence suggests that only membrane staining is a reliable sign of c-erbB-2 overexpression when IH staining is used.

Ki 67(proliferation)

Ki 67 is a nuclear protein seen in all phases of the cell cycle, except in G0 (Gerdes et al., 1984). A large proportion of cell nuclei stained for Ki 67, or a high S-phase fraction indicates a high proliferation rate in the tumour and thus a poorer prognosis (Sahin et al., 1991; Wintzer et al., 1991; Camplejohn et al., 1995). Today, DNA flow cytometric measurement of the S-phase fraction is clinically used to assess cell proliferation in many sites. Ki 67 may be a more reliable indicator of cell proliferation than the S-phase-fraction determination, though this is not an established fact (Van Dierendonck et al., 1989). In small and non-palpable tumours there may be difficulties to assess enough tumour-material to perform a flow cytometry. Furthermore, the S-phase-fraction gives us an averaged estimate of the whole tumour. Heterogenicity within the tumour with parts exhibiting high proliferation indicating a poor prognosis can be overlooked. The value of S-phase-fraction and Ki 67 as prognostic tools in small invasive and in situ carcinomas of the breast is not clearly established (Clark et al., 1989; Clark et al., 1992).

Angiogenesis

In the last decade, much attention has been given to tumour angiogenesis as a strong predictor of poor prognosis in breast cancer patients (Weidner et al., 1992; Toi et al., 1993; Gasparini et al., 1994), however, a few reports cannot verify this tendency (Khanuja et al., 1993; Visscher et al., 1993). Even in DCIS, there has been reports of cases with an augmented angiogenesis (Guidi et al., 1994; Lee et al., 1997). The importance of angiogenesis as a prognostic factor in DCIS is still obscure. CD 31 (or JC 70) is an antibody that stains endothelial cells. This antibody is a more sensitive marker of endothelial cells than F VIII-ab, which was used earlier

(Parums et al., 1990). The methods of quantification of angiogenesis in breast cancer are still much debated (Fox et al., 1995) and for now there is no standard method used for both DCIS and invasive breast cancer. In our study, we used a semi-quantitative method and then classified the amount of microvessels as high or low.

Hormone receptors

Many studies indicate that among patients with node positive breast cancer, those with oestrogen and progesterone receptor positive tumours have a better outcome (Parl et al., 1984; Klintenberg et al., 1986; Mansour et al., 1994). Patients with a high level of hormone receptors are also thought to be more likely to respond to anti-hormonal treatment (Litherland et al., 1988). However, the significance of hormone receptors in node negative women is more controversial (Fisher et al., 1988; Silvestrini et al., 1995). In small tumours, IH staining seems to be the method of choice for hormone receptor determination (Blomqvist et al., 1997). Analogous with the S-phase fraction determination, it may be difficult to provide enough tumour material for biochemical receptor analyses in small invasive and in situ carcinomas.

Bcl-2

The Bcl-2 protein, expressed by the Bcl-2 gene (B cell leukemia/lymphoma-2 gene), is a protein belonging to a family of apoptosis-regulatory gene products (Boise et al., 1995; Kroemer, 1997). Bcl-2 is thought to prevent apoptosis. Loss of Bcl-2 expression occurs in poorly differentiated invasive and in situ carcinomas of the breast. Some evidence is available to suggest that loss of Bcl-2 expression may be related to negative ER status and to positive p53 and positive c-erbB-2 status (Silvestrini et al., 1994; Quinn et al., 1998). Its relation to prognosis, however, is not fully confirmed (Joensuu et al., 1994).

Aims of the study

<u>General aims</u>

The study consists of two main parts: One part involves the use of an epidemiological approach to investigate risk factors for subsequent invasive breast carcinoma and breast cancer death after primary carcinoma in situ of the breast. The second part concerns tumour biology in which small invasive and in situ breast carcinomas were systematically examined in order to study tumour progression.

<u>Specific aims</u>

I.   To investigate the risk of new invasive breast cancers (ipsi- and contralateral) and to determine the risk of dying of breast cancer in a large Swedish population-based cohort of women with a previous CIS.

II.   To investigate risk factors (age, mode of detection, tumour characteristics and therapy) associated with the development of invasive breast cancer or breast cancer death after a previous DCIS in a case-control study nested within a population-based cohort.

III.   To estimate the standardised incidence ratio (SIR) of developing an invasive breast cancer for women with a previous DCIS or LCIS.

IV.   To evaluate new classification systems of DCIS.

V.   To compare histopathological grading and expression of tumour markers in pure DCIS, small invasive lesions and lesions with both an invasive and an in situ component in an effort to find biological markers of the progression from in situ to invasive carcinoma.

Materials and methods

Paper I.

From all 4,661 women registered to the SCR for a first CIS (1960-1992), we selected a cohort of 3,398 women who were diagnosed between 1980 and 1992. The recruitment period was chosen in accordance with the reporting routines for the registry. Up to 1980, DCIS was often reported as invasive cancer. We followed the cohort for the events of interest (subsequent invasive carcinoma of the breast and breast cancer death) until December 1992. We were particularly interested in time trends, since the period of diagnosis covers the 1980s when mammography screening and BCS were introduced. We also studied age at diagnosis as a determinant for poor prognosis. In this cohort, we could not differentiate DCIS from LCIS. The medical records were collected in women whose breast cancer was entered in the CDR as the direct or indirect cause of death or who later developed invasive breast cancer reported to the SCR. The 500 controls from the case-control study (Paper II) that were diagnosed between 1980 and 1992 were used as a sample to validate our cohort data. We studied the correctness of registration and estimated the proportion of LCIS, of BCS and of mammographically-detected lesions over time.

Paper II.

From a cohort including all 4,661 women registered to the SCR for a first CIS (1960-1992), we selected as cases all women with a DCIS who later died of breast cancer (n=39) or who developed a subsequent invasive cancer in either breast (n=118). From this cohort, we also selected up to four controls matched to the cases by year of diagnosis and health care region. Only controls with primary DCIS were included; women with LCIS or signs of invasiveness were excluded from the study. We investigated age at diagnosis, mode of detection, tumour characteristics and primary therapy as risk factors for developing ipsilateral invasive breast cancer, contralateral invasive breast cancer or death as a result of breast cancer. We collected all medical records from the time of the initial diagnosis, including the original histopathological reports for all cases and controls. Furthermore, we collected all medical records from the time of the event for the cases.

Paper III.

We estimated the SIR for invasive breast cancer developing in a cohort of 3,455 women with primary CIS reported to the SCR from 1980 through 1992. The distribution of DCIS and LCIS in the cohort was estimated by using the controls (n=500) from a case-control study nested within the cohort (Paper II) as a sample from the cohort (Paper II). All medical records, including the original histopathological reports, were collected for all cases and controls in the case-control study to determine the specific type of CIS. The expected number of breast carcinomas was calculated using the SCR data for an age- and calendar year-specific Swedish female population.

Paper IV.

The histopathological specimens from 195 women with primary DCIS, consecutively diagnosed from 1986 to 1994 at the University Hospital of Uppsala and at the Central hospital in Västerås, were re-classified by two independent observers. We used two new histopathological classification systems: a system proposed by an EORTC working group (Holland et al., 1994) and the Van Nuys system (Silverstein et al., 1995). The relapse free survival (RFS) by histopathological subgroup was estimated for women treated with BCS (n=149). In a separate analysis, we analysed nuclear grade only, which is the accepted system for classification of DCIS by a European Union Work Group on Breast Cancer Screening Pathology in 1996 (European Community, 1996).

Paper V.

We examined the distribution of tumour markers in three cohorts of women with breast carcinoma: Pure DCIS (n=194), small invasive breast carcinomas ≤10 mm (n=127) and breast carcinomas that contained both an in situ and an invasive element (n=305). Of these three cohorts, two were recruited from the catchment areas of Uppsala and Västerås hospitals during the years 1986 to 1994. The first of these two cohorts was the group with pure DCIS, as described above (Paper IV); the second included the cohort with all invasive breast carcinomas with a diameter of 10 mm or less and without signs of an in situ component. The third cohort contained all women with an invasive breast carcinoma, having a DCIS component according to the original histopathological report. These women were diagnosed in Uppsala County between the years 1986 and 1995.

We hypothesised that tumours comprising both DCIS and an invasive component constituted a group in which malignant transformation was currently in progress. We further assumed that small invasive carcinomas (≤10 mm) without any in situ component made up a group in which only a small proportion of the tumours had gone through a stage of DCIS before becoming invasive. Women with pure DCIS served as a reference group in which the crucial malignant transformation to invasiveness was presumed not to have taken place.

All histopathological specimens were re-evaluated according to the EORTC and the Elston-Ellis classification systems. New slides were prepared for IH staining of paraffin embedded material for each tumour marker separately (p53, c-erbB-2, Ki 67, ER, PR, Bcl-2 and CD 31). In lesions with both an invasive and an in situ element, the histopathological classification and the evaluation of the staining were done in both parts separately.

The antibodies used were p53 clone DO-7, DAKO, c-erbB-2 poly rabbit, DAKO, Ki 67 MIB-1, Immunotech, KEBO, ER 6F11, Novocastra, PR 1A6, Novocastra, Bcl-2 clone 124, DAKO and CD 31 clone JC/70A, DAKO. The staining was performed in an automatic staining machine (Ventana Medical Systems, Inc.).

Statistical methods

Paper I.

Life-table plots of overall survival, breast cancer-related suvival and RFS were constructed for the cohort. Cox regression models were made to study the effects of age, time of diagnosis and screening activity on the risk of succumbing to breast cancer as well as the risk of developing subsequent invasive breast cancer. The age groups chosen were <40, 40-49, 50-59, 60-69, 70-79 and >79 years. The time of diagnosis was divided into four periods: 1980-1982, 1983-1985, 1986-1988 and 1989-1992. In the analyses on the effects of screening, we compared regions having fully developed screening with those having no screening programs, for each of the four study periods. Because not all women were offered screening in counties where randomised studies of mammography screening took place, these counties were excluded in the analyses of screening effects. We included

women only between the ages 40 and 69 years in the analysis of screening effects since women in this age range were most often included in the screening programs. We studied models of age and time of diagnosis and age and screening activity; we did not study screening and time of diagnosis together, however, because of colinearity (in the latest period 1989 to 1992 the screening was almost nation-wide). For these analyses, 95% confidence intervals (CIs) were used. SAS software was employed in all statistical calculations.

Paper II.

Using the SAS procedure -- PROC PHREG -- we calculated odds ratios (ORs) and the 95% CIs from univariate and multivariate conditional logistic regression models. The analyses were based on the matched case-control sets to associate risk factors to subsequent invasive breast cancer, either ipsilateral or contralateral, as well as to breast cancer death (SAS Institute, 1997).

Paper III.

To estimate the SIR for women with DCIS and LCIS, we used a variant of the case-cohort design with external comparison (Wacholder et al., 1987). Specifically, we calculated person-years at risk for each woman in the cohort, starting at the date of the primary diagnosis of CIS and ending at the date of diagnosis of a subsequent invasive breast cancer, at the end of follow-up, or at the date of death. We calculated expected numbers of subsequent invasive cancer cases from 5-year age-specific and calendar year-specific incidence rates in the Swedish female population and enumerated the observed numbers of cases from the case-control study. Finally, we calculated 95% CIs of the SIR using the stratified ratio method (Wacholder et al., 1987).

Paper IV.

Kaplan-Meier estimations were performed for RFS for each histopathological subgroup. For comparison of the Kaplan-Meier estimates a Log-Rank test was used. When we compared proportions and mean values, the Chi-square test and Student´s T-test, respectively, were applied. Statistical significance was set to $p<0.05$. The Microsoft Statistica software package was used for all statistical calculations.

<u>Paper V.</u>

The association between the staining of the different tumour markers in the DCIS and the invasive components of mixed lesions was described as the proportion of lesions in which both parts stained positive or both parts stained negative. The statistical trend of staining by histopathological tumour grades for the different tumour markers in invasive and in situ carcinomas was calculated with logistic regression models that used grade as an ordinal variable and estimated ORs with 95% CIs. The Microsoft Statistica and SAS software packages were used for all statistical calculations.

Results

<u>Paper I.</u>

During the follow-up period (mean 4.3 years, 14,699 person-years of observation), 78 deaths were reported to be caused by breast cancer. We classified 30 of these deaths as caused by breast cancer and 45 as due to other causes. In three cases, the individual medical records could not be traced; these three cases were included in the analyses as breast cancer deaths. During the follow-up, 115 women developed subsequent invasive breast cancer.

Overall survival and survival related to breast cancer deaths were 81.6% (CI 95%, 79.2-84.0) and 97.4% (CI 95%, 96.3-98.5), respectively, at the 10-year follow-up. The yearly risk of subsequently developing invasive breast cancer was stable during the postoperative follow-up, being similar for ipsi- and contralateral events (accumulating to approximately 4% on each side after 12 years).

The prognosis was observed to improve over time (Table 1), with survival being statistically significantly better in the last period (i.e., 1989 to 1992). The risk of developing subsequent invasive cancer also tended to be lower in the latest periods. The risk of death resulting from breast cancer was highest in the youngest and the oldest age groups, but these differences were only statistically significant in relation to the reference group in women over 70 years of age. The risk of subsequent invasive cancer

increased with age at diagnosis though this finding was not statistically reliable (Table 1).

A separate analysis was performed to study the influence of mammography screening. Women under 40 and over 69 years of age and women in counties where randomised trials of screening were being undertaken were excluded from this analysis. In the subgroup of 2,121 women, 14 died from breast cancer and 62 developed invasive breast cancer. The risk of death due to breast cancer was five times lower in women from regions where mammography screening was fully developed in comparison with those in regions without a screening program; however, the estimate was not statistically significant (relative hazard [RH] 0.2 with 95% CI, 0.0-2.1). The risk of developing subsequent invasive breast cancer was almost the same in women from regions with mammography screening programs compared with women from the regions without screening programs (RH 0.9 with 95% CI, 0.5-1.7).

Table 1. Relative hazards from Cox regression models of breast cancer death and of invasive breast cancer subsequent to breast carcinoma in situ by age group and time of diagnosis.

Age and time periods	Breast cancer death RH (95% CI)	Subsequent invasive cancer RH (95% CI)
<40 years	1.4 (0.3-7.6)	0.5 (0.2-1.3)
40-49 years	1.6 (0.5-5.3)	0.9 (0.5-1.6)
50-59 years	Ref	Ref
60-69 years	2.4 (0.7-8.0)	1.4 (0.9-2.4)
70-79 years	4.0 (1.2-13.0)	1.3 (0.7-2.4)
≥ 80 years	5.5 (1.2-25.0)	1.2 (0.5-3.2)
1980-82	Ref	Ref
1983-85	0.7 (0.3-1.6)	0.7 (0.4-1.1)
1986-88	0.5 (0.2-1.3)	0.5 (0.3-0.8)
1989-92	0.1 (0.0-0.9)	0.5 (0.3-0.9)

Abbreviation: Ref=reference group

<u>Paper II.</u>

After exclusion of all patients who were not eligible for the analyses, 570 women remained in the final sample, including 70 cases with subsequent ipsilateral cancer, 48 with subsequent contralateral cancer, 39 cases in which death was due to breast cancer and their corresponding controls.

Univariate logistic regression

Tumour size ≥25mm and multifocality were found to significantly increase the risk for breast cancer death. The 50-59 year-old group had a non-significantly lower risk for breast cancer death than either younger or older age groups. Mode of detection, palpability, free margins and type of treatment did not appear to affect this risk.

Compared with BCS *without* XRT, mastectomy and BCS *with* XRT significantly lowered the risk for new ipsilateral invasive cancers. Tumour palpability also lowered this risk by half, although the upper 95% confidence limit for the OR just exceeded 1.0. In contrast to the pattern for breast cancer deaths, large tumour size and multifocality did not affect the risk for ipsilateral cancer, neither did age group, mode of detection, nor free margins affect this risk.

Unclear margins and BCS *with* XRT markedly increased the risk for new contralateral invasive cancers. Tumour palpability lowered this risk but not significantly so. No other risk factors appeared to affect the risk for new contralateral invasive cancers.

Multivariate conditional logistic regression

The final models included age group, tumour size, and treatment category. Tumour size ≥25mm was the most important prognostic factor that increased the risk for breast cancer death (Table 2).

In the models for ipsilateral invasive breast cancer, tumour size and type of treatment were probably mutually confounding because adding the variable tumour size to the models enhanced the protective effect of mastectomy and XRT on ipsilateral cancer occurrence.

In the models for contralateral invasive breast cancer, tumour size and type of treatment were mutually confounding because adding tumour size to the

models substantially diminished the effect of BCS *with* XRT on contralateral invasive cancer occurrence (Table 2).

Table 2. Variables associated with breast cancer death and development of invasive cancer in either breast after primary DCIS. Multivariate analysis.

Variable	Breast cancer death OR (95% CI)	Ipsilateral cancer OR (95% CI)	Contralateral cancer OR (95% CI)
Age:<50 years	1.6 (0.3-8.3)	0.7 (0.2-2.4)	0.7 (0.2-2.7)
50-59 years	Ref	Ref	Ref
>59 years	1.2 (0.2-7.5)	0.9 (0.2-3.4)	1.2 (0.3-4.3)
Tumour size:≥25mm vs.<25mm	2.9 (0.8-10.1)	2.3 (0.7-7.0)	1.7 (0.5-5.1)
Treatment: BCS	Ref	Ref	Ref
BCS + XRT	1.4 (0.1-18.1)	0.1 (0.0-1.0)	3.6 (0.3-43.5)
Mastectomy	1.8 (0.4-7.6)	0.1 (0.0-0.5)	0.7 (0.2-2.9)

Abbreviation: Ref=reference group

Paper III.

During the follow-up, 102 women with primary DCIS and 14 women with primary LCIS had subsequent invasive breast cancer reported to the SCR. In nearly half of these cases, the cancer appeared in the contralateral breast. After primary DCIS, 48% of the invasive cancers were ipsilateral, 33% contralateral and 19% either bilateral or metastatic beyond the breast. After primary LCIS, 36% were ipsilateral, 57% contralateral and 7% bilateral. The SIR for subsequent invasive breast cancer after primary DCIS was 4.5 (CI 95%, 3.7-5.5) and after primary LCIS it was 4.0 (CI 95%, 2.1-7.5). The SIRs did not differ by age at diagnosis.
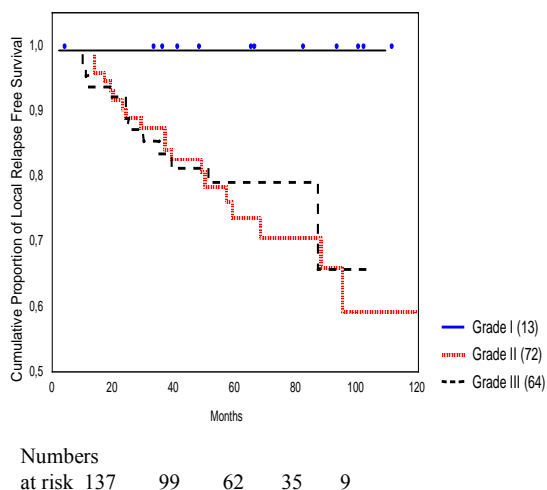
Paper IV.

According to the EORTC and Van Nuys classification systems, overall agreement was achieved in the histopathological grading between the

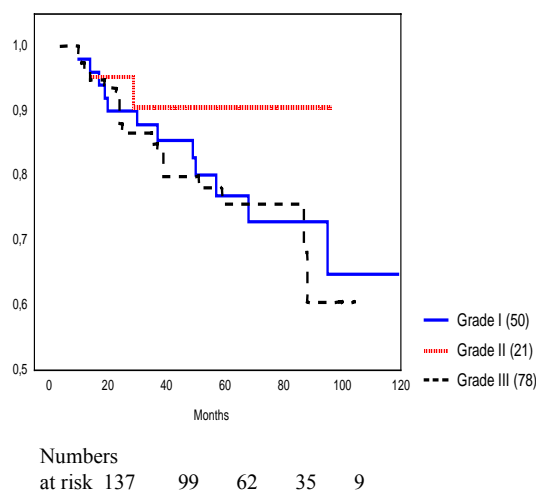observers in 79% (EORTC system) and 64% (Van Nuys system) of the cases.

In all, 32 local recurrences occurred among 149 women treated with BCS during the follow-up (mean, 59 months). No distant recurrences or breast cancer deaths were reported. The women in a small group (9%) with the highest differentiation, based on the EORTC classification, had no recurrences. RFS did not differ appreciably between the two other groups. This lack of difference also held after stratification for radiotherapy. We found no statistically significant difference in RFS between the three groups either in the Van Nuys classification or in the nuclear grade system. (Figure 1).

Figure 1.
(A) EORTC classification

Numbers
at risk 137   99   62   35   9

(B) Van Nuys classification

Numbers
at risk 137   99   62   35   9

(C) Nuclear grade

Numbers
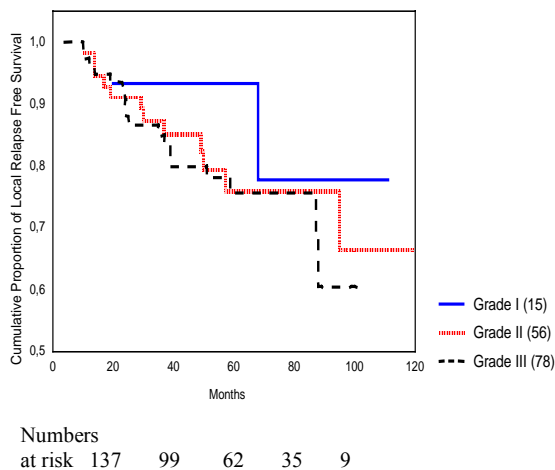at risk 137   99   62   35   9

Figure 1. RFS by histopathological subgroup in women with DCIS and treated with BCS.

<u>Paper V.</u>

Among the 626 women included in this study, we could not find paraffin embedded material in 30 cases (5%). These 30 women were included in analyses using the van Gieson staining technique only. In another 46 (7%) cases one or more of the IH staining instances was not performed, which was mainly due to a small tumour size that did not allow us to prepare all the slides that were needed.

The proportions of lesions showing positive staining for the different tumour markers and the distribution of lesions according to histopathological grade in the different cohorts are summarised in Table 3.

Table 3. DCIS was classified in accordance with the EORTC system (grades A-C) and invasive breast carcinoma based on the Elston-Ellis system (grades I-III).

	DCIS pure	**Invasive breast carcinoma ≤10mm** *without* **an in situ component**	**Invasive breast carcinoma** *with* **an in situ component**
P53	40%	25%	28%
C-erbB-2	55%	41%	49%
Ki 67	19%	21%	27%
ER+	68%	78%	71%
PR+	43%	52%	52%
Bcl2+	48%	52%	57%
CD 31	35%	27%	35%
Grade A-I	6.7%	51.2%	56.3%
Grade B-II	50.5%	43.9%	35.4%
Grade C-III	42.8%	4.9%	8.3%

An unexpected finding was the "more malignant" picture in the DCIS cohort compared with the invasive tumours. However, the distribution based on grade was quite different in DCIS and invasive lesions. DCIS and the invasive carcinomas were graded using different histopathological

systems. Since grade may be a more general and composite marker for tumour progression and strongly associated with the other markers, we analysed the staining for the tumour markers by grade. These results are presented in Table 4.

Table 4.

Grade	DCIS pure			Invasive breast carcinoma ≤10mm *without* an in situ component			Invasive breast carcinoma *with* an in situ component		
	A	**B**	**C**	**I**	**II**	**III**	**I**	**II**	**III**
P53	0%	25%	63%*	13%	21%	67%*	17%	33%	71%*
C-erbB-2	31%	27%	75%*	35%	33%	83%	43%	53%	70%*
Ki 67	0%	9%	33%*	5%	23%	67%*	15%	41%	52%*
ER+	92%	85%	46%*	81%	71%	17%*	77%	70%	30%*
PR+	83%	54%	25%*	46%	46%	0%	57%	56%	21%*
Bcl2+	83%	54%	37%*	54%	41%	33%	66%	48%	37%*
CD 31	25%	22%	52%*	13%	40%	100%*	25%	42%	68%*

*Statistically significant trend of staining for the tumor markers based on grade in the different groups.

Going from lower to higher grades, almost every biological event that the different staining represented had a statistically significant trend of a "more malignant" picture in both the in situ and the invasive cohorts (Table 4). We looked at the in situ component of the mixed lesions and when the analysis was contingent on grade, it revealed the same pattern of a "more malignant" picture for all markers. The staining of the invasive and in situ components was highly correlated for all tumour markers in the mixed tumours, which is expressed in Table 5.

Table 5. The correlation of the staining between the invasive and the in situ part in mixed breast carcinomas for different tumour markers. The percentage of lesions with either both parts positive or both parts negative are given.

	P53	C-erbB-2	Ki 67	ER+	PR+	Bcl2+	CD 31 stroma
Invasive breast carcinoma *with* in situ (n=301)	86%	93%	89%	93%	84%	89%	74%

General Discussion

Papers I-III.

Women treated for DCIS run a higher than normal risk of developing invasive breast cancer in the same breast (Millis et al., 1975; Rosner et al., 1980; Ringberg et al., 1991; Bellamy et al., 1993; Eusebi et al., 1994). In retrospective studies of small series of patients, a re-evaluation of biopsies judged to be benign led to a re-classification of lesions to DCIS. The risk of developing a subsequent ipsilateral invasive or in situ cancer in these studies was up to 50% after approximately 10 years (Betsill et al., 1978; Rosen et al., 1980; Page et al., 1982). In studies of the outcome after breast-conserving treatment for DCIS, the cumulative incidence of new ipsilateral events varies from 4-45% with a follow-up of 3 to 8 years (Bradley et al., 1990; Price et al., 1990; Graham et al., 1991; Swain, 1992; Fisher et al., 1993; Solin et al., 1996). Approximately 50% of the new ipsilateral events are invasive cancers (Graham et al., 1991; Fisher et al., 2000). Postoperative radiotherapy has been shown to considerably lower the risk of a new ipsilateral event (Fisher et al., 1993). The risk of ipsilateral recurrences after mastectomy is low: 2-5% after up to 9 years of follow-up (Fisher et al., 1986; Kinne et al., 1989; Silverstein et al., 1991; Harris et al., 1992).

Few of the studies concerning prognosis after treatment for DCIS have published data on contralateral breast cancer events, but the NSABP group reported that 2.3% of 790 patients had a new contralateral event after a

mean follow-up of 43 months, with 61% of these classified as invasive cancers (Fisher et al., 1993). In a register study from Washington the cumulative incidence of contralateral breast cancer was 2.4% and 6.1% after 5 and 10 years, respectively, in 1,929 patients with DCIS. Of these cancers, 66% were invasive (Habel et al., 1997).

The risk of dying of breast cancer after a primary diagnosis of DCIS is largely unknown. In a meta-analysis that included 585 patients treated with mastectomy and 308 patients treated with local excision, the overall survival rate was 97-99% after a mean follow-up of about 7 years (Bradley et al., 1990). In the NSABP B-17 study the mortality rate due to breast cancer was 1.6% among 623 women after an 8-year follow-up (Fisher et al., 1999). The generally good prognosis after treatment for DCIS requires large patient series and long-term follow-ups to evaluate the risk of death caused by breast cancer. In a recent population-based Norwegian study the breast cancer corrected survival after DCIS was almost 100% after follow-up of 10 years (Wang et al., 2000).

Even less is known about the natural history of LCIS. LCIS is thought to be a general risk factor for invasive breast cancer, with similar risks for both breasts (Rosner et al., 1980; Bradley et al., 1990; Posner et al., 1992; Frykberg et al., 1993). The risk of a subsequent invasive cancer is reported to be 7 to 11 times greater than the risk of breast cancer in the general female population (Rosen et al., 1978; Haagensen et al., 1978; Page et al., 1991; Harris et al., 1992). No estimates with respect to the risk of dying of breast cancer after primary LCIS have been reported.

The key finding in our cohort study (Paper I) was that women with cancer in situ of the breast had a better prognosis if diagnosed in the period 1989 to 1992 in comparison with those diagnosed in 1980 to 1983. To understand how changing diagnostic and treatment policies influence the change in prognosis, the study provides information similar to that from an ecological study. We have no individual data. However, the analysis comparing counties and diagnostic periods with different screening policies suggests that screening may contribute to improved prognosis. During the 1980s, participation in screening programs was high, well over 80% in all programs. Consequently, it is probable that detection with mammography is the chief contributor to incident cases in counties with mammography screening programs. On the other hand, if screening is responsible for much of the improvement in prognosis, we cannot say whether we observed (i) a lead time effect, (ii) a natural history of cancer in situ of a more benign

type than the one detected in an ordinary clinical setting, or (iii) a really improved prognosis. We could not determine whether the histopathological diagnostic criteria changed during the study period, but the cohort study was completed at a time when reporting routines to the cancer registry were uniform. The increasing use of breast conservation over the years may indeed increase the risk of subsequent invasive cancer events, but such an effect was not detected at a group level. It may also have been counteracted by the increased use of postoperative radiotherapy.

Another important outcome of the cohort study (Paper I) was that we could substantiate the findings that although these women ran a considerably higher risk of developing invasive breast cancer than a normal background population, their prognosis was good (Bradley et al., 1990; Wang et al., 2000), with a breast cancer corrected survival of more than 97% at 10 years.

To study the effects of different tumour characteristics and treatment on prognosis, we designed a case-control study nested within the population-based cohort (Paper II). The use of the population-based cohort allowed an unbiased selection of controls. We allocated the entire cohort with all primary CIS reported to the SCR during the period 1960 to 1992; only DCIS cases and controls were included. Matching controls to cases on year of diagnosis and health care region minimised the possible effects of periodic or local differences in reporting. Within each health care region, regional breast cancer working groups have established guidelines for treatment since the end of the 1970s. Thus, within each region and time period, treatment protocols have been similar since that time.

Tumour size is an important, clinically relevant prognostic factor for breast cancer death. The strong protective effect on ipsilateral cancer recurrence from XRT or mastectomy was expected. However, XRT and mastectomy did not protect against contralateral cancer or breast cancer death. Thus, bilateral mammography remains necessary during follow-up.

As in most observational studies, the estimates of the effect of different treatment strategies must be interpreted with extreme caution. Confounding by severity or other selection mechanisms may have influenced these estimates, particularly because the multivariate models indicated that tumour size and choice of treatment were mutually confounded. The decision to administer radiotherapy at a time when this was not a routine practice was probably based on patient characteristics that could have

influenced the prognosis. However, for confounding alone to explain very low (0.25 or below) or high (4.0 or above) ORs, the effect of this confounding on the outcome must be very strong (Bross, 1967; Day et al., 1979).

The pattern of risk by treatment category for ipsilateral new invasive cancers differed from that for breast cancer death and contralateral cancer. This difference challenges the notion that the primary path from primary DCIS to breast cancer death runs from local progression in the breast to invasiveness, then to metastases and, ultimately, to death. Several possible alternative explanations would be worthwhile to pursue in other systems or laboratory settings. It should be noted that most of the deaths may arise from patients who actually had an invasive cancer from the start, but where the invasiveness was never diagnosed. The probability of overlooking an invasive component of the tumour increases with increasing tumour size. Remnants of DCIS in the remaining breast or the mastectomy scar may progress to invasiveness and still be capable of metastasising without giving any clinical signs of local recurrence. In general, however, these explanations are difficult to reconcile with the very low propensity of DCIS to metastasise to axillary lymph nodes (Lagios et al., 1982; Silverstein et al., 1991).

In accord with other reports on invasive breast cancer (Adami et al., 1986; Host et al., 1986), we found the same biphasic risk pattern regarding age at diagnosis and breast cancer death (Papers I-II). However, we could not corroborate the reports of another study on DCIS (Van Zee et al., 1999) that younger age at diagnosis entailed a higher risk of local recurrence.

Although DCIS and LCIS both increased the risk for invasive breast cancer, the extent of their risks did not differ and high proportions of these invasive cancers were contralateral after both DCIS and LCIS (Papers I and III). This challenges the notion that their natural histories -- in terms of risk of invasive breast cancer -- should differ. The old dogma asserts that the cancer morbidity after DCIS is almost solely due to local recurrences, where LCIS is "only" a marker of susceptibility to cancer (Rosner et al., 1980; Posner et al., 1992; Frykberg et al., 1993). The ratio of contra- to ipsilateral invasive cancers is obviously influenced by the use of BCS and of contralateral prophylactic mastectomy. Increasing use of adjuvant radiotherapy to the remaining breast after BCS, however, should result in local control similar to that with mastectomy (Fisher et al., 1993). Prophylactic mastectomy was used in only a few cases during the studied

period and we estimated that a prophylactic contralateral mastectomy was performed in 1.2% of the cohorts in Papers I and III.

Finally, this study has several implications for further research. Women with cancer in situ of the breast should be analysed separately in clinical studies (e.g., in studies of screening). The time of diagnosis, mode of detection and age at diagnosis are all likely to confound comparisons of treatments and risk factors in non-randomised studies or in studies comparing different series of patients. The margin for therapeutic improvement is small and very large numbers of patients are needed to detect modest therapeutic effects.

Papers IV-V.

DCIS is typically viewed as a precursor of invasive breast cancer. A model for the progression from adenoma to adenocarcinoma is suggested for colon cancer (Fearon et al., 1990). The genetic steps necessary for malignant transformation in breast cancer, however, are not known (atypical ductal hyperplasia – DCIS – invasive cancer) and we do not yet know if these steps are reflected in the presence or absence of known prognostic tumour markers (Rosai, 1991; Steeg et al., 1996; Gupta et al., 1997). Autopsy studies strongly suggest that not all DCIS lesions progress to invasiveness during a patient's lifetime (Andersen et al., 1985) and it would be relevant to be cognisant of which lesions have a high risk of progression.

Some investigators have claimed that subgroups of DCIS differ in their biological behaviour; e.g., the comedo-type lesion is reported to have a higher risk to progress to invasive cancer (Lagios et al., 1989; Silverstein et al., 1990). The comedo-type lesion presents with a higher thymidine labelling index, a higher grade of stromelysin-3 and is more often c-erbB-2 positive than other subgroups (Meyer et al., 1986; Van der Vijver et al., 1988; Bartkova et al., 1990; Basset et al., 1993). These findings are related to DCIS, classified in accordance with the histopathological architectural pattern. Because of the disadvantages with low reproducibility and because of a lack of clear criteria for the different subgroups based on the architectural patterns, we wanted to find a reliable and clinically useful histopathological classification system as a base for further studies (Paper IV).

In our view, the classification proposed by the EORTC working group (Holland et al., 1994) is the most promising. It was easy to learn and one small group with well-differentiated lesions and with very good prognosis was easily discernible. Two reports using the EORTC classification have been published (Bobrow et al., 1994; Zafrani et al., 1994). Regrettably, prognosis was not reported in these studies and we can not disclose if their well-differentiated groups had a prognosis comparable to that in our study. There are indications that the subgroups, based on the Van Nuys system proposed by Silverstein, have a different likelihood of local recurrence after BCS (Silverstein et al., 1995). However, in our study there was no statistically significant difference regarding local RFS between the subgroups using this classification system (Figure 1). The Van Nuys system divides DCIS into three groups. First, between high and non-high nuclear grade and, second, the non-high grade lesions are further divided by the presence or absence of comedo-type necrosis. If we classified the lesions on the basis of nuclear grade only, excluding necrosis as a criterion, then we had a better separation between the RFS curves, although not to a statistically significant degree. When we used the Van Nuys system, we obtained a large number of lesions (n=45) with intermediate nuclear grade but no necrosis and which therefore were classified as grade I, based on the Van Nuys system and as grade II, on the basis of nuclear grade. As 10 of those 45 women had a local relapse, this shift of grade might explain the difference in RFS for the different groups according to the two systems (Figure 1).

In Paper V, we studied the distribution of tumour markers in three cohorts of women with breast carcinoma: Pure DCIS, small invasive breast carcinomas ≤10 mm and breast carcinomas that contained both an in situ and an invasive element. Our goal was to determine if the distribution of some commonly used prognostic markers could distinguish a group of DCIS patients with a high probability of progression to invasiveness or if any change in tumour markers between DCIS and invasive lesions could yield important clues about tumour biology.

We also studied the distribution of tumour markers relative to histopathological grade. Several studies have indicated that high grade DCIS might be a precursor to high grade invasive breast cancer, likewise, low grade DCIS is thought to be a precursory indication of low grade invasive breast cancer. Both histopathological grade and DNA contents have been reported to be similar in the invasive and in the in situ

components of mixed breast carcinomas (Iglehart et al., 1995; Douglas-Jones et al., 1996; Gupta et al., 1997; Buerger et al., 1999).

The cohort with pure DCIS exhibited a "more malignant" pattern for all tumour markers, except proliferation. One possibility is that the invasive carcinomas might be more easily detected (clinically or mammographically) than DCIS and, therefore, they were detected at an earlier point in time relative to their inception. DCIS, then, may have had time to progress, both regarding the studied tumour markers and the histopathological grade before becoming detectable. However, invasive carcinomas with an in situ component did not differ clearly from invasive carcinomas without an in situ component in regards to tumour markers and histopathological grade. Proliferation seemed to be more pronounced in invasive carcinomas than in DCIS, but it was also strongly correlated to grade in all cohorts.

Similar to other studies, the overexpression of c-erbB-2 in DCIS was most frequently seen in low-differentiated lesions. C-erbB-2 overexpression was also more frequently found in the cohort with pure DCIS than in the cohort with invasive lesions (Allred et al., 1992b; Somerville et al., 1992; Leal et al., 1995). Yet, if we compared each grade separately, c-erbB-2 overexpression was more common in invasive carcinomas (Table 3). Contrary to Allred's suggestion (Allred et al., 1992b), the present results do not support the hypothesis that overexpression of c-erbB-2 decreases within individual tumours as they evolve from in situ to invasive carcinomas. As in our study, Igelhart found that the expression of c-erbB-2 was similar in both growth phases of lesions with an invasive and an in situ component (Iglehart et al., 1995).

The angiogenesis in DCIS and invasive carcinomas was not classified in exactly the same way. However, we did compare the angiogenesis by grade in the different cohorts separately. When we compared the angiogenesis in the two growth stages of the mixed lesions, DCIS and invasive carcinoma, we found a statistically significant correlation between the components though not as strong as among the other tumour markers.

We used separate histopathological classification systems for DCIS and invasive carcinomas since there is no single system dealing with both entities. In the mixed lesions the grade of the two components, in situ and invasive carcinoma, was basically, but not totally, corresponding. When we studied the expression of the different tumour markers, however, the

expression of the markers in the two components was very similar. These findings corroborate the theory that a high-differentiated DCIS progresses to a high-differentiated invasive cancer and a low-differentiated DCIS progresses to a low-differentiated invasive cancer. Consequently, it is probable that the biological step between invasive and in situ carcinoma occurs independently of tumour grade. A substantial proportion of small invasive carcinomas does not have detectable areas of in situ carcinoma. In these carcinomas it is possible that some crucial genetic changes have occurred early in the process. Thus, it is probable that invasive breast cancer can also occur de novo.

Grade, from a prognostic perspective, is important within the in situ and invasive groups. The other biological markers we studied -- reflecting more of singular entities than grade -- are all strongly correlated with grade. In in situ carcinomas high grade entails a higher risk of local new events (of which 50% are invasive); in invasive carcinomas high grade is also associated with risk of distant metastases. It is intriguing that patterns of grade or the other markers did not seem to mark the transition from in situ to invasive carcinoma, a step believed to be crucial for the tumour's ability to metastasise. We postulate that factors that signal the tumour's ability to invade may be powerful prognostic factors and that they may be searched for in a setting comparable to ours.

General Conclusions

- Overall, the prognosis after CIS was very good. The risk for death due to breast cancer or subsequent invasive breast cancer after primary CIS was lowered if the CIS was diagnosed late in the studied period (1980-1992). Screening mammography may have contributed to the improvement of prognosis over this period. The increased use of breast conservation was not associated with a poorer prognosis in the cohort as a whole.

- Large tumour size and multifocality increased the risk for breast cancer death after primary DCIS. Postoperative radiotherapy and mastectomy lowered the risk for ipsilateral invasive breast cancer. The driving forces behind local recurrence and generalised disease may differ.

- The SIR for subsequent invasive breast cancer after primary DCIS was 4.5 and after primary LCIS, 4.0. The sizes of their risks did not differ and, a high proportion of these invasive cancers was contralateral after both DCIS and LCIS. This finding challenges the notion that their natural histories -- in terms of risk of invasive breast cancer -- should differ.

- A small group of DCIS with highly differentiated lesions and an excellent prognosis was defined with the EORTC classification system. Further classification into intermediately and low-differentiated lesions did not help predict RFS.

- The expression of different tumour markers (p53, c-erbB-2, Ki 67, ER, PR, Bcl-2 and CD 31) strongly correlated with tumour grade in both DCIS and invasive breast cancer. The expression of the tumour markers in the two components of lesions with both an invasive and an in situ component was very similar. Apparently, a high-differentiated DCIS progresses to a high-differentiated invasive cancer and a low-differentiated DCIS progresses to a low-differentiated invasive cancer.

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