UMEÅ UNIVERSITY

DCIS of the breast
Aspects on treatment and prognosis

Thesis for doctoral degree

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Umeå 2018

Dissertation for PhD
ISBN: 978-91-7601-899-6
ISSN: 0346-6612
New Series Number 1972
Cover design: Ella Wadsten
Electronic version available at: http://umu.diva-portal.org/
Printed by: Print & Media, Umeå University, 901 87 Umeå
Umeå, Sweden 2018

To Mats, Anna, Carl, and Ella

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Abstract

Breast cancer is the most common cancer form and a leading cause of death in women worldwide. Ductal breast carcinoma in situ (DCIS) is characterized by a proliferation of malignant cells confined within the mammary ducts and is a potential precursor of invasive breast cancer. The risk estimations of a DCIS to develop into invasive cancer over a 10 year period range from 30-50%. In the past 25 years, concomitant with the implementation of screening mammography, the incidence of DCIS has increased dramatically and presently almost 1 000 women are diagnosed with DCIS each year in Sweden. The increased incidence poses concerns of overtreatment and current research aim at identifying clinical or pathological markers that can reliably distinguish hazardous from harmless DCIS.
The overall aim of this thesis was to explore the prognostic significance of clinical and tumourbiological characteristics of DCIS and to assess the benefits and harms of adjuvant treatment.

In a population-based cohort of 2 952 women with primary DCIS, we analysed trends in incidence, treatment and outcome over a 20-year period (**paper I**). Information was obtained from the regional breast cancer register in Uppsala-Örebro healthcare region between 1992 and 2012. A validation of 300 randomly selected women revealed high overall completeness and reliability of most key variables, whereas follow-up data were of moderate quality with only 65% of the recurrences reported to the register.
The major finding of the study was a trend towards more intensified treatment over time. The frequency of mastectomy increased from 23.0% to 39.0% and the proportion of patients receiving adjuvant radiotherapy after breast-conserving surgery increased from 30.1% to 67.6%. This did not, however, translate into any noteable improvements in outcome. Relative survival was >97% after 10 years with no significant variation over time. In conclusion, these results may reflect adequate treatment selection, but may also indicate a significant overtreatment.

In **paper II** and **III**, a nested case-control study was conducted from a cohort of 6 964 women with primary DCIS to identify clinical characteristics in DCIS associated with subsequent breast cancer death. Ninety-six women who later died from breast cancer were compared to 318 controls selected by incidence density sampling. Information was obtained from medial records and histopathology reports.
Tumour size over 25 mm or multifocal DCIS (OR 2·55; 95%CI 1·53 to 4·25), a positive or uncertain margin status (OR 3·91; 95%CI 1·59 to 9·61) and detection outside the screening programme (OR 2·12; 95%CI 1·16 to 3·86) increased the

risk of death from breast cancer. In the multivariable analysis, tumour size (OR 1·95; 95%CI 1·06 to 3·67) and margin status (OR 2·69; 95%CI 1·15 to 7·11) remained significant. More extensive treatment was not associated with lower risk, which may be due to confounding by indication, or indicate that some DCIS have an inherent potential for metastatic spread.

In **paper III**, to further explore the association of tumour biology and risk of breast cancer death, archival tumour blocks were collected. Freshly cut hematoxylin and eosin (H&E) stained sections of the primary DCIS were histopathologically evaluated for nuclear grade, presence of comedonecrosis and lymphocytic infiltration (LI). Tissue microarrays were constructed for immunohistochemical analysis (IHC) of oestrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki67. Using the results of the IHC analyses, tumours were classified into surrogate molecular subtypes.
Presence of intense periductal LI was associated with an increased risk of subsequent breast cancer death (OR 2.25; 95%CI 1.02 to 4.99). None of the other biomarkers were individually related to breast cancer death, nor were there any statistically significant differences in risk between the molecular subtypes. In multivariable analysis, stepwise adjusting for age, tumour size and treatment, PR negativity in combination with LI; PR negativity, LI and presence of comedonecrosis and the combination of PR negativity, LI, comedonecrosis and HER2 positivity were all independently associated with increased risk of breast cancer death. The significance of features in the peritumoral stroma need further investigation and may have implications for targeted treatments.

In **paper IV**, we studied the risk of ischemic heart disease (IHD) after treatment for DCIS. Postoperative radiotherapy (RT) in DCIS reduces recurrence rates by half but confers no benefits in terms of survival. It is thus of major importance to consider long-term adverse effects. Left-sided breast irradiation may involve exposure of the heart to ionising radiation with an associated risk of subsequent cardiovascular disease. The cumulative incidence of IHD was analysed in a population-based cohort of 6270 women with DCIS compared 31 257 women without a history of breast cancer. Of the women with DCIS, 38.9% had received adjuvant RT.
After a median follow-up of 8 years, there was no increased risk of IHD for women with DCIS versus the comparison cohort. The risk was lower for women with DCIS allocated to RT compared to non-irradiated women and to the comparison cohort, probably due to patient selection. Comparison of RT by laterality did not show any over-risk for irradiation of the left breast. These results are reassuring, but longer follow-up may be warranted considering the continuously increasing use of RT in DCIS management.

List of publications

I

Wadsten C, Heyman H, Holmqvist M, Ahlgren J, Lambe M, Sund M, Wärnberg F (2016): A validation of DCIS registration in a population-based breast cancer quality register and a study of treatment and prognosis for DCIS during 20 years. Acta Oncol 55:1338–1343. doi: 10.1080/0284186X.2016.1211317

II

Wadsten C, Garmo H, Fredriksson I, Sund M, Wärnberg F (2017): Risk of death from breast cancer after treatment for ductal carcinoma in situ. Br J Surg 104:1506–13.

III

Wadsten C, Hartman J, Fredriksson I, Garmo H, Sund M (2018): Biomarkers in DCIS associated with breast cancer related death. *In manuscript*.

IV

Wadsten C, Wennstig AK, Garmo H, Nilsson G, Blomqvist C, Holmberg L, Fredriksson I, Wärnberg F, Sund M (2018): Risk of ischemic heart disease after radiotherapy for ductal carcinoma in situ. Breast Cancer Res Treat. May 5; 171:95–101.

Abbreviations

BC	Breast Cancer
BRCA 1, BRCA 2	Breast Cancer susceptibility gene 1,2
TDLU	Terminal ductal-lobular units
UDH	Usual Ductal Hyperplasia
ADH	Atypical Ductal Hyperplasia
DCIS	Ductal Carcinoma in Situ
IHC	Immunohistochemistry
EMT	Epithelial to mesenchymal transition
MRI	Magnetic resonance imaging
BCS	Breast conserving surgery
RT	Radiotherapy
SNB	Sentinel node biopsy
EBCTCG	Early Breast Cancer Trialists' Collaborative Group
NSABP	National Surgical Adjuvant Breast and Bowel Project
EORTC	European Organization for Research and Treatment of Cancer
UK/ANZ	The UK, Australia and New Zealand DCIS trial
SweDCIS	The Swedish DCIS trial
CT	Computed tomography
Gy	Gray
IHD	Ischemic heart disease
SEER	Surveillance, Epidemiology, and End Results
ER	Oestrogen Receptor
PR	Progesterone Recepter
HER2	Human Epidermal Growth Factor Receptor 2
CK	Cytokeratin
Cox2	Cyclooxygenase 2
SCR	The Swedish Cancer Register
ICD	International statistical classification of diseases
BCQR	Breast cancer quality register
CDR	The Cause of Death Register
NPR	The National Patient Register
LISA	Longitudinal Integration Database for Health Insurance and Labour Market Studies
BCBase	Breast Cancer database Sweden
TMA	Tissue Microarray
H&E	Hematoxylin and Eosin
CCI	Charlson Comorbidity Index
OR	Odds ratio
CI	Confidence Interval
LCIS	Lobular cancer in situ
HR	Hazard ratio
LI	Periductal lymphocytic infiltration

Sammanfattning (summary in Swedish)

Bröstcancer är den vanligaste tumörformen hos kvinnan. Duktal Cancer in Situ (DCIS) är en typ av bröstcancer där de elakartade tumörcellerna är begränsade till bröstets mjölkgångar. Obehandlad kan DCIS progrediera till invasiv cancer, vilket enligt studier sker i knappt hälften av fallen. Under de senaste 25-30 åren, i samband med införandet av mammografisk hälsokontroll, har incidensen DCIS ökat dramatiskt och för närvarande diagnostiseras nästan 1 000 kvinnor med DCIS varje år i Sverige. I nuläget saknas kunskap om hur vi säkert ska kunna skilja ut farlig från ofarlig DCIS hos den enskilda individen, vilket leder till en ganska omfattande överbehandling med kirurgi och strålterapi.

Det övergripande syftet med denna avhandling var att undersöka det prognostiska värdet av kliniska och tumörbiologiska egenskaper hos DCIS och att värdera fördelar och risker med tillägg av strålbehandling.

I **delarbete I** analyserades trender i incidens, behandling och utfall över tid i en kohort omfattande 2 952 kvinnor med primär DCIS mellan 1992 och 2012. Information erhölls från det regionala kvalitetsregistret för bröstcancer i Uppsala-Örebro regionen. En validering av data på 300 slumpmässigt utvalda kvinnor i registret visade generellt hög täckningsgrad och god tillförlitlighet av de flesta variabler, medan uppföljningsdata var av måttlig kvalitet, 65 % av återfallen var rapporterade till registret.
Studien visade en trend mot intensivare behandling över tid. Andelen kvinnor där hela bröstet avlägsnades ökade liksom andelen patienter som fick tillägg av strålbehandling efter bröstbevarande operation. Mer behandling medförde dock ingen signifikant förbättring vad gäller återfall eller överlevnad över tid vilket kan tolkas som att omfattningen av kvinnor som överbehandlas ökar.

I **delarbete II** och **III** studerades kvinnor med primär DCIS som senare avlidit i bröstcancer i en fall-kontrollstudie. Av 6 964 kvinnor med primär DCIS i Stockholm, Uppsala-Örebro och norra regionen mellan 1992 och 2012 identifierades 96 fall och dessa jämfördes med 318 slumpmässigt valda kontroller ur samma kohort.
DCIS upptäckt utanför screeningprogrammet, stor tumörstorlek, multifokalitet eller icke radikal kirurgi ökade risken för bröstcancer död. Mer omfattande behandling minskade inte risken vilket delvis kan bero på behandlingsselektion men kan också tyda på att en del DCIS har en mer aggressiv karaktär med benägenhet för spridning där behandling med kirurgi och strålbehandling är otillräckligt.

I **delarbete III** samlade vi in tumörmaterial från fallen och kontrollerna för att undersöka eventuell association mellan tumörbiologi och risk för bröstcancerdöd. Nya snitt från tumörklossarna analyserades och vävnadsstansar samlades i en sk tissue microarray (TMA). Immunhistokemiska färgningar av olika tumörmarkörer såsom hormonreceptorer (ER och PR), proliferationsmarkörer (Ki67) och en tillväxtfaktor receptor (HER2) utfördes. Tumörerna klassificerades i molekylära subgrupper med hjälp av dessa infärgningar.
Analyserna visade att DCIS med periduktal lymfocytinfiltration ökade risken för senare bröstcancer död. Ingen annan markör kunde enskilt relateras till ökad risk, men kombinationer av negativt progesteron uttryck tillsammans med lymfocytinfiltration, med eller utan förekomst av nekros eller HER2-överuttryck var relaterat till en ökad risk. Betydelsen av ansamling av lymfocyter vid DCIS är än så länge väldigt lite utforskat och kan vara av intresse för utveckling av framtida målstyrda behandlingar.

Syftet med **delarbete IV** var att undersöka risken för kranskärlssjukdom efter strålbehandling mot bröstet vid DCIS. Strålbehandling efter bröstbevarande kirurgi vid DCIS minskar återfallsrisken med hälften men har inte visats medföra någon överlevnadsvinst. Det är därför viktigt att överväga långsiktiga biverkningar. Vänstersidig bröstbestrålning innebär exponering av joniserande strålning mot hjärtat med risk för skador på hjärtats kranskärl. Vi analyserade förekomst av kranskärlssjukdom i en populationbaserad kohort av 6 270 kvinnor med DCIS jämfört med 31 257 kvinnor utan DCIS. Av kvinnorna med DCIS hade 38,9% fått strålbehandling.
Efter en median uppföljningstid på 8 år fanns ingen ökad risk för kranskärlssjukdom för kvinnor med DCIS jämfört med jämförelsekohorten. Risken var snarast lägre för de kvinnor som fått strålbehandling jämfört med icke-bestrålade kvinnor och jämförelsekohorten, troligen på grund av selektionsmekanismer. Vi såg heller ingen ökad risk vid strålbehandling mot vänster bröst jämfört med höger bröst. Resultaten är betryggande men längre uppföljningstid kan behövas för att säkert kunna avgöra att strålbehandlingen är helt riskfri.

Background

Breast cancer Epidemiology

Breast cancer is the most common form of cancer and a leading cause of death in women worldwide (1). Incidence rates have increased primarily due to increased screening, changes in reproductive patterns and increased use of hormonal replacement therapy (1). In 2015, close to 9000 new breast cancers were diagnosed in Sweden and it is estimated that about one in nine women will be diagnosed with breast cancer in her lifetime (2).

Although the incidence increases, breast cancer mortality has declined (Fig 1.) (1). The continued improvement in prognosis may be attributable to both screening and treatment effectiveness.

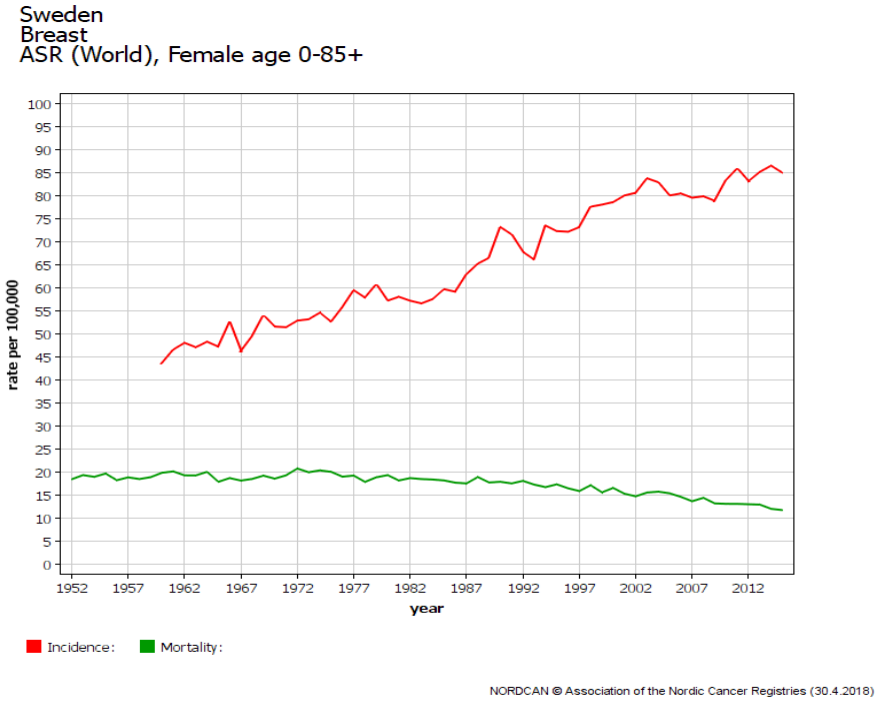

Figure 1 *Breast cancer incidence and mortality for women in Sweden. Data from NORDCAN april 2018.*

Aetiology

The aetiology is most likely multifactorial. Although breast cancer can occur

early in life, it is in general a disease of ageing (3).

After age and female sex, factors associated with a genetic predisposition or a prior history of a proliferative breast lesion are among the strongest risk factors (3). Having a mother or a sister with breast cancer doubles the risk (3,4). For women with an inherited disorder in the tumour suppressor genes BRCA 1 or BRCA 2, the life-time risk of developing breast cancer range from 45-80% (4,5). Heritability of mammographic density is emerging as one of the most important risk factors with a five times greater risk for women with the highest degree of breast density compared to women with little or no breast density (6–8).
A prior history of proliferative breast disease entails about 1.5- to 1.9-fold increased risk for breast cancer, whereas presence of atypical hyperplasia is associated with an up to five-fold increased risk (9,10). Moreover, the joint occurrence of family history and atypical hyperplasia have a strong synergistic effect (9).

Factors such as reproductive history, menstrual history, menopausal status and exogenous hormone use correlate to breast cancer risk, although these have a more modest influence on risk than the factors discussed above (3).

Anatomy of the breast and breast cancer types

The mammary gland consists of lobules (milk producing glands) and branching ducts (milk channels). The ends of the ducts are termed the terminal ductal-lobular units (TDLUs). The TDLUs consist of two types of epithelial cells: the inner luminal epithelial cells and the outer myoepithelial cells. Luminal epithelial cells line the normal breast duct and have secretory properties. Myoepithelial cells have both contractile muscle and epithelial properties (11,12). The basement membrane surrounds the epithelial cells and works as a mechanical barrier. Its function is to anchor the epithelial layer to the connective tissue underneath.

Most breast cancers arise in the TDLUs (13,14). Ductal carcinoma, currently referred to as invasive carcinoma of *no special type,* is the most common histologic type comprising about 75 % of all invasive breast cancers (15). The second most common is lobular carcinoma accounting for 5-15 % (16–18). Other less common types of invasive breast carcinomas include tubular carcinoma, mucinous carcinoma, metaplastic, papillary and medullary carcinomas.

Breast carcinogenesis

Breast carcinogenesis is a complex molecular process initiated by an accumulation of mutations in genes. Amplification of oncogenes and mutation or loss of tumour suppressor genes will affect DNA repair and disturb the balance between proliferation and cell death (apoptosis) (19). The progression from normal epithelial cells to invasive breast cancer develops through multiple stages with a number of proliferative lesions seen; from benign *usual* ductal hyperplasia (UDH), to borderline *atypical* ductal hyperplasia (ADH) and ductal carcinoma in situ (DCIS). Immunohistochemical (IHC) analyses reveal differences in these lesions indicating that UDH is a hyperplastic process, whereas ADH and DCIS are neoplastic, with a clonal proliferation of luminal epithelial cells (20,21). The progression from ADH to DCIS marks the transition between benign and malignant disease.

Invasion

Through the stages of carcinogenesis, the epithelial tumours undergo changes summarised as an epithelial to mesenchymal transition (EMT) by which epithelial cells gain migratory and invasive properties (22). Tumour invasion is a milestone in the evolution of breast cancer (Fig 2). Three general mechanisms responsible for the breach of the basement membrane have been proposed; increased mechanical pressure arising from proliferation, increased motility of tumour cells (EMT) and the release of proteolytic enzymes causing degradation of the underlying basement membrane (22,23). Once the basement membrane has been invaded, cancer cells gain access to the periglandular stroma and this paves the way for interactions with the stromal cells, growth factors and the immune system. Analyses of the tumour microenvironment during breast cancer progression suggest that these cells participates in tumorigenesis even before tumour cells invade into the stroma (24,25).

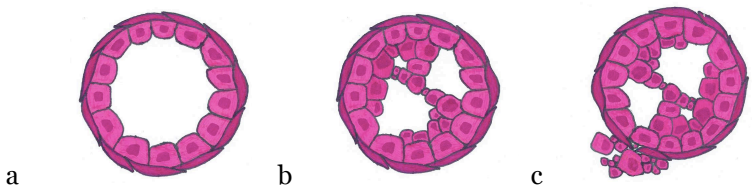

Figure 2. *Schematic illustration of a) The mammary duct lined by normal epithelial cells, b) Ductal Carcinoma in Situ and c) Invasive breast cancer*

Metastasis

Access to lymph and blood vessels allows for cells to pass on to lymph nodes where they can form locoregional metastases, as well as to the bloodstream to form distant metastases.

The prevailing theory that metastasis is a late event in disease progression has been challenged by recent research (26). Metastatic dissemination may in fact be an early event supported by findings that tumour cells can be detected in bone marrow in 20-60% of breast cancer patients without manifest metastasis (27). Emerging data suggest that molecular changes occur before morphologic alteration during progression, hence, some preinvasive lesions may have an inherent potential for metastatic spread and most relevant biological features of breast cancer are probably determined at an early stage (28–30). Preinvasive lesions tend to progress while maintaining their morphologic differentiation status, usually referred to as "progression within grade", thus well differentiated DCIS tends to progress to well differentiated invasive cancer etc. Even distant metastases are usually of the same grade as the primary tumour (31–33).

DCIS

Ductal carcinoma in situ (DCIS) is defined by a proliferation of malignant cells confined within the lumen of the breast ductal system. The term *in situ* means *in place* and was first introduced in 1932 by Broders (34). Before the introduction of public mammographic screening programs DCIS was a rare diagnosis, but since then the reported incidence has increased substantially (35–39). Today, DCIS accounts for approximately 10 % of all breast cancers in Sweden (2).
Risk factors for DCIS are similar to those for invasive breast cancer (40) implicating that DCIS is a true precursor to invasive cancer. However, not all invasive breast cancers are clearly preceded by DCIS and not all DCIS lesions will progress to invasive cancer. In autopsy specimen, the prevalence of DCIS ranges between 6% and 14% (41–43), suggesting some lesions would never have been of clinical importance. Estimates of the risk of progression from *in situ* to invasive breast cancer has been obtained from patients with previously misdiagnosed benign breast disease who received no treatment and for whom subsequent evaluation of biopsy specimens revealed DCIS. Progression rates between 14-53% have been reported from these studies (44–47).
Several studies have aimed to assess prognostic factors to characterize and classify DCIS lesions and their risk of invasive potential.

Classification

Traditionally, DCIS was classified according to the predominant architectural pattern in which comedo, solid, cribriform, micropapillary growth patterns were recognized (48). This classification has its limits in lack of reproducibility, mainly because of heterogeneity of the disease (49). In the last decades a classification based primarily on cytonuclear differentiation has been adopted (50). This differentiates the lesions into grades I, II and III, where grade III represents the most aggressive type characterized by marked nuclear pleomorphism, large nuclei size, irregular chromatin and mitoses. Grade I DCIS cells show small, monomorphic nuclei with diffuse finely dispersed chromatin. The intermediate grade II is defined as neither grade I nor grade III (50).

Detection and diagnosis

More than 90% of DCIS lesions are identified on mammography as suspicious microcalcifications (51). This explains the rapid increase in incidence with the introduction of mammography screening. In Sweden, invitational screening was implemented between 1974 and 1997 (52), whereas most other countries in Europe and north America started screening in the early 90's (53). A fivefold increase in incidence of DCIS has been reported in several studies (35,36,38,53), but after the initial rapid increase, the incidence has remained stable (36,38). Between 70-80% of all DCIS are estimated to be detected by screening (54–60). The main limitation of mammography is that the extent and the number of tumour foci in patients with multifocal disease often are underestimated. Contrast-enhanced magnetic resonance imaging (MRI) has higher sensitivity but low specificity, which may lead to unnecessary additional biopsies or more extensive surgery than required (61). Currently, MRI is only used in selective cases and there is no evidence that the use of MRI improves outcome in patients with DCIS.
The DCIS diagnosis is confirmed by either stereotactic or ultrasound-guided core biopsy. Over the past ten years, larger-gauge vacuum-assisted needle biopsies have developed. These are particularly useful in breast lesions of uncertain malignant potential (B3 lesions) as an alternative to surgical excision (62).

Surgery

Surgical management of DCIS involves either mastectomy or breast conserving surgery (BCS) with or without radiotherapy (RT). After a mastectomy, reported recurrence rates are as low as 1 to 2% after 10 years of follow-up (63,64). Recurrence rates after BCS range between 20% to 30 % after 10 years of follow-up (56,57,65,66). There are no randomized studies comparing BCS with

mastectomy, but in population-based studies survival is similar (63,67), potentially due to appropriate selection of treatment for each patient. Currently, the majority of women with DCIS are treated with BCS with postoperative RT to the conserved breast.
There are on-going trials investigating conservative management of DCIS where surgery is completely omitted in women with a low-risk DCIS profile (68–71).

The incidence of lymph node metastasis in pure DCIS is extremely low. Axillary lymph node dissection is not recommended due to its associated risk of morbidity. It is generally suggested that a sentinel node biopsy should be considered in patients undergoing mastectomy and in patients with a high-risk of occult invasive disease (72,73). These recommendations have been questioned recently however, considering the extremely low incidence of nodal involvement (74). In 10-33 % of cases with a pre-operative diagnosis of DCIS by core needle biopsy the diagnosis is upgraded to invasive disease on the final post-operative histopathology report (72).

Adjuvant treatment

Radiotherapy (RT) has been used as part of the adjuvant treatment for breast cancer since the 1940s. Ionizing radiation causes damages to the DNA of cancerous tissue leading to cellular death and the rationale is that the rapidly proliferating cancer cells are inferior compared to normal cells in repairing the DNA damages (75). In invasive breast cancer, adjuvant RT has been shown to reduce the risk of local recurrence by 50% after BCS, and to reduce breast cancer mortality by about a sixth after 15 years of follow-up (76).

In the DCIS setting, four randomized trials have compared adjuvant postoperative RT versus surgery alone for DCIS (Table 1) (77–80).

Table 1. *Randomized trials comparing radiotherapy versus not after breast conserving surgery for DCIS*

Study	Year	Women randomized	Follow-up (years)	HR (CI) for local recurrence after surgery+RT versus surgery alone
NSABP B-17 (65)	1985-1990	818	15	0.48 (0.33-0.69)
EORTC 10853 (81)	1986-1996	1010	15	0.52 (0.40-0.68)
SweDCIS (57)	1987-1999	1067	10	0.40 (0.30-0.54)
UK/ANZ (66)	1990-1998	1030	12	0.41 (0.30-0.56)

HR=Hazard Ratio, CI=Confidence Interval, RT=Radiotherapy

An overview of these trials published by EBCCTG in 2010 showed that postoperative RT approximately halved the rate of ipsilateral breast events (82). At 10 years after randomization the absolute reduction in risk was 15,2% (12,9% vs. 28,1%). Radiotherapy was effective in all analysed subgroups of patients regardless of type of DCIS, grade and mode of detection but resulted in a larger proportional reduction in recurrent events for women aged more than 50 years than for younger women.
Half of the recurrences in both groups were invasive cancer and half were DCIS, but RT did not influence overall or breast cancer specific survival.

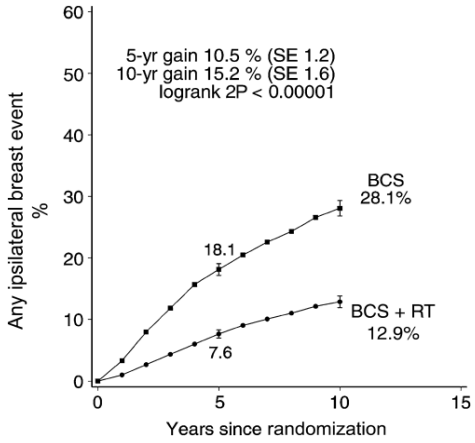

Figure 3. *Effect of radiotherapy after breast conserving surgery (four trials, start dates 1985-1990, 3 729 women): 10-year cumulative risks of any ipsilateral breast event.*

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RT is, however, known to have potential harmful side effects, such as increased risk of cardiotoxicity and induction of second malignancies with long-term follow-up (83–85). In the earliest RT trials the reduction in deaths due to breast cancer was counterbalanced by an excess of deaths due to heart disease after 10 years (86). The radiation dose is measured in Gray (Gy) defined as the absorbtion of one joule of radiation energy per kilogram of matter. The use of three-dimensional radiation planning by computed tomography (CT) was adopted in the early 1990´s and has led to a substantial improvement in dose estimations to targets (75,87). As an example, the mean heart dose in tangential RT to the left breast was estimated to 13.3 Gy in the 1970's compared to 2.3 Gy in 2006 (87). There are, however, still concerns regarding exposure of the anterior part of the heart to radiation thus causing damage to the coronary arteries and studies imply an increased incidence of ischemic heart disease (IHD) after left-sided RT compared to right-sided RT, even with modern RT technique (84,88,89).

Radiation is also a well-documented carcinogen, shown both epidemiologically from populations exposed to an atomic bomb (90), but also after medical therapeutic radiation (91). Data on the risk of secondary malignancies after breast irradiation are contradictory. Some studies have shown a significantly increased risk of lung cancer, leukemia and contralateral breast cancer after RT (83,92,93), while others have not (94,95).

Randomized trials assessing adjuvant hormonal treatment in DCIS have shown that addition of tamoxifen for patients with an ER positive DCIS reduces the risk of ipsilateral and contralateral events (65,66). These studies found no evidence, however of risk reduction regarding distant metastasis and no difference in overall survival. A recently published randomized trial demonstrated further improvement in reducing local events with anastrazole treatment compared with tamoxifen, but survival benefits are still uncertain (96).

Swedish guidelines do not recommend any form of systemic adjuvant treatment for DCIS.

Clinical and histopathological prognostic markers

Young age is recognized as an adverse prognostic factor associated with a higher risk of both invasive recurrence (65,97–99), distant metastasis (100) and breast cancer death (67,101). It seems the risk of recurrence not only decreases linear with age, those in the youngest group (<40 years) are at particularly high risk (100). In an observational study from the Surveillance, Epidemiology, and End Results (SEER) database, the hazard ratio for mortality was 2.16 (95% CI 1.54-3.02) in women younger than 35 years compared with women who were diagnosed at an older age (67).

A comprehensive meta-analysis of DCIS tumour characteristics and their relationship to recurrence risk was performed in 2011 by Wang et al (102). The pooled risk estimates for ipsilateral breast recurrence were increased by symptomatic DCIS (as opposed to screen-detected), large tumour size, multifocality, high grade (nuclear grade III), presence of comedonecrosis, and positive margin status (102).

Multifocality is defined as separate foci of DCIS within the same ductal system. However, multifocality may arise as an artefact of the two-dimensional sectioning of an arborizing lesion and the reported incidence therefore varies. Large and multifocal DCIS are more likely to harbour occult foci of microinvasion, defined by extension of cancer cells beyond the basement membrane into the adjacent tissues but with no single focus larger than 1 mm in

greatest dimension (103,104). The clinical significance of microinvasion is not clear but in two recent reports microinvasion was a significant adverse prognostic factor for survival (105,106).

High nuclear grade is both associated with a higher probability of ipsilateral invasive recurrence (55,107–110), increased risk of distant metastasis (111,112), and death (67,113) compared to low-grade DCIS.
Comedonecrosis refers to ducts plugged with atypical cells and necrosis. Rapid proliferation of the malignant cells leads to insufficient nutrition supply resulting in characteristic necrotic debris with calcifications in the lumina. Comedonecrosis indicates biological aggressiveness and is associated with both increased risk of recurrence (108,111) and breast cancer death (56).

Several studies support that margin status is an important prognostic factor for recurrence (55,56,58–60) but controversy exists on how to define a free margin. In a meta-analysis, including 7 564 patients, a 10 mm threshold had the lowest odds ratio for local recurrence compared to thresholds of 0 mm, 2 mm and 5 mm (114). This is in contrast to the results of another meta-analysis, including 7 883 patients, where there was no benefit of margins wider than 2 mm (115). Vicini et al suggested that margin status alone may be suboptimal in defining excision adequacy. They found that although the local recurrence rate generally decreased as margin distance increased, these differences did not achieve statistical significance unless the volume of excision was taken into consideration (116).

Biomarkers

The expression rates of biological molecular markers are quantified by immunohistochemical (IHC) staining of paraffin sections using antibody panels. The most well-established breast molecular markers with prognostic and/or therapeutic value are ER, PR, Ki67 and HER2. These analyses are routinely performed in invasive breast tumours, but not, as of yet, in DCIS.
Oestrogen and progesterone are steroid hormones involved in the normal development of the ovary, the uterus and the mammary gland. Oestrogen controls the early ductal morphogenesis of the breast, whereas progesterone controls ductal branching and alveolar development during puberty and pregnancy (117).

Oestrogen receptor

The oestrogen receptor (ER) is considered as one of the most valuable markers in breast cancer. Expression of ER is a strong predictor of response to hormonal therapy, it is generally higher in well-differentiated lesions and is

associated with a favourable prognosis (118,119). The definition of ER positivity is somewhat controversial. The most commonly used cut-off is when 10 % or more of the tumour cells show positive nuclear staining in IHC analysis. American guidelines recommend a threshold of 1 % (120), but women with 1-9% ER positivity tumours do not seem to benefit from endocrine therapy and survival rates between patients with 1–9% ER-positive tumours and ER-negative tumours do not differ significantly (121,122).

Progesterone receptor

The progesterone receptor (PR) is an oestrogen-regulated gene; expression is thus dependant on ER activation and indicates a functioning ER pathway (123). It has been claimed by some that the ER-/PR+ phenotype does not exist and that the ER-negativity in these cases is due to inadequate tissue fixation or technical failure of the immunohistochemical assay (124). Others claim that it represents a distinct although rare subtype, more often affecting young women and with similar responsiveness to hormonal treatment in comparison to ER positive cancer (125). PR negativity has been demonstrated to be an independent negative prognostic factor for breast cancer survival, even for patients with ER positive breast cancer receiving endocrine treatment (119,126,127).

In DCIS, the mean expression rate of ER is 68.7% and of PR 59.6% (128).

Ki67

The proliferative rate of breast tumours may be assessed by IHC using monoclonal antibodies to antigens found in proliferating cells. The Ki67 protein is present only in proliferating cells, i.e. during all active phases of the cell cycle, but is absent in resting cells (129). Ki stands for Kiel University in Germany and 67 refers to the original clone number on a 96-well plate. There are issues of assessment, scoring and interpretation of cut-off values, which is why this biomarker is not considered optimal for comparisons between clinical trials (130). In the StGallen guidelines, Ki67 is recommended to be used to distinguish between low-proliferative Luminal A tumours and high-proliferative Luminal B tumours with a cut-off at 14% (131). The American (ASCO) guidelines on the other hand, do not recommend the use of Ki67 due to the issues stated above and lack of reproducibility (132). In DCIS, the proportion of tumours that are classified Ki67-positive is highly variable (128). Increased levels are associated with high nuclear grade and comedo necrosis (128,133).

HER2

Human epidermal growth factor receptor 2 (HER2) is a tyrosine kinase receptor located on the cellmembrane. Overexpression arises from the amplification of the HER2 gene and is strongly associated with increased disease recurrence and a poor prognosis (134). HER2-positive status is defined by overexpression of the HER2 protein ($\geq$ 3+) analysed by IHC, or by gene amplification (HER2 copy number $\geq$ 5 or HER2/CEP17 ratio $\geq$ 2.0) analysed by in situ hybridization (ISH) (135).
In normal breast tissue or benign lesions, HER2 is generally not expressed, but overexpression is more common in DCIS than in invasive cancer (136,137). This suggests that HER2 amplification may be lost during tumour progression and it has therefore been hypothesized that HER2 plays a more important role in initiation rather than in progression of cancer (136).

Cytokeratin 5/6

Cytokeratins (CK) are fibrous structural proteins within epithelial cells. CK 5/6 are expressed in the basal/myoepithelial cells of the normal breast. In breast cancer, expression of this protein implies a basal-like molecular phenotype associated with poor clinical outcome (138) and is often encountered in BRCA1-related breast cancers (139). The prevalence of the basal-like subtype is much lower in DCIS than in invasive breast cancer, which might be a result of rapid progression in invasive cancers (140,141).

Molecular subtypes

Microarray gene profiling of invasive breast cancer is increasingly relevant in defining cancer biology. Intrinsic molecular subtypes based on gene expression patterns are strongly predictive for recurrence and survival (142). IHC biomarkers can be used to classify tumours into surrogate molecular subtypes as proposed by the StGallen international expert consensus as follows (131):

- Luminal A (ER and/or PR positive, HER2 negative and Ki67 <14%)
- Luminal B/HER2- (ER and/or PR positive, HER2 negative and Ki67 ≥14%),
- Luminal B/HER2+ (ER and/or PR positive, HER2 positive),
- HER2+ (non luminal) (ER and PR negative and HER2 positive),
- Triple Negative (basal-like) (ER, PR and HER2 negative)

DCIS can be classified in a similar manner (137,141,143–146), but much less data on survival after different subtypes is available in the DCIS setting.

Other biomarkers

Other biomarkers of interest include the tumour suppressor genes involved in the cell cycle, for example, p53 and p16. Expression of p53 is generally associated with high proliferation (128). In breast cancer, approximately 30% display p53 gene mutation, but this frequency fluctuates from more than 80% in basal-like to less than 15% in luminal A subtypes (142). In DCIS mean p53 expression is estimated to 40% (128). Overexpression of p16 is also more often manifested in basal-like tumours (147,148). Bcl-2 is an apoptosis regulatory protein and overexpression is generally considered to be a favourable prognostic factor (31,133,149). Cyclooxygenase 2 (Cox-2) is an enzyme regulating tumour growth, invasion and metastasis. Overexpression of Cox-2 has been shown to be associated with an aggressive DCIS phenotype (128,150).

The tumour microenvironment

It is becoming increasingly apparent that the tumour microenvironment strongly influences tumour behaviour and clinical outcome (24,151). Myoepithelial cells in DCIS differ substantially from those found in normal breast tissue (151) and activated fibroblasts in the peritumoural stroma correlates with poor clinical outcome (152). Both fibroblasts and immune cells seem to be active mediators in tumour development. The clinical relevance of an immune response in DCIS and invasive breast cancer is not completely clear, as it may both represent a protective host response to tumour, but also stimulate tumour growth by releasing proteolytic enzymes and angiogenic factors (153). DCIS with periductal lymphocytic infiltration and fibrosis has in clinical trials been reported to be associated with a more aggressive biological phenotype (109,154,155).

Prognostic tools

The University of Southern California Van Nuys Prognostic Index was one of the first nomograms created (156,157). This index incorporates lesion size, margin width, pathologic classification and patient age to stratify patients into three groups. Excision alone is recommended for patients with total scores of 4-6, excision and radiotherapy for scores of 7-9 and mastectomy for scores of 10-12. The main drawback is that the variables included are based on a retrospective single-institution register and lacks independent validation (158,159).

The Memorial Sloan Kettering Cancer Center nomogram takes into account ten clinicopathological variables: age at diagnosis, family history of breast cancer, presentation (clinical vs. radiologic), adjuvant RT, adjuvant endocrine therapy, nuclear grade, necrosis, surgical margins, number of surgical excisions, and year

of surgery (60). These predictors are combined in a nomogram to estimate the probability of recurrence at 5 and 10 years. In an external validation the nomogram was however not conclusive, suggesting that clinical parameters alone may be insufficient (160).

The Oncotype DX DCIS score was created in 2013 (161). A panel of 12 genes are included in the assay. The analysis generates a score of 0-100 and has been shown to predict the 10-year risk of developing DCIS recurrence or invasive cancer in individuals with low-risk DCIS treated by BCS alone (161,162). The usefulness of the score in intermediate- and high-risk DCIS has however not been explored (163).

Aims of Thesis

The overall aim of this thesis was to explore the prognostic significance of clinical and tumour biological characteristics of DCIS and to assess the benefits and harms of adjuvant treatment.

- To analyse trends in treatment and prognosis for DCIS during 20 years in a Swedish cohort and to validate the registration of DCIS in a regional breast cancer quality register
- To identify patient or tumour related risk factors for breast cancer death in women with primary DCIS
- To investigate biomarkers in DCIS associated with the risk of breast cancer death
- To assess the risk of ischemic heart disease after adjuvant radiotherapy for DCIS

Materials and Methods

Data sources

The Swedish Cancer Register

Nationwide information on cancer incidence in Sweden has been available since 1958. Since that year, both physicians and pathologists are required to submit reports on all new cases of malignant disease detected on clinical and histopathological grounds to the Swedish Cancer Register (SCR) (164). Diagnoses are based on morphological findings in 98 % of cases. The register contains information on diagnosis, SNOMED tumour morphology codes, International Classification of Diseases (ICD) code, basis for diagnosis, examination of tumour specimen (pathology or cytology) and whether the tumour was diagnosed at autopsy. The coverage is estimated to 98 % (165,166).

The regional quality registers of breast cancer and INCA

The regional registers in Stockholm, Uppsala/Örebro and Northern health care regions were started in the late 1970s, in 1992 and in the early 1980s, respectively. These three regions altogether cover a source population of 4,8 million people, representing about 50% of Sweden´s total population. The registers are regularly linked to SCR and capture more than 98-99% of all newly diagnosed, biopsy confirmed breast cancers in these three regions. INCA is a national network for cancer care established all over Sweden in 2008 where new incident cases of breast cancer are reported online. The primary data completion rate is 98.1% *(167)*.

Cause of Death register

Information about deaths was first systemically registered in Sweden in 1749. The Swedish cause of death register (CDR) held by the National Board of Health and Welfare contains data from 1961 and is updated annually (168). Information is collected about all deceased individuals that have been registered in Sweden whether they have died in Sweden or abroad. The register contains data on date of death, underlying cause of death, contributing cause(s) of death, and information on whether an autopsy was performed or not (169).

The National Patient Register (NPR)

NPR has records of all hospital discharges in Sweden since 1987 and contains data on main diagnosis and up to eight secondary diagnoses. The register has

been validated and is estimated to capture about 99 % of all hospitalisations (170). The NPR also contains hospital-based outpatient care since 2001.

Longitudinal Integration Database for Health Insurance and Labour Market Studies (LISA)

This is an annually updated register integrating data from the labour market, the educational and social sectors. The register includes data on various socioeconomic variables for all residents in Sweden, such as marital status, income, place of employment (county, municipality) and highest level of education (171).

Breast Cancer Database Sweden (BCBase)

The breast cancer quality registries in Stockholm, Uppsala-Örebro and Northern health care regions have been merged together and linked to a number of national population-based registries, creating BCBase. To this a comparison cohort of women without BC has been added in a ratio of 5:1 matched by year of birth and county of residence. Eligible for inclusion were women free of BC at the end of the year of diagnosis of the index case. Using the method of incidence density sampling, the women in the comparison cohort may have been selected for more than one case and were also allowed to become a case after the date of diagnosis of the index case.

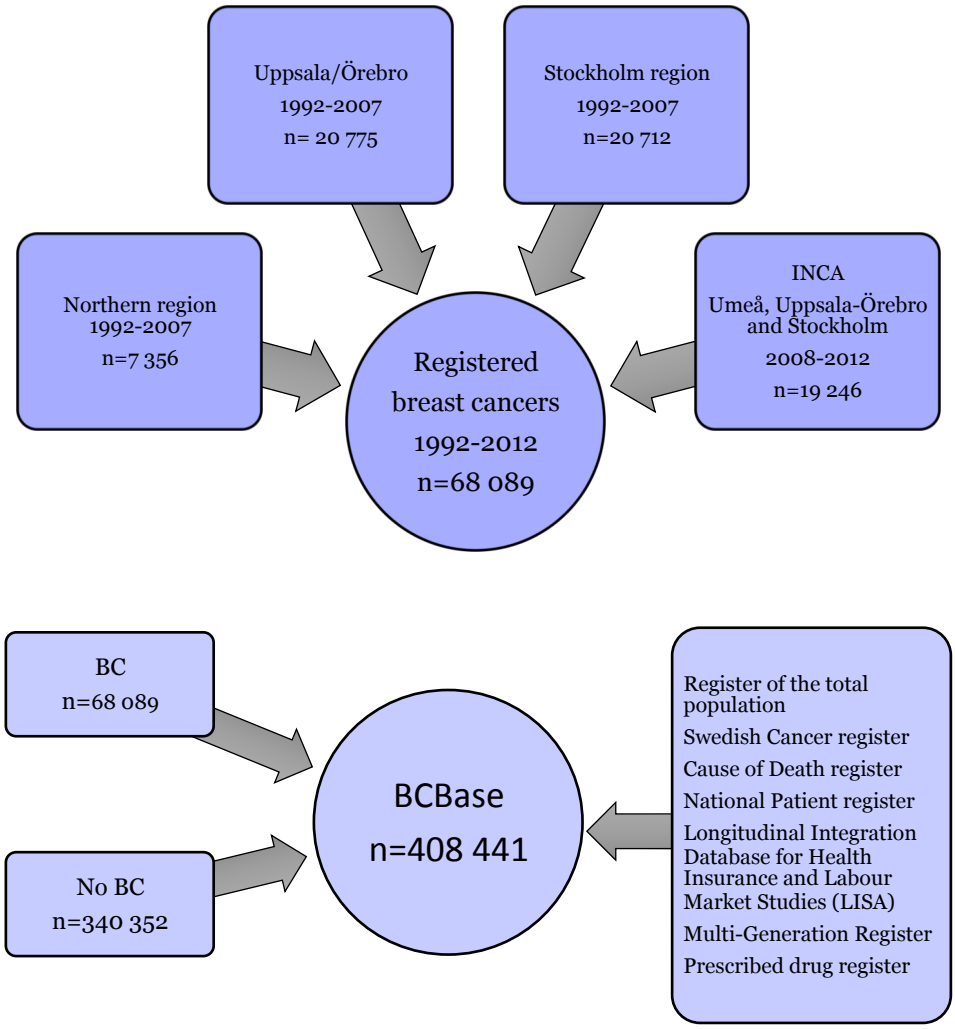


Figure 4: *The creation of BCBase including 68 089 women with breast cancer diagnosed between 1992 and 2012 and a comparison cohort of women without history of breast cancer.*

Methods

In **paper I**, women reported with a primary DCIS in the breast cancer quality registry of the Uppsala-Örebro healthcare region between 1992 and 2012 were included. Information on date of diagnosis, age at diagnosis, mode of detection, size of DCIS, nuclear grade, type of surgery, planned adjuvant treatment and reported subsequent breast cancer events was collected.

To validate the data on DCIS in the register, medical records of 300, randomly selected women (10% of the cohort) were obtained.

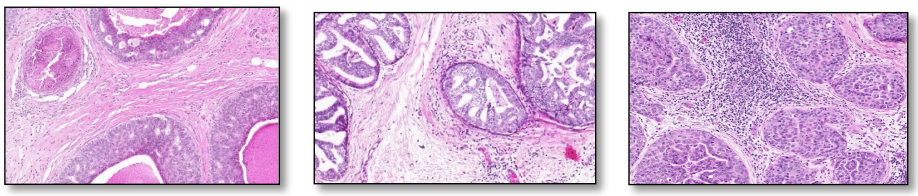
In **paper II and III**, a nested case-control study was conducted. The regional breast cancer registries in Stockholm, Uppsala-Örebro and Northern health care regions were linked to the cause of death register to identify women registered with a primary DCIS between 1992 and 2012 who later died from breast cancer. For each case, four controls were selected at random using incidence density sampling (172). All women with a primary DCIS diagnosed from 1992 onwards who were alive at the time of death of the corresponding case were eligible as controls.

Medical records were collected for both cases and controls. The actual cause of death was verified for the cases. Information on mode of detection, primary treatment, tumour size, multifocality and nuclear grade was obtained from the medical records and the original histopathology reports.

Re-evaluation of grade and generation of Tissue Microarrays

In **paper III**, one to three paraffin-embedded tissue blocks were retrieved for each patient and were used to construct tissue microarrays (TMA). Appropriate areas of DCIS were identified and two cores with a diameter of 1.0 mm were drilled from the tissue blocks and mounted into the recipient TMA block. TMA construction was performed manually at one laboratory, one laboratory used the TMA Grandmaster (3DHistech Ltd., Budapest, Hungary) system and one the Alphelys Minicore^{R} TMA (Alphelys, Plaisir, France) system.

Haematoxylin and Eosin (H&E) staining was done using freshly cut sections from the primary DCIS and these were re-evaluated and re-graded by an experienced breast pathologist. Comedonecrosis was noted as present or absent. Lymphocytic infiltration in the periductal stroma was scored as absent, mild or intense.



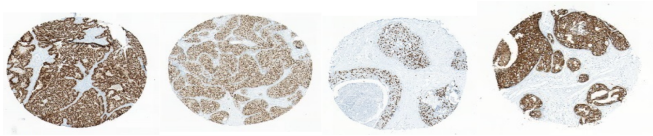
a) b) c)

Figure 5. *Periductal lymphocytic infiltration a) none, b) mild, and c) intense*

Immunohistochemistry of molecular markers

Immunohistochemical staining (IHC) on 4 µm sections was performed for Oestrogen Receptor (ER), Progesterone Receptor (PR), Human Epidermal growth factor Receptor 2 (HER2), and Ki67 on a Ventana BenchMark Ultra automated stainer (Ventana Medical Systems, Inc, Tucson, AZ). The following antibodies were used: For ER an RTU (Ready To Use) dilution of rabbit monoclonal antibody SP1 (catalog no. 790-4324, Ventana/Roche), for PR an RTU dilution of rabbit monoclonal antibody 1E2 (catalog no. 790-2223, Ventana/Roche), for HER2 an RTU dilution of rabbit monoclonal antibody 4B5 (catalog no. 790-4493, Ventana/Roche) and for Ki67 an RTU dilution of rabbit monoclonal antibody 30-9 (catalog no. 790-4286). The incubation time was 16 minutes for the antibodies against PR and 32 minutes for the remaining antibodies.

Cut off for ER and PR was defined as 10 % or more tumour cells showing nuclear staining. Membrane expression of HER2 was scored on a 0-3+ intensity scale (1+=weak and incomplete membrane staining, 2+=moderately intense and complete membrane staining, and 3+=strong/intense and complete membrane staining), and 2-3+ were considered positive. Proliferation was considered high if 20 % or more of the tumour cells showed Ki67 positivity (131).



ER PR Ki67 HER2

Figure 6. *Cores of DCIS with positive immunostaining for ER, PR, Ki67 and HER2.*

Using the results of the IHC analyses, tumours were classified into five subtypes according to the St. Gallen consensus statement (131,173).

In **paper IV**, BCBase was linked to the NPR to assess the incidence of ischemic heart disease in women with DCIS treated with postoperative radiotherapy or surgery alone versus women without a history of DCIS. IHD was defined by the International Classification of Disease (ICD) 9th edition codes 410-414 or ICD-10 codes I20- I25.

Table 2. *ICD codes and definitions of ischemic heart disease*

Diagnosis	ICD-9	ICD-10
Angina pectoris	413	I20
Acute myocardial infarction	410	I21
Other ischemic heart disease	411-412,414	I22-I25

ICD= International statistical classification of diseases

Incidence rates were adjusted for educational level and comorbidity. Classification of comorbidity was performed according to the Charlson comorbidity index (CCI) using three comorbidity levels; 0 (no comorbidity), 1 (mild), and 2 (severe comorbidity) (174).

Statistics

Paper I

In the validation, positive prognostic values were calculated for accuracy of primary registration and sensitivity analyses were performed for reported recurrences.

Chi 2-tests and Fisher's exact test were used for testing differences between variables. Cumulative risk for breast cancer events and relative survival were calculated by the Kaplan-Meier method.

Relative survival was calculated in Stata 13. Statistics were performed using SAS 9.4 software and R 9.4.

Paper II and III

Conditional logistic regression was used to estimate the univariable and multivariable odds ratios (OR) of breast cancer death and their 95% confidence intervals (95% CI).

Age at diagnosis was categorized into younger than 50, 50–60 or older than 60 years. Tumour size was categorized in two ways: in three categories (smaller than 20 mm, between 20 and 50 mm, or larger than 50 mm) and in two categories (smaller than 25 mm, or 25mm or larger including tumours recorded as multifocal but without a clear size measurement). In paper II, to enable statistically efficient use of the data and avoid bias by excluding cases with missing information, multiple imputation was used with five imputation data sets using the full conditional specification method (175).

All analyses were adjusted for year of diagnosis and time at risk.

The different multivariable analyses included tumour-related, treatment-related, and both tumour- and treatment-related variables.

In **paper III**, the regression analysis first included each histopathological characteristic and biomarker individually and then in various combinations. These were based on the results of each variable or on previously reported predictors of invasive recurrence (111,128,147,176–178). All analyses were adjusted for year of diagnosis and time at risk. In the multivariable analyses, individual or combinations of markers that were statistically significantly associated with breast cancer death in the univariable analysis were analysed by successive adjustment for age, mode of detection, tumour size, type of treatment, and margin status.

SPSS® version 23 (IBM, Armonk, New York, USA) was used for all analyses.

Paper IV

Hazard ratios for risk of IHD were estimated by Cox proportional hazards regression analysis. Only events requiring a hospital admission were captured and only the first event recognized for each subject. Time at risk started at DCIS diagnosis and ended at date of IHD event, date of invasive breast cancer event in either the ipsilateral or the contralateral breast, death, or end of the year of 2013, whichever came first. Risk of IHD was investigated by comparing women with DCIS to women in the comparison cohort, women with DCIS treated with surgery and RT to those having surgery alone, and women receiving left-sided RT to those with right-sided RT. Risk estimates were adjusted for previous cardiovascular events, CCI, and educational level. The CCI score was modified by removing IHD in order to avoid duplicate adjustment for this covariate. Cumulative incidence of IHD was calculated by the Kaplan-Meier method.

Analyses were performed using the statistical software R (179).

Results

Paper I

Validation of register data

Of the 300 women randomly selected for validation, 264 were found to have pure DCIS and could be validated for primary data and reported recurrences. Of the excluded cases, 21 had primary invasive breast cancer (7 %), eight had lobular cancer in situ (LCIS), two were local recurrences and five medical records were unavailable (Figure 7)

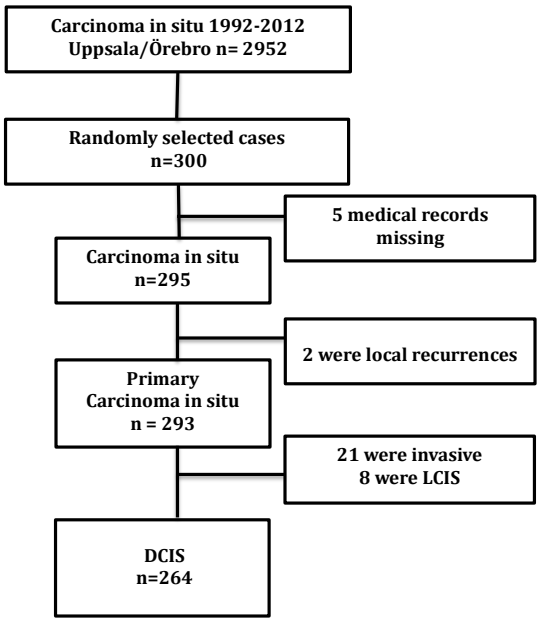


Figure 7 *Selection of DCIS cases validated in the Uppsala-Örebro regional breast cancer quality register 1992 to 2012.*

The overall completeness and validity of variables was good, 91–99%. There were a total of 31 local recurrences of which 20 were reported (65 %). Eighteen of the recurrences were invasive cancer and 13 were DCIS. Of 12 cases with distant metastasis, seven events had been reported to the register (58 %).

Incidence and mode of detection

The incidence of DCIS increased over time, but the proportion of DCIS to all

reported breast cancers was stable (Table 3). There was a trend of increasing tumour size over time. Between 1992 and 1997, 36.4% of the lesions were 15 mm or larger compared to 64.8% during 2008–2012.

Table 3. *Distribution of cases and tumour characteristics for patients registered with DCIS in Uppsala-Örebro 1992-2012 by time period.*

	1992-1997	**1998-2002**	**2003-2007**	**2008-2012**	**P value**
Patients	693	628	835	796	<0.001
Proportion DCIS of all breast cancer (%)	9.6%	8.6%	10.6%	9.2%	0.22
Age(median,range)	56(28-76)	57(33-74)	58(26-76)	60(31-94)	0.96
Mammography detected	462(66.7%)	426(67.8%)	542(64.9%)	591(74.3%)	<0.001
Nuclear grade					
I	13 (72.2%)	15 (45.5%)	40 (23.7%)	78 (11.3%)	not done
II	3 (16.7%)	15 (45.5%)	85 (50.3%)	254 (36.9%)	
III	2 (11.1%)	3 (9.1%)	44 (26.0%)	357 (51.8%)	
DCIS Size					
< 15 mm	272 (39.3%)	287 (45.7%)	339 (40.6%)	235 (29.5%)	<0.001
≥ 15mm	252(36.4%)	257(40.9%)	449(53,8%)	511(64,2%)	
Missing	169(24.4%)	84(13.4%)	47(5.6%)	50(6.3%)	

Treatment

The mastectomy rate increased over time from 23.2% during the first time period to 39.3% in 2008–2012 (Table 4). The proportion of women who were treated with adjuvant RT after BCS also increased over time from 30.1% during the first time period to 67.6% in the last time period. The frequency of axillary node clearance declined over time from about 10% to almost none. SNB, however, was not performed before 1998, but then increased rapidly. In the last period, 54.9% of the patients with DCIS underwent a SNB.

Table 4. *Distribution of type of surgery and radiotherapy for patients registered with DCIS in Uppsala-Örebro 1992-2012 by time period.*

	1992-1997	1998-2002	2003-2007	2008-2012	P value
Mastectomy	161 (23.2%)	150 (23.9%)	323 (38.7%)	313 (39.3%)	<0.001
BCS	519 (74.9%)	468 (74.5%)	506 (60.6%)	476 (59.8%)	
Missing	13(1.9%)	10(1.6%)	6(0.7%)	7(0.9%)	
BCS+RT	156/519 (30.0%)	178/468 (38.0%)	347/506 (68.6%)	322/476 (67.6%)	<0.001

Outcome

The net probability of a reported local recurrence was 3.5% at 5 years and 9.7% at 10 years. There were significantly more reported recurrences in the group treated with BCS compared with the mastectomy group, 12.0% versus 7.0% after 10 years, but no difference in recurrence rate whether adjuvant radiotherapy was added or not after BCS, 11.0% versus 13.0% after 10 years. Relative survival was 99.0% and 97.0% at 5 and 10 years respectively, with no clear trend over time (Figure 8).

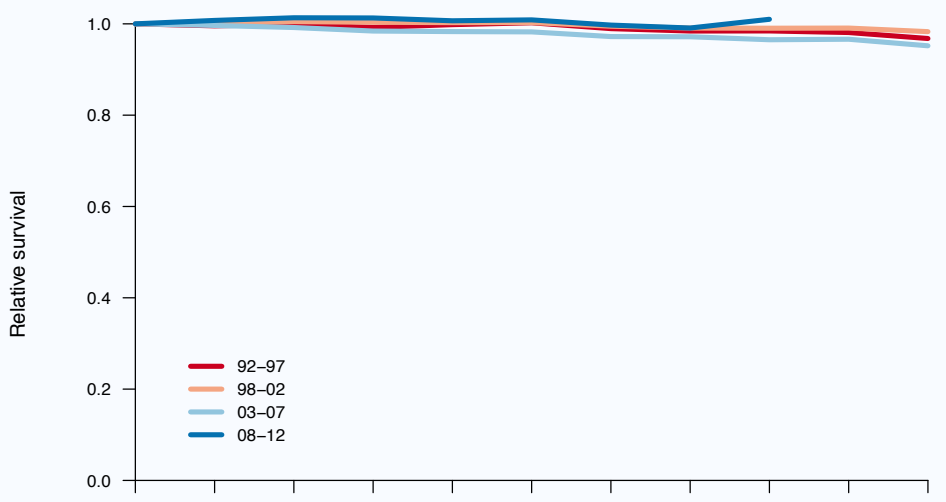


Figure 8 *Relative survival among registered patients with DCIS in Uppsala-Örebro 1992-2012, by time period.*

Paper II

In the cohort of 6 964 patients with DCIS from the three included health care regions, 228 were registered as having died of breast cancer as an underlying or contributing cause of death. After review of their medical records 132 were excluded leaving 96 cases for the final analysis. To these, 384 controls were randomly selected of which 66 patients were excluded as shown in the flowchart of inclusion and exclusion of cases and controls (Figure 9):

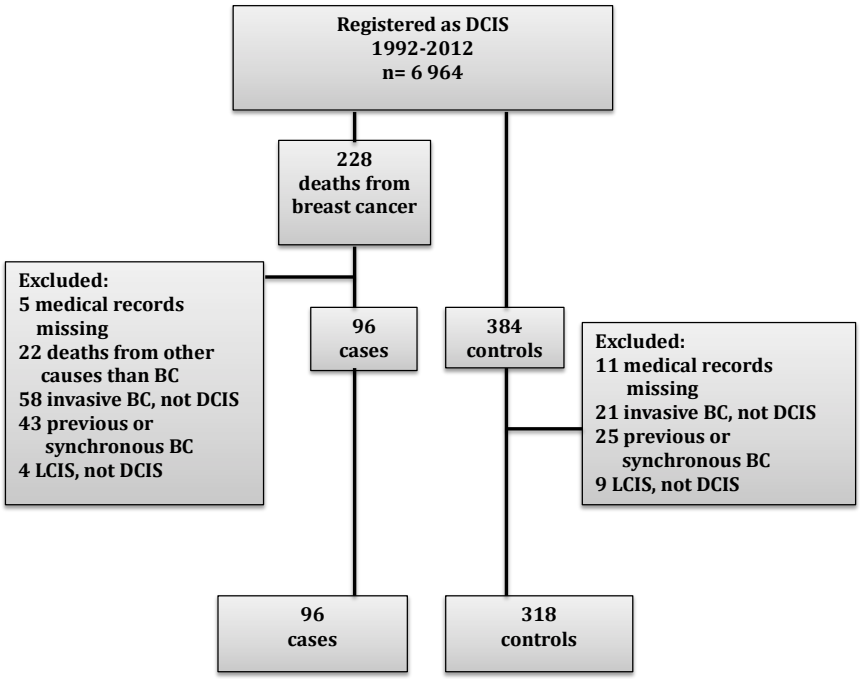


Figure 9 *Flowchart of inclusion of cases and controls from a populationbased cohort of women registered with DCIS 1992-2012.*

Clinical, pathological and treatment characteristics of the cases and controls are presented in table 5.

Table 5. *Baseline characteristics of patients with primary ductal carcinoma in situ*

	Cases n=96		***Controls n=318***		***OR(95% CI)****
Age at diagnosis					
< 50 years	33	(34.3)	86	(27.0)	1.38 (0.77-2.50)
50-60 years	34	(35.4)	123	(38.7)	1.0 (ref)
> 60 years	29	(30.2)	109	(34.3)	1.06 (0.58-1.93)
Mode of detection					
Screening	50	(52.1)	233	(73.3)	1.0 (ref)
Non-screening	33	(34.4)	72	(22.6)	2.12 (1.16-3.86)
Missing	13	(13.5)	13	(4.1)	
Laterality					
Right	34	(35.4)	176	(55.3)	1.0(ref)
Left	62	(64.6)	142	(44.7)	2.12(1.30-3.45)
Tumour size					
<20mm	26	(27.0)	167	(52.5)	1.0(ref)
20-50mm	43	(44.8)	93	(29.2)	2.88(1.60-5.21)
>50mm	13	(13.5)	18	(5.7)	3.96(1.85-8.51)
Missing	14	(14.6)	40	(12.6)	
Focality					
Unifocal	66	(68.8)	270	(84.9)	1.0(ref)
Multifocal	30	(31.2)	48	(15.1)	2.35(1.36-4.07)
Tumor size category					
< 25mm	37	(38.5)	192	(60.4)	1.0 (ref)
$\geq$ 25 mm or multifocal	52	(54.2)	108	(34.0)	2.55 (1.53-4.25)
Missing	7	(7.3)	18	(5.6)	
Grade					
I	5	(5.2)	37	(11.6)	1.0 (ref)
II	13	(13.5)	60	(18.9)	2.22 (0.64-7.67)
III	36	(37.5)	102	(32.1)	2.68 (1.04-6.90)
Missing	42	(43.8)	119	(37.4)	
Margin status					
Negative	82	(85.3)	305	(95.9)	1.0 (ref)
Positive/ uncertain	12	(12.6)	9	(2.8)	3.91 (1.59-9.61)
Missing	2	(2.1)	4	(1.3)	0.95 (0.14-6.32)
Microinvasion					
No	89	(92.7)	308	(96.9)	1.0 (ref)
Yes/Suspected	7	(7.3)	10	(3.1)	1.73 (0.62-4.82)
Breast surgery					
BCS	57	(59.4)	231	(72.6)	1.0(ref)
Mastectomy	30	(31.3)	56	(17.6)	2.32 (1.32-4.10)
Mastectomy with reconstruction	9	(9.4)	31	(9.7)	1.31 (0.57-3.03)

Axillary surgery					
None	67	(69.8)	229	(72.0)	1.0(ref)
SNB	6	(6.3)	47	(14.8)	0.59(0.21-1.65)
ALND	23	(24.0)	42	(13.2)	1.67(0.90-3.02)
Radiotherapy					
No	69	(71.9)	217	(68.2)	1.0(ref)
Yes	27	(28.1)	101	(31.8)	0.99(0.60-1.64)
Treatment category					
BCS	30	(31.2)	132	(41.5)	1.0 (ref)
BCS+RT	27	(28.1)	99	(31.1)	1.46 (0.81-2.63)
Mastectomy	39	(40.6)	87	(27.4)	2.29 (1.29-4.06)

OR= Odds ratio, CI=Confidence Interval, BCS= Breast Conserving Surgery, SNB= Sentinel node biopsy, ALND= Axillary lymph node dissection, RT= Radiotherapy

Oddsratio for death from breast cancer

Detection outside the screening programme (OR 2·12; 95%CI 1·16 to 3·86), large tumour size or multifocal DCIS (OR 2·55; 95%CI 1·53 to 4·25) and positive or uncertain margin status (OR 3·91; 95%CI 1·59 to 9·61) significantly increased the risk of death from breast cancer. The risk was not affected by age.

Margin status was positive or uncertain in ten of 57 cases treated by BCS, and in two of 39 treated by mastectomy, with or without reconstruction. Among the controls, margins were positive or uncertain in six of 231 women treated by BCS and three of 87 treated by mastectomy.

In the multivariable analyses, tumour-related variables were built in the model and, after controlling for year of diagnosis and time of exposure, tumour size remained a significant risk factor. In the analysis of treatment-related variables, the risk of death from breast cancer in women with positive or unknown margins was increased regardless of treatment. Finally, in the analysis that included both tumour-related and treatment-related variables, the type of treatment did not affect the risk, whereas tumour size (OR 1·95; 95%CI 1·06 to 3·67) and positive or unknown margin status (OR 2·69; 95%CI 1·15 to 7·11) remained significant (Table 6).

Table 6. *Variables associated with death from breast cancer in multivariable conditional logistic regression of tumour-related variables, treatment-related variables and all variables*

	Odds ratio* (95 % Confidence Intervals)		
	Tumour-related variables	Treatment-related variables	All variables
Age (years)			
< 50	1.03(0.53-1.98)		0.98 (0.51-1.90)
50-60	1.00 (ref)		1.00 (ref)
> 60	1.12 (0.59-2.11)		1.06 (0.55-2.03)
Mode of detection			
Screening	1.00 (ref)		1.00 (ref)
Clinical	1.66 (0.83-3.30)		1.79 (0.89-3.61)
Tumour size (mm)			
<25	1.00 (ref)		1.00 (ref)
>25 or multifocal	2.15 (1.24-3.71)		1.95 (1.06-3.67)
Grade			
I	1.00 (ref)		1.00 (ref)
II	2.19 (0.61-7.92)		2.35 (0.72-7.64)
III	2.28 (0.83-6.28)		2.46 (0.82-7.40)
Microinvasion			
No	1.00 (ref)		1.00 (ref)
Yes/suspected	1.35 (0.43-4.23)		1.35 (0.42-4.30)
Treatment			
BCS		1.00 (ref)	1.00 (ref)
BCS+RT		1.28 (0.69-2.39)	0.98 (0.48-2.00)
Mastectomy		1.66 (0.87-3.15)	0.96 (0.42-2.15)
Margin status			
Negative		1.00 (ref)	1.00 (ref)
Positive		2.83 (1.16-6.89)	2.69 (1.15-7.11)

Paper III

Tumour tissue was available for 66 of the 96 cases (69 %) and 195 of the 318 controls (61 %). Complete IHC analysis could be evaluated for 44 cases and 124 controls. There was no statistically significant difference in distribution of nuclear grade between cases and controls (Table 7). Presence of comedonecrosis was more frequent in tumours among the cases than the controls (78.8% vs. 64.6%, p=0.03) as was periductal lymphocytic infiltration (75.7% vs. 63.6%, p=0.07).

ER expression was positive in 64.4% of the cases and 73.0% of the controls. PR expression was highly correlated to ER expression, but slightly lower with positivity in 46.7% of the cases and 62.1% of the controls. HER2 expression was very similarly distributed between cases and controls (44.4% and 43.7% respectively) as was expression of Ki67 (45.5% of the cases had high Ki67 compared to 42.7% of the controls).

Classification into intrinsic subtypes by IHC showed a distribution as follows: Among the cases tumours were 31.8% Luminal A, 20.5% Luminal B, 11.4% HER2 Luminal, 31.8% HER2 non-luminal and 4.5% Triple negative. Among the controls tumours were 42.1% Luminal A, 13.5% Luminal B, 19.8% HER2 Luminal, 23.8% HER2 non-luminal and 0.8% Triple negative (p= 0.16).

Presence of intense periductal lymfocytic infiltration was associated with an increased risk of subsequent breast cancer death (OR 2.25; 95%CI 1.02 to 4.99) (Table 7). None of the other biomarkers were individually related to breast cancer death, nor were there any statistically significant differences in risk between the molecular subtypes. When selected variables were combined, some risk groups could be identified (Table 8). LI combined with PR negativity was statistically associated with an increased risk (OR 2.96; 95%CI 1.12 to 7.79). The combination of LI, PR negativity and comedonecrosis increased the risk further (OR 4.02; 95%CI 1.70 to 9.49).

Table 7. *Distribution of histopathological features and immmunohistochemical markers in patients with primary DCIS.*

	Cases n= 66[a]	***(%)***	***Controls n= 195[a]***	***(%)***	***OR (95% CI)[b]***
Grade					
I	10	(15.2)	43	(22.1)	1.0(ref)
II	28	(42.4)	78	(40.0)	1.26 (0.54-2.96)
III	28	(42.4)	74	(37.9)	1.63 (0.70-3.83)
Comedonecrosis					
Absent	14	(21.2)	69	(35.4)	1.0 (ref)
Present	52	(78.8)	126	(64.6)	1.93 (0.97-3.81)
LI					
None	16	(24.2)	71	(36.4)	1.0(ref)
Mild	22	(33.3)	65	(33.3)	1.75 (0.79-3.89)
Intense	28	(42.4)	59	(30.3)	2.25 (1.02-4.99)
ER					
≥ 10 %	29	(64.4)	92	(73.0)	1.0(ref)
< 10 %	16	(35.6)	34	(27.0)	1.74 (0.71-4.30)
PR					
≥ 10 %	21	(46.7)	82	(62.1)	1.0(ref)
< 10 %	24	(53.3)	50	(37.9)	1.97(0.80-4.81)
HER2					
0-1+	25	(55.6)	71	(56.3)	1.0(ref)
2-3+	20	(44.4)	55	(43.7)	1.10(0.47-2.59)
Ki 67					
< 20 %	24	(54.5)	71	(57.3)	1.0 (ref)
≥ 20 %	20	(45.5)	53	(42.7)	1.19 (0.52-2.75)
Molecular subtype					
Luminal A	14	(31.8)	53	(42.1)	1.0 (ref)
Luminal B	9	(20.5)	17	(13.5)	1.69 (0.47-6.09)
HER2 Luminal	5	(11.4)	25	(19.8)	0.66 (0.17-2.55)
HER2Non-luminal	14	(31.8)	30	(23.8)	1.82(0.60-5.59)
Triple neg	2	(4.5)	1	(0.8)	3.66(0.18-73.57)

[a] Complete IHC analysis available in 44 cases and 124 controls

[b] All analyses adjusted for year of diagnosis and time at risk.

OR= Oddsratio, CI= Confidence Interval, LI= Lymphocytic infiltration, ER= Oestrogen Receptor, PR= Progesterone Receptor, HER2= Human Epidermal growth factor Receptor 2

Table 8. *Univariate results of IHC markers and histopathological characteristics associated with risk of breast cancer death*

	OR (95% CI)[a]
LI	
Present	1.98 (0.98-4.02)
Absent	1.0 (ref)
LI/ER	
Present/Negative	2.05 (0.88-4.79)
All other groups	1.0 (ref)
LI/PR	
Present/Negative	2.96 (1.12-7.79)
All other groups	1.0 (ref)
LI/PR/Comedonecrosis	
Present/Negative/Present	4.02 (1.70-9.49)
All other groups	1.0(ref)
LI/PR/Comedonecrosis/HER2	
Present/ Negative/Present/Negative	2.11 (0.52-8.47)
Present /Negative/Present/Positive	3.51 (1.46-8.43)
All other groups	1.0(ref)

[a] All analyses adjusted for year of diagnosis and time at risk.

OR= Oddsratio, CI= Confidence Interval, LI= Lymphocytic infiltration, ER= Oestrogen Receptor, PR= Progesterone Receptor, HER2= Human Epidermal growth factor Receptor 2

Multivariable analysis of breast cancer related death

The multivariable analysis was performed by stepwise including age at diagnosis, tumour size, margin status and treatment (Table 9). In the final model with all variables included, PR negativity in combination with LI (OR 4.40; 95%CI 1.20-16.14), PR negativity, LI and presence of comedonecrosis (OR 5.48; 95%CI 1.71-17.57) and the combination of PR negativity, LI, comedonecrosis and HER2 positivity (OR 7.54; 95%CI 2.00-28.43) were all independently associated with increased risk of breast cancer related death.

Table 9. *Multivariable results of IHC markers and histopathological characreistics associated with risk of breast cancer death.*

Variable	OR (95% CI)	
LI/PR	**Present/Negative**	**All other groups**
Crude [a]	2.96 (1.12-7.79)	1.0 (ref)
+age	3.01 (1.13-7.99)	1.0 (ref)
+tumour size[b]	2.60 (0.91-7.44)	1.0 (ref)
+margin status[c]	2.67 (0.92-7.80)	1.0 (ref)
+treatment[d]	4.40 (1.20-16.14)	1.0 (ref)
LI/PR /Comedonecrosis	**Present/Negative/ Present**	**All other groups**
Crude [a]	4.02 (1.70-9.49)	1.0 (ref)
+age	3.90 (1.65-9.26)	1.0 (ref)
+tumour size[b]	3.40 (1.34-8.59)	1.0 (ref)
+margin status[c]	3.84 (1.43-10.31)	1.0 (ref)
+treatment[d]	5.48 (1.71-17.57)	1.0 (ref)
LI/PR / Comedonecrosis/HER2	**Present/Negative/ Present/Positive**	
Crude [a]	3.51 (1.46-8.43)	1.0 (ref)
+age	3.51 (1.45-8.47)	1.0 (ref)
+tumour size[b]	3.62 (1.27-10.30)	1.0 (ref)
+margin status[c]	3.95 (1.32-11.82)	1.0 (ref)
+treatment[d]	7.54 (2.00-28.43)	1.0 (ref)

[a] All analyses adjusted for year of diagnosis and time at risk

[b] Tumour size categorized into < 25mm or $\geq$ 25mm or multifocal

[c] Free margin versus positive or uncertain

[d] Treatment categorized into three categories; mastectomy, breast conserving surgery or breast conserving surgery followed by radiotherapy

LI= Lymphocytic infiltration, PR= Progesterone Receptor, HER2= Human Epidermal growth factor Receptor 2, OR= Odds ratio, CI= Confidence Interval

Paper IV

The study cohort consisted of 2978 women with right-sided DCIS and 3239 with left-sided DCIS, and 31 527 women without a history of breast cancer.

Women with DCIS had a higher level of education compared to the women in the comparison cohort (32.9 % versus 28.1% in the highest level of education category) and they were generally healthier (89.9 % versus 88.7 % with no comorbidity according to CCI score). Of the women with DCIS, 38.9 % received adjuvant RT (Table 2).

Patient characteristics and treatment did not differ significantly between right- and left sided DCIS.

Risk of IHD for women with DCIS

There were a total of 269 IHD events among women with DCIS and 1450 IHD events in the comparison cohort (Table 10). The risk of IHD was not increased for women with DCIS versus women in the comparison cohort (unadjusted HR 0.93; 95%CI 0.82 to 1.06 and adjusted HR 0.96; 95%CI 0.85 to 1.10). In the comparison of IHD risk in relation to treatment of DCIS (radiotherapy versus surgery alone) and using the comparison cohort as reference, the risk was lower for women receiving RT (HR 0.77; 95%CI 0.60 to 0.98) and at a very similar level after adjusting for CCI and educational level (HR 0.79; 95%CI 0.62 to 1.01). A comparison by laterality showed no increased risk of IHD from RT to the left breast (HR 0.85; 95%CI 0.53 to 1.37) versus the right breast.

Table 10. *Hazard ratio of IHD with 95%CI in women irradiated or not for DCIS versus women without a history of DCIS.*

	No. of events	**HR**	**CI**	**Adjusted HR** [a]	**CI**
No DCIS	1450	1.0 (ref)		1.0 (ref)	
DCIS	269	0.93	0.82-1.06	0.96	0.85-1.10
DCIS right	129 [b]	0.97	0.81-1.16	0.99	0.83-1.19
DCIS left	135 [b]	0.92	0.77-1.10	0.95	0.80-1.13
No DCIS	1450	1.0 (ref)		1.0 (ref)	
DCIS no RT	201	1.01	0.87-1.17	1.04	0.90-1.21
DCIS RT	68	0.77	0.60-0.98	0.79	0.62-1.01
DCIS RT right	36	0.84	0.60-1.16	0.86	0.62-1.20
DCIS RT left	32	0.72	0.51-1.02	0.74	0.52-1.06

[a] Adjusted for educational level, CCI and previous ischemic heart disease [b] 5 events in women with bilateral DCIS or unknown laterality.

IHD= Ischemic heart disease, DCIS= Ductal Carcinoma In Situ, No. = Number, HR= Hazard ratio, CI=Confidence Interval, Ref= reference, RT= Radiotherapy, CCI= Charlson Comorbidity Index

The cumulative probability of IHD in women treated with adjuvant RT or surgery alone versus women without history of DCIS is visualized by a Kaplan-Meier analysis. Up to 16 years after treatment, the incidence of IHD for women with DCIS, whether irradiated or not, did not exceed that for the women in the comparison cohort (Figure 10).

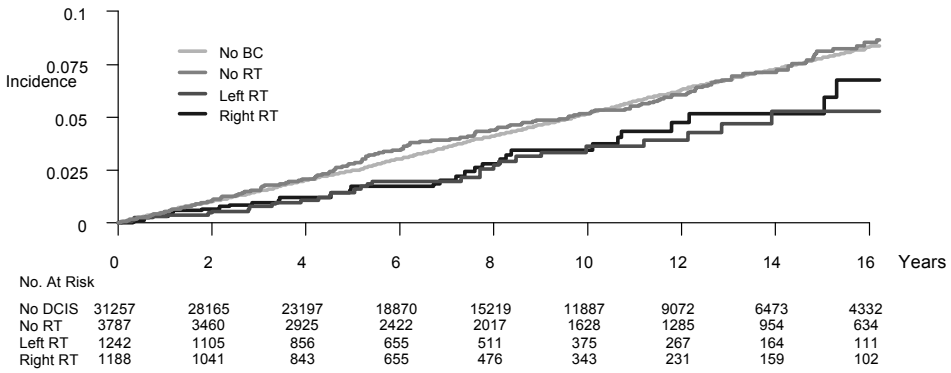


Figure 10. *Cumulative incidence of IHD in women treated with adjuvant RT or surgery alone versus women without history of DCIS*

Discussion

The management of DCIS is challenging due to its heterogeneous nature. Most women with DCIS have an excellent prognosis, but a minority will develop invasive disease and a few will ultimately die from breast cancer. At present almost 1 000 women are diagnosed with DCIS every year in Sweden (167). The vast majority are treated with surgery and overall about 40% of these women will also receive RT. The increased incidence poses concerns of overtreatment. In order to identify a subgroup of women for whom RT and its associated risks could be avoided and to differentiate DCIS with an aggressive potential, the identification of patient or tumour related characteristics, a biomarker or a combination of markers with prognostic and predictive information is essential.

Paper I

The aim of this study was to analyse trends in incidence, treatment and outcome of DCIS over a 20-year period in a Swedish health-care region. At this time, mammography screening was established practically all over this region. We found a slightly increasing incidence of DCIS over time, but the proportion of DCIS to all breast cancer was stable. This is in line with other reports and implies that the increased incidence mainly is due to screening and not to other risk factors.

The proportion of tumours in the larger size category increased over time. A possible explanation for this is the more widespread use of large histological tissue sections along with improvements in mammography.

Overall, about 67% of the women in the register were treated with BCS. Interestingly, there was a statistically significant increasing use of mastectomy over time, from about 25 % to about 40 %. This is in contrast with most other population-based studies, where the mastectomy rate generally is decreasing (36,37,53,180–185). Historically, mastectomy was the routine procedure in DCIS treatment. However, although mastectomy results in very low recurrence rates (63,64), it confers no survival advantage compared to BCS in observational studies and is considered overtreatment in most patients with DCIS. Mastectomies should presumably be reserved for extensive or multifocal DCIS where a breast conserving radical excision not is feasible. Quite contradictory to the reported increasing use of BCS is a concurrent increased rate of bilateral mastectomy for DCIS in the United States, from 0% to 8.5% between 1991 and 2010, a trend likely driven by prophylactic mastectomy rather than by bilateral DCIS (183,186). The ideal proportion of BCS versus mastectomy is hard to

define. Maybe more radical surgical intervention is motivated in groups of women with low RT efficacy. The results of the SweDCIS randomized controlled trial indicated that younger women had a relatively lower protective effect of RT after BCS (57), and there may be additional, so far unknown tumour biological properties that can affect RT responsiveness.

The use of adjuvant postoperative RT increased substantially over time as also reported by others (37,180,183,184,187) and follows with the results of the randomized trials published in the early 1990's. RT reduces ipsilateral recurrent events by half but has not been shown to influence distant metastasis or death (82). In the trials of RT after BCS for early invasive BC, about one breast cancer death was avoided by year 15 for every four recurrences avoided by year 10 (76). Thus, theoretically, RT might have a small beneficiary effect on survival also in DCIS with long-term follow-up. However, in the 20 year follow-up of the SweDCIS study, RT did not influence breast cancer death or overall survival (188). RT reduced recurrences by 37.5%, but the absolute reduction for invasive recurrences was only 2.0%. Furthermore, there were an increased number of contralateral events in the RT arm compared to the control arm, which may have prevented improved survival.

Axillary management in DCIS has been intensely debated. Indications for a sentinel lymph node biopsy (SNB) are based on the risk for occult invasive disease which, according to literature, is found in up to 25-30 % of excision specimens after a diagnosis of DCIS on core biopsy (189,190). The indications for SNB vary among published guidelines. The Swedish national guidelines were updated in 2007 and recommend SNB to be considered for DCIS nuclear grade III and larger than 20 mm (191). In the present study, SNB increased rapidly from 0% to 54.9% while axillary node clearance (ALND) dropped from 10% to almost none. Although the side effects of SNB are minor compared to ALND, they are not negligible. To do a SNB in more than 50% of patients with a final diagnosis of primary DCIS is overtreatment and needs to be addressed. It seems reasonable to recommend a SNB in patients planned for a mastectomy, but a more restrained management for patients treated with BCS needs to be adopted since a SNB still can be performed afterwards, if final histopathology reveals invasive breast cancer.

Recurrence rates in the present study were similar after BCS with or without RT, probably due to selection bias by indication. The gradually increasing treatment intensity over time did not translate into any further improvements to the already high recurrence-free survival observed. This possibly also reflects a selection bias, but it could be seen as a significant overtreatment.

Validation

The collection of data in the form of registers gives access to information on a large scale at a relatively low cost. It also provides the opportunity to study trends over time. It is of importance, however, to know the limitations and random or systemic errors that may exist. The value of a register relies heavily on the underlying quality of its data and the quality control procedures in place. Bray et al have presented four key aspects in addressing quality of register data (192).

- *Comparability* describes the importance of registering data in a standardized manner concerning classification and consistency in definitions of incidence, such as rules for the recording of multiple primary cancers occurring in the same individual
- *Completeness* measures how close incidence rates and survival proportions are to their true value
- *Validity* or *accuracy* refers to the proportion of cases in the registry with a given characteristic that truly have that attribute
- *Timeliness* describes the actuality of the register

The regional breast cancer quality register in Uppsala-Örebro was found to have a high overall completeness for primary data, but included a proportion of misclassified patients with invasive cancer and LCIS. For some of these patients, a lesion consisting of both an invasive and an in situ component was registered as two different primary tumours. Today, the guidelines for registering in Sweden are clearer and state that patients with both an invasive and an in situ component are to be reported as an invasive cancer only. This could thus be regarded as a systematic error where registering improved over time. However, the low number of cases falsely reported as DCIS should not seriously bias the data. The validity of key variables was between 91-99% and timeliness was good. The proportion of reported subsequent events was disappointingly low, only 65% of local recurrences and 58% of distant metastasis were reported. No similar comprehensive validation of a DCIS registration in a population-based register has been reported earlier, and comparisons with other register data is therefore not possible. These results emphasize the necessity to validate register data on a regular basis.

Paper II

In this nested case-control study in a population-based cohort of 6 964 women with DCIS, 96 women who died from breast cancer were identified and

compared with a group of 318 controls.

Current knowledge of risk factors for breast cancer death after primary DCIS is very limited. Numerous studies have been performed to define predictors for recurrence (55,58–60,102,107,108,111,193), but they are generally not powered to detect differences in mortality. A non-invasive recurrence has no impact on survival whereas an invasive recurrence entails a markedly increased risk, rendering a 15-year breast cancer specific survival just over 60 % (67,81,194). It therefore seems appropriate to focus on predictors for *invasive* recurrences. However, it is not clear whether death from breast cancer is the direct consequence of an invasive recurrence or if an invasive local recurrence is a marker for a more aggressive potential (195). Factors that predict recurrence may be different from factors that predict death.

We analysed oddsratios with 95% confidence intervals for breast cancer death in this cohort. Detection outside screening, large tumour size, multifocality, and positive or unclear margin status were associated with a higher risk. The risk was not affected by age or type of treatment.

Detection outside screening included asymptomatic DCIS detected by mammography but not within the population-based screening programme. Hence, comparison was not made directly between symptomatic and asymptomatic DCIS. Nevertheless, women with non-screening DCIS were at higher risk. Clinical presentation is one of the most important factors in predicting invasive recurrence compared to non-invasive recurrence (55,97). Moreover, symptomatic DCIS is usually larger, is more likely to harbour occult invasive disease (72,189) and has been shown to have a poorer overall prognosis than screen-detected DCIS (196,197).

The strongest predictor of cancer-related death was tumour size and multifocality. This corroborates with the results from an earlier case-control study including 39 women with primary DCIS who died from breast cancer (198). The risk remained significant in the multivariable analysis and after adjustment for treatment received. Tumour size is an established risk factor for local recurrence, although according to studies comparing risk factors for different types of recurrence, the risk is more likely associated with *in situ* recurrences than invasive recurrences (97,199). One explanation to this could be a higher risk of residual disease after surgery in larger lesions. Perhaps more importantly, tumour size is one of the most important predictors of presence of occult (micro) invasion (72,104,189,200). Sopik and colleagues showed that the ratio of distant metastasis to local recurrence (which they suggested as an index of metastatic potential) increases with increasing tumour size. They speculated that fast-growing cancers are inherently more likely to metastasize – that

tumour aggressiveness predicts tumour size (113).

A few women, both among cases and controls had verified or suspicious foci of microinvasion. The risk of breast cancer death was not increased by this variable and the exclusion of these women did not alter the results in our study. This is in line with the work by Narod et al (67), but has been contradicted in a recent study by the same research group (106). They found that the 20-year actuarial breast cancer-specific mortality rate was 3.8% for women with pure DCIS compared to 6.9% for women with microinvasion, rendering a hazard ratio of 2.0 (95% CI 1.75-2.26) for microinvasive DCIS compared to pure DCIS.

High Nuclear grade (grade III) has been reported to increase the risk of recurrence (55,107,108), although not specifically of invasive recurrence (67,111,113,201). Importantly, both Bijker et al and Narod et al found that for those women who encountered an invasive recurrence, nuclear grade III in the primary lesion was associated with increased risk of subsequent distant metastasis and breast cancer mortality (67,111,202). We also noted an increased risk of breast cancer death by nuclear grade III, but in the multivariable analysis, after adjusting for other tumour related variables, it was no longer statistically significant. Unfortunately, information of nuclear grade was missing in about 40% of the patients in our study cohort.

One important finding was that type of treatment did not affect the risk which is in line with a meta-analysis of the results from both randomized and observational studies (203). In the work by Narod et al including more than 100 000 women to estimate breast cancer mortality after a diagnosis of DCIS they also concluded that prevention of ipsilateral invasive recurrences did not prevent death from breast cancer (67). This points out that an invasive local recurrence maybe should be considered a marker of risk for, rather than a cause of, distant metastasis (195). It could be speculated that RT might not prevent tumours that are destined to cause distant metastases from metastasizing or that RT may be less efficient in certain subgroups. Sagara and colleagues examined the benefit of RT stratified by factors associated with risk of recurrence (204). They used a Patient Prognostic Score including age, tumour size and nuclear grade and showed that in women with a higher risk score, breast cancer survival was significantly better after BCS and RT compared with BCS alone. This improvement was not observed among women without these negative prognostic factors and implies that, at least in some patients, local control does make a difference. In our study, positive or uncertain margin status increased the risk of breast cancer death. This was statistically significant both whether treatment included BCS or a mastectomy and remained significant also in the multivariable analysis.

Young age has been reported as an adverse prognostic factor associated with a higher risk of invasive recurrence (65,97–99), distant metastasis (100) and breast cancer death (67,101). This could not be supported by the results in our study. The comparison between studies is complicated by that the definition of young age has varied widely among investigators, ranging from younger than 35 years of age to younger than 50 years of age. We performed regression analyses both with age as a continuous variable as well as by different age categories, but no significant correlations between age and breast cancer mortality was found (data not published).

In order to distinguish hazardous from harmless DCIS it is relevant not only to study risk factors for recurrence, but risk factors for breast cancer death, especially as they may differ. There are, however, several other issues that influence survival. Early detection and definitive treatment of an ipsilateral invasive recurrence have an important impact on prognosis. A few of the women in the present study were quite old at the time of recurrence, which meant that local and systemic treatment of the relapse had to be modified due to comorbidity. Moreover, death may be preceded by a contralateral cancer, in which case the tumour properties of this cancer probably is more important than the characteristics of the primary DCIS. We performed a separate analysis after excluding women in whom an invasive contralateral breast event was diagnosed after the primary DCIS and excluded also their corresponding controls, but this did not alter the results significantly.

The main drawback of this study is the incomplete data in the histopathology reports. A more standardized assessment of size, focality and surgical margins along with complete information of nuclear grade and microinvasion would potentially have improved the ability to draw firm conclusions.

Continuous efforts are needed to identify tumour biological markers or markers in the tumour microenvironment, that can distinguish DCIS lesions inherently more prone to metastasize, and/or less sensitive to radiation.

Paper III

This work aimed to investigate tumour biomarkers in DCIS associated with aggressiveness. Tumour biological features associated with risk of invasive recurrence and metastasis may already be present at the pre-invasive stage. DCIS with intense lymphocytic infiltration (LI) was associated with a statistically significant increased risk of breast cancer related death in the univariable analysis. None of the other biomarkers assessed were individually related to increased risk. PR negativity, however, when combined with presence of LI, was an independent prognostic factor after adjustment for age, tumour

size and treatment. Combining PR negativity and LI with presence of comedonecrosis further increased the risk.
To date, no single histopathological or molecular marker has been identified that may serve as an individual predictor for progression from DCIS to invasive disease (128,176,205). Studies investigating various combinations of biomarkers in relation to prognosis have led to inconsistent results and are generally not powered to detect differences in survival. Factors that predict recurrence may be different from those that predict death. Interestingly, PR status was recently reported as an independent strong prognostic factor for mortality in DCIS and early breast cancer, but not for local recurrence (113). Moreover, negative PR status has been shown statistically significantly associated with detection of disseminated tumour cells in DCIS and small invasive breast cancers (206).

Previous trials have shown that infiltration of specific subsets of immune cells in DCIS is related to recurrence (109,207). The overall significance of inflammation in breast cancer is controversial. Inflammation may represent an immune response against the tumour, but inflammation may also stimulate tumour growth by releasing proteolytic enzymes and angiogenic factors (153). Moreover, animal studies imply a role for macrophages in mediating resistance to radiotherapy (208). Studies assessing the relationship of lymphocyte infiltration to prognosis in invasive breast cancer show improved survival in ER negative and HER2 positive tumours, but not in ER positive tumours (209). The underlying mechanism for this is unknown but may be due to differences in the specific types of immune cells. The exact role of the immune system during the progression of ductal carcinoma in situ needs further investigation. There may be clinical implications with options to find targeted therapies.

Paper IV

This study addressed the issue of radiation induced ischemic heart disease after adjuvant postoperative RT in DCIS.

As mentioned earlier, none of the four randomized trials comparing BCS with postoperative adjuvant RT to surgery alone could demonstrate any benefits in terms of survival (82). In the EBCTCG overview, overall mortality and mortality from heart disease were actually slightly higher for women allocated to RT. In addition, improvements in imaging and assessment of margins potentially have led to a lower rate of ipsilateral breast events without RT now when compared to the era in which these studies were performed. Consequently, it is of utmost importance to evaluate potential hazards with radiation.

In this analysis 6 270 women with DCIS and a comparison cohort of 31 257 women were included. The risk of IHD was lower for women with DCIS allocated to RT compared to non-irradiated women and to the comparison cohort, probably due to selection mechanisms. It has been established previously that women with breast cancer, in particular screen-detected breast cancer are generally healthier (89,101,210,211) and treatment has likely been adjusted to avoid radiation for women with comorbidity. Comparing heart disease in irradiated women with left-sided and right-sided breast cancer is an unbiased approach since it is unlikely that treatment choice would differ by tumour laterality. We showed that irradiation of the left breast did not confer any over-risk compared to irradiation to the right breast. These results are important, for the reasons stated above. The strength of the study is the adjustments of the analyses for comorbidity and educational level, variables which otherwise could have underestimated the risks.

One of the issues when analysing hazards from RT is that there is a continuous improvement in targeting to reduce radiation exposure to organs at risk but at the same time, long follow-up is required. In a meta-analyses of long-term risks of coronary heart disease after RT, the risk increase started within the first 5 years and continued into the third decade after RT (88). The highest relative risk occurred between 10 to 14 years after the diagnosis of BC. Uncertainties about the duration of risk remain, as radiation-related mortality risks have been shown to be larger after 10 to 20 years after exposure than within the first decade (86,212–214).

The results of the present study are reassuring in that adjuvant RT with modern RT technique to the conserved breast after surgery for DCIS did not show any increase of IHD in the first eight years of follow-up. Nevertheless, the use of RT in DCIS management is increasing. Even small increases in risk of IHD are thus of importance and longer follow-up of these women may be warranted.

Methodological considerations

Important aspects of research include the possibility to generalize the observations made in a sample to other populations. Internal validity refers to the accuracy of the conclusions within that particular study sample, while external validity refers to whether or not the results of a particular study are relevant to a more general population.

Two types of errors, random errors and systemic errors, affect internal validity. A random error, as the name suggests, is random in nature and very difficult to

predict. Systemic errors are commonly referred to as biases. For example, when the selection of study subjects is selected in a non-randomized way (selection bias) or when there is an error in measurement or classification (information bias). Confounding is another type of systemic error and may be considered as a confusion of effects. The characteristic of a confounder is that it coincides with exposure and that it itself contributes to the disease.

Paper I and **IV** in this thesis include women from population-based registers with high documented coverage, ensuring high external validity. In **paper IV**, there was an expected selection bias in relation to the outcome of this study, as women with DCIS presumably are at lower risk of heart disease than women in general. This was accounted for in the study design by comparing hazards between left-sided and right-sided radiation. In both these studies there are potentially a risk of systemic errors due to changes in classification or registration over time, but any errors are most likely at random.

A major strength of the nested case-control design used in **paper II** and **III** is that the source population from which the cases and controls are derived is defined and every individual in the cohort has an equal chance of being included. The most challenging part of a case-control study is appropriate selection of controls that serve as a reference group to which the cases are compared. One sampling method is cumulative incidence sampling in which controls are selected from non-cases at the end of the follow-up period. This method is however sensitive to bias, as there may be differences between individuals who are lost to follow-up and those who remain in the cohort.

Incidence density sampling is the least biased method for control sampling (172). Here, a control is randomly selected from all individuals at risk at the time of the index case occurrence. A selected control is still eligible to be selected again as a control for another case and may also become a case at a later time in follow-up.

Sometimes the controls are matched to the cases with the intention to control confounding, but in case-control studies matching introduces bias instead (172). One of the reasons for this is that matching on one or more factors related to the disease makes the controls more similar to the cases and this may reduce the specificity of the results in the study. We selected controls randomly, completely without matching. A major difference in follow-up time between the cases and controls was encountered by this, which was adjusted for in the regression analyses. An alternative might have been to select controls matched by time of diagnosis and thereby providing identical time at risk for both groups.

Missing data can reduce the statistical power of a study and can produce biased estimates, leading to invalid conclusions. Data can be missing at random or not at random. Information on nuclear grade was missing in a fairly large proportion of the cases and controls in **paper II**, but the missing data was evenly distributed between the two groups and, as far as we know, at random. Imputation is a process where missing data is replaced by estimated values. In multiple imputations several imputed datasets are created, in our case five, and this is considered the most appropriate method of handling missing data (175).

In **paper III**, some patients were excluded due to unavailable tumour specimen. More controls than cases were excluded as the tumours of the controls on average were smaller. Nevertheless, this should not bias estimates as there were up to four controls sampled for each control from start.

Conclusions

- The regional breast cancer quality register in Uppsala-Örebro has valid information on most parameters in women registered with DCIS but data on follow-up is incomplete. These results address the necessity to validate register data on a regular basis.
- Treatment of DCIS in the Uppsala-Örebro healthcare region has intensified over the last 20 years. This has however not translated into any significant improvement in outcome. Increasing mastectomy rates and use of postoperative radiotherapy may reflect overtreatment and long-term adverse effects as well as costs need consideration.
- The risk of breast cancer death in women with primary DCIS was increased for DCIS detected outside the screening program, large tumour size, multifocality and positive margin status. Our results implicate that tumour size is a measure of disease aggressiveness.
- DCIS with periductal lymphocytic infiltration (LI) or the combination of PR negativity and LI was statistically significantly associated with risk of breast cancer related death. Combining biomarker expressions in DCIS with features in the peritumoural stroma may be useful tools for prognostication.
- The risk of ischemic heart disease was not increased for women with DCIS compared to women without a history of breast cancer after median 8 years of follow-up. No increased risk was seen either after comparing treatment with radiotherapy versus surgery alone or when analysing RT by laterality.

Future implications

Several questions regarding DCIS remain unanswered. We need better tools to discuss treatment options with the well-informed woman in the clinical setting. A mastectomy is overtreatment in most cases, but maybe there are cases who would benefit more from this approach.

- How can we identify women with less responsiveness to RT, who might be better off with a mastectomy (with immediate breast reconstruction)?
- How can we select women for whom adjuvant radiotherapy and/or surgery can be safely omitted? The ongoing trials investigating outcome after active surveillance only in low-risk DCIS may elucidate these questions.
- Markers need to be defined and validated to identify women who are at high or low risk of subsequent invasive cancer. Integrating clinical, histopathological and biomarker data may provide tools for risk stratification.
- The interaction of biomarkers with the microenvironment needs to be further explored.
- The increasing use of radiotherapy in DCIS management requires further evaluation of long-term adverse effects such as radiation induced sarcomas and other secondary malignancies in large population-based cohorts with longterm follow-up.

Acknowledgement

I would like to express my sincere gratitude and appreciation to all those who have supported me in my work on this thesis: In particular, I wish to thank:

My main supervisor, **Malin Sund**, for guidance and support, and for sharing your excellent skills in research. You're absolutely brilliant! Most of all, thank you for being such a good friend!

My co-supervisor, **Fredrik Wärnberg**, for your always so positive attitude, creativity, and never-ending enthusiasm. I´m so glad that you´ve inspired me to explore the fascinating field of DCIS!

The BCBase research group in Uppsala,

Lars Holmberg, for initiating this inspiring and creative research environment and for sharing your immense wisdom.

Hans Garmo, for your incredible patience, support and guidance in statistics and epidemiology.

Calle Blomqvist, for sharing your vast research experience and enthusiasm.

Greger Nilsson for sharing knowledge and intellectual thoughts in our discussions and for excellent skills in reviewing manuscripts, into the last detail!

Stefan Emdin for introducing me into the world of breast cancer research.

Anna-Karin Wennstig, my fellow PhD student, for invaluable friendship, sharing ups and downs on train rides, in "R" and manuscript submissions! I sincerely hope we will continue our tradition of sharing one or two glasses of wine at Uppsala train station!

Hanna Heyman, for fruitful collaboration in paper I

Marit Holmqvist, for statistical assistance in paper I

Irma Fredriksson, coauthor and a dear friend. Thank you for support and invaluable contributions to the manuscripts.

All the staff at the Pathology departments throughout Sweden, for helping me gather histopathological slides and tissue blocks. Special thanks to **Anette**

Berglund and **Pernilla Andersson** at Umeå research lab, **Tor Halle,** Uppsala research lab, **Helena Hermelin,** Dalarna research lab, **Gregory Winn** and **Åsa Edvardsson,** Karolinska Institute for helping me with the laboratory work, constructing TMA.

Egle Tolockiene for helping me with the slides and generously sharing your knowledge in histopathology.

Johan Hartman for your invaluable effort in reviewing all the histopathological slides.

Leif Israelsson, my boss during 15 years, for your support and for giving me space to work on this thesis.

My dear colleagues at the breast-endocrine surgery team **Jan Kågström**, **Ann Nordeman** and **Helene Lindfors.** My breast nurses **Ann Torstensson, Ann Dannerfjäll** and **Anna Dyrander.** Thank you all for keeping business as usual in my absence.

All my other present and former colleagues at the Dept of Surgery and Urology, Sundsvall Hospital. A special thanks to **Elisabet Skagius** for long and lasting friendship and, in advance, for helping me with the dissertation party!

My father **Bo**, my late mother **Barbro** and my dear brothers **Olle** and **Bengt** with families for love, support and encouragement throughout my life.

In the end, the most important people in the world, **Anna, Carl** and **Ella** (and **Nicke** of course!) You mean everything to me!

And **Mats,** my husband, my love, and my best friend in life! You inspired me to do this!

This thesis was supported by grants from the Department of Research and Development, Vasternorrland County Council, Vasterbotten County Council, the Swedish Breast Cancer Association, Stiftelsen Emil Anderssons fond för medicinsk forskning, and Stiftelsen JC Kempes Minnes Stipendiefond.

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