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CONTRALATERAL BREAST CANCER

- Risk and Prognosis

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Till Mamma och Pappa, Jonte och Nicke.

Illustrations

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Och dock, om du i tvivel sjunkit ner
och dröjer dystert grubblande vid vägen,
du griper åter ditt baner
och bär det genom öknen oförvägen.
Vad mer, om spejarblicken ser,
hur bort från fästet tusen solar fejas?
Vad mer, om stjärneskördar mejas
som gyllne säd av tidens lie ner?
Vad rätt du tänkt, vad du i kärlek vill,
vad skönt du drömt, kan ej av tiden härjas,
det är en skörd, som undan honom bärgas,
ty den hör evighetens rike till.
Gå fram, du mänsklighet! var glad, var tröst,
ty du bär evigheten i ditt bröst.

Viktor Rydberg (1828-1895)

FOREWORD

Half of science is putting forth the right questions.

Sir Francis Bacon (1561-1626)

Today, many millions of women worldvide are 'breast cancer survivors', most of these women will luckily have long lives after their diagnosis, both due to lower breast cancer mortality and to longer life expectancy in general. Women surviving breast cancer are, however, at increased risk of several diseases, partly because they are a selected subpopulation; a diagnosis of breast cancer indicates proneness for cancer, thus increased risk of other cancers. In addition; adjuvant cancer therapy increases the risk of secondary cancers, as well as e.g. heart disease and potentially also serious infections. Part of the increased risk of different diseases in this population could probably also be accounted for by the more intense surveillance of breast cancer patients as compared to healthy women. One of the most common and serious diseases after breast cancer is *contralateral breast cancer* (CBC); a new primary cancer in the opposite breast. With this cancer added to the first, these women have significantly worse prognosis compared to women with only one breast cancer. The incidence of breast cancer is very high and as the mortality after breast cancer keeps improving, CBC will be of increasing importance.

My rationale to study CBC is twofold; it is a potentially deadly but woefully understudied disease that will affect an increasing number of women and is thus worth to study in its own right, to potentially improve the reality in the clinic for these patients. Also, in the rich part of the world, breast cancer patients are followed up after their diagnosis and treatment to a substantial cost, both measured in inconvenience and anxiety for the patients and in resources for the society, therefore, CBC-risk stratification should also be seen as an important goal. Further, I believe that by studying CBC we might, in the long run, also have a possibility to draw conclusions about breast cancer in general. For example, to study how endocrine therapy affects the risk, development and characteristics of CBC will most likely give an indication of how endocrine chemoprevention will affect unilateral breast cancer, which might otherwise be difficult to study as the population at risk is cancer-free women.

The lightning actually does strike twice in the same place…
Let's learn something from it.

ABSTRACT

"Therefore, since brevity is the soul of wit, and tediousness the limbs and outward flourishes, I will be brief"

William Shakespeare (1564-1616)

The objective of this thesis was to investigate different aspects of *contralateral breast cancer*. This disease is of increasing importance as the mortality of breast cancer is decreasing while the incidence remains very high, consequently the population at risk for non-simultaneous (metachronous) contralateral breast cancer is increasing. Further, both simultaneously occurring (synchronous) contralateral breast cancer and non-simultaneous (metachronous) contralateral breast cancer have a worse prognosis than breast cancer in general. The population-based cohort used in all four studies includes all patients diagnosed with contralateral breast cancer in the Stockholm region during 1976-2005 (N=1422).

In the first paper, which investigates the timing of diagnosis of contralateral breast cancer, we conclude that the diagnostic work-up has not improved over the last 25 years; the second cancers are neither found earlier, nor at smaller tumor size. In our second study we investigate the effect of adjuvant radiotherapy for the first breast cancer and conclude that radiotherapy seems to worsen tumor characteristics (TNM-stage and differentiation grade) and prognosis after contralateral breast cancer. In analyzing estrogen receptors of the two breast tumors we, in our third study, show how estrogen receptors of both tumors taken together have an important prognostic value. We also find several indications of endocrine therapy resistance in patients with two metachronous estrogen receptor positive tumors. The fourth and final study investigates mammographic density as a risk factor for contralateral breast cancer, studying both mammographic density at diagnosis of the first cancer and changes in density following the first cancer. We find no effect on the risk of contralateral breast cancer by mammographic density at the time of diagnosis of the first cancer; however, there is a significant risk decrease for patients who experience a decrease in breast density during follow-up after the first cancer.

LIST OF PUBLICATIONS

I. **Diagnostic work-up of contralateral breast cancers has not improved over calendar period**
Maria EC. Sandberg MSc, Mikael Hartman PhD, Gustaf Edgren PhD, Sandra Eloranta MSc, Alexander Ploner PhD, Per Hall PhD, Kamila Czene PhD
Breast Cancer Res Treat. 2010 Aug;122(3):889-95

II. **Aggressiveness of contralateral breast cancer is influenced by radiotherapy for the first tumor**
Maria EC. Sandberg MSc, Sara Alkner MD, Mikael Hartman PhD, Sandra Eloranta MSc, Lisa Rydén PhD, Alexander Ploner PhD, Hans-Olov Adami PhD, Per Hall PhD, Kamila Czene PhD
Manuscript submitted

III. **Prognostic implications of estrogen receptor pattern of both tumors in contralateral breast cancer**
Maria EC. Sandberg MSc, Mikael Hartman PhD, Daniel Klevebring PhD, Sandra Eloranta MSc, Alexander Ploner PhD, Per Hall PhD, Kamila Czene PhD
Breast Cancer Res Treat. 2012 Jul;134(2):793-800

IV. **Change of mammographic density predicts the risk of contralateral breast cancer**
Maria EC. Sandberg MSc, Jingmei Li PhD, Per Hall PhD, Mikael Hartman PhD, Isabel dos-Santos-Silva PhD, Keith Humphreys PhD, Kamila Czene PhD
Manuscript submitted

CONTENTS

LIST OF ABBREVIATIONS

CBC	Contralateral breast cancer
ER	Estrogen receptor
TNM	Tumor size, nodal status, metastatic status
CPM	Contralateral prophylactic mastectomy
SBCR	Stockholm breast cancer register
ICD	International classification of disease
MRI	Magnetic resonance imaging
MLO	Media-lateral-oblique
CC	Cranio-caudal
HRT	Hormone replacement therapy
IRR	Incidence rate ratio
OR	Odds ratio
RR	Risk ratio
CI	Confidence intervals
SE	Standard error

1 INTRODUCTION

1.1 EPIDEMIOLOGY

It could be said for epidemiology, with respect to disease etiology and prevention, what is frequently said about democracy as a system of government: they both have many problems and weaknesses, but they still represent the best available approach for the achievement of their respective objectives.

Prof. Dimitrios Trichopoulos (1938-), Harvard School of Public Health

Epidemiology can be broadly defined as the knowledge *how and why diseases occur*. Naturally, such short definition needs clarification; *how* refers to questions of descriptive epidemiology; in which areas are the disease more common, in which age groups, in which periods, etc. *Why* refers to questions of association, which almost always aim towards a causal interpretation; to assess what factor/s cause the disease. Further; not all epidemiology study *diseases*, not even when we define it broadly, e.g. a disease being 'metastasis after breast cancer'. Oftentimes epidemiologists are interested in, if not disease, then at least health-related outcomes, e.g. 'attitudes towards cancer screening' but sometimes the outcome studied is not related to health, like 'occurrence of left handedness' or even to physical traits, e.g. studies of emigration.

I believe most epidemiologists would agree with the above quoted Professor Trichopoulos; there is no perfect epidemiological study, they all have some defect. As an epidemiologist, but also as a consumer of epidemiological research, it is of uttermost importance to be able to spot the weaknesses of a study and make up your mind about whether the weakness render the findings irrelevant, or whether credibility can still be given to the findings, despite the weakness of the study. Every time a researcher makes a too generous judgment of his/her own study and publish the findings, the creditability that is lost in the eye of the public potentially harms the entire field of epidemiology. On the other hand, to quote another man of great formulations; Sir Winston Churchill have said *"'Nothing prevails but perfection' can be spelled shorter; paralysis"*. To that we can add that in order to make progress new and innovative ideas should be tested, and tested hard, preferentially by different researchers, in different settings by different methods. In order to make this possible also imperfect studies have to be published so that the findings can be brought up for discussion and testing, but the key is to not over interpret, nor under interpret, the findings.

As in most research, there is somewhat of a difference between epidemiological studies aiming primarily towards understanding the underlying etiology/biology of the outcome and studies aiming towards results that can immediately improve the real-world handling of the outcome. Preferentially there should be elements of both these types of questions in all studies; an epidemiological finding without any type of plausible underlying mechanism should be regarded with caution, on the other hand; if the study findings have no potential connection to a clinical reality perhaps time and resources could be better spent.

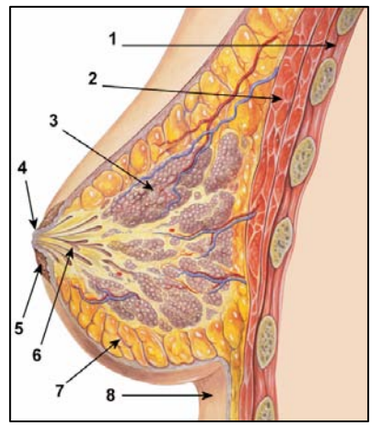
1.2 BREAST

"Your breasts are like two fawns, twin fawns of a gazelle grazing among the lilies."
Songs of Salomon 4;5

The English word *breast* derives from the Proto-Indo-European base *bhreus* (to swell, to sprout). In this thesis 'breast' refers to the human mammary glands. These are made up mainly of lobular cells, which constitute the alveoli, where milk production takes place, ductal cells, which constitute the ducts for leading the milk from the alveoli to the nipple, fat tissue and connective tissue. The anatomical parts of the female breast can be seen in Figure 1.

Figure 1: Anatomy of the female breast.

1	Chest wall
2	Pectoralis muscle
3	Lobules
4	Nipple
5	Areola
6	Milk ducts
7	Fat tissue
8	Skin



Normal breast development takes place in several distinct phases during a woman's life. In the prenatal phase, about 6 weeks after conception, ectodermal cells form two vertical ridges along the front of the torso, these later involute, leaving cells only in the pectoral region[1]. This development is the same for both male and female embryos[2]. In puberty, under the influence of estrogen and progesterone, the female breast grows by further development of ducts and thereafter secretory glands; lobules, in the end of the ducts, and by deposition of fatty tissue. This development, *thelarch*, leads to permanent breasts before pregnancy, which differentiates humans from other mammals, in which the mammary glands are developed only during pregnancy and lactation. The adult human breast before pregnancy consists of approximately 80% stroma cells, though there are large differences in the distribution of cell types, both between the two breasts of the same individual and between individuals[2]. During pregnancy, the epithelium proliferates and differentiates into secretory cells, which can synthesize and secrete milk. Several hormones are involved in this process, among them are estrogen, progesterone, prolactin and oxytocin. When breast feeding is discontinued the breast involutes by apoptosis and phagocytosis of the secretory cells, but the ducts remain mainly unchanged[2]. However, during menopause, the ceasing excretion of hormones from the ovaries leads to reduced numbers of both ducts and lobules, which are mostly replaced by fat tissue and in consequence the density of breast decreases[3]. Further, this process, in combination with the relaxation of the ligaments of Cooper will also change the physical appearance of the breast; it will sit lower on the torso and the nipple will point downward, an aging process known as ptosis[1].

1.3 CANCER

"Nothing in biology makes sense except in the light of evolution"
Christian Dobzhansky (1900-1975), geneticist and biologist

Cancer is defined as a mass of cells that grows without the boundaries and stop signals that regulates the growth and division of healthy cells. Cell division is a dangerous procedure, and under the evolution strong mechanisms have developed to regulate this and keep it under strict control. When these mechanisms are circumcised cancer occur. Our understanding of carcinogenesis is still very much under development, but what is known is that cancer develops as a consequence of mutations and epigenetic changes in the genome. Before a cancer can start growing in earnest, several control mechanisms need to be overthrown; apoptosis needs to be evaded, endless capacity for cell division needs to be acquired, stop signals for cell divisions must be overcome, the cell must be self sufficient in growth signals, must have a sufficient supply of blood and must finally obtain the ability to grow without contact to the basal membrane and invade other tissues. This means that a large number of genomic changes have to occur for all these individual mechanisms to be affected. For inherited cancers one of these changes might already present in every one of the cells of the body, therefore, one less change have to happen in any given cell before cancer can develop and inherited cancer therefore often develop early. Environmental exposures also induce damage to the genome, oftentimes by mutations, but also via epigenetic changes; structural reversible changes of the DNA molecule. The cell is most vulnerable for these changes when it is dividing, therefore, although cancer can occur in most tissues, it is much more common in cell types that divide often, and factors that lead to cell division in that particular tissue type are also often risk factors for cancer in that tissue type.
In all tissue stam cells and pluripotent cells; cells with the ability to regenerate and the potential of developing into all types of cells (stam cells) or several different types of cells (pluripotent cells), are present. The current understanding of cancer development states that probably only these cells, and not the fully differentiated cells, can undergo the changes required to become a cancer cell.
Cancer cells divide constantly and the normal mechanisms to stop cell division and/or make the cell go into apoptosis in the event of irreparable damage to the DNA are put out of function. A cancer cell will thus accumulate mutations much faster than a normal cell. This was thought to mean that a cancer will always develop from less aggressive to more aggressive, from less invasive to more invasive, but recently indications have been found that invasive cancer might go into a dormant state, or even regress. Regardless, the effect will be that a cancer tumor consists of different clones of different characteristics and different level of aggressively, so when the microenvironment changes a cancer tumor has a large potential of adapting, simply by that the clone best fitted to the new environment will take over.
The first recording of cancer is from 2300 BC in ancient Egypt. At that time, and for a long time to come, cancer was a certain death sentence, and today 7.5 million people die each year from cancer worldwide (0.5 millions of the deaths are due to breast cancer)[4], but why? There are two main explanations as to why cancer is potentially

deadly; the growth of cancer cells might decrease the function of the organ in which the cancer is situated and if the organ is vital this might be deadly, e.g. cancer or metastasis in the brain, liver or lung. However, less intuitive but probably at least as important is the fact that cancer cells in constant division are incredibly energy consuming and the cancer growth starves the rest of the body of nutrition until life cannot be sustained. This can be seen in the way cancer patients wither away in the last stages of metastatic disease. Metastasized cancer is still mainly without cure, and the treatment given is palliative, though research for new treatments is constant and some of them show promise in trials[5].

2 BACKGROUND

To know that we know what we know, and to know that we do not know what we do not know, that is true knowledge.

Nicolaus Copernicus (1473-1543)

2.1 BREAST CANCER

"Cancer is a word, not a sentence"

John Diamond (1953-2001), journalist (and cancer patient)

2.1.1 Occurrence

In Sweden 7917 patients were diagnosed with breast cancer in 2010, which equal an incidence of female breast cancer of 168 cases/100 000 women years[6].
Brest cancer incidence has continuously increased since the cancer register started in 1958 until today[6], although the increase have seemed to subside in the last 10 years[7]. Breast cancer is much more common in high-income countries[4], this is most likely due to lifestyle risk factors, screening routines as well as the genetic makeup of the different populations. About 35 cases of male breast cancer are diagnosed each year (0.71 cases / 100 000 male person-years)[6]. These men are in general diagnosed at a later stage compared to women, which leads to worse prognosis[8].

2.1.2 Risk factors

Two oftentimes not mentioned risk factors, perhaps since they are not modifiable, are sex and age. Age has an interesting effect on breast cancer risk; in general, cancer becomes more common as a population grows older, and this was how it looked for breast cancer before 1980[6]. In the case of breast cancer this age dependency also included a feature known as *Clemmensen hook*; the increasing incidence dips around age 50, i.e. during menopause, but then continues to increase[9]. Then, from the 1990s, the incidence increased in the younger ages, until breast cancer was almost equally common in all age groups from 55 years and up[6, 9]. This shift has been attributed to the wide spread use of hormonal replacement therapy (HRT) during and after menopause (though this is difficult to study due to other changes that also affect the age-specific incidence[10]) and since HRT use have decreased significantly the age-specific incidence of breast cancer can be expected to return to the earlier pattern.
Next to age, sex and having had breast cancer (or benign breast disease), mammographic density is probably the most important risk factor for breast cancer. On a radiological picture of a breast, a mammogram, epithelia and connective tissue will appear white, while fat tissue will appear dark. Mammographic density is defined as the white proportion of the mammogram. A meta analysis of 14 000 breast cancer patients and 226 000 non-cases showed that women with >75% mammographic density had almost 5 times the risk of breast cancer compared to women in the lowest density group (<5%)[11]. Further, decreasing mammographic

density in healthy women has been shown to correspond to decreasing breast cancer risk[12].

A part of the risk factor profile for breast cancer consists of genetic factors; four high penetrance genes have been identified for breast cancer; BRCA-1 and BRCA-2, PTEN and TP53[13]. Both BRCA-1 and BRCA-2 genes encode for DNA double-strand break repair proteins. An inherited mutation in BRCA-1 confers a 65% risk of developing breast cancer before the age of 70, the corresponding risk for BRCA-2 mutation carriers are 45%[14]. However, these inherited mutations are very rare in the population, and even among breast cancer patients they are only found in about 5%[15]. In addition to these genes, about a handful medium-penetrance genes have been found[13] and the hunt for single-nucleotide polymorphisms (SNPs) is still very intense. So far approximately 50 SNPs have been consistently found to be associated with breast cancer[16], each of them confer a very small risk increase.

Among the environmental risk factors for breast cancer hormonal exposure plays an important part. Regarding endogenous hormones it has long been known that factors that decrease the numbers of menstruation cycles; e.g. late menarche, pregnancy and early menopause, decrease the risk of breast cancer[17]. For pregnancy the relationship is dual; overall parous women have lower risk of breast cancer than nulliparous women, and this is further enhanced with each subsequent birth[18]. However, during pregnancy and for the subsequent 10 years the risk of breast cancer for parous women, compared to nulliparous women, is increased[19], possibly due to the effect of pregnancy-associated hormones on preexisting malignancies, this effect is however small in comparison to the long-term protective effect. Further; age at first full-term birth[20], and potentially also breast feeding[21], is associated with breast cancer risk, hypothetically linking the maturation/differentiating of the breast tissue to breast cancer risk[18, 20]. For exogenous hormones it is well proven that hormone replacement therapy[22] after menopause increase the risk, and a small risk increase has also been shown for current use of oral contraceptives[23].

Regarding other life style factors, alcohol intake has a modest, but consistently shown, risk increase, estimated in a meta analysis to 7% per each additional drink/day[24] and no risk increase is seen by cigarette smoking[24]. Many attempts have been made to investigate the effect of diet on the risk of breast cancer, but this is a difficult field and no effect have been consistently shown to date[25]. Physical activity have been shown to decrease the risk of postmenopausal breast cancer, but only weak evidence exists for any effect on premenopausal breast cancer[26]. Body mass index is related to breast cancer risk in a rather complex web; fat tissue is the most important source of estrogen in postmenopausal women[27] and consequently, obesity is a risk factor for breast cancer after menopause[28, 29], for premenopausal women, on the other hand, it seems that obesity is protective[28]. Body height has been consistently associated with breast cancer risk; taller women have a modestly increased risk compared to shorter women[30]. The causation behind this risk increase is still unclear; taller women tend to have larger breast which puts more cells at risk of developing into cancer cells, further; growth hormones *in utero* or childhood energy intake has been proposed as underlying factors[31].

Ionizing radiation is a well-known carcinogen, of the same reason as it is a useful anticancer therapy; it induces DNA double-strand breaks, low doses lead to

mutations that increase the risk of cancer, while high doses lead cell death[32]. The effect of low-dose ionizing radiation have been studied extensively and it has been shown consistently to increase the risk for breast cancer, the effect depends on the age at exposure; the highest risk was shown in women exposed before their teens, and on the dose, where a strong dose-relationship has been found[33].

2.1.3 Diagnosis and treatment

2.1.3.1 Diagnosis

Breast cancer is diagnosed either *clinically*; by a lump discovered in the breast (usually by the patient herself) or by some other diagnostic sign, like nipple discharge, tenderness or redness, or *sub-clinically*, though screening mammography or, in rare cases, by other imaging techniques, like ultrasound or MRI. Breast cancer screening by mammography every 18-24 months is recommended since 1986 to all women in Sweden between 40-74 years[34], and has been shown to decrease breast cancer mortality[35], (which however has been put into question lately[36]). Once a cancer is suspected a conclusive diagnosis is reach by the *triple diagnostic approach,* which include mammography, palpation and cytological investigation by fine needle aspiration. If at least one of these diagnostic tools gives reason to suspect malignancy further investigation by surgery is undertaken.

2.1.3.2 Surgery

The oldest recognized, and most important, treatment of breast cancer is surgery, where the tumor is removed and the margins of the resection is microscopically investigated to confirm that they are clear of malignant cells. The surgical techniques are presently of two main types; *modified radical mastectomy*, where the entire breast is removed (but not the pectoralis muscle, in contrast to the previously widely used *radical mastectomy*) and *partial mastectomy/breast conservative therapy*, where only the tumor (with margins) is removed. In combination with post-surgical radiotherapy patients with partial mastectomy is shown to have the same survival as patients with modified radical mastectomy[37] and with a cosmetically much better result. This technique is however contraindicated if the tumor is very large or multifocal, when radiotherapy cannot be given or when the probability of local recurrence is deemed to be high[38].
Over 75% of the lymphatic drainage of the breast is though lymph nodes in the axilla and this is the primary route of metastatic spread of cancer[1]. Therefore, lymph nodes are removed from the axilla during surgery, for prognostic reasons. Previously the aim was to remove as many lymph nodes as possible, at least 10, but unfortunately this increases the risk of lymphedema in the arm[39]. In the end of the 1990's the *sentinel node* technique was developed, where a dye or a radioactive marker is injected in the tumor during surgery and is used to distinguish which lymph node/s that drains the tumor. These nodes are removed and only if cancer cells are found a complete axillary dissection is performed, this way, only patients who benefit from this prognosticator will have the increased risk of lymphedema.

Recently, however, a large randomized trial of patients with positive sentinel node excision showed no survival benefit for of complete axillary dissection[40].

2.1.3.3 Adjuvant therapy

Adjuvant therapy is given in order to decrease the risk of the cancer recurring, the objective is to eliminate any cancer cells that might have spread outside the tumor; in the breast, axilla or distant sites. Radiotherapy targeted against the remainder of the breast is a standard part of the breast conservative therapy, the dose is usually 50 Gy, given in fractions of 2 Gy. If the cancer was spread to the axilla lymph nodes also the axilla is included in the radiation field, regardless of type of surgery. Radiotherapy decrease the risk of local/regional recurrences and by that also decreases the breast cancer-specific mortality[41, 42]. Further decrease of breast cancer mortality is achieved by systemic adjuvant therapy, targeted at the potential spread of cancer cells to distant sites. In the case of chemotherapy, however, the side effect are severe and the cost needs to weight against the benefit. The probability of spread to distant sites is much increased if the cancer was spread to the axilla lymph nodes, which is therefore one of the most important criteria to give systemic adjuvant chemotherapy. This is currently also recommended if the tumor is large or otherwise aggressive, and especially if the patient is young (<60). If the tumor shows over expression of estrogen receptors, endocrine therapy is potentially beneficial. The side effects of endocrine therapy is less severe and the administration is easier than for chemotherapy, therefore the treatment indication is wider, today endocrine therapy is given to almost all breast cancer patients with estrogen receptor over expression[34]. Endocrine therapy primary consists of a competitive inhibition of either the estrogen receptor (Tamoxifen) or of the enzyme *aromatase* (which converts androgens to estrogens in peripheral tissues) (Anastrozole). During the study period of the studies included in this thesis Anastrozole was used very little, but is now the primary choice for post-menopausal women, due to its higher efficacy and less side effects[43]. Likewise, in the end of the study period, the monoclonal antibody Trastuzmab (Herceptin™) was developed and showed to improve survival for patients with over expression of the HER2/neu receptor[44].

2.1.3.4 Prophylactic mastectomy

The most radical approach for decreasing the risk of CBC at time of the first breast cancer is to surgically remove also the healthy breast, a procedure known as *contralateral prophylactic mastectomy* (CPM). During the last 10-15 years an increasing trend of CPM has been shown, primarily in the US, where 5-15% of all unilateral breast cancer patients now have a CMP[45, 46]. This trend is somewhat worrisome since, while CPM does decrease the risk of CBC, no clear survival benefit has been shown. On the other hand, women who underwent CPM generally have a better quality of life after, compared to before, the surgery, and also compared to a control group[47]. Both patient factors (such as high socio-economical status, high level of education and ethnic group) and risk factors for CBC have been shown to be predictors of CPM[46, 48].

2.1.4 Prognosis

Approximately 1500 women die from breast cancer in Sweden each year, or about 30/100 000 person years. This number has been rather constant over calendar period, despite the increasing breast cancer incidence, due to immense improvements in mortality rate. Among breast cancer patients diagnosed 1999-2003 86% of the women survived 5 years or more, compared to a 5-year survival of 64% for women diagnosed 1969-1973[49]. This improvement is most likely due to both improved early diagnosis by screening and adjuvant therapy. However, in likeness with prostate cancer, but in contrast to most other cancers, breast cancer has no *cure point*[50]. This means that regardless of how long after diagnosis, breast cancer patients will always have a higher risk of death than women of the same age without breast cancer, metastasis might be diagnosed 20, or even 30, years after initial breast cancer diagnosis. The prognosis for women with metastasized breast cancer is unfortunately very poor, as shown in studies from both Sweden[51] and the US[52], and has not improved over calendar period. The median survival is about 15 months and the 5-year survival after diagnosis of distant metastasis is around 15%[51]. The improved survival of breast cancer can thus be attributed to fewer women developing distant metastasis. In our material the most common site of first distant metastasis after breast cancer is the skeleton, followed by lung and liver, see Figure 2. It is presently unknown how prognosis is affected by the site of first distant metastasis.

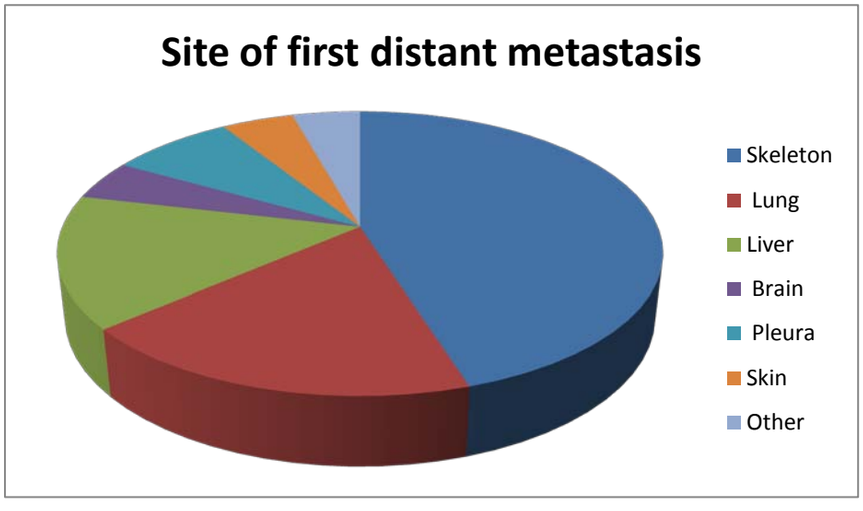


Figure 2: Site of first distant metastasis, based on the 5002 patients in the Stockholm breast cancer register diagnosed with distant metastasis and for whom the site of metastasis was recorded (=90% of all patients with distant metastasis).

Tumor- or patients characteristics that predict prognosis; *prognosticators*, are important, primarily to decide the adjuvant therapy regimen. Among the important prognosticators for breast cancer are TNM-stage[53], age at diagnosis (which is an U-shaped relationship where both very young and old women have worse prognosis)[54], histological grade[55], estrogen- and progesterone-receptor over expression[56] , Her2-neu over expression[57] and S-phase[58]. Over expression of

estrogen receptor and HER2-neu are also *predictors*, i.e. factors that predict the response of a certain therapy[59, 60]. The hunt for new prognosticators and predictors is an important, very large and quickly changing field of research, which it unfortunately is impossible to give an account of in the thesis.

2.2 CONTRALATERAL BREAST CANCER

The reason lightning doesn't strike twice in the same place is that the same place isn't there the second time.

Willie Tyler (1940-), comedian

Cancer is not evenly distributed in the population and having had one cancer does not protect against being diagnosed with another. Rather the opposite actually; people who have had one cancer are at increased risk of having yet another cancer [61]. Secondary malignancies is naturally most apparent among patients with a first cancer of favorable prognosis, so it should not be surprising that one fourth of all second primary cancers arise among breast cancer patients [61]. Breast cancer patients have about 25% increased risk of non-breast secondary primary cancers compared to healthy persons [62], and their risk to develop yet another breast cancer is much higher, approximately twofold [63].

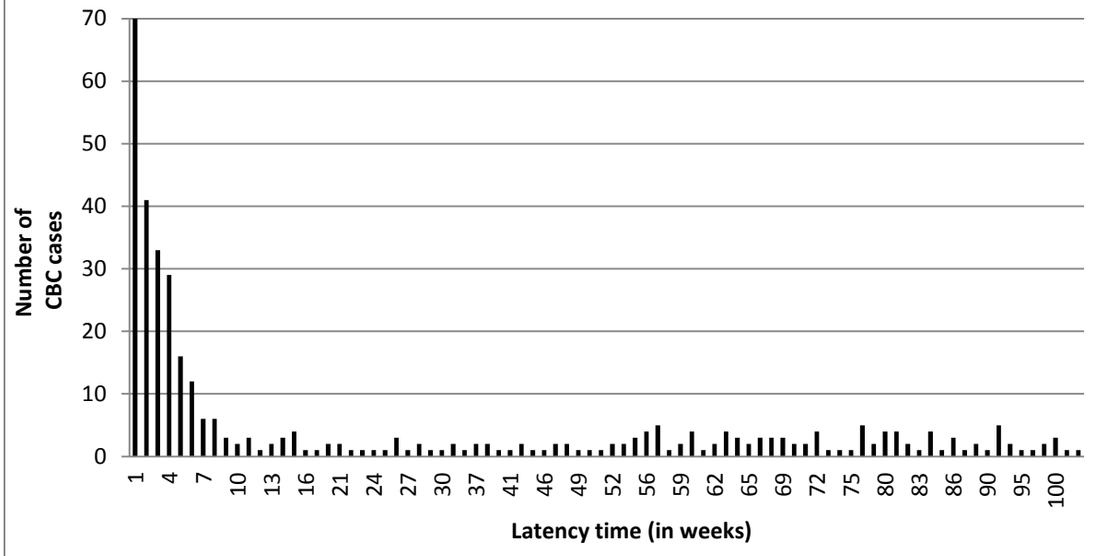
In 1921 Alson R. Kilgore (1987-1959) published the first scientific paper reporting on CBC, called "The Incidence of Cancer in the Second Breast After Radical Removal of One Breast for Cancer"[64]. The introduction states his two reasons for studying this disease and the main obstacle; the women developing CBC is of interest as they seem to constitute a susceptible subpopulation and, in practice, the risk need to be determined in order to give recommendations for prophylactic mastectomy, which was the only prevention available at the time. The main obstacle was to determine whether the cancer in the opposite breast was a new primary or a distant metastasis of the first cancer. (An attempt to answer this question using modern literature will be offered in *Background - Occurrence 2.2.1.1)*

The two cancers of CBC-patients might be diagnosed at the same time; *synchronous* CBC, or separated in time; *metachronous* CBC. What these definitions are meant to do is to separate the cancers that *developed* simultaneously from those that did not, unfortunately we do not know when the cancers developed, we only know when they were diagnosed. This gives rise to the need for a cutoff, for allowing for the possibility that one of the cancers were more difficult to diagnose/took longer to become registered so that the two cancers were diagnosed separated in time even though they did developed simultaneously.

This cutoff has been set quite differently in the literature of CBC, from one month[65, 66] of latency time to 1 year[67, 68], but 3 or 6 months is most commonly seen. We chose to use the cutoff of 3 months (92 days), mainly due to two reasons; in the unified health care system of Sweden there is good routines both for diagnostic procedures and registering of cases into the cancer register, so we do not see any reason for a prolonged cutoff due to fear of delay in reporting. At the same time it has been of interest to study the effect of treatment of the first cancer on the metachronous

cancer, and in those instances we want to have a cutoff that is sufficiently long to allow such effects to take place. All taken together we ended up with the Swedish *lagom* (translates to intermediate, just right) alterative of 3 months. When investigating the number of CBCs diagnosed per week of latency time in our CBC-cohort we found that the absolute majority of the synchronous CBC cases are diagnosed the same week as the first cancer, the number of CBC diagnosed then rapidly drops and from the ninth week the number of diagnosed CBC are down to a level which is kept for the two years we investigated. See Figure 3.

Figure 3: Number of CBC-cases in relation to time between cancers



*303 cases diagnosed the first week. Our cutoff was set at 12 weeks (92 days) of latency time.

2.2.1 Occurrence

The risk of CBC has repeatedly been shown to be 0.6-0.7% yearly [65, 69-73] translating into approximately 10-15% of all breast cancer patients being diagnosed with CBC during the first 20 years after initial diagnosis [71, 74]. This risk has been consistently shown during 50 years of CBC research, but comparison between these studies might be difficult due to methodological issues, e.g. different definitions of CBC or of synchronous/metachronous CBC, as outlined in a review by Chen et al[75].
In most of the studies investigating the risk or incidence of CBC there has been some attempt to exclude the synchronous cancers, since the population at risk for synchronous cancer is healthy women, while the population at risk for metachronous CBC is breast cancer patients (see *Discussion-CBC specific challenges 7.1.1*). We can consequently regard the risk of 0.7% or the incidence of 7/1000 person years to be the incidence of metachronous CBC. For synchronous CBC the incidence is much lower; 1.6/100 000 person years[76] and constitutes between 0.9 – 1.9% of all diagnosed breast cancers[77-80].

2.2.1.1 Temporal trends

Metachronous CBC have gotten attention as an emerging clinical problem, since the population at risk is increasing, as a consequence of the steeply increasing breast cancer incidence of the past decades, and improving breast cancer mortality. Despite increasing absolute numbers of CBC-patients, studies both in Sweden and in the US have observed decreasing incidence of metachronous CBC over calendar period[76, 81]. In the US study this decrease was shown to be mainly seen after ER-positive first cancer, and the authors suggest that this might be due to the widespread use of endocrine therapy.
Regarding synchronous CBC, on the other hand, our material show an increasing incidence over calendar time, in accordance with the increasing incidence of unilateral breast cancer (the population at risk for unilateral breast cancer and synchronous CBC is the same). These two opposing temporal trends of synchronous and metachronous CBC is a tentative explanation for the constant CBC-risk mentioned in the previous paragraph and was the rational for Study I.

2.2.1.2 Age trends

The age trend for synchronous CBC follows the same pattern as for unilateral breast cancer (and indeed for most other cancers); synchronous CBC increase steeply with age[82].
For metachronous CBC the story is somewhat more complicated; the risk for metachronous CBC is shown to be constant[72, 83, 84] (or almost constant[85]) from diagnosis of the first cancer during the rest of the patient's life. Many studies show that the level of risk is determined by the age at diagnosis of the first cancer[72, 83, 85, 86] (though this is not un-disputed[87, 88]); patients diagnosed with their first cancer when they were young are at a constantly higher risk than patients who had their first cancer when they were older.
This leads to the observation that younger patient groups have a higher incidence of CBC than older patient groups[63, 89]. Even if all of the individual patients are at their constant risk, the younger patient group will constitute only of patients with a high risk, while a older patient group will constitute of a group with mixed risks; higher for those that have lived a long time since their first cancer (i.e. got the first cancer when they were young) and lower for those who just had their first cancer (i.e. got the first cancer when they were old).

2.2.1.3 The more we look, the more we find?

As previously mentioned, 0.9 – 1.9% of all women with breast cancer are found (by palpation and/or mammography) to have cancer also in the other breast at time of first diagnosis; synchronous CBC. However, it seems the more we look the more we find; if the opposite breast is investigated with ultrasound in addition to palpation and mammography, an additional 2% is found[90], and if MRI is added to palpation and mammography an additional 4% is found[91] (including both invasive and in-situ lesions). Further, two studies have made on so called blind biopsies, where a sample of 2-3cm^3 breast tissue was surgically removed from the contralateral breast (that

was clinically and mammographically free of cancer) at the time of the primary breast cancer surgery. In 6-7% of the patients invasive or in-situ cancers were found[92, 93] and the one study that also included benign lesions of precancerous type could diagnose an additional 9% of the investigated patients with this condition. An autopsy study of pre/peri-menopausal women showed that close to 20% had clinically undetected (invasive/in-situ) breast cancer[94].

2.2.1.4 Is CBC a true primary cancer?

A central question in CBC-research is whether the second cancer is actually a new primary cancer, or if it is a metastasis from the first cancer. According to the 'seed and soil' hypothesis, first proposed by Fuchs and Paget[95], cancers will tend to metastasize in organs that are similar to that where the first tumor developed, hence metastases to the contralateral breast would be a very plausible idea. However, today most agree that the most likely scenario is a combination of the "seed and soil" hypothesis and another hypothesis, proposed by James Ewing[96], that suggests that the organs to which the cancer metastasize are determined by anatomical and mechanical routes. In light of that hypothesis it is of interest to note that, in contrast to sites to which breast cancer commonly metastasizes, the contralateral breast is not highly vascularized. Traditionally, criteria of the presence of an in-situ component in the second cancer or of different histology at the two cancers, have been used to distinguish between true second primary cancers and metastases, this method however has serious drawbacks; since 80% of all breast tumors in general are of ductal histology[97] and 40% lacks in-situ component[98], the risk of falsely categorized tumors as metastasis is very high.
Several studies have been performed to answer this question by molecular means, unfortunately they are all quite small, but the message is, despite this, rather clear. Janschek et al. compared mutations in the p53-gene in 33 patients with synchronous and metachronous CBC, for 13 patients this analysis was informative and among these only one patient had the same mutation[99]. Imyaitov et al. investigated the allelic imbalance profiles in 28 synchronous and metachronous CBCs, they found evidence for different origin of the two tumors in 23 cases, among the remaining 5 cases the clinical and histopathological information indicated different origin of the two tumors in 4 cases, leaving one case unanswered[100]. Brommesson et al investigated 8 synchronous CBC tumors by comparative genomic hybridization and found 1 case that had genomic similarities.
Indications from the epidemiological perspective also speak against that metastases should contribute significantly to the group of CBC cancers. If the second cancer would be a metastasis then TNM-stage of the first cancer should be the most important risk factor for CBC, but despite extensive research of the question there is still no distinct conclusion whether higher TNM-stage is at all a risk factor for CBC. Further, the prognosis of CBC is worse than for unilateral breast cancer, but the prognosis for patients with metastasized breast cancer is much worse still (See *Background –Prognosis 2.1.4*). There is no obvious reason that a metastasis in the contralateral breast should be so much less harmful than a metastasis in e.g. the skin or the skeleton. Finally, among patients with distant spread, metastasis in only one site is rarely seen, therefore, if CBC was a metastasis

to the opposite breast one would expect that CBC without distant metastasis also was a rare scenario, which it is not.
In conclusion, evidence from different areas of medical research taken together indicate that CBC, in the overwhelming majority of the cases, indeed is a new primary cancer, though the phenomenon that one breast cancer metastasize to the opposite breast does occur. Judging from the genetic studies referred to above (albeit small) the proportion of CBC that are not true primaries are approximately 6% of all CBCs diagnosed, and probably less if using the same definition of CBC as in the studies included in this thesis; that the second cancer should be diagnosed without distant metastasis.

2.2.2 Risk factors

Only three risk factors have been consistently shown to increase the risk of CBC; young age at the first cancer (See *Background- Age trends 2.2.1.2*), family history of breast cancer and lobular histology of the first cancer.
Having at least one first-degree relative with breast cancer approximately doubles the risk of CBC, and having both mother and sister affected by breast cancer confers a 5 times increased risk[101]. Some studies also indicate that the risk is increased if the relative had early disease onset[102].
About 85% of all invasive breast tumors arise in the milk ducts, so called *ductal* cancer, 10% arise in the lobuli, so called *lobular* cancer, the remaining 5% have a mixed, or other, histology[103]. Breast cancers of lobular histology have a more favorable prognosis than ducal cancers[104], but also have approximately double the risk of CBC[87].
An intuitive hypothesis when it comes to lifestyle risk factors for CBC could be referred to as *more of the same*; that women with CBC have more of the risk factors that have been characterized for unilateral breast cancer. This has been researched extensively; studies investigating established hormonal risk factors for unilateral breast cancer; age at menopause, breast feeding, hormone replacement therapy and oral contraceptives have consistently shown no association with risk of CBC [87, 105-109], while the results have been inconstant for number of children[86, 87, 106-109], age at parity[86, 87, 106, 107, 109, 110] and age at menarche[87, 106, 107, 109, 110]. Regarding other life style risk factors as smoking, alcohol consumption, obesity and social-economical status results are also mixed [87, 106, 108, 110-112], but indications are that alcohol consumption is a risk factor for CBC [108, 111, 112] and smoking is not[87, 106, 112].
As previously mentioned (See *Background –Risk factors 2.12*), the breast is sensitive to ionizing radiation, therefore it has been a concern for many years whether adjuvant radiotherapy for breast cancer increases the risk of CBC. Overall, the results indicate that the risk is not increased in the general population of breast cancer patients[71, 113], but a risk increase can be detected among patients that were young at irradiation, or genetically susceptible [114, 115].

2.2.3 Diagnosis and treatment

Since the high risk of CBC for breast cancer patients is well recognized in the healthcare system the contralateral breast is closely examined at time of first breast cancer diagnosis. After the initial treatment period all breast cancer patients in Sweden are followed-up with clinical examinations and mammography during 5 years, one of the main aims of this follow-up program is the early detection of potential CBCs[38].

The close follow-up (in particular by mammography) together with the heighted awareness among the patients are a plausible explanation for the fact that the second cancer of metachronous CBC is more likely to be diagnosed at smaller tumor size and at a more favorable stage, compared to unilateral breast cancer[116]. CBC-patients who have their second cancer detected by mammography have been shown to have better survival than patients with the second cancer diagnosed by clinical examination[117], this seem to be mostly, but not entirely, due to the cancers being diagnosed at a better stage and younger age[118].

Regarding synchronous CBC, since they are diagnosed, and presumably developed, simultaneously, it is not possible to distinguish which cancer was the 'first' and which was the 'second'. However, oftentimes the larger and/or more spread cancer will give rise to symptoms that will lead to the diagnosis of that cancer and also a sub-clinical cancer in the opposite breast. Therefore, the tumor that is recorded as 'second' cancer is often smaller and of more favorable stage also when the cancers are diagnosed only days apart, this could however be seen as an artifact.

In general, there is a lack of specific treatment directives when it comes to adjuvant therapy after metachronous CBC, the patients will most likely be treated as any (unilateral) breast cancer patient, while naturally taking potential earlier therapy into account when calculating maximum dose of chemotherapy or the fields of radiotherapy. Neither for synchronous CBC exists any specific directives, most commonly the adjuvant therapy regimen will be decided by the aggressiveness of the most advanced cancer, however, endocrine therapy will probably be given if any of the cancers are estrogen receptor positive.

2.2.4 Prognosis

During many years the question of whether CBC-patients had worse prognosis than unilateral breast cancer patients remained unanswered, most likely due to inherited problems in studying survival after secondary cancers (See *Discussion -CBC-specific challenges 7.1.3*). Though still not completely uncontroversial, we can now say with some certainty that the prognosis of CBC is indeed worse than for unilateral breast cancer, but that this is highly dependent on the latency time[76]. Figure 4 shows a Kaplan-Meier plot of percentage survival over time (in years) since CBC-diagnosis for patients with synchronous CBC (red), CBC with short latency time (<5 years) (green) and CBC-patients with long latency time (>5years) (black), this can be compared to the survival for unilateral breast cancer patients (blue). This plot is based on the cohort of CBC-patients included in this thesis, and is in close agreement to what has been show by Hartman et al.[76], though it has lower power.

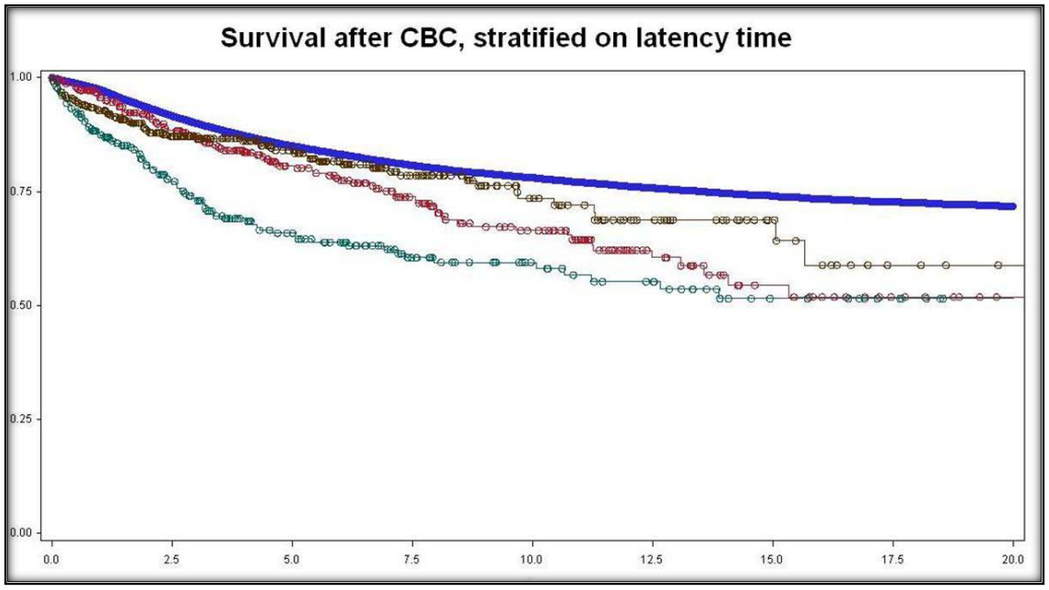


Figure 4: Kaplan-Meier plot of survival after breast cancer (blue) and CBC. Synchronous CBC (red), CBC with short latency time (<5 years) (green) and CBC-patients with long latency time (>5years) (black). The time scale is in years.

Given that breast cancer metastasis might be diagnosed 20 or 30 years after initial breast cancer diagnosis, in the case of a CBC-patient dying in breast cancer, how do we know which cancer the metastases came from? The answer is that we do not. Metastasis are seldom biopsied, if they were, it might be possible to distinguish a larger genomic similarity with one of the primary cancers, though it is not certain, since the cancer is likely to have mutated further from initial diagnosis until diagnosis of the metastases.
For synchronous cancers it is more likely that the larger/more advanced cancer is the cancer setting metastases, for metachronous cancer, the situation is somewhat more complicated. Even though breast cancer can metastasize long after initial diagnosis, it is still more likely within the first few years[50], thus it should be less likely that the first cancer is the metastasizing cancer. This indication is further enhanced by the fact that to be able to get a second cancer, the patient has to survive the first cancer long enough, it therefore follows that the first cancer is less aggressive and less likely to set metastases.

3 AIMS OF THIS THESIS

Leave this world a little better than you found it.
Sir Robert Baden-Powell (1857-1941), founder of the Scout Movement

The overall aim of this thesis has been to increase the biological understanding and clinically applicable knowledge about contralateral breast cancer.

More specifically, the aims of the four studies were to investigate the following:

- To what extent the increasing proportion of synchronous CBCs can be explained by improvements in the diagnostic work-up of breast cancer patients.

- If adjuvant radiotherapy for the first cancer can affect the aggressiveness of the second cancer and explain the bad prognosis for CBC patients with short latency time.

- How estrogen receptor status of the first and second cancer relates, and how these estrogen receptor patterns affect the prognosis after CBC.

- Whether mammographic density at diagnosis of the first cancer, or its changes over time, can be used to predict the risk of CBC.

4 MATERIALS

"Data! Data! Data!" he cried impatiently. "I can't make bricks without clay."
Sir Arthur Conan Doyle (1859–1930), author of *Sherlock Holmes*

4.1 REGISTERS

One of the often brought up advantages about epidemiological research in Sweden is the many high-quality health registers. Sweden has five main health registers, one of these registers was used in this thesis, namely the Swedish cancer register. The Swedish cancer register has close to complete coverage of the population and close to complete follow-up[119, 120]. Sweden also has a number of smaller registers for specific conditions, usually not covering the whole country and constructed primary for internal quality control. These registers constitute a hugely underused source of information for epidemiological research. Information from the Stockholm breast cancer register (SBCR) was used extensively in this thesis.

4.1.1 Swedish cancer register

Since 1958 all cancers diagnosed in Sweden are reported to the Swedish cancer register, every tumors is reported separately, resulting in that the same person might have several posts in the register, if that person suffered from more than one primary cancer. Also, each cancer is reported both by the diagnosing pathology/cytology laboratory and by the treating physician, to ensure completeness. The information recorded in the register is the International Classification of Disease (ICD)-code, the date of diagnosis and the personal identification number and vital/emigration status. From the ICD-code the laterality of the breast cancer can be derived, which is of great importance in this thesis, as CBC is defined as two primary cancers in *opposite* breasts. From the personal identification number date of birth and sex can be derived (additionally; the personal identification number can be used to link the patients to the other health registers and to medical records, though this functionality has not been used in this thesis)

4.1.2 Stockholm breast cancer register (SBCR)

The Stockholm Breast Cancer Register (SBCR) was started 1970 to register breast cancer patients that participated in clinical trials. Gradually the registry was expanded to cover all breast cancer patients and from 1976 there is a close to complete coverage of all breast cancer patients in the Stockholm-Gotland Health care region (which presently has a catchment area of 1.9 million people). As of December 2008 SBCR contained information on 32 153 breast cancer patients. The register is maintained by the Oncological Center in Stockholm and the aim is quality control of the breast cancer care in the region, even though SBCR also has been used somewhat for breast cancer research.

Information recorded in SBCR includes personal identification number, date of diagnosis, family history of breast cancer, tumors characteristics, details of surgery and, from 1990, adjuvant therapy. The register is continuously updated with information on local recurrence and distant metastasis, as the patients are clinically followed up, and SBCR and the Swedish Cause of Death Register are regularly linked so that information on cause of death for the deceased patients is available.

4.2 CBC-COHORT

4.2.1 Inclusions

When collection of medical records for this thesis started we made a selection of all patients with contralateral breast cancer (CBC) in the SBCR that fulfilled our criteria for CBC-diagnosis. The following inclusion criteria were used:

- Diagnosed with two primary, invasive breast cancers, one in each breast.

- Not diagnosed with any other malignancy before the second breast cancer.
- No distant metastasis diagnosed at time of the first breast cancer diagnosis.

  The last two criteria was set primarily to increase the probability that the second breast cancer was indeed a new primary cancer, and not metastatic spread from the first breast cancer or any other malignancy, to the second breast. It has also been of importance when studying effect of treatment of the first cancer, to ensure that the patients have not received cancer therapy for any other cancer.

As the selection was made from SBCR the same restrictions in time and place as for that register applies also to the CBC-cohort;

- Both breast cancers diagnosed in the Stockholm-Gotland health care region.
- Both cancers diagnosed during 1970-2005.

4.2.2 Exclusions

When the medical records were collected we applied additional criteria for the CBC-cohort; we had selected all CBC-patients from the SBCR, but since the register was only complete from 1976 we decided to not include CBC-patients diagnosed before then.
Also, obviously, patients for whom we could not find the medical record could not be included, but these were mercifully few, only 17 patients (1%). Further we excluded patients who were diagnosed with distant metastasis before diagnosis of the second breast cancer and patients who had the second breast cancer diagnosed via axilla lymph node metastases. This was done to decrease the probability that the second cancer was a metastasis of the first breast cancer. Finally, for some patients the information in the medical records was different than in the registers, e.g. 19 patients had a malignancy at another site recorded in the medical record, for 4

patients the cancer recorded in the medical record was non-invasive (in-situ). See Figure 5 for exclusions.

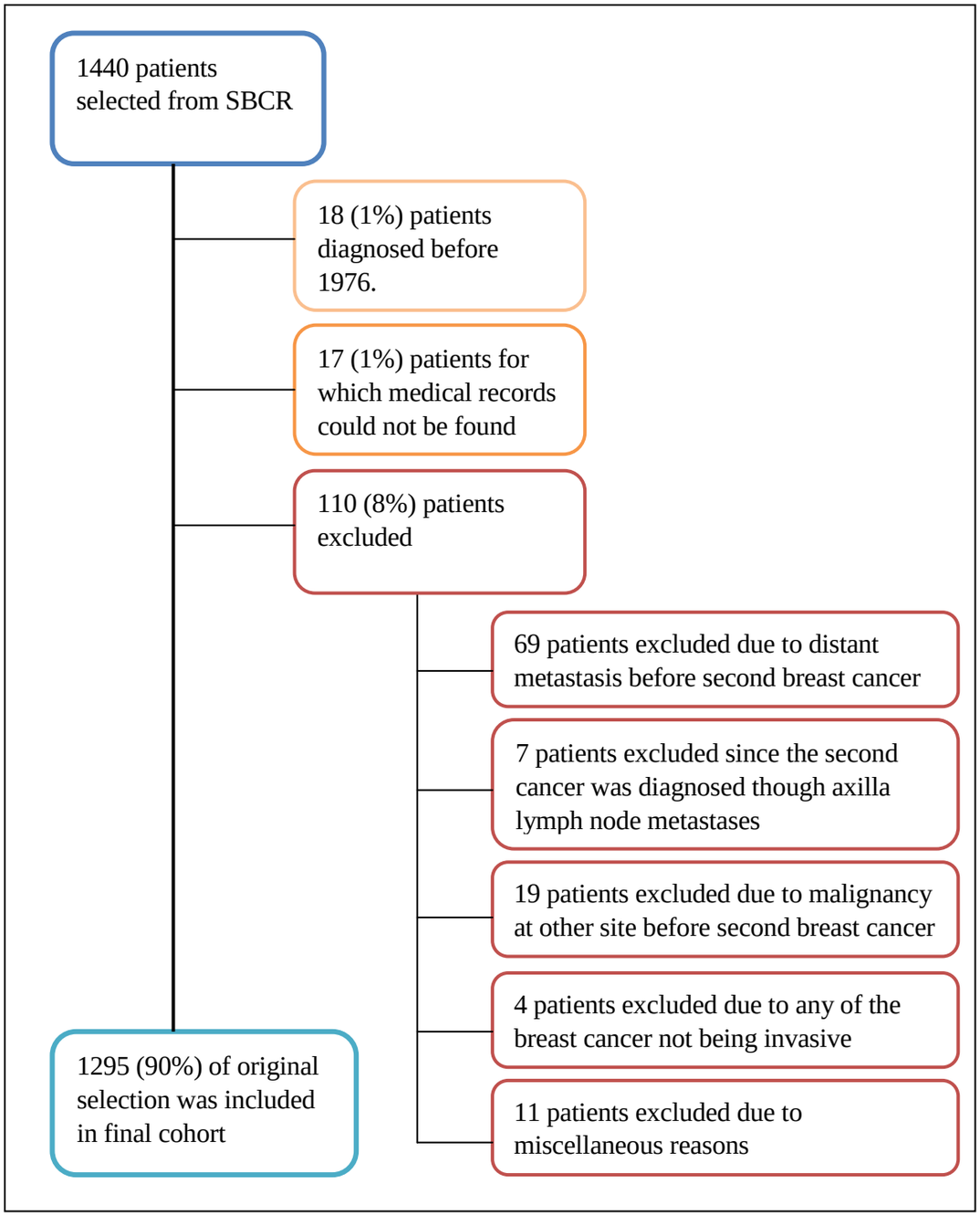


Figure 5: CBC cohort, with exclusions.

4.2.3 Populations for study I-IV

The four studies in this thesis all used the CBC-cohort, but due to the different research questions different subsets were used, as explained in Figure 6.
Study I included all CBC-cases with three years or less between the cancers, with the aim of including all CBCs that were likely to have been sub clinically present in the

opposite breast at time of the first diagnosis. Study II included all CBC-cases with more than three months between the cancers, with the aim of including all CBCs that might potentially have been affected by adjuvant therapy for the first cancer. Study III used all CBC-cases, here the aim was to evaluate the impact of estrogen receptor status and compare synchronous and metachronous CBC. Finally, Study IV used all CBC-cases with more than one year between the cancers, with the aim of including all CBCs (and selected controls with unilateral breast cancer) with at least two mammograms far enough apart for a potential change of mammographic density to be possible to evaluate.

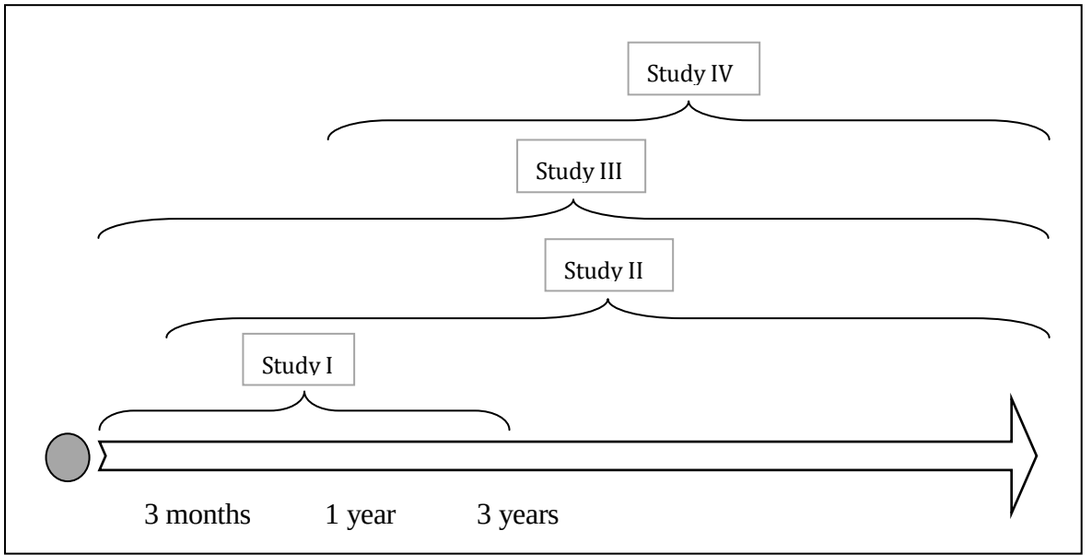


Figure 6: Populations used for Studies I-IV.

4.2.4 Medical records

Medical records were collected from the archives of the oncology and surgical clinics of the hospitals in the Stockholm region. The retrieval rate was very good (99%), a fact that is most likely attributable to the unified health care system of Sweden. The medical records were read and the predefined variables were extracted, the pathological-anatomical reports from all breast cancer surgeries were also copied and collected. The variables extracted were anthropological measures, preexisting diseases (thyroid disease, diabetes, benign breast disease and schizophrenia), breast cancer family history and hormonal factors (e.g. pregnancy, parity, oral contraceptives use, menopause, hormonal replacement therapy) at time of the first breast cancer. For both cancers we also collected mode of detection, all available tumor characteristics (size, hormone receptor status, histological grade, and lymph node status), type of surgery and type of adjuvant therapy (with details on dose and duration). We further collected information on local/regional recurrence and date and site of distant metastasis, but no treatment or follow-up after diagnosis of distant metastasis. For some of the variables, comparison was possible between the Stockholm Breast Cancer Register and the data collected from medical records; overall the concordance was good (e.g. menopause status:96%, estrogen receptor status: 98%, adjuvant treatment 85%), the data from the medical records was

however regarded as the gold standard. Regarding the adjuvant treatment a significant difference is that the information in SBCR is from the time of treatment conference (so called 'intention to treat') while the information collected from the medical records was the actual treatment given.

4.2.5 Mammograms

A mammogram is radiological picture of a breast, the breast is compressed and the mammogram is taken either in cranio-caudal fashion (vertically), media-lateral (from the side) or media-lateral-oblique (diagonally), these are different so-called *views*. The media-lateral-oblique (MLO) view has been shown to be the most useful[121], partly since it better includes the upper-lateral quadrant of the breast, where many breast cancers occur[122]. For breast cancer screening the MLO view was predominantly used, while for diagnosis and follow-up (the setting mostly relevant to Study IV) both the cranio-caudal (CC) and MLO view were used, but of the above mentioned reasons we preferentially used the MLO view. In routine follow-up after breast cancer mammograms are oftentimes taken every 12 months, after 5 years the patient is usually referred back to the screening program, where mammograms are taken every 18-24 months until the patient is no longer in the screened age interval.

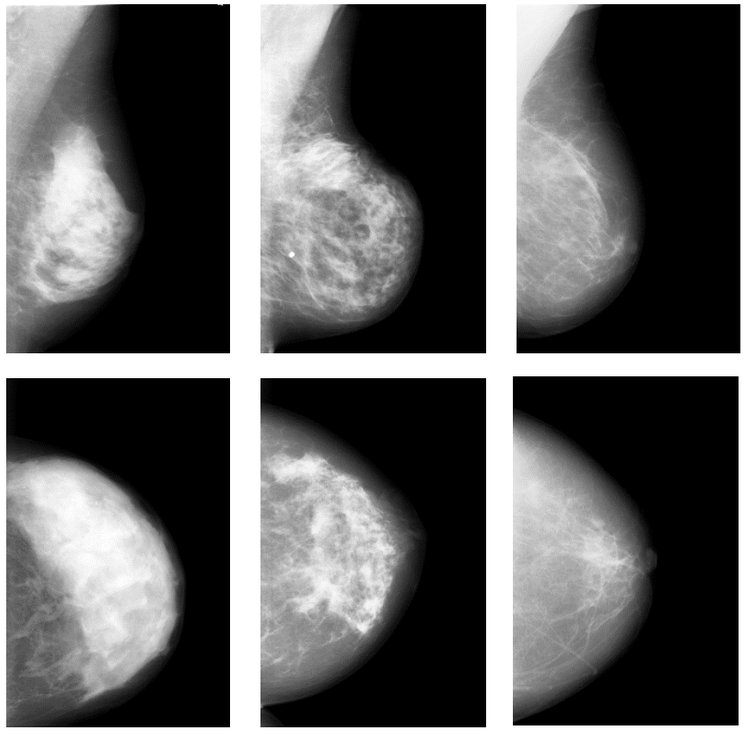


Figure 7: Examples of mammograms. The top row shows mammograms taken in the MLO view, the bottom row shows mammograms in the CC view.

The mammograms to the left show breasts with high mammographic density, the middle pictures show intermediate density and the mammograms to the right show low density breasts.

For Study IV we collected mammograms from the time of diagnosis of the first cancer and until 6 months before the second cancer for the CBC-cases. In this study we also selected controls (patients with unilateral breast cancer), five unilateral breast cancer patients were selected to each case. The controls were individually matched to the cases on calendar period and age of diagnosis, adjuvant therapy for the first cancer and follow-up time. The reason for selecting five controls when we only planned to collect the mammograms for one of them was to allow for the event

that no mammograms were found for a particular patient. For the controls mammograms were collected from time of breast cancer diagnosis until 6 months before the cutoff date (the date corresponding to second breast cancer diagnosis for the individually matched case). To be able to investigate the effect of factors as hormone replacement therapy, estrogen receptor status at first cancer, menopause, etc, this information were collected from the medical records of the controls, this was done after collection of the mammograms. The collected variables were however only a small selection of the variables collected from the medical records of the CBC-patients. The retrieval rate of mammograms was markedly worse than for medical records, partly due to different regulations regarding archiving.
The mammograms were digitized using an Array 2905HD Laser Film Digitizer (Array Corporation, Tokyo, Japan). Mammographic density was measured using an automated thresholding method[123] which incorporates the knowledge of a trained observer, by using measurements obtained by an established user-assisted threshold method - Cumulus[124] - as training data. The externally validated results showed a high correspondence between our automated method and the established used-assisted thresholding method; Cumulus ($r_{\text{percent mammographic density}}$ = 0.88 (95% CI: 0.87-0.89). Kallenberg et al[125] have developed a similar automated method, which also extracted a number of features from the pixels in the mammograms and used them to train, and validate, a measurement of mammographic density against Cumulus (the latter taken as the ground truth); this automated method has performed similarly ($r_{\text{percent mammographic density}}$ =0.90 with Cumulus).

5 METHODS

In respect of military method, we have, firstly, Measurement; secondly, Estimation of quantity; thirdly, Calculation; fourthly, Balancing of chances; fifthly, Victory.

Sun Tzu (~500 BC), author of *The Art of War*

5.1 DESIGN AND STATISTICAL ANALYSIS

Do not try to bend the spoon — that's impossible. Instead, only try to realize the truth: there is no spoon.

The movie *Matrix* (1999)

Epidemiological studies are either *experimental studies*, in which the researcher influence the investigated exposure, e.g. as in a randomized clinical trial, or *observational studies*, where the exposure and outcome is merely observed and causal interpretation is attempted through adjustments. Observational studies are either *retrospective* or *prospective*, in a retrospective study information about the exposure is gathered after the outcome has occurred, this might be a problematic approach since it is, at least theoretically, possible that the case-control status influences the assessment of exposure, e.g. due to *recall bias*[126]. In a prospective study the exposure is assessed before the outcome occurs, this can be done either by actually starting the study before the outcome happens, or by going back to records of the exposure which were documented before the outcome (e.g. mammograms taken before CBC, as in Study IV).
All studies in this thesis are prospective observational studies, Study I-III are cohort studies and Study IV is a case-control study. Within each design several statistical/analytical approaches were used.

5.1.1 Cohort design

Historically, cohort designs have been described as investigating "one exposure, many outcomes", however, to choose only one exposure is a thing of the past and now the best description might be that in a cohort study no restriction is made on the prevalence of the exposure or the outcome. A sample of the population which the study is aimed to target is investigated and the prevalence of exposure and outcome is assumed to be the same as in that population, which enables the calculation of baseline risk. In some instances however, the population of a cohort study might be selected in a way that predetermines the prevalence of exposure; a *matched cohort study*[126], which is done in order to increase the power of the study and/or prevent confounding[127].
Statistical models are used in order to calculate the effect on the outcome of different explanatory variables independent of each other, usually one explanatory variable is regarded as the main exposure, and the others are regarded as potential confounders or effect modifiers. In cohort studies, the outcome might be *continuous*, in which case a linear regression model is usually used, or it might be *binary/categorical*, in which case a logistic regression model is oftentimes used. In

both these instances the measure of the effect of the exposure is a *risk ratio*. *Linear regression* might be regarded as the model underlying all others, here the equation of a straight line is used to estimate the average change of the outcome (dependent) variable for a one-unit change of the exposure (independent) variable. The dependent variable in a linear regression is a continuous measure that can take any value from minus infinity to plus infinity. (Oftentimes, however, the application of the model restricts the possible values of the outcome variable).
For binary outcomes (e.g. being diagnosed with the disease or not) the aim of the study is usually to calculate the risk, and since a risk only takes values from zero to one, the outcome variable has to be transformed mathematically so that it may be modeled by the straight-line equation. The most common is to log transform the outcome, the resulting value; the *logit*, can then be modeled as the dependent variable; a *logistic regression*. In Study I logistic regression was used to calculate the effect of a certain calendar period on the probability that a second cancer was diagnosed synchronously, as opposed to metachronously. In Study II we used logistic regression to calculate the effect of adjuvant radiotherapy on the odds that a CBC-patient has worse tumor characteristics at her second cancer than at her first. Finally, in the forth study, logistic regression was used in a nested case-control setting to calculate the odds ratio of CBC as an effect of mammographic density and its changes.
Further, it might be of interest to calculate the *rate* with which an outcome occurs and the corresponding *rate ratio* between the exposed and unexposed. For this purpose a cohort study can be used to follow the study persons over time to determine whether they will have the outcome or not. This is known as *survival analysis*, (or *time-to-event-analysis*) and the focus is, not on the number of persons at risk, but on the *person time* at risk. The study persons contribute with person time from the time they enter the study until they either have the outcome or are not at risk of the outcome any longer, i.e. they die, are lost to follow-up or the study ends. Survival analysis can be performed by Cox regression or Poisson regression. Poisson regression may be preferential since it enables a straight forward estimation of the baseline risk. The benefit of Cox regression, on the other hand, is that the hazard over time is modeled as a smooth function (and not in a stepwise fashion, like in Poisson) which may be more biologically plausible. Both Poisson and Cox assume *proportional hazards*; that the ratio of the incidence rate/hazard rate of the exposed and unexposed is the same during all follow-up time. Survival analysis was used both in Study II and Study III, in which we investigated the effect of the different exposures on the incidence rate ratio of distant metastasis, by fitting Poisson regression.

5.1.2 Case-control design

Case-control studies were designed for cost-effectiveness when investigating rare outcomes, assuming that data collection (e.g. medical records, mammograms, blood analysis or DNA sequencing) is required for the study, in which case economical and/or practical reasons prevent using a cohort. E.g. the reason that we, in Study IV, opted to do a case-control study was that mammograms needed to be collected, for pure register-based studies, on the other hand, there are few reasons to use a case-control design.

In a case-control study, in contrast to a cohort study, the outcome status is known at the time of enrollment into the study; persons with the disease of interest (cases) and persons without the disease of interest (controls) are included. Often one control is selected for each case, but to increase precision up to four controls might be selected, to use more than four controls has been shown to increase the precision only marginally[128]. The aim is that all cases within the study population should be included and that the included controls should be a random selection of all eligible persons, so that the distribution of exposure among the controls is the same as the distribution within the whole study population without the disease of interest, but who are at risk of having it.

In a case-control study neither the baseline risk nor baseline odds of disease, nor the risk ratio of exposed over unexposed can be calculated, since the ratio of cases to controls is predetermined by design. What can be used, on the other hand, is the *odds ratio*; the odds of exposure among the cases over the odds of exposure among the controls. Since the distribution of exposure is not predetermined by the design and since odds ratios are mathematically symmetrical, the odds of exposure can be calculated and not only interpreted as *the odds of being exposed if being a case*, but also as *the odds of being a case if exposed*. If cases and controls have been at risk for the same length of time and if the outcome of interest is rare the odds ratio will be a good approximation of the risk ratio. But if the *rare disease assumption* is not fulfilled the odds ratio will overestimate the risk ratio (if the association between exposure and outcome is positive) since, at the end of follow-up, the distribution of exposure in the controls will not reflect the distribution in the study population. This follows logically as the exposed were more likely to have the outcome and thus be a case, not a control.

To reduce this potential problem *nested case-control* design may be used, the study population is then required to be a specified cohort from which the cases and controls are selected, the case-control study is said to be *nested* within a cohort. The nested design makes it possible to match the controls to the cases on follow-up time; *density sampling*. In such a design, the controls are selected from the individuals at risk when the corresponding case had her outcome, the same individual can become the control to several different cases and will also herself become a case if she later develops the disease. Through this matching, the probability of being selected as a control is proportional to the person time the individual contributed with, ensuring that the distribution of exposure is the same among the controls as it is in the full cohort in which the case-control study is nested. Thus, person time is taken into account and the odds ratio will now be a very good approximation of the *rate ratio/hazard rate*, even if the outcome is not rare. However, it is still an approximation, and if the disease is very common, it might not be a very good approximation, despite the nested-case-control design. With population registers like Sweden's, almost all case-control studies can be said to be nested, which gives the opportunity for density sampling.

Case-control studies very often have binary outcome and therefore logistic regression is used to estimate the odds ratio, even though it is possible to have categorical outcomes as well, and then *ordinal logistic regression* or *multinomial logistic regression* would be used, depending on the nature of the outcome. Almost

all density sampled case-control studies are individually matched (See *Methodological considerations 7.1.1*), since they are matched on survival time in small intervals. For these studies *conditional logistic regression* is used, which is basically the same estimation as is done for odds ratio in a regular case-control study, but here the ratio is of the *number of strata* where the case is exposed and the control is not over the *number of strata* where the control is exposed and the case is not. Since the case and control in the same stratum is identical regarding the matching variables these obviously do not need to be included in the model, they are already taken into account.

5.2 ANALYTICAL APPROCH

The symbol and the metaphor are as necessary to science as to poetry.
Jacob Bronowski (1908–1974), author of *Science and Human Values*

The pink ribbon has been used for several decades as a symbol for breast cancer and as a sign to promote breast cancer awareness. The double pink ribbon in Figure 7 was created by the author, in collaboration with Adina L. Feldman, and has been used throughout the thesis projects and presentations, to symbolize CBC; double breast cancer
Figure 7: The double pink ribbon, a symbol for CBC.

5.2.1 Study I

Study I is a prospective cohort study, with the aim to study the diagnostic work-up of CBC over calendar period. Figure 8 describes the original hypothesis; that the increase in incidence of synchronous CBC and decrease in metachronous CBC over time could be explained by second cancers being diagnosed closer to the first cancer, because of better diagnostic methods.

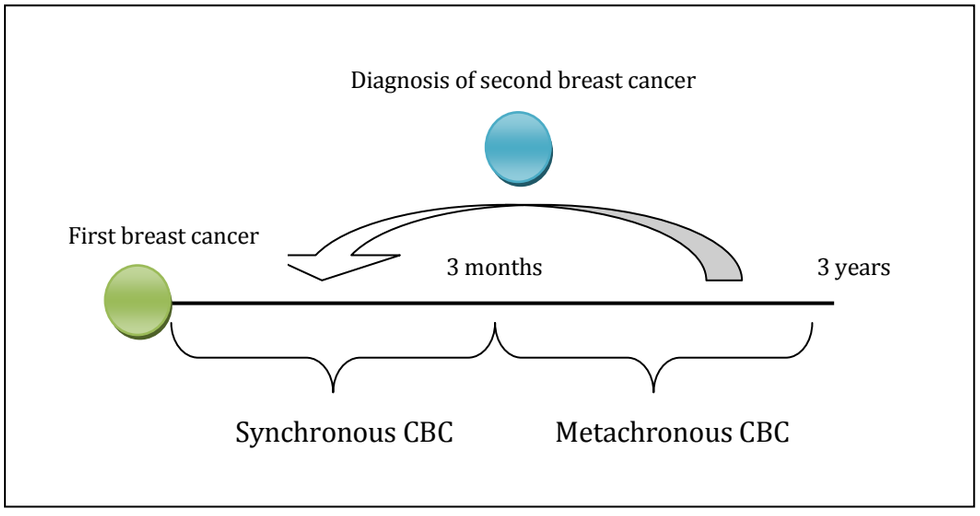


Figure 8: Analytical approach of Study I.

The correlation between calendar period of the first diagnosis and time between the first and second cancer (number of days) was calculated. Logistic regression was used to estimate the effect of calendar period on the odds of being diagnosed synchronously. The model was subsequently adjusted for mode of detection of the second cancer, to try to elucidate whether the effect of calendar period might be mediated through this.

5.2.2 Study II

Study II is a prospective cohort study with the aim to analyze the aggressiveness of the second cancer as an effect of adjuvant radiotherapy for the first cancer in a cohort of CBC-patients. Figure 9 describes the study approach, in which CBC-patients exposed to radiotherapy for their first cancer were compared to CBC-patients who were not exposed, with respect to whether they later developed distant metastasis and whether they had increased odds of worse tumor characteristics. Survival analysis (Poisson regression) was used to determine the risk of distant metastasis and logistic regression was used to estimate the odds of worse TNM- stage and the odds of worse histological grade. The main model, estimating the relative risk of distant metastasis, was adjusted for age and calendar period of first diagnosis, latency time between the cancers, stage and grade of both cancers and adjuvant therapy for the second cancer.

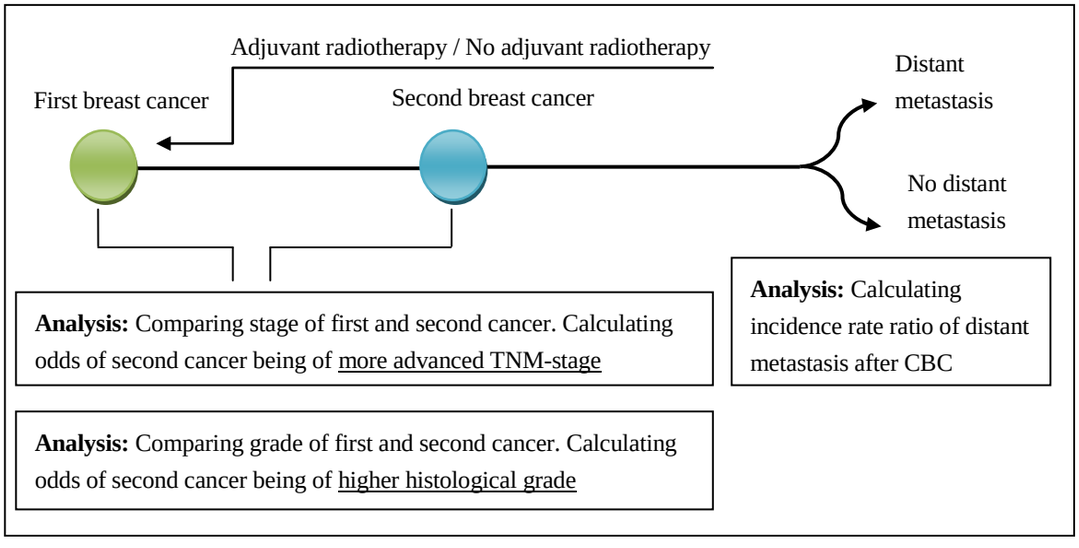


Figure 9: Analytical approach of Study II.

5.2.3 Study III

Study III is a prospective cohort study with the aim to evaluate the prognosis after CBC with respect to estrogen receptor (ER) status of the two tumors. Figure 10 illustrates the four possible combinations of ER-status of the two cancers, the prognosis for these four groups of patients was compared to the prognosis of patients with unilateral breast cancer. Latency time was an important factor in these analyses, partly due to the potential effect of therapy given at diagnosis of the first cancer. The measure of prognosis was risk of distant metastasis and survival analysis (Poisson regression) was used to estimate this as an effect of ER-status. We

also calculated the concordance of ER-status of the two tumors of the same women and compared to the concordance that would be expected if the cancers were unrelated. The main analysis was adjusted for calendar period at diagnosis, age at diagnosis and TNM stage of the (first) cancer for unilateral breast cancer patients and synchronous CBC patients and calendar period, age and TNM stage of the second cancer for metachronous CBC patients.

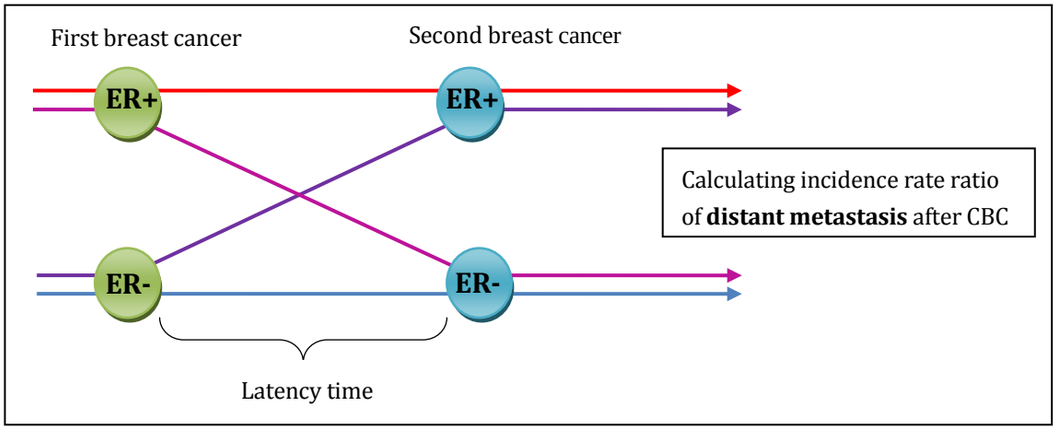


Figure 10: Analytical approach of Study III.

5.2.4 Study IV

Study IV is a prospective nested case-control study with density sampling, matched on age, calendar period and adjuvant therapy of the first cancer. Figure 11 illustrates the two time points at which mammographic density was measured for cases and controls.

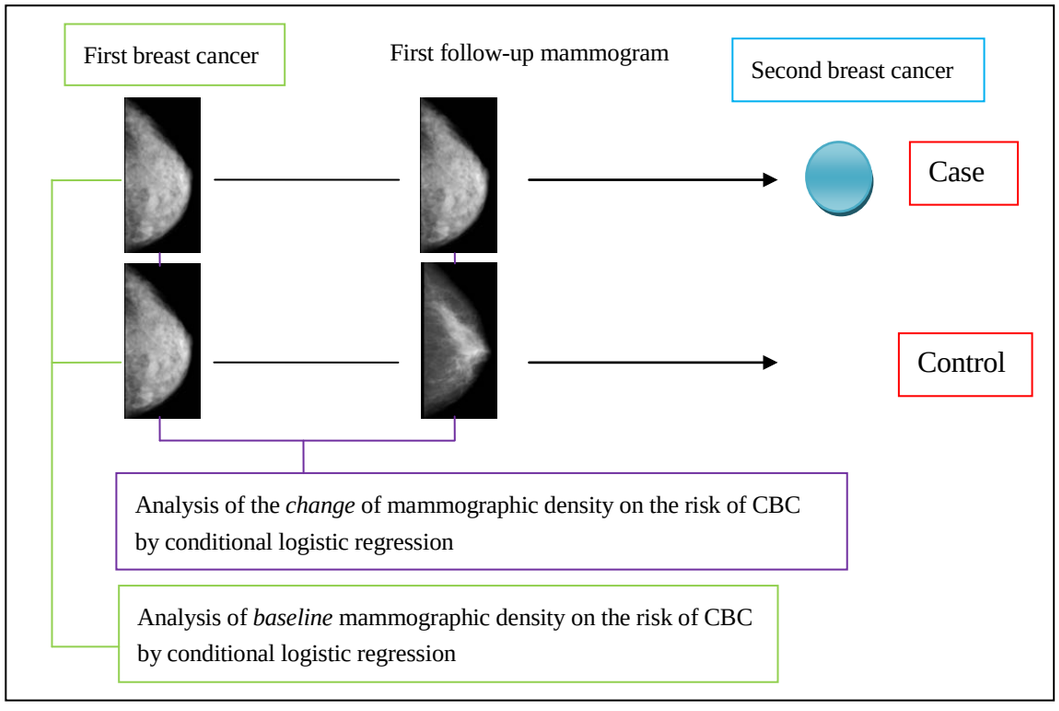


Figure 11: Analytical approach of Study IV.

The aim of Study IV was two-fold; to estimate the risk of CBC as an effect of density of the unaffected breast at time of the first breast cancer diagnosis and, secondly, to estimate the effect of change of mammographic density from diagnosis of the first cancer until the first follow-up mammogram. Both questions were analyzed by conditional logistic regression, the first analysis was adjusted for non-dense area of the breast, and the second analysis was adjusted for non-dense area of the breast and density at diagnosis of the first cancer.

6 RESULTS AND IMPLICATIONS

"However beautiful the strategy, you should occasionally look at the results."
Winston Churchill (1874-1965)

6.1 STUDY I: DIAGNOSTIC WORK-UP OF CBC

This study was designed to investigate the fact that the incidence of metachronous CBC had been decreasing over calendar period while the incidence of synchronous CBC had been increasing. It was hypothesized that this change was due to improvements of diagnostic work-up (diagnostic tools and/or routines) so that cancers that previously would have been discovered later, and thus labeled metachronous, was discovered closer to the first cancer, and thus labeled synchronous. An alternative explanation would be that the use of adjuvant therapy decreased the incidence of metachronous CBC while, simultaneously, the incidence of synchronous CBC increased, in parallel with the increasing incidence of unilateral breast cancer. Two population-based cohorts were used; all CBCs in Sweden during 1976-2004 (N: 2932), and all CBCs in Stockholm during 1976-2005 (N: 626), both cohorts were restricted to CBCs with a maximum of three years between the cancers.

6.1.1 Results

We found no statistically significant correlation between latency time (time between first and second cancer) and calendar period of the first cancer (P=0.20). Furthermore, the proportion of CBCs with both cancers diagnosed on the same day was similar through the period, ranging from 37% in 1976-78 to 45% in 2000-2002. When using the Stockholm cohort we found the odds of CBC being diagnosed synchronously, as compared to metachronously, increased by 27% by every five years later the first cancer was diagnosed (OR: 1.27[95% CI, 1.13 to 1.42]). This increased odds for later calendar period was not affected by the clinical work-up of the second cancer, but diminished when adjuvant hormone therapy was included in the model (Table 1).

	Odds ratio of being diagnosed synchronously			
	OR 95% CI	**OR[a]** 95% CI	**OR[b]** 95% CI	**OR[c]** 95% CI
Effect of calendar period (per each 5-years later)	1.27 1.13 - 1.42	1.23 1.10 - 1.38	1.02 0.89 - 1.17	1.24 1.11 - 1.40

Model adjusted for
[a] **mode of detection of second BC:** Clinical workup inc. mammographic screening
[b] **adjuvant treatment of first BC:** Hormone therapy vs. No hormone therapy
[c] **adjuvant treatment of first BC:** Chemotherapy vs. No chemotherapy

Table 1: Odds ratio of being diagnosed synchronously.

We further investigated the proportion of CBCs detected by clinical work-up over calendar period of the first cancer and could not detect any increasing proportion when excluding mammographic screening as part of the diagnostic work-up, when including screening there was a decreasing trend (Figure 12).

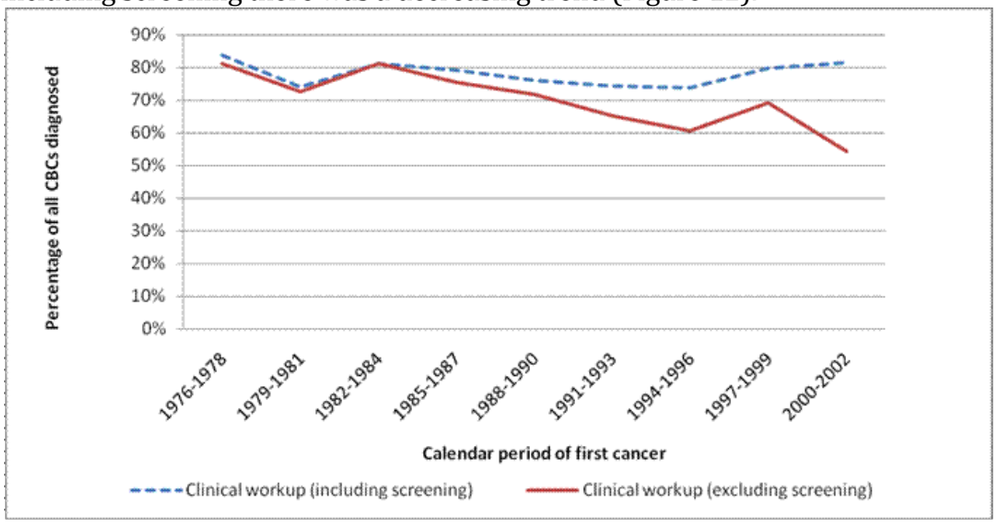


Figure 12: Percentage of CBCs diagnosed through clinical work-up.

Finally, we used tumor size of the second tumor as an alternative way of investigating the efficiency of the diagnostic work-up, tumor size of the second tumors was not found to be any smaller in the later period (P= 0.84).

6.1.2 Implications

With some confidence, due to the several different and independent measures used, we can refute the hypothesis that the increasing incidence of synchronous CBC and decreasing incidence of metachronous CBC is due to improved clinical workup of the opposite breast of newly diagnosed breast cancer patients. This seemingly lack of improvement over the last 25-30 years is somewhat surprising, it is however difficult to judge whether the interpretation should be that the clinical work-up now is *equally bad* as in the 1970s, or if it was *already so good* in the 1970s so that no further improvement could occur.
The alternative hypothesis; that the decreasing proportion of metachronous CBC is due to adjuvant therapy for the first cancer is supported by our results. We find two possible explanations. Firstly, the use of adjuvant therapy for the first cancer has increased over calendar period [129, 130] and has been shown to decrease the risk of metachronous CBC. The alternative explanation is that with calendar period, the probability of receiving adjuvant (hormonal) therapy for the first cancer increased to a larger extent for patients with synchronous CBC than for unilateral breast cancer patients (the population at continued risk for metachronous CBC), thus creating an association between being diagnosed synchronously and receiving adjuvant therapy. In our material we see a tendency towards this uneven distribution of adjuvant therapy, and this makes causal inference about the association between adjuvant hormonal therapy and the odds of being diagnosed synchronously less reliable.

6.2 STUDY II: AGGRESSIVENESS OF CBC AFTER RADIOTHERAPY

Radiotherapy is well known to protect against local recurrence and improve breast cancer specific survival [41, 42]. In Sweden approximately 60% of all breast cancer patients receive adjuvant radiotherapy [131]. The common dose of 48-50 Gy following surgery[38] entails scattered radiation to the contralateral breast, estimated to 2-3 Gy [113, 132, 133] or about 5% of the dose to the treated breast[134]. Because such doses may be carcinogenic, the risk of CBC following adjuvant radiotherapy for breast cancer has been studied for several decades with inconclusive results [42, 71, 113, 132]. However, no studies have investigated if prognosis after the second cancer and/or malignant features of the second cancer is associated with adjuvant radiotherapy for the first cancer. We used two large and independent cohorts of metachronous CBC-patients to investigate the question of whether ionizing radiation in the form of radiotherapy might affect subclinical cancers/pre-malignant lesions in the opposite breast.

6.2.1 Results

The two cohorts analyzed in this study contributed with 809 CBC patients from the Stockholm cohort and 750 patients from the South Swedish cohort (In Table 2 the results for the fully adjusted model for the Stockholm cohort is shown). This study showed that patients treated with radiotherapy for their first cancer had a borderline significant increased risk of distant metastasis after the second cancer, interaction analysis showed this effect to be confined to the second cancers diagnosed in the first five year after the first cancer, where the risk of distant metastasis was highly significantly increased to about twice the risk of untreated patients in the same latency group. When contrasting the patients treated by radiotherapy only to patients entirely without adjuvant therapy (model II)) we found very similar results as when contrasting patients who were treated with radiotherapy, potentially in combination with any other adjuvant therapy to patients not treated with radiotherapy but potentially with any other adjuvant therapy (model I).

Exposure	Events (N)	IRR	95% CI
Model I. Adjuvant radiotherapy			
Any latencies	111	1.32	0.99 - 1.77
Latency ≤ 5 years	76	1.88	1.28 - 2.76
Latency >5 years	35	0.81	0.52 - 1.27
No adjuvant radiotherapy	92	1.00	Ref.
Model II. Adjuvant radiotherapy only			
Any latencies	60	1.54	1.04 - 2.27
Latency ≤ 5 years	42	2.42	1.45 - 4.03
Latency >5 years	18	0.78	0.42 - 1.45
No adjuvant therapy	52	1.00	Ref.

Table 2: IRR of distant metastasis after CBC.

The risk estimates did not change substantially when we restricted adjustments to calendar period, age at first diagnosis and latency time. Our findings were confirmed when analyzing the south Swedish cohort.

Due to lack of information it was impossible for us to investigate any dose-response relationship, however; the second tumors arising in the medial part of the breast would have been exposed to higher radiation dosage than the tumor arising in the lateral part of the breast. We therefore stratified our analysis of the risk of distant metastasis for patients with short latency time on location of the second tumor. We found higher excess risk following radiotherapy among women with medial tumors (IRR: 3.03 [95% CI: 1.10-8.32]) than with lateral tumors (IRR: 1.46 [95% CI: 0.70-3.02]). This analysis was adjusted for calendar period and age at first diagnosis.
When proceeding to analyzing the odds of worse TNM-stage/histological grade of the second cancer compared to the first, we again found about twice the odds of worse TNM-stage and similarly increased odds for histological grade (Table 3). This association was even more pronounced when contrasting patients who received radiotherapy only to patients entirely without adjuvant therapy.

	TNM-Stage			**Histological grade**		
	Events (N)	**OR**	**95% CI**	**Events (N)**	**OR**	**95% CI**
Adjuvant radiotherapy						
Any latencies	65	1.36	0.89 - 2.09	119	1.16	0.76 - 1.78
Latency ≤ 5 years	35	2.16	1.13 - 4.11	62	2.00	1.08 - 3.72
Latency >5 years	30	0.96	0.55 - 1.67	57	0.75	0.43 - 1.32
No adjuvant radiotherapy	50	1.00	Ref.	78	1.00	Ref.

Table 3: OR of worse tumor characteristics at the second cancer.

6.2.2 Implications

Since our findings are confined to cancers arising within five years from the first cancer, clearly, the effect of radiotherapy seems to be on cancers that were sub clinically present at time of the initial diagnosis. Interestingly, studies on random biopsies of the opposite breast of unilateral breast cancer patients showed that 6-7% of the patients had invasive or in-situ malignancies and an additional 9% had premalignant lesions [92, 93]. In other words, approximately 15% of all breast cancer patients have lesions in the contralateral breast with the possibility to progress into CBC. We hypothesize that if such lesions are exposed to a carcinogenic stimulus, such as low-dose ionizing radiation, this can enhance the rate of mutations and thereby accelerate tumor progression. This study was not aimed or designed to investigate the induction of new cancers, not least due to that radiation-induced development of cancer is likely to take much longer[135] than our current follow-up time.
This study, as any non-randomized study that investigates treatment, is potentially subject to *bias by indication* (see *Discussion- Methodological considerations 7.1.1*). We had several different approaches to handle this; first, naturally, all survival analysis was adjusted for the most important prognosticators; age at diagnosis, calendar period of diagnosis, TNM-stage and histological grade. Secondly, we designed the analysis of tumor characteristics to

take into account that some women have host factor that might make them prone to have more aggressive cancers than others; we compared the probability of the second cancer to be of worse tumor characteristics than the first cancer, for the same women. Thirdly; we used two different exposure groups; 1) women with radiotherapy, alone or in combination with any other adjuvant therapy compared to women without radiotherapy, either entirely without adjuvant therapy or with any other therapy except radiotherapy and 2) women treated with radiotherapy only, compared to women entirely without adjuvant therapy. Fourthly; our finding that tumors in the medial part of the breast have a worse prognosis if exposed to radiation than if not exposed, and tumors in the lateral part of the breast have less of this effect, is in accordance with the aforementioned biological hypothesis but hardly with bias by indication. Fifthly and finally; if bias by indication was in play we would expect to see a worse prognosis also for patients whose second tumor was treated by adjuvant radiotherapy. This was investigated but no sign of any worse prognosis was seen (in fact, for patients with latency time <5years, we saw the opposite; IRR of distant metastasis: 0.39 [95% CI: 0.24-0.63]).
We believe that adjuvant radiotherapy given for the first cancer and affecting a subclinical cancer in the opposite breast might be part of the explanation for the finding that CBC patients with short latency time have worse prognosis, both compared to synchronous CBC as well as to patients with long latency time. The clinical implication of this finding is obviously *not* to refrain from adjuvant radiotherapy, but to minimize radiation to the contralateral breast (possibly by using accelerated partial breast irradiation [136] when appropriate) and to take the given therapy into account in the management of a second cancer.

6.3 STUDY III: ESTROGEN RECEPTOR PATTERN IN CBC

Approximately 80% of all breast cancer tumors have increased levels of estrogen receptors (ER) on the surface of the cell, transmitting increased number growth signals. Presence or absence of estrogen receptors is an important factor for breast cancer survival in its own right[137, 138], but it is also the most important predictor for endocrine therapy response [59], since endocrine therapy function depends on the estrogen receptor. Very few studies have addressed how ER-status of the second tumor affects prognosis of CBC patients [139, 140] and the combined influence of ER-pattern of both tumors has never been investigated previously. As a consequence, clinical guidelines for how to evaluate prognosis for CBC patients with respect to ER-status are lacking. We performed a cohort study, including all synchronous and metachronous CBCs with known ER-status (N=933).

6.3.1 Results

Having both tumors ER-positive (Double ER-positive) was the most common ER-pattern among the CBC patients (70%), followed by two tumors of different ER-statuses (ER-discordant) (21%) and both tumors ER-negative (double ER-negative) (9%) (Table 4). The observed and expected distribution of ER-pattern

was significantly different for both types of CBC (p-value <0.001 for synchronous CBC and p-value <0.001 for metachronous CBC). The ER-pattern of metachronous and synchronous CBC was moreover also significantly different from each other (P<0.001).

	N	Expected proportions %	Observed proportions %	95% CI
Synchronous CBC:				
Double ER-positive	263	61.1	78.7	74.0 - 83.0
ER discordant	45	34.1	13.5	10.0 - 17.6
Double ER-negative	26	4.8	7.8	5.2 - 11.2
Metachronous CBC:				
Double ER-positive	390	61.1	65.1	61.1 - 68.9
ER discordant	151	34.1	25.2	21.8 - 28.9
Double ER-negative	58	4.8	9.7	7.43 - 12.3

Table 4: Observed and expected proportions of ER-positive and ER-negative CBC.

When analyzing the risk of distant metastasis of the different ER-pattern groups stratified on synchronous/metachronous CBC a significant effect was found among patients with synchronous CBC. Among metachronous CBC-patients, however, the prognosis is equally bad for patients with ER-double positive CBC as for patients with double ER-negative CBC; approximately 3 times the risk of a patient with unilateral breast cancer (Table 5).

	IRR	95% CI	P-value
Unilateral breast cancer	1.00	Ref.	
Synchronous CBC			
ER-double positive	1.25	0.88 - 1.76	0.01
ER discordant	2.19	1.18 - 4.08	
ER-double negative	3.95	1.77 - 8.81	
Metachronous CBC			
ER-double positive	2.95	2.39 - 3.64	0.18
ER discordant	2.23	1.61 - 3.09	
ER-double negative	2.88	1.83 - 4.52	

Table 5: IRR of distant metastasis after CBC.

In line with current clinical practice we also analyzed the risk of distant metastasis for patients with metachronous CBC and found no difference between patients with ER-positive second cancer (IRR=2.67 ([95% CI: 2.20-3.25]) and ER-negative second cancer (IRR= 2.87 [95% CI: 2.11-3.89]).
We found the bad prognosis for patients with double ER-positive metachronous CBC surprising, and hypothesized that it might be due to endocrine therapy resistance. One could argue that an ER-positive second cancer that develops during, or shortly after, endocrine therapy has escaped the therapeutic effect and therefore might have particularly aggressive characteristics. If therapy resistance produces particularly aggressive cancers, the worsened prognosis should diminish for cancers diagnosed in the post-therapeutic period. We found

the risk of distant metastasis to decline steadily as time between the first and second cancer increased. When the time exceeded 9 years the risk of distant metastasis was the same as for women with synchronous double ER-positive CBC (Figure 13).

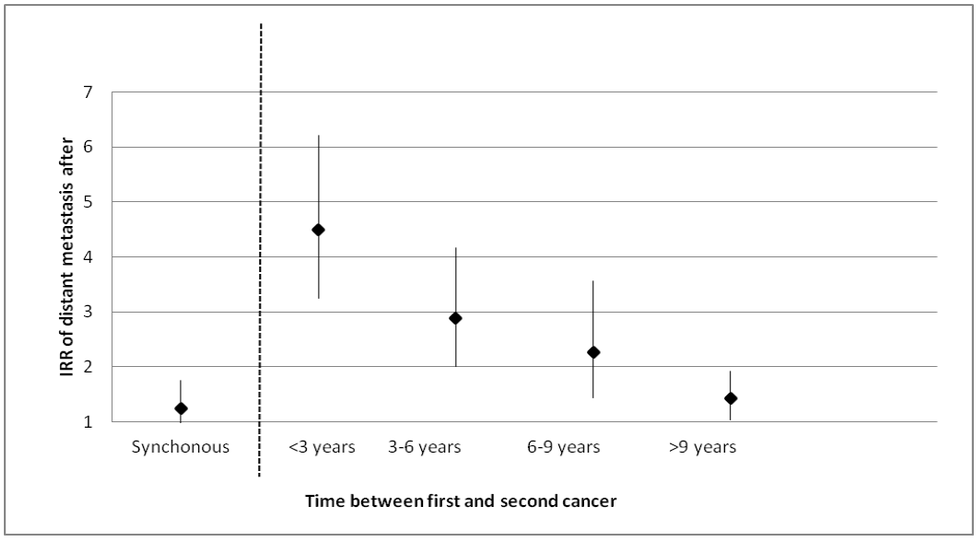


Figure 13: IRR of distant metastasis after CBC, as an effect of latency time.

To further investigate the hypothesis regarding endocrine therapy response we stratified the patients on endocrine therapy of the first cancer. Double ER-positive metachronous CBC patients treated with endocrine therapy for their first cancer had a four-fold higher risk (IRR=4.42; 95% CI: 2.91- 6.71) compared to women with unilateral breast cancer treated with endocrine therapy. For women not treated with endocrine therapy the risk was not statistically significantly increased compared to patients with unilateral breast cancer. (IRR = 2.03 (95% CI: 0.95-4.35).

6.3.2 Implications

The proportion of CBC patients with discordant ER-status was significantly smaller than would be expected if tumor development would be independent, this could conceivable be due to host factors leading to particular women being more likely to have cancers of a certain ER-subtype and significant concordance have been shown repeatedly in previous studies [77, 140-145]. Notably, double ER-positive cancers were more prevalent than expected in synchronous disease but not in metachronous disease. One possible explanation for this finding could be that endocrine therapy, normally only administrated to patients with ER-positive cancer, is decreasing the probability of ER-positive metachronous second cancer, thereby, as a consequence, decreasing the proportion of metachronous patients with double ER-positive cancers.
For synchronous CBC, ER-pattern carries a significant prognostic value and might be helpful when deciding on adjuvant therapy regimen. For metachronous CBC, this study establish no difference between patients with ER-positive and ER-negative second cancer, this can thus not be used as a prognosticator, and

neither can ER-pattern (both ER-statuses combined), as the prognosis among patients with double ER-positive cancers is equally bad as among those with double ER-negative cancers. This study indicate that this unexpected result is driven by patients who develop yet another ER-positive cancer during or shortly after endocrine therapy and might thus lend support to the hypothesis of endocrine therapy resistance. Our results imply that these patients probably should have a more aggressive therapy than currently recommended, particularly if the time between first and second cancer is short.

6.4 STUDY IV: MAMMOGRAPHIC DENSITY AFFECTS RISK OF CBC

Mammographic density is, as is described in the background, a well known and strong risk factor for breast cancer[11], recently attempts have been made to use also changes in mammographic density to predict breast cancer risk[12, 146]. It has however previously been unknown whether either of these findings for unilateral breast cancer is also applicable to CBC. The aim of this study was to investigate whether the mammographic density (of the unaffected breast) at time of the first breast cancer diagnosis, or its changes during the time after the cancer diagnosis, could be used for predicting the risk of CBC. This was done in a nested case-control study with CBC-patients (cases) individually matched to unilateral breast cancer patients (controls) on calendar period of (first) breast cancer diagnosis, age at (first) breast cancer diagnosis, adjuvant therapy and follow-up time, so that the control had survived without distant metastasis (or CBC) at least as long as the time between the first and second cancer of the corresponding case. We measured mammographic density with an automated thresholding method[123] and used both percent mammographic density and dense area as measures of density.

6.4.1 Results

For 211 case-controls pairs we could locate and measure both a base-line mammogram and mammogram taken at least a year after diagnosis. We found that mammographic density at (first) breast cancer diagnosis does not affect the risk of CBC, adjusted for non-dense area at diagnosis (Table 6).

Density at diagnosis							
Percent density	**N**	**OR**	**95% CI**	**Area density**	**N**	**OR**	**95% CI**
≤ 5 %	24	1.00	Ref.	≤ 20 cm^2	106	1.00	Ref.
> 5 - 25 %	174	0.78	0.32 - 1.92	>20 - 40 cm^2	144	0.74	0.43 - 1.27
> 25 - 50 %	184	0.89	0.34 - 2.33	>40 - 60 cm^2	96	0.93	0.50 - 1.71
> 50 %	40	0.36	0.10 - 1.31	> 60 cm^2	76	0.94	0.49 - 1.79

Table 6: OR of CBC, comparing patients of different mammographic density.

Regarding change of mammographic density we found that women who experience an absolute decrease of mammographic density of at least 10%, or at least 10 cm^2, have approximately 50% lower risk of CBC, compared to women with stable density (Table 7). No effect was seen for women who increased in mammographic density.

This analysis was adjusted for non-dense area of the breast at diagnosis and of baseline mammographic density.

Post-diagnostic change of density			
Percent density	**N**	**OR**	**95% CI**
Absolute decrease ≥10%	96	**0.45**	**0.24 - 0.84**
Stable (<10% decrease to <10% increase)	243	1.00	Ref.
Absolute increase ≥10%	17	0.83	0.24 - 2.87
Area density	**N**	**OR**	**95% CI**
Absolute decrease ≥10cm^2	108	**0.54**	**0.30 - 0.99**
Stable (<10cm^2 decrease to <10cm^2 increase)	197	1.00	Ref.
Absolute increase ≥10cm^2	33	0.71	0.30 - 1.69

Table 7: OR of CBC, comparing patients with changing and stable mammographic density.

Further, women before menopause are known to have higher mammographic density and are more likely to experience a decrease in the years after diagnosis (as they pass through menopause, either naturally or artificially, due to therapy[147]). In our study women who are premenopausal at (first) diagnosis decreased 5.9 %-units from diagnosis to first follow-up mammogram, this was significantly more than women who were postmenopausal at diagnosis. When the analysis of change of mammographic density was stratified on menopausal status at diagnosis of the first cancer pre-menopausal women who decreased in density was found to have an odds of CBC of 0.29 (95% CI: 0.09-0.92) compared to the premenopausal women of stable density. For postmenopausal women the corresponding odds was 0.49 (95% CI: 0.16-1.45).

6.4.2 Implications

The finding that mammographic density at baseline did not influence the risk of CBC in breast cancer patients might be somewhat unexpected; it is in contrast to the effect of mammographic density on the risk of unilateral breast cancer among healthy women. However, an analogy can be made to the effect of established hormonal/reproductive risk factors for breast cancer [87, 108, 109], which increases the risk of breast cancer [148, 149] but not the risk of CBC [87, 108, 109]. An alternative explanation for the lack of association in this study between mammographic density and risk of CBC could be that there was a systematic difference in mammographic density of the unilateral breast cancer patients (the controls) in this study compared to unilateral breast cancer patients in general. To investigate this concern we studied the effect of mammographic density on the risk of breast cancer in our selected unilateral breast cancer patients compared to healthy women and reassuringly found the expected strong association between mammographic density and the risk of breast cancer.
The decrease in risk of CBC that was seen following a decreasing mammographic density of 10% or more can, if confirmed, be used to predict the risk of CBC, and can thus contribute to decision making in follow-up routines and adjuvant treatment

regimens as well as provide reassurance to the patients at decreased risk. The 10% cutoff has been previously shown as the minimum change that could be reproducibly detected visually [146] and we believe that this finding might therefore be clinically useful, 23% of the women participating in this study experienced a decrease of this magnitude. Furthermore, the effect of decreasing mammographic density on risk of CBC was independent of therapy given for the first cancer. The indication of a stronger effect among the pre-menopausal women leads us to hypothesize that the change of density occurring during menopause is more important than changes in density due to other factors or during other periods of life.

It can also be noted that this study is one of the two first studies to make use of a new method to measure mammographic density. This method is fully automated and well adapted for use in large-scale studied regardless of whether the mammograms are digital or digitalized, and have been described in detail in a recent publication[123]. We use mammographic density measured both as percent density and as dense area and we can show that they both yield very similar results. Further, the analysis is adjusted for non-dense area of the breast, rather than body mass index which has been previously regarded as an important confounder but has recently been shown to be inferior to adjusting for non-dense area of the breast [150].

7 DISCUSSION

The truth is rarely pure and never simple.

Oscar Wilde (1854-1900)

7.1 METHODOLOGICAL CONSIDERATIONS

7.1.1 Validity

Doubt is the offspring of knowledge: the savage never doubts at all.

Winwood Reade (1838-1875), explorer and philosopher

Whether the results of a study are valid is assed in two steps, first; the *internal validity*, which describes whether the association that is found is correct within the studied population. Threats to the internal validity are *systematic errors*; *bias* and *confounding*. Secondly: the e*xternal validity* or *generalizablity* is the question of whether the findings of a study are valid for all populations that the researcher indented to target. Obviously, internal validity is a prerequisite for external validity.

Systematic errors

A *confounder* is a factor that causes both the exposure and outcome in the study, if such a variable is not taken into account, the results of the analysis might be misleading in that they seem to suggest a causal relationship between the exposure and outcome where there actually is none (See green part of Figure 14). E.g. in Study II, TNM-stage of the first cancer is a potential confounding factor; high TNM-stage increase the risk of distant metastasis (the outcome) and it also potentially increase the chance of receiving adjuvant radiotherapy (the exposure). Thereby; the incidence of distant metastasis would be higher among the radiotherapy treated than among the non-treated, even if radiotherapy would not cause distant metastasis after CBC. In many studies adjustments are made for factors that are not directly causing the exposure/outcome, but are used as proxies for the underlying, often unknown, factors. E.g. in Studies II, III and IV we adjust for calendar period, since prognosis has improved over period and most other factors can also be expected to have changed. However, obviously, it is not calendar period by itself that has caused the changes, but rather treatment, routines, public awareness, etc. A note on a specific type of confounding; namely *confounding by indication* (or *bias by indication*), this is of particular interest in observational studies of treatment effects. The underlying problem is that aggressive subtypes of diseases tend to get more aggressive treatment than less aggressive subtypes of the same diseases, thereby potentially leading to a non-causal association between an aggressive treatment and unfavorable outcome after the disease. E.g. in Study II, bias by indication is a potential problem, since, hypothetically, more aggressive cancers could be more likely to be treated by radiotherapy and also more likely to metastasize. Several strategies were employed to determine whether such bias was present, including (obviously) to adjust for markers of aggressively, as the example of TNM-stage above.

A *mediator* is a factor that is in the causal pathway between the exposure and outcome of interest (See orange part of Figure 14), e.g. estrogen level in the blood is in the suggested causal pathway between hormone replacement therapy and breast cancer. It might be tempting to adjust for a mediator, as a way to investigate whether that is the only causal pathway (in which case no effect would be seen in an adjusted analysis) or if there is other causal pathways between the exposure and outcome (in which case an effect would remain also after adjustment). However; this strategy has to be approached with caution, since adjusting for a mediator potentially open up for confounding by underlying factors causing both the mediator and the exposure or outcome. Thus, mediator analysis should only be attempted if the existence of such underlying factors is deemed to be very unlikely.
An *effect modifier* is a factor by which the effect of the exposure on the outcome varies, e.g. in Study II we find that the effect of radiotherapy is only apparent if the time between the first and second cancer is less than 5 years, if the time between the cancers is more than 5 years we find no effect. In contrast to confounders, which are non-biological 'problems' of a study, which need to be adjusted away, effect modifiers are true effects. Investigating these effects might however be problematic of several reasons; firstly it is a matter of discussion on what scale interaction should be investigated (ratio or difference). In almost all cases interaction will be present on one scale and not on the other and biological arguments can be made for both scales. This is only unproblematic if an effect is seen in one stratum, and not at all in the other/s, in this particular case both scales will show interaction. Secondly; interaction analysis tend to be very exposed to the problem of multiple testing; with the currently most used α-level of 5% one of every 20 statistical tests (on average) will show a statistically significant effect even if there is none, purely by chance. This, in combination with the fact that many studies investigate a large number of interaction terms (without any clear hypothesis) might make such studies difficult to interpret.

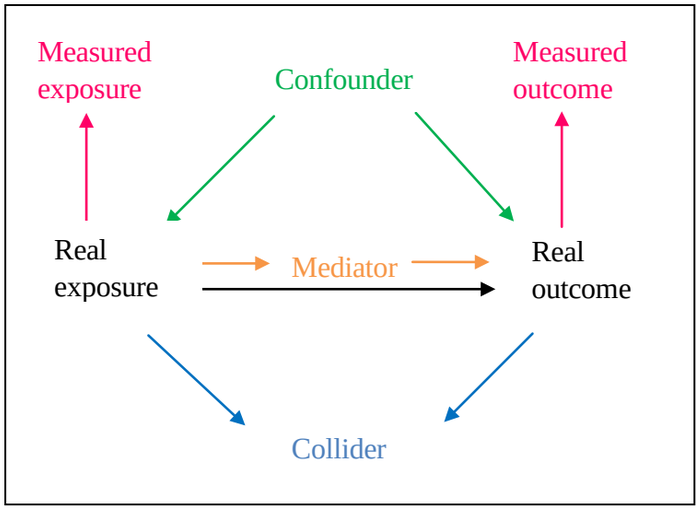


Figure 14: Illustration of epidemiological concepts.

Information bias arises when the measure of the exposure is not exactly corresponding to the real exposure and/or when the measure of the outcome is not exactly corresponding to the real outcome (See red part of Figure 14). E.g. it is likely

that not all cancer metastases are recorded in the Stockholm breast cancer register, some women will not come to the hospital even though they have metastasis, some registration forms will be lost and so on. If we then assess distant metastasis (the outcome) through the register, some patients with the outcome will be missed; we will have information bias. It is easily realized that some degree of information bias exist in virtually all epidemiological studies, and it is of importance to evaluate how large it is and in what direction it might affect the estimate. When the measured variable is binary, the effect of information bias is *misclassification*; some study participants who are recorded as unexposed are really exposed and vice versa, and some study participants that are recorded as having the outcome actually did not have it, and vice versa. The next step is assessing whether the misclassification is *differential*.

Differential misclassification arises when the probability of misclassification of exposure is different depending on the outcome status of that patient, or the other way around. E.g. in Study III; if patients with ER-negative cancers would be more carefully followed-up, then their metastases would be more likely to be recorded in the register and using diagnoses of distant metastasis from the register as outcome would lead to differential misclassification. (Fortunately for our study, ER-status is not taken into account in the follow-up routines, therefore differential misclassification unlikely in this case.) Differential misclassification might lead to an overestimation or underestimation of the effect, depending on whether patients with the outcome are more likely to be misclassified as exposed (overestimation), or unexposed (underestimation). In other words, it might lead to type 1 error (to reject a hypothesis that is actually true) as well as type 2 error (failing to reject a hypothesis that is actually false). If the unexposed patients (in a study investigating a potentially harmful exposure) are more likely to be misclassified as having the outcome, the effect would be to underestimate the effect, sometimes called *bias towards the null*. On the other hand, if the exposed patients are more likely to be misclassified as having the outcome, the effect would be an overestimation of the investigated association. Non-differential misclassification is often regarded as less of a problem, as it would only lead to a dilution of the effect, but, naturally, this increases the risk of type 1 error. In study IV we use a rather new measure of mammographic density, it is likely that the measured mammographic density does not correspond exactly to the 'real' mammographic density, but there is no reason to believe that the measurement error would be different for future CBC-patients and patients who will not develop CBC in the future. Thus we expect to have non-differential misclassification, and, depending on the size of the measurement error, the true effect might be larger than the one shown in the study.

Selection bias in a general sense affects the external validity by including only a subset of the population which the study was intended to target. Therefore it might be difficult to extrapolate, or *generalize*, the findings of the study to a population that was actually not included. E.g.: many of the studies of breast cancer risk factors are performed on populations of high-income countries, the findings of these studies may or may not be generalizable to populations of other countries, with different genetic, cultural and environmental backgrounds. Another common type of selection bias is *self selection*; e.g. in a study of breast cancer risk, where the exposure is family history of breast cancer it can be expected that women with breast cancer in the

family are more likely to volunteer to be in the study, and the risk of breast cancer is also increased in this group, therefore the association found in the study might not be applicable for the whole population (and the prevalence of the exposure will certainly be distorted). However, a particular type of selection bias; *Berksonian,* may affect also the internal validity. Berksonian selection bias arises when the women included in the study are selected on some factor that is caused by both to the exposure and the outcome, a *collider* (see blue part of Figure 14). A collider is not problematic unless the model is adjusted for it, but if this is done an association arises that might be mistaken for causal, similar to the association seen in a study that suffers from unadjusted confounding. Selection bias is not the only scenario which might lead to problems with colliders; it might also be that a factor, that turns out to be a collider, is chosen to be include in the model.

7.1.2 Precision

It is the mark of an educated mind to rest satisfied with the degree of precision which the nature of the subject admits and not to seek exactness where only an approximation is possible.

Aristotle (384-322 BC)

Since epidemiological studies investigate only a sample of the population of which inference is made *random error* has to be taken into account. Random error refers to the uncertainty of the estimates that is a result of the sample randomly not reflecting the full population perfectly. To increase the study size is an obvious way to decrease the random error (thereby increasing the precision). Since the prime determinant of study power is the number of cases/outcomes an alternative way to increase precision, while keeping the study size constant, is to use a case-control design, instead of a cohort study. The trade-off naturally being that since the ratio between cases and controls is predefined (in order to increase the proportion of cases) the study can no longer be used to determine the underlying risk of the outcome, and the risk ratio can thus not be calculated. Instead the odds ratio is used, which is usually, but not always, an appropriate approximation (See *Methods – Design and statistical analysis 5.1.2*).
Both case-control studies and cohort studies may use *matching* as a way to increase power. Simplistically; statistical adjustment can be viewed as performing the analysis in strata of the adjustment variable and weighting the estimates. When regarded as such, it is easy to understand that matching on an adjusting variable increases power; the confounder is by definition unequally distributed between cases and controls. Therefore, when stratifying on the confounding variable, strata in one end of the spectrum might contain almost only cases and strata in the other end might contain almost only controls. The consequence, in an unmatched analysis, is a lack of precision in those strata and, consequently, in the study. Matching can be done as *frequency matching*; when the cases and controls are sampled so that there are equal numbers of both in strata of the adjusting variable, or by *individual matching* (as in study IV); when a case and the corresponding control/s are selected so that they match each other on all the matching variable. Individual matching is by

necessity used in nested case-controls studies (See *Methods –Design and Statistical analysis 5.1.2*).

7.1.3 CBC-specific challenges

Every honest researcher I know admits he's just a professional amateur. He's doing whatever he's doing for the first time. That makes him an amateur. He has sense enough to know that he's going to have a lot of trouble, so that makes him a professional.

Charles F. Kettering (1876-1958), engineer and inventor

Contralateral breast cancer have presented a variety of interesting epidemiological problems, below is a brief account these and how they were handled in studies in this thesis.

7.1.3.1 Population at risk

It might be of interest to compare the risk of metachronous and synchronous CBC; this is however complicated by the fact that the *populations at risk* of these diseases are different. The population at risk is an epidemiological concept describing the entire population in which a person diagnosed with the disease would be recorded as a case. Controls for a case-control study should be selected from this group of people. In the case of metachronous CBC the population at risk is women with unilateral breast cancer. However, for synchronous CBC, which we regard as being diagnosed simultaneously (even though for practical reasons a time margin is used, in this thesis; three months) the population at risk is healthy (breast-cancer free) women.

The population at risk posed another problem in Study IV; the question was whether to match on survival time (density sampling) or not. This matching would ensure that that the matched unilateral breast cancer patient; the control, had survived at least as long as the CBC-patient; the case, had. As a consequence, the breast cancer patients selected as controls in this study have, at average, longer survival than breast cancer patients in general. On the other hand, if the controls would not be matched on survival time the consequence would be that the controls would, on average, have a worse prognosis than the cases, actually; that the selected controls would not belong to the population at risk (since one cannot get CBC unless one survives the first cancer). It is true that the nested case-control/density sampling design leads to that the finding of the study is not generalizable to all breast cancer patients, but it is generalizable to those that are alive (which tend to be group for which we are interested in predicting the risk of CBC).

A final note of population at risk of metachronous CBC; an argument has been made in the literature[82] that when comparing the risk of CBC among breast cancer patients to the risk of breast cancer among breast-cancer free women we might actually think of the number of *breasts* at risk, rather than the number of *women* at risk. In that case, the common finding that breast cancer patients have twice the risk of yet another breast cancer compared to a healthy woman's risk of breast cancer would

instead be four times the risk. While I believe this to be theoretically true and that it further supports the notation of CBC-patients as a selected subpopulation worthy of particular interest, it is not of relevance for clinical or public health decisions.

7.1.3.2 Measure of prognosis

Prognosis after cancer can be (and is) studied using many different measures; 5-year survival, overall survival, rate of death, rate of breast cancer-specific death, rate of distant metastasis, etc, all with their advantages and disadvantages. 5-year survival is less useful in breast cancer, compared to other cancers, since breast cancer does not have a cure rate (See *Background –Prognosis 2.1.4*). Overall survival is a suboptimal measure when studying etiology, as it does not take into account whether the women died from breast cancer or something else (it is however the preferred measure in randomized clinical trials). On the other hand; breast cancer specific survival also has problems; cases might be missed; a women with breast cancer might die from her cancer, without this being recorded on the death certificate, this might be a relatively larger problem among older women. In the studies in this thesis investigating prognosis (Study II and Study III) we have used the incidence rate of distant metastasis. The survival for women with diagnosed distant metastasis is very poor; in our cohort of CBC-patients the median survival is 2 years, which makes distant metastasis a good proxy for breast cancer specific death, with less risk of missing cases than one would have when using the death certificates. (It also makes the need of further research of metastasized breast cancer very urgent, it is however outside the scope of this thesis.) It can also be argued that the time from breast cancer diagnosis to diagnosis of distant metastasis is more determined by the characteristics of the cancer, while the survival from diagnosis from distant metastasis might be more determined by host factors; the general health and strength of the patient. However, this mechanism, if present, does not seem to be very important as we, in Study II, found similar results when using breast cancer specific death as outcome as when using distant metastasis.

7.1.3.3 Survival time

When using survival analysis (e.g. to study prognosis) a starting point needs to be determined and thereby an underlying time scale, whereby the survival is counted. The choice is made according to the most important confounding time variable, by which the effect is expected to vary. For example; a study of the risk of cancer by age would probably start at birth, or at an age when the risk of cancer is sufficiently high to be measured, and have the underlying time scale of *age*, a study of an exposure that changes with calendar time, like air pollution, would start at a certain year, say 1950, and measure time by *calendar period*. Though both age and calendar period is important when investigating prognosis after cancer, the most logical starting point is often thought to be the diagnosis, and the most important time scale is *time since diagnosis.* It is perfectly doable to have more than one underlying timescale, but it is not uncommon that the other time scales that are of importance are adjusted for in the analysis as a constant values, e.g. age at diagnosis and calendar period of diagnosis, while the risk is allowed to vary over time that has elapsed since the diagnosis. Since all these timescales are interconnected (one year after diagnosis the

patient is also a year older and the calendar period is one year later) the adjustment is usually sufficient.
Now, how to count survival time for CBC-patients, especially if one wants to compare the prognosis of CBC-patients to the prognosis of patients with unilateral breast cancer (as in Study III)? One could start at time of the first diagnosis for CBC-patients (and at the only diagnosis for patients with unilateral breast cancer), this would however lead to what is known is *immortal time bias*; in order to have CBC the patient has to survive the first cancer, thus CBC-patients who have lived long enough to develop a second cancer would be compared to unilateral breast cancer patients of which some could have died shortly after diagnosis. The survival time would be artificially prolonged for the CBC-patients, leading to the conclusion that CBC-patients do not have worse survival than unilateral breast cancer patients, the published literature contains examples of this approch[68]. I believe this to be not entirely accurate and instead we start counting survival time at time of the second breast diagnosis for CBC-patients, and compared this to the survival time for breast cancer patients counted from their (only) breast cancer diagnosis. This approach is better, but is not unproblematic, as breast cancer has no cure rate (See *Background – Prognosis 2.1.4*). For CBC, this has an important implication; for some of the patients who are diagnosed with distant metastasis after the second cancer it will actually be the first cancer that metastasized. Since the risk of a breast cancer patient to be diagnosed with metastasis decrease by time since diagnosis it is however more likely to be the second tumor, but currently there is no routine procedure to determine for certain, neither is there any clinical use of the information.

7.1.3.4 Correlation of tumor characteristics

In Study II, where we wanted to investigate the potential effect of treatment of the first cancer on the tumor characteristics of the second cancer we ran in to a problem; host factors of the patient will influence the tumor characteristics, making two tumors of the same patient more likely to be similar than two tumors of two different patients. Also, the tumor characteristics of the first cancer, to some extent, influence the treatment decision, thereby feigning an association between certain tumor characteristics (worse) and a certain treatment (more aggressive), even without any causal link between treatment and tumor characteristics of the second cancer. This is the underlying reason why we choose to, instead of calculating the odds of advanced TNM-stage or low differentiation at the second cancer, we calculated the odds of patients having worse TNM-stage/differentiation at the second cancer *than at the first.* Since, while a tendency to have bad tumor characteristic is associated with treatment decision, there is no such association between treatment decision and the tendency to have worse tumor characteristics at the second cancer than at the first.

7.2 FINDINGS AND INTERPRETATION

At the heart of science is an essential balance between two seemingly contradictory attitudes; an openness to new ideas, no matter how bizarre or counterintuitive they may be, and the most ruthless skeptical scrutiny of all ideas, old and new.

Carl Sagan (1934-1996), astrophysicist and author

In addition to the implications of each study per see, which are being discussed briefly in *Results and Implications (6.1-6.4)* and at some length in the articles/manuscripts, there are also some questions for which findings from several of the studies are relevant.

7.2.1 Why does short latency time infer bad prognosis?

One of the important reasons to study CBC is that the prognosis for these women are worse than for women with unilateral breast cancer, in particular for patients with metachronous CBC with short (< 5 years) time between the cancers (See *Background –Prognosis 2.2.4*). To elucidate the reasons behind this phenomenon has been an underlying aim for several of the studies in this thesis. Part of the explanation is naturally inherited in the definition of the sub group; short latency metachronous CBCs have, per definition, developed from a subclinical state to a detectible cancer during a relatively short period of time and is consequently fast growing, a characteristics that is usually a bad prognostic marker.
However, adjuvant therapy given for the first cancer, which is administered to almost all breast cancer patients nowadays, could conceivable also have an effect. One possible mechanism for such an effect is selective pressure; that the adjuvant therapy acts on subclinical second cancers, eliminating less aggressive tumors and leaving the most aggressive unaffected. This would lead to a scenario where the therapy would decrease the incidence of CBC but worsen the prognosis after CBC, should it anyway occur. This pattern is indeed what has been seen when we investigated the effect of ER-status and endocrine therapy. Endocrine therapy decreased the risk of ER-positive CBC (but not ER-negative CBC, which is in accordance with the biological mechanism of endocrine therapy). For patients with endocrine therapy and ER-positive second cancer, however, the prognosis was significantly worse than for patients with ER-positive second cancer but who had not been treated with endocrine therapy for the first cancer. A similar pattern has also been seen after chemotherapy for the first cancer in several previous studies[76, 151, 152].

Another mechanism that could lead to worse prognosis after adjuvant therapy for the first cancer, but without any decrease in incidence, is investigated in Study II. The current view on cancer development (See *Introduction-Cancer 1.2.2*) tells us that it is a multi-clonal disease, i.e. that every cancer tumor consists of many different clones, some less aggressive and some more aggressive. However, they all have mutations that make the cells likely to keep going through cell division despite these, and other, mutations. We hypothesize that if such instable genome was exposed to low-dose ionizing radiation this could further deteriorate the genome, making the

cancer more aggressive. Large doses of ionizing radiation, like the doses to the breast during breast cancer treatment, would introduce enough DNA damage to kill the cancer cells, which explain the reduced risk of local recurrence after radiotherapy. However, small doses, like the scatter doses to the contralateral breast, might, on the other hand, act as carcinogens, which explain the increased risk of CBC seen for certain patients subgroups after radiotherapy (for breast cancer patients overall no effect is seen, probably due to that most breast cancer patients are relatively old and a cancer initiated during radiotherapy would not have time to be clinically detectible during the patient's life time) (See *Background- Risk factors 2.1.2 and 2.2.2*), and also our finding of worse prognosis of short latency metachronous CBC after radiotherapy, without any decrease the incidence of CBC[153].

7.2.2 What can estrogen receptor status tell us?

Breast cancer is a heterogeneous disease and estrogen receptor (ER) status was one of the first measures of this. In Study III we investigate the concordance of ER-status between the two cancers of CBC-patients, and it is shown to be significantly higher than what would be expected by chance, i.e. if it would be entirely random which ER-status the tumor had. A high degree of ER-concordance in CBC tumors has been previously shown [141, 142, 144, 145], and this is believed to be due to host factors, which are still largely unknown. Previous studies have indicated that ER-positive and ER-negative breast cancers have different risk profiles[154, 155] and we have shown, in a related manuscript (under review), that the risk of ER-positive metachronous CBC after ER-positive first cancer is almost doubled, compared to the corresponding risk after ER-negative first cancer and a similar increase is seen for the risk of ER-negative CBC after ER-negative first cancer. However, it must be kept in mind that ER-status is not a constant feature, as shown by a recent study in which distant metastasis of ER-positive cancer often did not over express ER and, less certain, distant metastasis after ER-negative breast cancer gained ER over expression[156]. Also, it is well-known that many breast tumors consists of both ER-positive and ER-negative clones simultaneously, which invites hypothesizing about development of therapy resistance. Studies have shown an increased risk of ER-negative CBC after endocrine therapy[157], and in our related study there was an indication, though not significant, of this. Such effect would not be surprising, considering the above mentioned clonal hypothesis. A likely explanation is that for many breast cancer patients with endocrine therapy and a subclinical CBC, all clones in the subclinical CBC are ER-positive and are eliminated by the endocrine therapy of the first cancer, resulting in lower overall CBC incidence. However, in some cases, the subclinical CBC contains also ER-negative clones, which may be promoted by selective pressure of the endocrine therapy and emerge as an ER-negative CBC, instead of being a small ER-negative part of an ER-positive CBC. This might also be part of the explanation for the finding that distant metastasis have lost ER over expression; that it may have been an ER-negative clone that metastasized.

7.2.3 Is synchronous/metachronous CBC different diseases?

The studies in this thesis, and other related studies, have taught us that synchronous and metachronous CBCs behave very differently; they are different when it comes to

risk, prognosticators and prognosis, is it then valid to say that they are different diseases? It is true that they are different, but it is also true that we often see metachronous cancers of different latency time behaving very differently. I think it would be a mistake to simplify reality by subdividing CBC into (only) two sub groups. Instead I believe that we need to remember that a cancer is not a fixed entity; it develops and adapts to its environment, not only during cancer initiation, but also later in cancer development. If the second cancer has been exposed to adjuvant treatment of the first cancer, it will have adapted to this, if the cancer has developed during decreasing hormonal levels (e.g. due to menopause) it is likely that it has, in some fashion, adapted to this. Latency time is important to take into account; whether it is likely that the second cancer was sub clinically present in the breast when the woman was exposed will have implications for how likely an adaption is. So in conclusion; we do not see that metachronous and synchronous CBCs are unexpectedly or inexplicable different, rather we see that all cancers are affected by the environment, and that this needs to be taken into account in clinical practice.

7.2.4 How should metachronous CBC be handled?

As has been pointed out earlier in this thesis; as long as breast cancer survival continues to improve, metachronous CBC will be an important problem, the question is how to best handle it. The important aspects would be to; 1) do an accurate risk stratification at time of the first diagnosis, in order to 2) give adjuvant therapy to decrease the risk for women at high risk, and finally 3) if a CBC diagnosed, predict the prognosis accurately in order to give appropriate adjuvant therapy for the second cancer.

1) In study IV we investigate one possible factor for risk stratification at time of breast cancer diagnosis; mammographic density of the unaffected breast. This unfortunately did not turn out to be a useful risk predictor. In a related study we investigated whether ER-status of the first cancer was a risk factor for CBC, and, again, this could not be used as a risk predictor. This is a familiar disappointment for researchers investigating risk factors for CBC as, despite a large numbers of studies, only three risk factors have been consistently shown (see *Background –Risk factors 2.2.2*). However, in study IV, we discover a potential way of distinguishing women at low risk. We find that women who decrease in mammographic density between diagnosis and first follow-up mammogram (approximately 1.5 years later) are at significantly decreased risk, independent of treatment. Thus, if this finding is confirmed, women who have not experienced any decrease in mammographic density during the first period after diagnosis should either start, or switch, adjuvant therapy to decrease a risk that is known to be high. For women who experience a decrease, clinical follow-up routines could be modified and certain reassurance to decrease anxiety could be given. In Study I we conclude that the clinical work-up of CBC at time of the first breast cancer diagnosis has not improved over the last 30 years; the tumors are neither found earlier, nor at smaller size. Recent studies have explored the possibility of using sonography[90] and/or MRI[158] to diagnose synchronous CBC, this is however not an uncomplicated question in light of the

recent debate of over diagnosis of breast cancer[36, 159] and is unfortunately outside the scope of this thesis.
2) Chemotherapy and endocrine therapy both decrease the risk of CBC, in addition to the more important treatment rational; decreasing the risk of recurrence and distant metastasis. Radiotherapy also decreases the risk of local recurrence which, in turn, decreases the risk of distant metastasis. Its effect on CBC is however uncertain, it might increase the risk, especially among very young or genetically predisposed patients (See *Background-Risk factors 2.1.2* and *2.2.2*). The results from Study II indicate that subclinical cancers in the unaffected breast are sensitive to scatter doses of radiation and the unaffected breast should be protected from this. Luckily, several new techniques for giving radiotherapy with decreased scatter doses are now available[136].
While the side effects of chemotherapy are too severe to consider this treatment for the sole purpose of decreasing the risk of CBC, endocrine therapy could be a useful prevention therapy. It has been used for healthy women at high risk of breast cancer (e.g. women with a family history of breast cancer)[160]. Endocrine therapy has unfortunately been shown to have low compliancy, sometimes as low as 50%[161], most likely due to side effects that might be perceived as minor from an outside perspective but that are obviously difficult enough. This is naturally worrisome, especially since there have been suggestions in previous literature supporting the intuitive idea that women experiencing the most side effect are also the women benefiting the most from the treatment, at least for Tamoxifen (basically because they have the highest levels of active metabolite)[162].
3) Finally; if a CBC occurs despite the preventive strategies, how should it then be handled? At the moment clinically useful models to predict prognosis after metachronous CBC are lacking. Study II indicates that radiotherapy for the first cancer worsens the prognosis after CBC. Other studies have shown a worse prognosis after chemotherapy of the first cancer and a significantly improved prognosis after adjuvant radiotherapy of the second breast cancer[163], we could confirm both these findings in our data. In Study III we have shown that ER-status is not an important prognosticator, at least not in presence of endocrine therapy. The study however gives indications that in the absence of endocrine therapy we would see the same effect of ER-status for metachronous CBC as we do for synchronous CBC, where ER-status is a significant prognosticator. In summary; in addition to the known prognosticators for breast cancer among CBC-patients latency time also needs to be taken into account. Adjuvant therapy, both radiotherapy, chemotherapy and, for ER-positive second cancers, endocrine therapy worsens the prognosis after the second cancer if the latency time is short. If confirmed, this warrants a more aggressive therapy regimen than an unilateral breast cancer with similar traditional prognosticators.

7.2.5 Did CBC teach lessons about general breast cancer?

It is stated in the foreword of this thesis that a long term goal of studying CBC is to be able to make inference about breast cancer in general, and though all the studies in this thesis were aimed at specific questions of the clinical reality of CBC-patients, there is still one general and one specific observation that might be applicable to

breast cancer in general. As is discussed in some detail paragraph *7.2.3 Is synchronous/metachronous CBC different diseases?* several of the studies conclude that CBC-tumors are adaptable and there is no reason to believe that this would be confined to the second tumor of CBC-patients. Rather, these findings add the growing literature and understanding of the ever changing nature of (breast) cancer tumors.

One particular aspect of the findings in Study III might be of interest for trials of primary chemoprevention of breast cancer (i.e. treating healthy women with high risk of breast cancer in order to decrease their risk, the treatment usually given is endocrine therapy of some kind). In study III we show that ER-positive cancers that developed during endocrine therapy were significantly more aggressive than ER-positive cancers that did not develop under the selection pressure of endocrine therapy. This is likely applicable also for women without any previous breast cancer, so if healthy women are treated with endocrine therapy, this would decrease their risk for ER-positive breast cancer, but should an ER-positive breast cancer anyway occur (and it will, no treatment is fully protective) there is reason to believe that this cancer would be more aggressive than it would have been without the treatment. Obviously, this is not an argument against treatment of these women; it is a caveat regarding the prognosis.

8 CONCLUSIONS

Truth in science can be defined as the working hypothesis best suited to open the way to the next better one.

Konrad Lorenz (1903-1989), Nobel Prize laureate in medicine

The clinical work-up of contralateral breast cancer has not improved over the last 25-30 years; the second tumors are neither found earlier, nor at smaller tumor size now than in the 1970s.

Adjuvant radiotherapy for the first breast cancer seems to, if a second cancer is diagnosed within five years from the first, result in a more aggressive second cancer, manifested by twice the odds of worse tumor characteristics and twice the risk of distant metastasis.

Adjuvant radiotherapy for the first breast cancer does not seem to affect aggressiveness of second cancers diagnosed more than five years from the first.

Estrogen receptor status of both tumors combined has a significant impact on the prognosis after synchronous (simultaneous) contralateral breast cancer.

For metachronous (non- simultaneous) contralateral breast cancer estrogen receptor status is not a significant prognosticator, neither separately nor combined.

The lack of prognostic power of estrogen receptor statues in metachronous contralateral breast cancer might be due to patients with two positive tumors doing worse than expected, especially if the patients received endocrine therapy for the first cancer.

Mammographic density of the unaffected breast at time of the first breast cancer diagnosis cannot predict the risk of subsequent contralateral breast cancer.

Breast cancer patients who experience decreasing mammographic density from diagnosis of the first cancer until the first follow-up mammogram, have a significantly decreased risk of subsequent contralateral breast cancer, the risk for these women is about half that of women with stable mammographic density.

9 FUTURE PERSPECTIVE

Luke: What is in there?
Master Yoda: Only what you take with you…
The Star Wars movie *The Empire Strikes Back* (1980)

In breast cancer research in general I sense two shifts of perspective and a new recognition of a problem that was thought to be solved years ago. First; we are in the middle of learning the consequences of a nowadays often heard statement; "Breast cancer is not *one* disease". There are many ways to characterize breast cancer; to divide them into smaller groups, and the more we find out the finer this characterization might become, until we reach the level dreamed about by the advocates of 'personalized medicine'; every cancer is its own kind. Already today we might realize that this is the case, we know that the genomic changes giving rise to cancer is different in every tumor, but at the moment we do not know which are the relevant differences, and more importantly, we do not have enough treatment alternatives to make use of a full sub grouping of breast cancer. The second shift of perspective seems to be the realization that cancers do not stop evolving at diagnosis, and why would they? For a long time, recurrence and metastasis of cancer have been assumed to be very similar to the primary tumor, but a recent study showed that estrogen- and progesterone- receptor status, as well as HER-2 status, differ between the primary tumor and its metastases in over 30% of the cases[156]. Perhaps this phenomenon is part of the explanation of the bad survival of metastasized breast cancer patients; the treatment is fitted to the first tumor, while the cancer have had a long time to mutate further and might not have the characteristics of the primary cancer any longer. This is luckily a finding that can be immediately implemented by incorporating biopsy of metastatic lesions in clinical practice. More problematic is the question of breast cancer screening by mammography; whether screening decreases mortality of breast cancer is a well-studied question, but unfortunately also one with many inherited problems, which has opened the debate anew.
Recent studies show that the cumulative incidence of an unscreened control group did not reach that of the corresponding screened group[164], and that the decreased mortality seen in a screened population was also present in the unscreened age intervals, which lead the authors to conclude that the improved mortality was mainly due to overall improvements (e.g. treatment and health care infrastructure)[36] and not primarily to screening. The findings regarding the cumulative incidence are difficult to explain without a hypothesis of breast cancer tumor regression, which is in contrast to what was previously thought to be known about tumor development.

In CBC-research specifically there are two main questions we need to ask ourselves; in what ways might studies of CBC help to answer questions about breast cancer, and what resources are already available for studies that might improve the clinical reality of CBC-patients?

Women with long latency CBC have not been the focus of this thesis, , it is however intriguing why some women are obviously prone to get breast cancer (as they get two) but are also prone to survive. Studies of this kind require a very long follow-up time, which is probably why so few studies are done. Sweden is likely one of few countries in the world where such studies might be performed, since full coverage population-based cancer registers can be assumed to be a criterion. One of the major issues in breast cancer care today is to distinguish the women who are treated unnecessarily aggressive, and studies of long latency CBC survivors could aid in this discussion.

When it comes to resources available for CBC-research it might be worth mentioning the great effort that was made to collect paraffin-embedded tissue for a subset of the CBC-patients included in the cohort described in this thesis. Due to lack of infrastructure and routines this proved to be somewhat challenging, but for a small sample of CBC-patients with latency time less than 5 years we have collected tumor tissue of both tumors, as well as normal tissue for these patients. These samples have now been sequenced with the aim of characterizing the second tumors as true primaries or metastases, based on their similarities/differences in copy number variation. This is a question that has been asked from the very beginning of CBC-research and few have had the possibility to answer. We could add a study that has the most well-characterized and homogenous patient sample and with a sample size that is in range of the few previous studies published.

Another great effort for breast cancer research, that could also be useful for CBC, is the KARMA study, a nationwide prospective study of breast cancer, which today includes over 40 000 healthy women and will keep recruiting to at least 100 000 participants. Among these women, approximately 250 incident cases of breast cancer will be diagnosed per year; of these about 1% will have synchronous CBC. In addition, as time moves on, there will also be a number of metachronous CBC-patients. For these women a wide variety of information will be available, including tumor tissue, DNA and information on life style factors. Perhaps might a study in this setting help characterizing a risk profile for CBC, which is currently largely lacking.

In research we are never ever finished; new ideas need to be tested, findings need to be replicated, and then, perhaps most difficult of all; the findings that withstand testing need to be taken into the real world and be made common knowledge or used to change policies and guidelines. As has been pointed out throughout this thesis, CBC is a problem that will continue to increase in the future, as we get better at curing a) breast cancer and b) most other diseases.

10 AFTERWORD

The only thing that one really knows about human nature is that it changes. Change is the one quality we can predicate of it.

Oscar Wilde (1854-1900)

Something big is about to happen when it comes to cancer (and most other non-communicable diseases); far less people in the world are very poor, and one of the first consequences of this is that communicable diseases decrease, but unfortunately the non-communicable diseases take their place. The theory of health transition of populations states that poor populations, those who lack pure water, food and sanitation, will suffer mainly from communicable diseases, as the standard of living improves diseases as diabetes, cardiovascular disease and cancer will be the main causes of death. In the richer parts of the world tremendous success has been made with the mortality of cardiovascular disease and, to a lesser extent, cancer. These diseases may still not be curable, but in many cases we may live with cancer or diabetes, rather than die from it. Instead, degenerative diseases and autoimmune diseases, e.g. Alzheimer's and MS increase in incidence.

Already more than half of the cancer cases in the world, and an even larger proportion of the cancer deaths, occur in low- and middle-income countries and by year 2030 the absolute numbers of people affected in the low-income countries will have doubled[165]. In the richer part of the world we have come a long way in medical research, but it would be naïve to believe that findings made in a certain society, with certain norms, prevalence of risk factors, economic resources and genetic makeup can be translated directly into a population where all those factors are different. Using breast cancer as an example; most researchers agree that breast cancer screening by mammography decreases the mortality of breast cancer, this however requires women to go to a screening facility, where there must be trained staff to interpret the mammograms, and a non-negligible percentage of the women has to be recalled for further investigation. This is an obvious impossibility in many poorer countries, in both economical and infrastructure terms[166]. Further, once a breast cancer has been diagnosed in e.g. Sweden, the patient has both economical and structural possibilities for treatment, but obviously, the same opportunities cannot be offered by majority of poorer countries.

I believe that the big challenge for the future is to figure out which research findings from the high-income countries that can be applied to the rest of the world and to investigate the other factors in the countries where the burden of disease will be in the near future. Attempts to start cohorts to study non-communicable diseases in low-income countries are ongoing[167] and new methods are being developed, e.g. giving one dose of radiotherapy at the time of surgery, instead of a smaller dose during a long period[136], which would be a great improvement in countries where just *getting to the hospital* is a barrier against correct care. However, much work remains to be done, both with etiological and clinical research, and that is where I would put the money and the effort if I were a professor…

11 SVENSK SAMMANFATTNING

Ärans och hjältarnas språk! Hur ädelt och manligt du rör dig!
Ren är som malmens din klang, säker som solens din gång
Vistas på höjdernas du, där åskan och stormarna tala

Esaias Tegnér (1782-1846)

Målet med den här avhandlingen har varit att undersöka olika aspekter av dubbelsidig bröstcancer (CBC), vilket definieras som två oberoende bröstcancertumörer, en i vartdera bröstet. Det är en sjukdom som blir viktigare allteftersom dödligheten i bröstcancer sjunker medan antalet som insjuknar fortsätter att vara högt. Dessa två faktorer leder en stor grupp sk. bröstcanceröverlevare, vilka har hög risk att drabbas av ytterligare en bröstcancer, i det motsatta bröstet. En ytterligare anledning att studera den här sjukdomen är att dödligheten för både samtidig och ickesamtidig (metakron) dubbelsidig bröstcancer är betydligt högre än för kvinnor med bara en bröstcancer.
Alla studier i den här avhandlingen använder sig av en populationsbaserad grupp av patienter, nämligen alla 1422 CBC-patienter som diagnosticerats i Stockholms Läns landsting under åren 1976-2005.
I den första studien, i vilken vi undersöker den diagnostiska upparbetningen av den andra brösttumören, kan vi dra slutsatsen att den andra tumören inte upptäcks vare sig tidigare eller när den är mindre nu för tiden, jämfört med för 25-30 år sedan. I den andra studien undersöker vi effekten av adjuvant (förebyggande) strålning för den första cancern och ser att den tycks leda till mer aggressiva tumöregenskaper för den andra cancern och sämre överlevnad, i de fall en andra cancer uppkommer. Genom att analysera östrogenreceptorer i de två tumörerna hos kvinnor med dubbelsidig bröstcancer visar vi, i vår tredje studie, att östrogenreceptorer för båda tumörerna har viktig inverkan på överlevnaden för samtidig CBC. För metakron CBC, å andra sidan, tycks kvinnor med två östrogenpositiva tumörer har lika dålig överlevnad som kvinnor med två östrogennegativa tumörer. Indikationer visar att detta kan bero på ökad aggressivitet som följd av anti-hormonell (endokrin) behandling för den första cancern. I den fjärde och sista studien undersöks mammografisk täthet (mängden bindväv och körtelvävnad i förhållande till fett på en mammografibild) som en riskfaktor för dubbelsidig bröstcancer. Vi utvärderar både mammografisk täthet vid diagnos av den första cancern och förändring av tätheten från den första diagnosen till den första uppföljningsmammografin. Vi hittar ingen effekt av mammografisk täthet vid diagnos men däremot; för de kvinnor som har en minskning i täthet ser vi också en minskad risk för dubbelsidig bröstcancer till ungefär hälften av risken för kvinnor med oförändrad täthet.
Implikationerna av dessa studier är, förutom att de bör replikeras i oberoende populationer eftersom resultaten alla är visade för första gången här, att förändring av mammografisk täthet bör vägas in när behandling för den första cancern bestäms samt att denna behandling bör tas med i beräkningen av aggressiviteten av en andra cancer, speciellt om tiden mellan cancrarna är kort.

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EPIDEMIOLOGY

Diagnostic work-up of contralateral breast cancers has not improved over calendar period

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Abstract Women who have been treated for breast cancer are typically followed up with regular mammography and palpation, with the aim of detecting recurrences and contralateral breast cancer (CBC). This study aims to investigate if the diagnostic work-up of breast cancer patients has improved over the last 25 years and resulted in earlier diagnoses of CBC. Two population-based cohorts were used; all CBCs in Sweden 1976–2004 (*n*: 2932), and all CBCs in Stockholm, Sweden, 1976–2005 (*n*: 626), both cohorts with a maximum of 3 years between the two cancers. Synchronous CBC was defined as two cancers <3 months apart, the remainder was defined as metachronous CBC. We calculated the odds ratio of being diagnosed synchronously, relative to metachronously, using logistic regression, adjusting for whether the second cancer was detected through clinical work-up or not. The odds of synchronous CBC were significantly increased: 1.27 (95% CI, 1.13–1.42) per 5-year period, compared to metachronous, and was not affected by detection mode, but seemed to be explained by adjuvant therapy. The proportion of CBCs detected by clinical work-up did not increase over the study period, and the mean size of the second tumor remained constant. We found an increase in the proportion of synchronous CBCs compared to metachronous, over calendar period, a change that was not associated with clinical work-up, but with adjuvant therapy. This study gives no indications that any improvement in diagnostic work-up of CBC have occurred over the last 25 years.

Keywords Contralateral breast cancer · Diagnostic work-up · Time trends · Mammography

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Background

A primary breast cancer in the opposite breast, contralateral breast cancer (CBC), is diagnosed in 1–2.6% of all women with breast cancer within 3 months of their first diagnosis, this subtype is referred to as synchronous [1–4]. CBCs diagnosed more than 3 months after primary diagnosis has a stable incidence of 0.5% per year throughout life and are referred to as metachronous [5–8].

As we have previously shown, the incidence of synchronous CBC has increased considerably during the 1970s and 1980s, while the incidence of metachronous CBC has decreased steadily during the past 30 years [7]. Possibly, this pattern reflects a shift towards earlier diagnosis of existing tumors, making cancers formerly classified as metachronous to be diagnosed earlier and consequentially fall within the 3-month definition of synchronous CBCs. Such a shift could have occurred due to improved diagnostic work-up and more intense follow-up of women with breast cancer, e.g., the opposite breast is examined more

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carefully and/or more often after a breast cancer diagnosis at present compared to earlier decades. An improvement like this could have several possible reasons: introduction of population-wide mammography screening [9, 10], improvements in mammographic technique [11], and implementation of nationwide follow-up routines of breast cancer patients. Also, it can not be excluded that increased public awareness of breast cancer has contributed. Another major change in the management of breast cancer during the last 25 years is the increased use of systemic adjuvant treatment [12–15], which may have decreased the incidence of metachronous CBC, thus further contributing to the observed incidence pattern.

The aim of the present study is to investigate to what extent improvements in the diagnostic work-up of breast cancer patients and change in use of systemic adjuvant therapy has contributed to an increasing proportion of synchronous CBCs.

Methods

Study population

The study population consists of women with CBC, we have restricted the group to patients with a maximum of 3 years between the diagnosis of their first and second breast cancer. The 3-year time limit was chosen arbitrarily, assuming that if the second cancer was diagnosed within 3 years, it was possible that it was present, though undetected, also at time of the first diagnosis. Patients with other malignant disease before or between the breast cancers were excluded. In one of the two cohorts used, where we also had the possibility to assess TNM stage, we did not include patients that had any of their cancers diagnosed in Stage IV. This was done to minimize the risk of the CBC being a metastasis of the first breast cancer, since it is not possible to assess from which cancer the metastasis originate from.

The two cohorts were assembled from population-based registers: the first cohort comprising all women with CBC in Sweden from 1976 to 2004, as ascertained from the nationwide and virtually complete Swedish Cancer Register [16, 17]. We retrieved information on dates of birth and cancer diagnosis for all patients. We refer to this as the national cohort.

We also established a second cohort, with more detailed information, consisting of all women with CBC diagnosed within 3 years between 1976 and 2005 in Stockholm county. The study participants in this cohort were selected from the Regional Oncological Center in Stockholm, where all new breast cancer cases in the Stockholm county health care region are recorded. The register, which covers a health care region of 1.9 million people, corresponding to approximately 20% of the Swedish population, provided us with information on date of birth, dates of diagnosis, stage and size of the tumors, as well as follow-up information for the patients. Below, we refer to this as the Stockholm cohort. For this cohort we also retrieved the medical records from the different oncology clinics where the patients were treated, and extracted information on detection mode of the two cancers, adjuvant therapy given for the first cancer and additional tumor characteristics. We also confirmed the diagnosis by reviewing all pathological reports.

For the purpose of calculating the proportion of breast cancer patients that develop CBC within 3 years of their first cancer, we used the Swedish Cancer Registry mentioned above, selecting all patients with primary breast cancer during 1976–2001 and excluding patients with other second malignancies except CBC. The reason for ending the study period at 2001 was to be able to calculate the proportion of women that develop CBC within 3 years, with full follow-up of all cases.

In the Stockholm cohort, mode of detection of the CBC was classified into four groups: (a) clinical work-up, which included CBCs detected through the follow-up program, by either mammography or palpation performed by health care personal, (b) self-palpation performed by the patient, (c) the national breast cancer screening program [10, 18], or (d) unspecified. The main comparison for our analysis: clinical work-up (a) versus the other modes (b–d), was expressed as binary variable 'clinical work-up' (yes/no). For further analysis, we also constructed an alternative binary variable 'clinical work-up including mammography screening' (yes/no), which contrasts modes a + c against b + d. Important to note in this context is that a woman with a primary breast cancer will not participate in the screening program for at least 5 years, during which she instead will be followed up by clinical mammography and breast palpation.

Adjuvant therapy can be given in a number of combinations, for the purpose of the analysis we have made three different comparisons. Firstly, we compared all CBCs patients that received any systemic adjuvant therapy for their first breast cancer (this includes hormone therapy, chemotherapy, or a combination of the two) with all who did not receive any such therapy (this group include patients who received radiotherapy only and those who did not receive any adjuvant therapy). The second comparison was made between patients who received chemotherapy (alone or in combination with any other adjuvant therapy) compared to those who did not. The third comparison was made between patients who received hormone therapy (alone or in combination with any other adjuvant therapy) and patients who did not receive hormone therapy.

Analysis

We used Spearman correlation to evaluate the possible correlation between latency time (time between cancers) and calendar period of first diagnosis and also between tumor size and calendar period of diagnosis. In the tumor size analysis we excluded all CBCs diagnosed on the same day, since in those 'first' and 'second' cancer can not be defined. Logistic regression was used to test for any trend over calendar period in the proportion of latency groups, the proportion of cancers diagnosed on the same day and the proportion of cancer diagnosed by clinical work-up.

To investigate the change in proportion of synchronous versus metachronous cancers over calendar period we used logistic regression and calculated the odds ratio, i.e., the odds that a cancer from the CBC-cohort was diagnosed synchronously as compared to metachronously. The logistic regression model included age at first diagnosis in 10-year groups, as a possible confounding factor. To asses the impact of each of the confounding factors on the changing proportion of synchronous/metachronous cancers, we sequentially adjusted our model for mode of detection of the second cancer and adjuvant therapy for the first cancer.

To investigate if the effect of calendar period on the timing of the CBC was different for younger/premenopausal women, compared to older/postmenopausal women we included an interaction term between calendar period and menopause status (defined as above/below 50 years of age, which is the mean age for menopause [19]). To allow for equal follow-up for all CBC patients we, in all analyses, excluded those that were diagnosed with their first cancer in the last 3 years of the follow-up period in both cohorts.

All data preparation and analysis was done using SAS statistical Package 9.1. This study was approved by the regional ethical review board at Karolinska Institutet, Stockholm, Sweden.

Findings

For the national cohort, we identified 2932 CBC patients diagnosed between 1976 and 2004. 58.1% were diagnosed synchronously (Table 1). The Stockholm cohort consisted originally of 691 CBC patients, 65 of whom were excluded either due to failing to fulfill inclusion criteria at medical records revision or that their medical record could not be found, thus leaving 626 cases in the cohort. 64.7% of the CBC patients were diagnosed synchronously and 68.6% had their second cancer diagnosed through clinical work-up, for 7% the mode of detection was unspecified.

In the national cohort, 3–4% of all breast cancer patients suffered a CBC within 3 years from their first diagnosis, this proportion remained constant over the calendar period. Among women with CBC, the proportion of synchronous cancers increased during the study period, constituting 49% of patients with their first cancer diagnosed in 1976–1978 and 67% of patients diagnosed at the end of the study period ($P < 0.0001$) (Fig. 1).

Table 1 Characteristics of the study cohorts

	National cohort	Stockholm cohort
Number of CBC patients	2932	626
Study period	1976–2004	1976–2005
Mean age at first breast cancer (years)	64.6	63.9
Median number of latency days	21	15.5
Synchronous CBC (%)	58.1	64.7
Number of CBC patients (%) in each period		
1976–1978	295 (10.1)	39 (6.2)
1979–1981	345 (11.8)	75 (12.0)
1982–1984	323 (11.0)	55 (8.8)
1985–1987	346 (11.8)	56 (8.8)
1988–1990	372 (12.7)	68 (10.9)
1991–1993	304 (10.4)	72 (11.5)
1994–1996	345 (11.8)	86 (13.7)
1997–1999	364 (12.4)	87 (13.9)
2000–2001	238 (8.1)	58 (9.3)
2002	–	31 (5.0)
CBC clinically detected (%)	–	68.6
First cancer treated with adjuvant therapy (%)	–	63.8

We found no significant correlation between latency time (number of days) and calendar period of first cancer ($P = 0.20$). Furthermore, the proportion of CBCs with both cancers diagnosed on the same day was similar through the period, ranging from 37% in 1976–1978 to 45% in 2000–2002 ($P = 0.41$).

For more in-depth analysis, we used the Stockholm cohort which, as expected, comprised approximately 20% of the national cohort. The risk of being diagnosed synchronously, as compared to metachronously, expressed as odds ratios, was statistically significantly associated with calendar period, OR = 1.27; 95% CI: 1.13–1.42, per 5-year period (Table 2). The association between calendar period and synchronous diagnosis persisted when we adjusted for mode of detection of the second cancer. However, adjusting for adjuvant therapy for the first cancer decreased the marked calendar period effect to a non-significant level (OR = 1.04; 95% CI: 0.90–1.20). We found furthermore that, when adjusting for chemotherapy and hormone therapy separately, the effect of calendar period

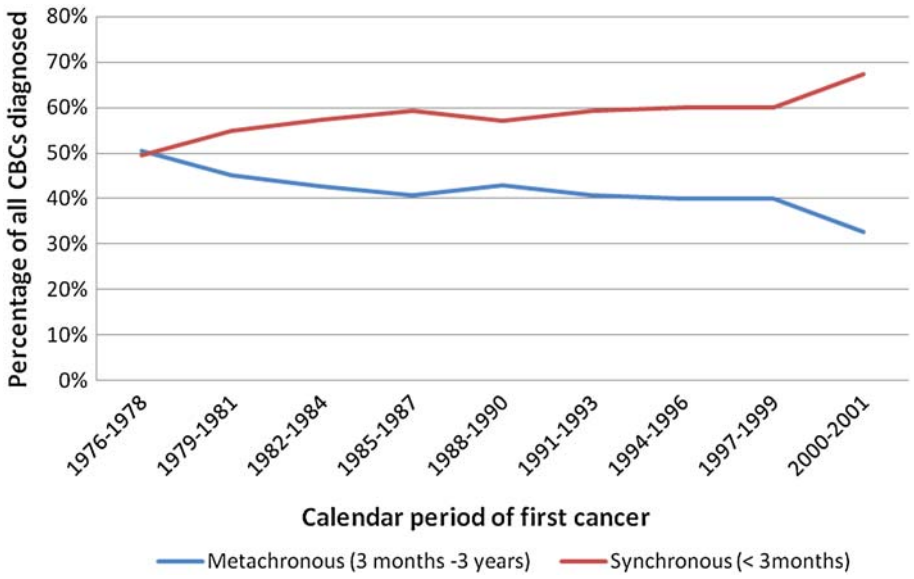

Fig. 1 Proportion of CBCs per latency category and calendar period of first diagnosis. Calculations for this graph use the national cohort. Note that these curves are each other's complements and add up to 100%

Table 2 Odds ratio estimate of being diagnosed synchronously, per 5-year period

	OR estimate	95% CI
OR	1.27	1.13–1.42
OR adjusted for mode of detection of second BC		
Clinical work-up	1.22	1.08–1.37
Clinical work-up including mammography screening	1.23	1.10–1.38
OR adjusted for adjuvant treatment of first BC	1.04	0.90–1.20
Hormone therapy vs. no hormone therapy	1.02	0.89–1.17
Chemotherapy vs. no chemotherapy	1.24	1.11–1.40

All odds ratios are adjusted for age at first diagnosis in 10-year groups. This analysis is preformed on the Stockholm cohort

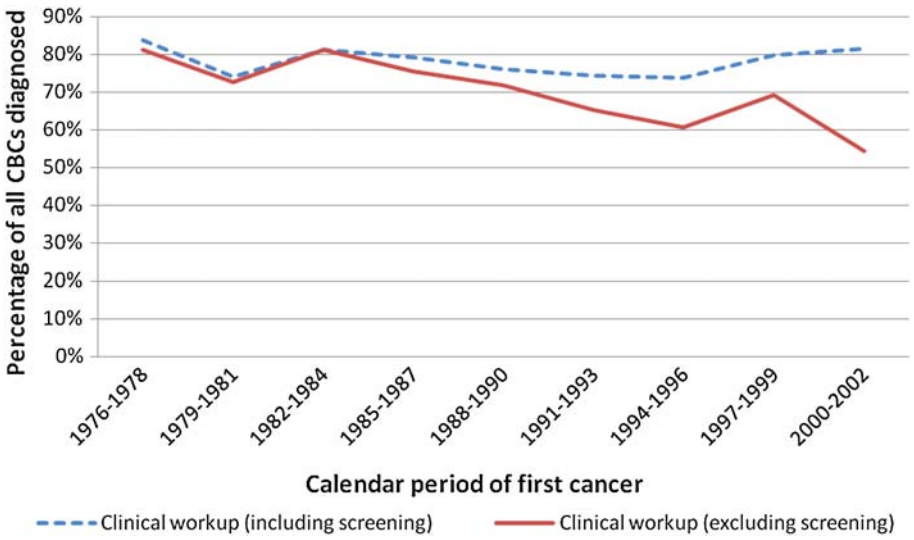

Fig. 2 Proportion of CBCs detected by clinical work-up over calendar period of first diagnosis. Calculations for this graph use the Stockholm cohort

was associated with hormone therapy, but not with chemotherapy. In addition, we found no significant interaction between menopause status and calendar period ($P = 0.23$).

When investigating the proportion of CBCs detected by clinical work-up over calendar period of the first cancer, we could not detect any increasing proportion neither when excluding, nor including, mammographic screening as part of the diagnostic work-up (Fig. 2). Using logistic regression we did not see any trend when including mammographic screening as part of the clinical work-up

Table 3 Mean tumor size (mm) in relation to calendar period of diagnosis

Calendar period of diagnosis	Mean size of first cancer	95% CI	Mean size of second cancer	95% CI
1976–1978	20.8	16.2–25.5		
1979–1981	22.5	19.6–25.3	15.0	11.6–18-3
1982–1984	23.9	18.2–29.7	12.9	10.3–15.6
1985–1987	24.1	18.9–29.3	12.3	9.14–15.5
1988–1990	23.7	17.1–30.3	15.0	11.7–18.4
1991–1993	22.6	15.3–29.9	18.3	12.9–23.7
1994–1996	23.0	19.1–26.9	14.8	12.3–17.3
1997–1999	24.1	19.2–29.0	12.8	10.7–14.9
2000–2002	25.9	17.8–33.9	14.8	11.7–17.8
2003–2005			17.7	10.2–25.1

Mean is calculated for first cancers in the calendar period of first diagnosis and for second cancers in the period of second diagnosis. This analysis is preformed on the Stockholm cohort. *CI* confidence interval

($P = 0.89$), and when not including mammographic screening, we found a significant decreasing trend ($P = 0.0003$).

Finally, as an alternative exploration of whether the sensitivity of the diagnostic work-up has improved, we compared the size of the second tumors across calendar period in the Stockholm cohort. The correlation analysis showed no association ($P = 0.84$) between size of the second tumor and calendar time of the diagnosis of the second cancer. The mean sizes ranged from 15.0 mm (95% CI: 11.6–18.3) in 1979–1981 to 17.7 mm (95% CI: 10.2–25.1) in 2003–2005 (Table 3). The analysis was repeated for synchronous and metachronous cancers separately, in neither of these groups could any decrease in size of the second tumors be shown (P-synchronous $= 0.63$; P-metachronous $= 0.78$). Also in the corresponding analysis of first tumor size and calendar time no association was shown.

Interpretations

Our original hypothesis was that the diagnostic work-up of breast cancer patients has improved over the study period, thereby shortening the latency time between the first and second cancer. We found support for this hypothesis in an increased proportion of synchronous cancers and corresponding decreased proportion of metachronous cancers (Fig. 1). Also, logistic regression analysis revealed that odds ratio of being diagnosed with synchronous CBC, compared to metachronous, significantly increased during the study period. However, we found that including clinical work-up in our regression model could not account for the increase in synchronous CBC over time, while including adjuvant therapy, and specifically hormone therapy, could. CBCs were not more likely diagnosed through clinical work-up later in the study period compared to the earlier years and the average tumor size of the second breast cancer has not decreased. Furthermore, we found no correlation between latency time and calendar period of first cancer.

In the Stockholm cohort, the odds of being diagnosed synchronously compared to being diagnosed metachronously has increased by 27% every 5 years (OR = 1.27 95% CI: 1.13–1.42). If this odds ratio was driven by improved clinical work-up, i.e., that the opposite breast is more closely or better examined after a breast cancer diagnosis at present compared to earlier, one would expect it to diminish when controlling for mode of detection, this was not the case in our study (Table 2). Instead, the increasing proportion of synchronous CBC is likely to be explained by adjuvant therapy for the first cancer. We find two possible explanations. Firstly, the use of adjuvant therapy for the first cancer has increased over calendar period [12, 13] and has been shown to decrease the risk of metachronous CBC. In agreement with our findings, some studies have reported that adjuvant hormonal therapy decreases the risk for CBC more than adjuvant chemotherapy [20, 21]. The alternative explanation is that with calendar period, the probability of receiving adjuvant therapy for the first cancer increased to a larger extent for patients with synchronous CBC than for unilateral breast cancer patients (the population at continued risk for metachronous CBC), thus creating an association between being diagnosed synchronously and receiving adjuvant therapy. In our material we see a tendency towards this uneven distribution of adjuvant therapy.

Clinical work-up through mammography might be less efficient for younger women, due to the reduced sensitivity in dense breasts [22, 23], this has been investigated in several studies, with conflicting results [24, 25]. This particular group of young women is also at higher risk of CBC [5]. However, we saw no difference between age groups in our finding of no association of clinical work-up with the increased proportion of synchronous CBCs.

The observed lack of improvement in clinical work-up could possibly be explained by the fact that clinical mammography already early had a high capacity and was frequently used in the breast cancer follow-up. Still, we believe other strategies for follow-up of the opposite breast of breast cancer patients might be considered. MRI and ultrasound examination are currently considered the most promising alternatives [26], with ultrasound having a higher specificity and sensitivity than mammography [27] and MRI is most likely even more efficient, but expensive

[28]. Both MRI and ultrasound have been used in studies of high risk populations (breast cancer patients and close relative of breast cancer patients), showing that they are both efficient in these particular setting [27, 28]. At present, in Sweden, both methods are used only to further investigate breast abnormalities found by mammography or breast palpation.

It has been shown earlier that tumor size is closely associated with mode of detection [29]. In our study the mean tumor size at diagnosis remained constant throughout the study period, supporting the notation that clinical work-up has not improved (Table 3). For comparison, we also show mean size of the first tumor of the CBC patients. It is known from earlier studies that the second cancer tend to be of smaller tumor size than the first cancer, likely due to that they have a different pattern of detection mode [29]. Still, we find the lack of decrease for size of the first tumor somewhat surprising, but possible explanations for this finding lies outside the scope of this paper.

While this investigation was based on the analysis of changing distributions of synchronous and metachronous CBC, it would have been possible to address the question of a changing pattern of CBC over time also by the analysis of incidence patterns. However, such an analysis would be both technically difficult and challenging to interpret because the population at risk is different for synchronous CBC (healthy women) and metachronous CBC (unilateral breast cancer patients). Even with the strategy we choose—an analysis of changing distributions—it would have been possible to express these distributions as a proportion of all breast cancers or as a proportion of all CBCs. Since our primary interest was the CBC-cohort we use the proportion of all CBCs as our main measure. Comfortingly, as the proportion of breast cancer patients that develop CBC within 3 years remained approximately constant over period, these two approaches should not differ. Strengths of our study include the population-based selection of cases, the full coverage of health care registers, which has allowed complete identification and follow-up of CBCs, as well as the almost complete retrieval of medical records (retrieval rate 98%). We also measure effectiveness of diagnostic work-up in two ways; firstly, all CBCs are categorized as either diagnosed by clinical work-up or not, secondly, we assess the time between the first and second cancer and size of the second cancer. These two measures are independent and assess different aspects of the success of the mode of detection; however, it seems pertinent to point out that neither of these measures can reflect the full complexity of diagnosing CBC. While we did adjust for mode of detection in the analysis of the association between synchronous CBC and calendar period, due to the structured guidelines for follow-up of breast cancer patients, there may be a more complicated interplay between timing of the second cancer and the mode of detection. Other limitations of this study are the limited sample size in the Stockholm cohort where we had access to complete information on mode of detection, tumor characteristics and treatment, and also the relatively large proportion of cases with unknown tumor size (14%).

We conclude that the change in proportion of latency groups can seemingly not be explained by earlier detection and a consequential shift from metachronous to synchronous CBCs. A more plausible explanation therefore seems to be a decreasing incidence of metachronous cancer [7] possibly due to adjuvant therapy for the first cancer. The change of latency proportions can be further explained by the increasing incidence of synchronous cancer during the 1970s and 1980s in Sweden [7]. This increase could be in agreement with increasing incidence of unilateral breast cancer. We have shown that the proportion of CBCs detected by clinical work-up is the same now as 25 years ago, and the second breast cancer is neither diagnosed any earlier, nor at any smaller tumor size. This might imply that the diagnostic work-up of CBCs has not improved significantly.

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Aggressiveness of contralateral breast cancer is influenced by radiotherapy for the first tumor

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Short title: Aggressiveness of CBC after radiotherapy for first breast cancer

Key words: contralateral breast cancer (CBC), adjuvant radiotherapy, prognosis, tumor characteristics, cancer epidemiology

Abstract

We aimed to investigate if characteristics of contralateral breast cancer (CBC) are influenced by adjuvant radiotherapy for the first breast cancer. Using information from population-based registers and medical records we analyzed two cohorts comprising all women with CBC diagnosed >3 months after their first cancer (809 patients in Stockholm 1976-2005 and 750 patients in South Sweden 1977-2005). We used Poisson regression to calculate risk of distant metastasis after CBC, comparing patients treated and not treated with radiotherapy for the first cancer. Logistic regression was used to estimate odds ratio of more aggressive tumor characteristics in the second cancer, compared to the first. For CBC-patients in Stockholm with < 5 years between the cancers radiotherapy for the first cancer conferred a nearly doubled risk of distant metastasis [IRR=1.91 (95% CI: 1.27-2.88)], compared to those not treated with radiotherapy. This was replicated in the South Swedish cohort (IRR=2.12 [95% CI: 1.40-3.23]). In Stockholm we found an increased odds that, following radiotherapy, a second cancer was of more advanced TNM-stage [OR 2.14 (95% CI: 1.12-4.07)] and higher histological grade [OR=2.14 (95% CI: 1.14-3.99)] compared to the first, for CBC-patients with < 5 years between the cancers. No effect on any of the investigated outcomes was seen for patients diagnosed with CBC > 5 years from the first cancer. In conclusion; patients diagnosed with CBC within 5 years had worse prognosis, and more aggressive tumor characteristics of the second cancer, if they had received radiotherapy for their first cancer, compared to no radiotherapy.

Novelty & Impact: While *risk* of contralateral breast cancer (CBC) after radiotherapy has been extensively investigated, the present study is the first to investigate whether *prognosis* after CBC is affected by radiotherapy. The study shows worse tumor characteristics and prognosis for women treated by radiotherapy for the first cancer (whereas no effect is seen for radiotherapy after the second cancer). If these finding are confirmed it has important implications, both for treatment decisions and for understanding tumor genesis.

Introduction

Approximately 10-15% of all breast cancer patients will be diagnosed with contralateral breast cancer (CBC) during the first 20 years after initial diagnosis[1, 2]. The prognosis of women with CBC has been shown to be worse than for patients with unilateral disease[3, 4]. Several studies have shown that CBC prognosis is highly dependent on time between first and second cancer[3, 5]; women diagnosed with CBC within 5 years from the first cancer have a particularly bad prognosis.

Prognosticators for CBC has not been studied in detail but seem to include tumor characteristics of the first and second cancer, age at diagnosis of first cancer and time interval between the first and second cancer[6, 7].

Radiotherapy is well known to protect against local recurrence and improve breast cancer specific survival[8, 9]. In Sweden approximately 60% of all breast cancer patients receive adjuvant radiotherapy[10]. The common dose of 48-50 Gy following surgery[11] have been very similar from 1980s' until the end of the study, and somewhat lower during the 1970's (29-45Gy)[12], such a dose entails scattered radiation to the contralateral breast, estimated to 2-3 Gy[13-15] or about 5% of the dose to the treated breast[16]. Because such doses may be carcinogenic, the risk of CBC following adjuvant radiotherapy for breast cancer has been studied for several decades with inconclusive results[2, 9, 13, 15]. However, no studies have investigated if prognosis after the second cancer and/or malignant features of the second cancer is associated with adjuvant radiotherapy for the first cancer.

We aimed to investigate the hypothesis that radiotherapy for the first cancer enhances the malignant features of the second cancer in the increasingly large group of CBC patients. To achieve this aim we analyzed two large, population-based cohorts of CBC patients for which we had accessed all medical records comparing all patients treated with radiotherapy to all patients not treated. Features of the contralateral cancer were measured in three ways; the risk of distant metastasis after CBC, the odds that the second cancer had more advanced TNM-stage and the

odds that the second cancer had higher histological grade at the second cancer, compared to the first.

Methods

Study population

The population-based Stockholm Breast Cancer Register records all incident breast cancers diagnosed in the Stockholm health-care region (a catchment area of 1.5 million inhabitants) since 1976 and contains information on date of birth, date of diagnosis of both tumors, TNM-stage and location of the tumors.

From the Stockholm Breast Cancer Register we selected all patients with CBC diagnosed more than 3 months after the first invasive breast cancer. Participants had to be female and diagnosed with both cancers within the Stockholm health-care region between the years 1976 and 2005. Both cancers had to be diagnosed in stage I-III with no other recorded malignancy before diagnosis of the second cancer. We excluded patients with stage IV breast cancers or patients with other prior malignancy to minimize the risk that metastatic disease was misclassified as a primary contralateral cancer. Altogether 936 patients fulfilled these inclusion criteria. Follow-up continued until date of distant metastasis, date of death or December, 31, 2006, whichever came first.

We reviewed the oncological medical records and the pathology reports from 99% of all eligible patients. We extracted additional information that was not available in the register (information on histological grade and adjuvant therapy for both cancers). For 109 patients (12%) the review of medical record and pathology reports revealed that the inclusion criteria were not met and they were excluded from the cohort; the majority of these, 77 (8%) had distant metastasis before diagnosis of the second breast cancer. For 10 cases (1%) the medical record could not be retrieved and they were also excluded. Finally, we also excluded 8 patients due to unknown treatment for first breast cancer. The final study cohort consisted of 809 patients with CBC.

To validate our results we analyzed a second cohort of CBC-patients. The South Swedish cohort included all women in the Southern health-care region of Sweden (a catchment area of 1.7 million inhabitants) diagnosed with the second primary breast cancer between 1977 and 2005. These patients were identified from the South Swedish Breast Cancer Register, and the surgical and oncological medical records as well as the pathology reports were reviewed, according to the same criteria as the Stockholm cohort. However, in the South Swedish cohort information on histological grade was available for less than one third of the patients, and no information was available on tumor stage. The final cohort consisted of 750 patients with information on treatment of their first breast cancer, age at first diagnosis, calendar period of first diagnosis and date of diagnosis of distant metastasis, death or date of end of follow-up.

Analyses

Our analytical approach was to investigate the risk of distant metastasis after CBC and the odds that the second cancer had more advanced TNM-stage or higher histological grade compared to the first (Figure 1). All CBC patients treated with adjuvant radiotherapy were compared to all CBC patients not treated with adjuvant radiotherapy, regardless of systemic adjuvant treatment such as endocrine- or chemotherapy. In our secondary analyses, we compared all patients treated with adjuvant radiotherapy only to patients not receiving any adjuvant therapy. Consistently we calculated our estimates for CBCs overall, and then separately for CBCs occurring within and beyond 5 years from the first cancer, by including interaction terms.

We used Poisson regression to estimate the incidence rate ratio (IRR) as a measure of risk, of distant metastasis following the second cancer, comparing patients treated to those not treated with adjuvant radiotherapy. The risk estimates were adjusted for calendar period (in 5-year categories) and age at diagnosis (in 10-year categories) of the first cancer, as well as histological grade and TNM-stage of both cancers, adjuvant therapy of the second cancer (radiotherapy, chemotherapy and endocrine therapy and combinations thereof) and time interval between the first and second cancer (latency time; ≤5 years and >5 years). The underlying time scale was time since the

Table 1 Characteristics of the patients in the Stockholm cohort and South Swedish cohort, showing the proportion exposed adjuvant radiotherapy for the first cancer, and the total cohort.

	Stockholm cohort				South Swedish cohort			
	Adjuvant radio-therapy treated (%)		**Total (%)**		**Adjuvant radio-therapy treated (%)**		**Total (%)**	
Number of patients	418		809		462		750	
Mean age (years) and SE	54.9	0.54	56.8	0.43	54.9		57.8	
TNM-stage of first cancer*								
I	239	58%	404	51%	-	-	-	-
II	135	33%	328	41%	-	-	-	-
III	39	9%	60	8%	-	-	-	-
Histological grade of first cancer*								
Low	40	14%	71	14%	-	-	-	-
Intermediate	113	39%	214	41%	-	-	-	-
High	138	47%	239	46%	-	-	-	-
Calendar period of first cancer								
≤ 1970	-	-	-	-	59	13%	62	8%
1971 - 1975	-	-	-	-	45	10%	55	7%
1976 - 1980	50	12%	134	17%	47	10%	88	12%%
1981 - 1985	69	17%	180	22%	85	18%	134	18%
1986 - 1990	92	22%	183	23%	84	18%	139	19%
1991 - 1995	108	26%	173	21%	77	17%	156	21%
1996 - 2000	76	18%	104	13%	50	11%	93	12%
2001 - 2005	23	6%	35	4%	15	3%	23	3%
Latency time								
< 5 years	196	47%	370	46%	171	37%	318	42%
> 5 years	222	53%	439	54%	291	63%	432	58%

second cancer. We further included an interaction term in the model, allowing the effect of adjuvant radiotherapy to vary over latency time.

Logistic regression was used to estimate the odds ratio (OR) of a more aggressive second cancer, adjusted for calendar period of first diagnosis (in 10-year categories), age at first diagnosis (in 10-year categories) and latency time, contrasting radiotherapy-treated patients to non-treated patients. Our outcomes of interest were more advanced TNM-stage at the second cancer, compared to the first and higher histological grade at the second cancer, compared to the first. If the first cancer was diagnosed in stage III it is not possible to have a more advanced stage of the second cancer. To accommodate this, we also defined our outcome as being diagnosed with the highest stage at both cancers. The same definitions were used for the analysis of histological grade.

All data preparation and analyses were done using SAS Statistical Package 9.2 and Stata 10.1. The studies were approved by the ethical committees at the Karolinska Institutet and Lund University, Sweden. The funding sources of this study had no part in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Descriptive data

Mean age at first diagnosis among the 809 CBC patients in the Stockholm cohort was 56.8 years, which was very similar to the 750 patients in the South Swedish cohort; 57.8 years (Table 1). The average follow-up time after second breast cancer diagnosis was 6.5 years (5.8 years for patients treated with radiotherapy and 7.3 years for patients without

radiotherapy treatment) in the Stockholm cohort and 6.2 years (6.0 years for radiotherapy treated patients and 6.4 years for untreated patients) in the South Swedish cohort. Overall, approximately half of the CBC-patients

received adjuvant radiotherapy (52% in Stockholm and 62% in the south of Sweden). Distant metastasis after the second breast cancer diagnosis were diagnosed in

210 patients (25%) and 226 patents (30%) in the Stockholm and South Swedish cohort respectively.

Risk of distant metastasis

In the Stockholm cohort, the risk of distant metastasis following the second breast cancer diagnosis was 43 % higher (IRR 1.43 [95% CI 1.04-1.95]) among patients who received radiotherapy as part of their first adjuvant treatment, compared to patients who did not receive radiotherapy (Table 2). This excess risk decreased with follow-up time as indicated by a significant interaction (P=0.02) between radiotherapy and latency time between the first and the second breast cancer. When the CBC was diagnosed within 5 years, radiotherapy increased the risk of distant metastasis by 91% (IRR 1.91 [95% CI: 1.27-2.88]), whilst no excess risk was seen when the time interval exceeded 5 years. The risk estimates did not change substantially when we restricted adjustments to calendar period, age at first diagnosis and latency time (Table 2). Our findings were confirmed when analyzing the south Sweden cohort (Table 2);. Further, the Stockholm cohort and south

Table 2 Incidence rate ratio of distant metastasis after CBC. Estimates calculated for both effect of adjuvant radiotherapy for all CBC-patients and the effect in each latency group (>5 years and ≤5 years) in the model allowing for interaction between latency time and radiotherapy. Analysis comparing CBC-patients treated with adjuvant radiotherapy versus CBC-patients not treated with adjuvant radiotherapy, regardless of other adjuvant therapy (I). In addition, a subset analysis comparing the patients treated with adjuvant radiotherapy only to those not treated with any systemic adjuvant therapy (II).

		Stockholm cohort				South Sweden cohort	
Exposure	**No. of events**	**IRR***	**95% CI**	**IRR****	**95% CI**	**IRR****	**95% CI**
I. Treated with adjuvant radiotherapy							
Any latencies	111	1.43	1.04 - 1.95	1.32	0.99 - 1.77	1.70	1.26 - 2.29
Latency ≤ 5 years	76	1.91	1.27 - 2.88	1.88	1.28 - 2.76	2.12	1.40 - 3.23
Latency >5 years	35	0.97	0.61 - 1.54	0.81	0.52 - 1.27	1.29	0.83 - 2.00
Not treated with adjuvant radiotherapy	92	1.00	Ref.	1.00	Ref.	1.00	Ref.
II. Treated with adjuvant radiotherapy only							
Any latencies	60	1.68	1.10 - 2.56	1.54	1.04 - 2.27	1.85	1.25 - 2.75
Latency ≤ 5 years	42	2.69	1.54 - 4.69	2.42	1.45 - 4.03	2.38	1.29 - 4.39
Latency >5 years	18	0.86	0.45 - 1.65	0.78	0.42 - 1.45	1.32	0.78 - 2.25
No adjuvant therapy	52	1.00	Ref.	1.00	Ref.	1.00	Ref.

IRR= Incidence Rate Ratio. CI=confidence Interval. CBC=Contralateral Breast Cancer. Both models investigating the group of patients with any latencies are also adjusted for latency time.
* Model adjusted for calendar period of the first cancer and age at diagnosis of the first cancer, grade of the first and second cancer, stage of the first and second cancer and adjuvant treatment of the second cancer.
** Model adjusted for calendar period of the first cancer and age at diagnosis of the first cancer.

Sweden cohort were combined and the IRR of distant metastasis for patients treated with radiotherapy compared to those not treated was 1.42 (95% CI: 1.16-1.74) (data not shown in Table). Among the patients with latency time <5 years the IRR was 1.92 (95% CI: 1.46-2.53) and for patients with latency time > 5 years the IRR was 0.96 (95% CI: 0.71-1.30). This combined analysis was adjusted for the factors for which we had information in both cohorts (age, calendar period and latency time).

Finally, we restricted both the Stockholm cohort and the south Sweden cohort to patients treated with radiotherapy only and patients with no adjuvant therapy, we found a similarly increased risk of distant metastasis for the radiotherapy treated patients, and the effect was again confined to the patients with short latency time (Table 2). In the Stockholm cohort, where we had information on histology, we also restricted the cohort to patients with ductal histology at the second cancer and found a similar pattern; IRR of distant metastasis for patients with latency time < 5 years: 1.81 (95% CI: 1.03-3.17) and for patients with latency time > 5 years: 0.96 (95% CI: 0.54-1.74) (data not shown in Table).

Doses of scattered radiation are higher to the medial than to the lateral part of the contralateral breast[13]. In the Stockholm cohort, we had valid information on location (medial/lateral) of the second breast cancer in a subset of 322 (40%) of the patients with second breast cancers occurring within 5 years. This information allowed us to analyze malignant features of the second cancer by location. The excess risk of distant metastases following radiotherapy was higher among women whose second cancer was located in the medial (IRR: 3.03 [95% CI: 1.10-8.32]) than in the lateral (IRR: 1.46 [95% CI: 0.70-3.02]) part of the breast. This analysis was adjusted for calendar period and age at first diagnosis and latency time between the cancers.

In all analysis above we have investigated the effect of adjuvant radiotherapy for the *first* breast cancer. To assess possible bias by indication we also calculated the IRR of distant metastasis after

Table 3 Odds of more aggressive tumor characteristics (TNM-stage / histological grade) at second cancer than at first cancer. Estimates calculated for both overall effect of adjuvant radiotherapy and, in the final model which allows for interaction between latency time and radiotherapy, also for the effect in each latency group (>5 years and ≤5 years). Analysis comparing patients treated with adjuvant radiotherapy versus patients not treated with adjuvant radiotherapy, regardless of other adjuvant therapy (I). In addition, a subset analysis comparing the patients treated with adjuvant radiotherapy only to those not treated with any systemic adjuvant therapy (II).

Exposure	Number of events (stage)	OR stage*	95% CI	Number of events (grade)	OR grade*	95% CI
I. Treated with adjuvant radiotherapy						
Any latencies	65	1.36	0.89 - 2.09	119	1.16	0.76 - 1.78
Latency ≤ 5 years	35	2.16	1.13 - 4.11	62	2.00	1.08 - 3.72
Latency >5 years	30	0.96	0.55 - 1.67	57	0.75	0.43 - 1.32
Not treated with adjuvant radiotherapy	50	1.00	Ref.	78	1.00	Ref.
II. Treated with adjuvant radiotherapy only						
Any latencies	33	1.24	0.70 - 2.20	51	1.07	0.58 - 2.00
Latency ≤ 5 years	20	3.15	1.31 - 7.58	25	2.48	0.95 - 6.49
Latency >5 years	13	0.58	0.26 - 1.26	26	0.61	0.27 - 1.34
No adjuvant therapy	31	1.00	Ref.	43	1.00	Ref.

OR= Odds Ratio. CI=Confidence Interval.
* Model adjusted for calendar period of the first cancer and age at diagnosis of the first cancer.

radiotherapy for the *second* breast cancer. We saw no evidence of a risk increase for the radiotherapy-treated patients, indeed, for patients with latency time <5years we saw a protective effect: (IRR: 0.39 [95% CI: 0.24-0.63]. This analysis was repeated for patients without systemic adjuvant therapy and neither among these patients did we see any risk increase.

Odds of worse tumor characteristics

The overall odds for higher stage of the second cancer following adjuvant radiotherapy was not significantly higher in the radiotherapy-treated group compared to the non-treated (OR=1.36 [95% CI: 0.88-2.09]; Table 3) but we observed evidence of interaction with latency time (p=0.05). Hence, patients with CBC diagnosed within 5 years from the first cancer were at a two-fold higher odds of being diagnosed in a more advanced stage if they had previously received radiotherapy (OR 2.16 [95% CI 1.13-4.11]), while we found no such association when the latency time was longer than 5 years. A similar pattern emerged when we compared histological grade of the first and the second cancer (Table 3); again with a significant interaction between latency time and radiotherapy (P= 0.02), and a statistically significantly increased odds (OR= 2.00 [95% CI: 1.08-3.72]) for treated vs. non-treated women diagnosed with CBC within 5 years. This association was even stronger both for more advanced stage (OR=2.48 [95% CI: 1.31-7.58] and higher grade (OR=3.15 [95% CI: 0.95-6.49]) in analyses restricted to women who received no systemic adjuvant therapy (Table 3).

Discussion

In two large, independent cohorts of CBC-patients we observed an increased risk of distant metastasis for CBC-patients treated with adjuvant radiotherapy for their first cancer, compared to CBC-patients not treated with adjuvant radiotherapy. This association was strongest among women diagnosed with the second cancer within 5 years from the first; for these women the risk was almost twofold. In the same group we also observed two-fold increased odds for the second cancer to be of more advanced stage and higher grade, compared to the first, for the treated patients.

Confounding by indication is a common problem in observational studies that investigate treatment effect. Such bias arise when cancers of more aggressive characteristics are treated more intensely, thereby feigning an association between the treatment and a worse prognosis. The decision to give radiotherapy is based not only on the aggressiveness of the cancer but also on the surgical technique, age of the patient and the patients' wishes. Still, to accommodate this potential bias we have adjusted our analysis of distant metastasis for TNM-stage and histological grade of the two cancers. Our analysis of TNM-stage and histological grade compares the tumor characteristics between the first and the second cancer *of the same patient*, to avoid the potential bias by indication that would lead to exposed patients overall having higher stage/worse grade compared to unexposed patients. Because the original comparison groups consist of a combination of different therapies, we also restricted our analyses to patients who received no systemic adjuvant therapy, and this did not change our findings. Our final approach to investigate bias by indication was to analyze the risk of distant metastasis in relation to radiotherapy for the *second* breast cancer. If more aggressive cancers are selected for radiotherapy and also have an increased risk of distant metastasis the same mechanisms should be in play for both cancers. Reassuringly, we found no evidence of increased risk of distant metastasis after

radiotherapy for the second breast cancer. Further; the potential bias by patient characteristics like socioeconomical status and co-morbidity would, if present, give rise to an association in opposite direction of what we observe. All in all, we do not believe that bias by indication could be the sole explanation for the findings presented in this study.

Strengths of this study include that the main results were replicated in two independent populations and that it was conducted in a country with a unified healthcare system and a population-based cancer register which enabled close to complete case identification and very good retrieval of medical records (99% in the Stockholm cohort and 83% in the south Sweden cohort).

Women with CBC are commonly reported to have a worse survival than women with unilateral breast cancer[3, 17-20]. A few studies indicated that the latency time of the metachronous CBC had an impact[3, 5, 19]. In the present study we show that the prognosis after CBC is worsened by radiotherapy for the first cancer, especially for CBCs with short latency time (Table 2). This association is independent of TNM-stage and histological grade, since our analysis is adjusted for those factors. Tumor stage of the first and second cancer has been well established to be associated with overall prognosis in CBC-patients[6, 20, 21]. Progesterone receptor status, size of the second tumor and histological grade of both cancers has also been related to prognosis[21]. In our analysis we have demonstrated a two-fold higher risk for a radiotherapy-treated patient, compared to a non-radiotherapy treated, to have higher stage and grade of the second cancer than of the first among patients whose second cancer occurred within 5 years from the first (Table 3).

The surgical and radiation techniques potentially affect the radiation dose to the contralateral breast. We lack information on the individual dose to the contralateral breast which precludes an analysis of a possible dose-response relationship. However, if radiotherapy affects the risk of distant metastases, we would expect this risk to be more pronounced for women having second tumors occurring in the medial part of the opposite breast, since the radiation dose is higher to that part, which is closer to the treated breast[13], and we indeed found indications of this.

Since our findings are confined to cancers arising within five years from the first cancer, radiotherapy does not appear to induce new cancers -since this process is likely to take much longer time[22] but rather to affect biologic features of subclinical cancers or pre-malignant lesions in the contralateral breast. Interestingly, studies on random biopsies of the opposite breast of unilateral breast cancer patients showed that 6-7% of the patients had invasive or in-situ malignancies and an additional 9% had premalignant lesions[23, 24]. In other words, approximately 15% of all breast cancer patients have lesions in the contralateral breast with the possibility to progress into CBC. We hypothesize that if such lesions are exposed to a carcinogenic stimulus such as low-dose ionizing radiation, this can enhance rate of mutations and thereby accelerate tumor progression and growth. Further, radiation might affect the prognosis of already existing cancer/premalignant lesions via stiffening the extracellular matrix. Radiation has been shown to have this effect and it is well known that rigidity of the extracellular matrix promotes cell migration and tumorigenesis[25, 26]. Furthermore, premalignant cells transplanted into mice with irradiated extracellular matrix grow into more and larger tumors compared to premalignant cells transplanted into non-irradiated mice[25].

In light of the current discussion on prophylactic mammary irradiation[27] this study illustrates an important aspect. Prophylactic mammary irradiation was first suggested in 2007 by Brenner et al.[27, 28] in order to lower the risk of second breast cancer. It is well known that radiation can both cure cancer by killing cancer cells and initiate cancer, dependent on the dosage given[29]. In addition, our study suggests that if a cancer/premalignant lesion is already present, but undetected, low dose radiation will on average, results in a more malignant phenotype.

In conclusion; patients treated with adjuvant radiotherapy for their first breast cancer and who are diagnosed with CBC within 5 years seem to have a more aggressive second cancer. The clinical implication of this finding is *not* to refrain from adjuvant radiotherapy, but to minimize radiation to the contralateral breast (possibly by using accelerated partial breast irradiation[30] when appropriate) and to take the given therapy into account in the management of a second cancer. A more intense treatment than indicated by the tumor characteristics of the second cancer might be called for. In the view of prophylactic mammary irradiation as an approach to decrease the risk of second breast cancer, it will be important to assess the validity of our findings in other populations and evaluate how the (potentially) decreased risk of second breast cancer relates to the (potentially) increased risk of metastasis, should a second breast cancer anyway occur.

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Conflict of interests

None.

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EPIDEMIOLOGY

Prognostic implications of estrogen receptor pattern of both tumors in contralateral breast cancer

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Abstract Estrogen receptor (ER) status is important for breast cancer survival, it is however unclear how prognosis of contralateral breast cancer (CBC) is affected by ER-status of the two tumors. We conducted a large, population-based study of ER-status of both tumors in CBC patients and its influence on prognosis. The cohort consisted of all women diagnosed with CBC in Stockholm, Sweden during 1976–2005, with information on ER-status from medical records ($N = 933$). Prognosis was modeled as incidence rates of distant metastasis via Poisson regression. The proportion of CBCs with both cancers of the same ER-status was significantly larger than expected by chance. For synchronous (simultaneous) cancers the prognosis was significantly affected by the combined ER-status of both tumors ($p = 0.01$). Compared to unilateral breast cancer patients the incidence rate ratio (IRR) for patients with double ER-positive tumors was 1.25 (95 % CI: 0.88–1.76), for ER-discordant tumors 2.19 (95 % CI: 1.18–4.08) and for double ER-negative tumors 3.95 (95 % CI: 1.77–8.81). For metachronous (non-simultaneous) cancers, women with double ER-positive tumors had similarly bad prognosis (IRR = 2.95; 95 % CI: 2.39–3.64) as women with double ER-negative tumors (IRR = 2.88; 95 % CI: 1.83–4.52). Both shorter time span between first and second cancer and endocrine therapy for the first cancer further worsened prognosis of women with double ER-positive metachronous CBC. For synchronous CBC patients, ER-pattern of both tumors is an important prognosticator, while among metachronous CBC patients, double ER-positive tumors confer equally bad prognosis as double ER-negative cancers. Our results indicate that this might be due to endocrine therapy resistance.

Keywords Contralateral breast cancer · Prognosis · Estrogen receptor

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Introduction

Since more than 30 years it has been known that estrogen receptor (ER) status is significantly affecting breast cancer survival. Breast cancer patients with ER-negative cancer have a shorter survival compared to patients with ER-positive cancer, a finding not influenced by lymph node status [1, 2]. ER-status is not only an important prognosticator, but also the only crucial predictor for endocrine therapy response, as shown in a recent meta-analysis of over 20,000 participants [3]. Patients with ER-positive breast cancer and tamoxifen treatment had a mortality reduction of one third during the first 15 year of follow-up, while patients with ER-negative disease had no effect of tamoxifen.

Women with breast cancer have, 20 years after their initial diagnosis, 10–15 % cumulative incidence of second

primary breast cancer in the opposite breast; contralateral breast cancer (CBC) [4, 5]. Given that the same genetic and/or environmental risk factors give rise to both tumors, concordance of ER-status is likely and has been shown in previous studies [6–8]. Differences in concordance between synchronous (simultaneous) and metachronous (non-simultaneous) disease could be due to treatment of the first cancer among the metachronous cancers and/or seen as an indication that the two CBC entities represent different cancer subtypes, which we have suggested earlier [9].

Women with CBC have considerably worse prognosis than women with unilateral breast cancer [10, 11]. Very few studies have addressed how ER-status of the second tumor affects prognosis of CBC patients [12, 13] and the combined influence of ER-pattern of both tumors has never been investigated previously. As a consequence, clinical guidelines for how to evaluate prognosis for CBC patients with respect to ER-status are lacking. We performed a comprehensive and thorough analysis of 933 women with CBC with the aim of assessing the ER-pattern of both tumors and its influence on prognosis of CBC, taking time between first and second cancer and endocrine therapy of the first cancer into account.

Methods

Study population

The study population originates from the Stockholm breast cancer register; a population-based register in which all breast cancer patients in the Stockholm health care region are registered. The register is complete from 1976 and we retrieved information on personal identification number, date of diagnosis, TNM stage of the cancers, and information on follow-up. From 1988 the register also provides information on adjuvant therapy, categorized in four categories; no adjuvant therapy/only radiotherapy/hormone therapy (with or without radiotherapy)/chemotherapy (with or without radiotherapy and hormone therapy). All women with contralateral invasive breast cancer were selected for the study. Women with a first primary invasive cancer other than breast cancer and women with breast cancer in TNM stage IV were excluded in order to minimize the risk of the CBC being misclassified metastases. 24,775 patients with unilateral breast cancer and 1,458 patients with CBC were identified, of these 17,272 unilateral breast cancer patients and 840 CB patients were diagnosed from 1988 onwards.

Additional information from oncological medical records and pathology reports was collected for all women with CBC to obtain information on ER-status of the two tumors and to verify the diagnosis (retrieval rate 99 %). Only women with known ER-status of both tumors were included. Following the review of pathology reports and medical records, 147 patients (10 %) did not fulfill the inclusion criteria and were excluded. The main reason for exclusion was distant metastasis (not recorded in the register) before the second breast cancer (84 patients). An additional 17 cases (1 %) were excluded since their medical records could not be retrieved. Our final study population for analysis included 933 women with CBC and known ER-status of both cancers (72 % of all eligible CBC patients).

The measurement of ER-status during the study period is naturally of crucial importance for this study. Prior to 1988 isoelectric focusing [14] was used to assess ER-status, from 1988 to 2003 an ELISA assay [15] (Abbott Laboratories kit) was used, and during the last 2 years of this study immunohistochemistry (DAKO Laboratories kit) was used. The measurement from these methods have been shown to be highly correlated [16, 17] and also to correlate well with more recent methods, like RT-PCR [18]. The ELISA assay measured fmole/μg DNA and any tumor with a concentration of ER $\geq$0.05 fmol/μg DNA was defined as ER-positive.

ER-pattern of both tumors among CBC patients was defined as follows; (1) CBC with both cancers ER-positive (double ER-positive), (2) CBC with cancers of opposite ER-status (ER-discordant), and (3) CBC with both cancers ER-negative (double ER-negative). In synchronous cancers it is not possible to determine which tumor is "first" and which is "second". Consequently to be able to compare the metachronous and synchronous cancers, we did not specify the two possible combinations of ER-discordant metachronous CBC (first cancer ER-positive, second cancer ER-negative/first cancer ER-negative, second cancer ER-positive) in our main analyses. Still, as a secondary analysis, we separated the two possible combinations when studying prognosis for metachronous CBC cancers.

Statistical analysis

We investigated the distribution of ER-pattern of both tumors in relation to what would be expected given the general probability of ER-positivity among women with unilateral disease. Further, following clinical practice, we investigated how the prognosis following metachronous CBC is affected by ER-status of the second cancer alone. Subsequently, we studied the influence of ER-pattern of both cancers on prognosis of CBC. Throughout the study we used distant metastasis following the second cancer as a measure of prognosis and we used all breast cancers in the Stockholm breast cancer register as a reference population. We defined synchronous CBC as two cancers diagnosed within 3 months of each other, this cutoff has been used previously by our group and others [11, 19, 20].

We compared the observed distribution of CBCs in the three ER-pattern groups to what would be expected if the ER-status of the two cancers were completely independent, using Chi-square tests. The expected distribution was calculated by assessing the ER-positivity among all breast cancers in the Stockholm breast cancer register ($N = 26{,}233$). We further constructed 95 % confidence intervals (CI) around the observed proportions of ER-pattern using the Clopper–Pearson exact confidence limits for proportions.

We used Poisson regression to calculate the incidence rate ratio (IRR) of distant metastasis, comparing prognosis between CBC patients of different ER-patterns and using unilateral breast cancer patients as a reference group. Survival time was defined as time from date of first breast cancer diagnosis in women with unilateral disease or from date of second breast cancer diagnosis in women with CBC, until the date of distant metastasis, date of death or December 31, 2005, whichever came first. This analysis was adjusted for calendar period at diagnosis, age at diagnosis and TNM stage of the (first) cancer for unilateral breast cancer patients and synchronous CBC patients and calendar period, age and TNM stage of the second cancer for metachronous CBC patients. A test of heterogeneity was used to assess the effect of ER-pattern in each group. All data preparation and analyses were done using SAS Statistical Package 9.2.

Results

Among all breast cancers in the register ($N = 26\ 233$) we identified 933 CBC patients for which we found the medical records, who fulfilled the inclusion criteria, and had known ER-status on both tumors (Table 1) 0.64 % of the women with CBC had metachronous disease and 36 % had synchronous disease. Mean age at first diagnosis of the CBC patients was 64.9 years; 66.7 years for synchronous and 64.0 years for metachronous CBC. 33 % of all CBCs had both cancers diagnosed in TNM stage 1 and 3 % had both cancers diagnosed in TNM stage 3.

Double ER-positive tumors was the most common ER-pattern among the CBC patients (70 %), followed by women with ER-discordant cancers (21 %) and those with double ER-negative cancers (9 %) (Table 2). The proportion of ER-positivity among all breast cancer in the Stockholm breast cancer register was 78.2 %. Assuming that ER-status of the two tumors are independent events, this percentage gives rise to the expected figures of ER-pattern shown in Table 2. The observed and expected distribution of ER-pattern was significantly different for both types of CBC ($p < 0.001$ for synchronous CBC and $p < 0.001$ for metachronous CBC). The ER-pattern of metachronous and synchronous CBC was moreover also significantly different from each other ($p < 0.001$). Due to the observed difference in distribution of ER-pattern in synchronous and metachronous cancers these groups were assessed separately in subsequent analyses.

We compared the prognosis of women with CBC to that of women with unilateral breast cancer and adjusted all our analysis for calendar period at diagnosis, age at diagnosis and TNM stage of the (first) cancer for unilateral breast cancer patients and synchronous CBC patients and calendar period, age and TNM stage of the second cancer for metachronous CBC patients. Women with synchronous CBC had a 51 % increased incidence rate of distant metastases (IRR: 1.51; 95 % CI: 1.14–2.00) (Table 3). The corresponding IRR for women with metachronous CBC was 2.73 (95 % CI: 2.30–3.22). Women with metachronous

Table 1 Characteristics of the CBC cohort with complete information on ER-status, in total and stratified by CBC subtype (synchronous/metachronous)

	Total (%)	Synchronous (%)	Metachronous (%)
No of patients	933	334	599
Mean age at second diagnosis	64.9	66.7	64.0
Standard deviation	12.6	12.5	12.5
Calendar period of second diagnosis			
1976–1985	120 (13 %)	66 (20 %)	54 (9 %)
1985–1995	297 (32 %)	109 (33 %)	188 (31 %)
1995–2005	516 (55 %)	159 (48 %)	357 (60 %)
TNM stage (first cancer + second cancer)[a]			
1 + 1	295 (33 %)	83 (26 %)	212 (37 %)
1 + 2	94 (11 %)	38 (12 %)	56 (10 %)
1 + 3	16 (2 %)	6 (2 %)	10 (2 %)
2 + 1	253 (28 %)	79 (25 %)	174 (31 %)
2 + 2	107 (12 %)	50 (16 %)	57 (10 %)
2 + 3	25 (3 %)	12 (4 %)	13 (2 %)
3 + 1	40 (4 %)	17 (5 %)	23 (4 %)
3 + 2	37 (4 %)	18 (6 %)	19 (3 %)
3 + 3	23 (3 %)	16 (5 %)	7 (1 %)
Endocrine therapy for first cancer[b]			
Yes	383 (65 %)	190 (75 %)	193 (57 %)
No	109 (18 %)	26 (10 %)	83 (25 %)
Unknown	99 (17 %)	37 (15 %)	62 (18 %)

CBC contralateral breast cancer

[a] 43 patients (15 with synchronous CBC and 28 with metachronous CBC) had unknown TNM stage of at least one of the cancers. Definition of first and second synchronous cancer was as registered by the Stockholm breast cancer register

[b] Information on endocrine therapy was available in the Stockholm breast cancer register only from 1988, thus this information can be given only on the 591 patients (63 %) that was diagnosed in this period

Table 2 Observed and expected proportion of ER-pattern groups, in total and stratified by CBC subtype (synchronous/metachronous)

	Number of patients	Observed proportion (%)	95% CI: observed proportion	Expected proportion (%)	Ratio observed proportion/ expected proportion	*p* Value*
All CBCs (any latency)						
Double ER-positive cancers	653	70.0	66.9–72.9	61.1	1.14	<0.001
ER-discordant cancers	196	21.0	18.4–23.8	34.1	0.62	
Double ER-negative cancers	84	9.0	7.2–11.0	4.8	1.88	
Synchronous CBCs						
Double ER-positive cancers	263	78.7	74.0–83.0	61.1	1.29	<0.001
ER-discordant cancers	45	13.5	10.0–17.6	34.1	0.40	
Double ER-negative cancers	26	7.8	5.2–11.2	4.8	1.63	
Metachronous CBCs						
Double ER-positive cancers	390	65.1	61.1–68.9	61.1	1.07	<0.001
ER-discordant cancers	151	25.2	21.8–28.9	34.1	0.74	
Double ER-negative cancers	58	9.7	7.43–12.3	4.8	2.02	

ER estrogen receptor, *CBC* contralateral breast cancer, *CI* confidence interval

*Chi-square exact test, testing if observed distribution follows expected *p* Value for metachronous distribution differing from synchronous distribution (Chi-square exact test): $p < 0.001$

CBC thus had a significantly worse prognosis, not only compared to women with unilateral breast cancer but also compared to women with synchronous CBC. In line with current clinical practice we investigated the effect of ER-status of the second cancer only, for women with metachronous CBC (data not shown in table). Compared to women with unilateral breast cancer, women with ER-positive second cancer (irrespective of ER-status of the first cancer) had an IRR of 2.67 ([95 % CI: 2.20–3.25] $N = 459$ CBC-cases) and CBC patients with ER-negative second cancer (irrespective of ER-status of the first cancer) had an IRR of 2.87 ([95 % CI: 2.11–3.89] $N = 140$ CBC-cases).

The ER-pattern of both tumors had a significant influence on prognosis of synchronous CBC ($p = 0.01$) (Table 3). Compared to women with unilateral cancer, patients with double ER-negative synchronous CBC had a risk of distant metastasis of 3.95 (95 % CI: 1.77–8.81). The corresponding figures for ER-discordant and double ER-positive cancers were 2.19 (95 % CI: 1.18–4.08) and 1.25 (95 % CI: 0.88–1.76), respectively. In patients with metachronous CBC very similar prognosis was seen for women with double ER-positive and double ER-negative tumors, the patients with ER-discordant cancers had slightly better prognosis but the risk estimates were not statistically different ($p = 0.18$).

As shown in Table 3, women with double ER-positive metachronous cancers had significantly poorer outcome compared to women with double ER-positive synchronous cancers. Although adjuvant therapy cannot formally be a confounder (adjuvant therapy cannot be a cause of [first] ER-status), it still importantly affects the prognosis. In order to investigate this, the cohort was restricted to patients diagnosed from 1988, since, for these women, we had information on adjuvant therapy and the analysis was repeated, adjusting additionally for adjuvant therapy (data not shown in table). The estimates were slightly increased but the pattern of risk increase was very similar, e.g., IRR for double ER-positive synchronous CBC: 1.55 (95 % CI: 1.04–2.32) and IRR for double ER-positive metachronous CBC: 3.52 (95 % CI: 2.58–4.81).

We further analyzed the prognosis for women with double ER-positive metachronous cancers with regards to time since first diagnosis and found decreasing risk of distant metastases as time between first and second cancer increased (Fig. 1). Next, we studied the influence of endocrine therapy of the first cancer on prognosis of these patients. Double ER-positive metachronous CBC patients treated with endocrine therapy for their first cancer had a fourfold higher risk (IRR = 4.42; 95 % CI: 2.91–6.71) of being diagnosed with distant metastases compared to women with unilateral breast cancer treated with endocrine therapy (Table 4). For women not treated with endocrine therapy the risk in patients with metachronous double ER-positive CBC was not statistically significantly increased compared to patients with unilateral breast cancer. (IRR = 2.03; 95 % CI: 0.95–4.35). The analysis for Table 4 was repeated using a cutoff between synchronous and metachronous cancer of 1 year instead of 3 months, to decrease the possibility that the metachronous cancers had not been exposed to endocrine therapy. The risk estimates were however very similar; among women treated with endocrine therapy the IRR for women with double ER-positive CBC was 4.17 (95 % CI: 2.69–6.45) ($N = 138$) compared to unilateral breast cancer patients while, among

Table 3 IRR of distant metastasis by ER-pattern groups, using unilateral breast cancer as reference level, all rates calculated for CBC overall and stratified by subtype (synchronous/metachronous)

	IRR of distant metastasis	95 % CI	*p* Value*
Unilateral breast cancer	1.00	Ref.	
Synchronous CBCs	1.51	1.14–2.00	
Metachronous CBCs	2.73	2.30–3.22	
Synchronous CBCs			
ER-double positive	1.25	0.88–1.76	0.01
ER-discordant	2.19	1.18–4.08	
ER-double negative	3.95	1.77–8.81	
Metachronous CBCs			
ER-double positive	2.95	2.39–3.64	0.18
ER-discordant[a]	2.23	1.61–3.09	
ER-double negative	2.88	1.83–4.52	

IRR incidence rate ratio, *ER* estrogen receptor, *CBC* contralateral breast cancer *CI* confidence interval

[a] When the metachronous ER-discordant CBC patients was separated into first cancer ER-positive and second cancer ER-negative and first cancer ER-negative and second cancer ER-positive the IRR was 2.85 (95 % CI: 1.89–4.31) and 1.63 (95 % CI: 0.97–2.77) respectively

**p* Value for the significance of ER-pattern within all synchronous CBCs, and all metachronous CBCs, respectively, without including the unilateral reference group in the analysis. This analysis was adjusted for calendar period at diagnosis, age at diagnosis and TNM stage of the (first) cancer for unilateral breast cancer patients and synchronous CBC patients and calendar period, age and TNM stage of the second cancer for metachronous CBC patients. The time scale is time from diagnosis for unilateral breast cancer patients and time from second diagnosis for CBC patients

women not treated with endocrine therapy, the IRR for women with double ER-positive CBC was 1.27 (95 % CI: 0.47–3.41) ($N = 34$), compared to women with unilateral breast cancer.

Discussion

We found that CBC patients are more likely to have the same ER-status in both tumors than would be expected by chance. In general, women with CBC have a worse prognosis compared to women with unilateral breast cancer. ER-pattern of both tumors significantly impacts the prognosis of women with synchronous CBC, where women with double ER-negative cancers have the worst prognosis. In contrast, in women with metachronous disease ER-pattern of both tumors was not significantly associated with prognosis. In fact, among patients with double ER-positive tumors, metachronous CBC patients have remarkably much worse prognosis compared to patients with synchronous CBC, this is further enhanced if the metachronous second cancer is detected shortly after the first and if the first cancer is treated with endocrine therapy.

Strengths of this study include that it was conducted in a country with a unified health care system and a population-based cancer register which enabled close to complete case identification. We reduced the risk of misclassification through exclusion of women diagnosed with a previous cancer other than breast cancer, women diagnosed with an initial TNM stage IV breast cancer and women diagnosed with distant metastasis prior to the second cancer. Further, to gain information on ER-status and to ensure correct CBC diagnosis we obtained all medical records and pathology reports of the CBC patients. A limitation of this study is that complete information of ER-status on both tumors was missing from the medical records in 28 % of the eligible CBC-cases. Further, misclassification of ER-status cannot be excluded since the measurements were preformed with different methods. However, this misclassification, if at all present, ought to be non-differential and would as such bias our results towards the null. In addition, a sensitivity analysis of the patients diagnosed from 1988 showed no indication of misclassification. Finally, we chose to not include analysis of progesterone receptor status, since the degree of missingness was markedly worse (43 %) than for ER-status, however, concordance between estrogen and progesterone receptor status in the same tumor was very high (76 %).

CBCs can be more similar in ER-status of the two tumors then expected by chance due to host factors leading to particular women being more likely to have cancers of a certain ER-subtype and significant concordance have been shown repeatedly in previous studies [6–8, 13, 21–23]. We found the proportion of CBC patients with discordant ER-status to be significantly smaller than would be expected if tumor development would be independent (Table 2). In both synchronous and metachronous cancers double ER-negative cancers were more prevalent than would be expected by chance. In contrast, double ER-positive cancers were more prevalent than expected in synchronous disease but not in metachronous disease. One possible explanation to this finding could be that tamoxifen, normally only administrated to patients with ER-positive cancer, is decreasing the probability of ER-positive metachronous second cancer, thereby, as a consequence decreasing the proportion of metachronous patients with double ER-positive cancers.

As we have shown previously [11], both synchronous and metachronous CBC patients have worse prognosis compared to unilateral breast cancer patients. The excess risk of distant metastasis in women with synchronous CBC (IRR: 1.51; 95 % CI 1.14–2.00) is similar to the increased risk for women with unilateral triple negative (negative for estrogen- and progesterone receptors and HER2) breast cancer [24, 25] or for breast cancer patients that are BRCA-1 mutation carriers [26]. For women with metachronous

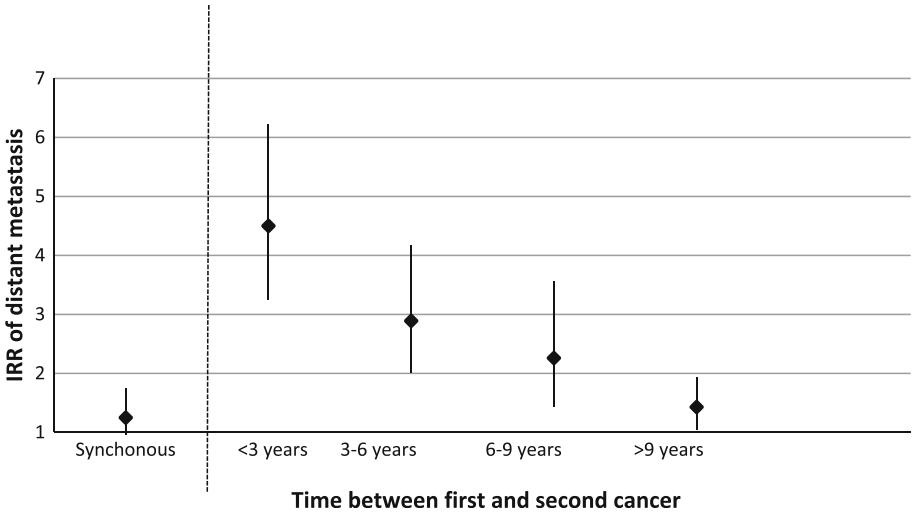

Fig. 1 IRR of distant metastasis for patients with double ER-positive CBC compared to unilateral breast cancer patients, stratified on time between first and second cancer. The reference level (1.00) corresponds to the risk of distant metastasis for unilateral breast cancer patients, the bars represents the 95 % confidence intervals for each value. This analysis was adjusted for calendar period at diagnosis, age at diagnosis and TNM stage of the (first) cancer for unilateral breast cancer patients and synchronous CBC patients and calendar period, age and TNM stage of the second cancer for metachronous CBC patients. The time scale is time from diagnosis for unilateral breast cancer patients and time from second diagnosis for CBC patients. *ER* estrogen receptor, *CBC* contralateral breast cancer, *IRR* incidence rate ratio

Table 4 IRR of distant metastasis for patients with double ER-positive metachronous CBC compared to unilateral breast cancer patients, stratified on endocrine therapy

	IRR	95 % CI
Double ER-positive CBC with endocrine therapy	4.42	2.91–6.71
Unilateral breast cancer with endocrine therapy	1.00	Ref.
Double ER-positive CBC without endocrine therapy	2.03	0.95–4.35
Unilateral breast cancer without endocrine therapy	1.00	Ref.

This analysis is restricted to patients diagnosed with their first cancer in 1988 or later, since there was no information on endocrine therapy available from the Stockholm breast cancer register before this year. This analysis was adjusted for calendar period at diagnosis, age at diagnosis and TNM stage of the (first) cancer for unilateral breast cancer patients and synchronous CBC patients and calendar period, age and TNM stage of the second cancer for metachronous CBC patients. The time scale is time from diagnosis for unilateral breast cancer patients and time from second diagnosis for CBC patients

ER estrogen receptor, *CBC* contralateral breast cancer, *IRR* incidence rate ratio, *CI* confidence interval

CBC the excess risk of distant metastasis (compared to unilateral breast cancer) is considerably higher (IRR: 2.73; 95 % CI: 2.30–3.22), compared to the excess risk for triple negative breast cancer, BRCA-1 breast cancers and synchronous CBC [24–26].

In clinical practice, normally only ER-status of the second cancer, not the first, is used as a prognosticator for patients with a metachronous CBC. Only two previous studies have investigated the prognostic impact of ER-status of the second cancer, they were in disagreement and both suffered from methodological limitations [12, 13]. When investigating this issue we did not find that ER-status of the second cancer influenced prognosis in patients with metachronous CBC.

The main aim of this study was to investigate the effect of ER-pattern of both cancers, which has not been done before. For synchronous CBC, ER-pattern carries a significant prognostic value. As expected, double ER-negative cancers have by far the worst prognosis, which probably corresponds to the worse prognosis among ER-negative unilateral breast cancer compared to ER-positive unilateral cancers [1, 2]. Further, patients with double ER-positive cancers had the best prognosis (in fact; not statistically significantly different from women with unilateral breast cancer) and women with discordant cancers had an intermediate prognosis. On the other hand, among the metachronous CBCs, the prognosis among patients with double ER-positive cancers is equally bad as among those with double ER-negative cancers and actually significantly worse than for patients with synchronous double ER-positive CBC.

The unexpectedly poor prognosis among women with double ER-positive metachronous CBCs could conceivably be due to endocrine therapy resistance. One could argue that an ER-positive second cancer that develops during or shortly after endocrine therapy has escaped the therapeutic effect and therefore might have particularly aggressive

characteristics. If therapy resistance produces particularly aggressive cancers, the worsened prognosis should diminish for cancers diagnosed in the post-therapeutic period. As can be seen in Fig. 1, we find a dose–response relationship; the prognosis is steadily improving with longer time between first and second cancer. For metachronous patients with double ER-positive cancers diagnosed more than 9 years apart the risk is very similar to that of patients with double ER-positive synchronous CBC. Further, when we compared the prognosis for women with double ER-positive metachronous CBC to women with unilateral breast cancer, stratified on endocrine therapy we found a fourfold increased risk of developing distant metastases among the endocrine-treated patients. The corresponding risk for non-endocrine-treated women was not statistically significantly increased (IRR = 2.03; 95 % CI: 0.95–4.35) (Table 4).

This study contains several novel findings that could have clinical implications. For synchronous CBC ($\sim$1.5 % of all breast cancer patients [9]) ER-pattern of both cancers should be taken into account when deciding the treatment regimen, as it has significant impact on prognosis. For metachronous CBC, ER-pattern does not significantly influence prognosis; patients with double ER-positive cancers have similarly bad prognosis as patients with double ER-negative cancers. Short interval between first and second cancer and endocrine therapy of the first cancer further deteriorates prognosis among double ER-positive metachronous CBCs and we speculate that our findings might be due to an acquired resistance to endocrine therapy given for the first cancer. Recent studies have shown that several different molecular pathways might be involved in the acquisition of resistance to tamoxifen (reviewed in Musgrove and Sutherland [27]). We believe that detailed molecular analysis of well annotated CBC tumors will be an important scheme for unraveling the mechanistic causes of resistance, and identify potential biomarkers. In the meantime our results imply that patients with metachronous double ER-positive cancers should probably have a more aggressive therapy than currently recommended, particularly if the time between first and second cancer is relatively short.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The ethical committee at Karolinska Institutet, Stockholm, Sweden, approved this study.

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Change of mammographic density predicts the risk of contralateral breast cancer

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Abstract

Purpose: Mammographic density is a strong risk factor for breast cancer, but it is unknown whether density at first breast cancer diagnosis (baseline), or changes during follow-up, influences risk of non-simultaneous contralateral breast cancer (CBC). **Methods:** We collected baseline and follow-up mammograms for CBC-patients; cases, and unilateral breast cancer patients; controls, individually matched on age and calendar period of first breast cancer diagnosis, type of adjuvant therapy and length of follow-up. Mammographic density (percent density and dense area) was measured in the breast unaffected by first cancer. The odds of CBC as a function of density at baseline, and of changes during follow-up, were investigated using conditional logistic regression, adjusting for non-dense area at diagnosis. **Results:** Mammographic density at baseline was not shown to affect the odds of CBC (p=0.27). In contrast; relative to patients who had little or no change in density from baseline to first follow-up mammogram (mean=1.6 (SD=0.6) years after diagnosis), those who experienced ≥10% absolute decrease in percent density had a 55% decreased odds of CBC (OR=0.45 95% CI: 0.24-0.84), whereas among those who experienced an absolute increase in percent density there was little change in the odds of CBC (OR=0.83 95% CI: 0.24-2.87). **Conclusion:** Decrease of mammographic density within the first 2 years after a first diagnosis is associated with a significantly reduced risk of CBC. This potential new risk predictor can thus contribute to decision making in follow-up routines and adjuvant treatment regimens as well as provide reassurance to the patients at decreased risk.

Introduction

Mammographic density is one of the strongest risk factors for breast cancer; a meta analysis of 14 000 cases and 226 000 non-cases showed that the women with >75% mammographic density have almost 5 times the risk of breast cancer compared to women in the lowest density group (<5%)[1]. Several hormonal factors affect mammographic density and changes of density have been shown to be associated with pharmacological therapies, such as hormone replacement therapy and tamoxifen/raloxifene[2,3]. When changes in mammographic density have been measured in healthy women, decreasing density has repeatedly been shown

to correspond to decreasing breast cancer risk[4,5].

Despite the well-known and strong association between mammographic density and unilateral breast cancer, the effect of mammographic density on the risk of a second primary breast cancer in the opposite breast, contralateral breast cancer (CBC), has to our knowledge not been investigated before. Breast cancer patients have approximately double the risk of CBC, compared to healthy women's risk of breast cancer[6] and this increased risk does not seem to decline with time after first diagnosis[7-9]. This translates into 10-15% of all breast cancer patients being diagnosed with CBC within 20 years of initial diagnosis[10,11]. When investigating hormonal risk factors for unilateral breast cancer no association with risk of CBC has been identified [12-14].

Trends of breast cancer incidence and breast cancer mortality indicate that CBC will increase in importance, since the population at risk of CBC is increaseing[15]. Since CBC also has far less well characterized risk factors[8] and considerably worse prognosis than unilateral breast cancer new tools for prediction of CBC would be of great clinical importance[16]. The aim of this matched nested case-control study was two-fold; to evaluate whether high mammographic density at initial diagnosis is a risk factor for CBC and secondly, to assess if change of mammographic density after first breast cancer diagnosis predicts a decreased risk of CBC.

Methods

Study population

The study was nested within the catchment population of the Stockholm Breast Cancer Register, a population-based register of all breast cancer patients diagnosed since 1976 in the Stockholm-Gotland health-care region (N>30 000). All women with CBC diagnosed more than one year after the first cancer (N=808) were identified as potential cases and patients with unilateral breast cancer in the same register were identified as potential controls. Women with a first primary cancer other than breast cancer and women with distant metastasis at first or second breast cancer diagnosis were excluded in order to minimize the risk of the CBC being a misclassified metastasis. For each case, one control was randomly selected matched to the corresponding case on calendar period of first breast cancer diagnosis (+/- two years), age at first breast cancer diagnosis (+/- two years), adjuvant therapy and follow-up time, so that the control had survived without distant metastasis or CBC at least as long as the time between the first and second cancer for the corresponding case. From the Stockholm Breast Cancer Register we retrieved information on menopausal status at time of the (first) breast cancer diagnosis and estrogen receptor (ER) status and TNM-stage of the (first) cancer, in addition to the matching variables. From the medical records of the cases and controls we retrieved information on hormone replacement therapy at time of the (first) breast cancer diagnosis as well as additional information on menopausal status and ER-status.

For each case-control set we retrieved all mammograms taken up to one year before diagnosis of the first cancer until 6 months before diagnosis of the second cancer for the case or 6 months before the equivalent censoring date for the control. The baseline mammogram was defined as a mammogram from the contralateral breast, i.e. the breast not affected by cancer, taken at any time during the year prior to diagnosis, or within 2 weeks after diagnosis, of the first cancer. Follow-up mammograms were defined as the first available mammogram from the unaffected contralateral breast which was taken at least one year, but no more than 5 years, after the first diagnosis after diagnosis. We defined sets of two individuals comprising of one CBC case and one matched control (unilateral breast cancer patient) and a total of four mammograms (one baseline and one follow-up mammogram for each patient) of the same view. The media-lateral-oblique (MLO) view of mammograms have been the preferred view in the Swedish screening program[17] and were therefore used as our primary selection. Sets using cranial-caudal (CC)-mammograms made up 14% of the final sample.

The baseline mammogram was available for 458 eligible CBC-cases; for 99 of these patients we could not locate any follow-up mammogram, while for 271 patients (59%) we had access to both the baseline and at least one follow-up mammogram of the unaffected breast from the same view. Among these patients we could trace the baseline and follow-up mammograms from the correct side and view for the corresponding control in 211 cases. These 211 case-control sets were thus included in the analysis sample. The patients excluded due to lack of eligible mammograms did not differ from those included in the analysis in relation to age, calendar period of first diagnosis or type of adjuvant treatment regimen administered.

For comparison with the risk of CBC as a function of baseline mammographic density (i.e. density at the time of first breast cancer diagnosis), we also examined the risk of unilateral breast cancer in relation to mammographic density. To achieve this we, for each unilateral breast cancer case, also retrieved mammograms from a healthy woman, taken in the same calendar period and at the same age as the corresponding unilateral breast cancer patient was at her first diagnosis. The healthy controls (n=142) were randomly selected from a breast cancer case-control study, extensively described elsewhere[18], for which all available mammograms had been previously collected.

Tabel 1: Distribution of CBC case and unilateral breast cancer controls by matching variables (age at diagnosis, calendar period of diagnosis, adjuvant therapy and follow-up time), **exposure variables** (dense area at diagnosis and change of dense area), **potential confounding variables** (non-dense area at diagnosis and time to first follow-up mammogram), **and stratifying variable** (menopause status).

		Cases	Controls	P-value*
Age at (first) diagnosis (%)	≤ 45 years	37 (18)	37 (18)	
	45-55 years	68 (32)	68 (32)	
	55-65 years	56 (27)	56 (27)	
	≥ 65 years	50 (24)	50 (24)	-
Calendar period of (first) diagnosis (%)	1976-1980	31 (15)	30 (14)	
	1981-1985	40 (19)	41 (19)	
	1986-1990	45 (21)	41 (19)	
	1991-1995	51 (24)	49 (23)	
	1996-2005	44 (21)	50 (24)	-
Adjuvant therapy (%)	No adjuvant therapy	39 (18)	39 (18)	
	Radiotherapy only	87 (41)	87 (41)	
	Hormone therapy (with/without radiotherapy)	57 (27)	57 (27)	
	Chemotherapy (with/without radiotherapy and hormone therapy)	28 (13)	28 (13)	-
Mean follow-up time in years		8.25	8.25	-
Percentage density at (first) diagnosis (%)	<=5%	13 (6)	11 (5)	
	5-25%	87 (41)	87 (41)	
	25-50%	97 (46)	87 (41)	
	>=50%	14 (7)	26 (12)	0.23
Change in dense area between diagnosis and first follow-up mammogram (%)	Decrease (>10%)	40 (19)	56 (27)	
	Stable density	164 (78)	143 (68)	
	Increase (>10%)	7 (3)	12 (6)	0.07
Dense area at (first) diagnosis (%)	Lowest quartile (≤ 20 cm^2)	55 (26)	55 (26)	
	25-50% quartile (20-34 cm^2)	44 (21)	56 (27)	
	50-75% quartile (34-53 cm^2)	56 (27)	49 (23)	
	Highest quartile (≥ 53 cm^2)	56 (27)	51 (24)	0.79
Non-dense area at (first) diagnosis (%)	Lowest quartile (≤67 cm^2)	42 (20)	60 (28)	
	25-50% quartile (67-93 cm^2)	58 (27)	43 (20)	
	50-75% quartile (93-127 cm^2)	50 (24)	55 (26)	
	Highest quartile (≥ 127 cm^2)	61 (29)	53 (25)	0.10
Mean time until first follow-up mammogram in years (SD)		1.56 (0.59)	1.54 (0.57)	0.56
Menopause status at diagnosis (%)**	Premenopausal	89 (42)	84 (40)	
	Postmenopausal	119 (56)	124 (59)	0.62

* P-value for Chi-square when testing categorical variables and Student's t-tests when testing continues variable (mean time until first follow-up mammogram).
** 6 patients had uncertain menopause status (e.g. hysterectomy).

The mammograms were digitized using an Array 2905HD Laser Film Digitizer (Array Corporation, Tokyo, Japan), which covers a range of 0 to 4.7 optical density. The density resolution was set at 12-bit spatial resolution. Mammographic density was measured using our automated thresholding method[19] which incorporates the knowledge of a trained observer, by using measurements obtained by an established user-assisted threshold method - Cumulus[20] - as training data. The externally validated results showed a high correspondence between our automated method and the established used-assisted thresholding method Cumulus ($r_{\text{percent mammographic density}}$ = 0.88 (95% CI: 0.87-0.89).

Statistical analysis

We estimated percent mammographic density as well as absolute size of the dense area and of the total area of the breast. Percent density and dense area have been used in previous studies and have both been shown to be important predictors of breast cancer risk[21]. Calculating the total breast area enabled us to adjust for non-dense area in the analyses; adjustment for this

Table 2: Mean absolute change of percent density (PD) from baseline until first follow-up mammogram calculated for cases and control combined, stratified on different patient characteristics.

	N	**Mean** decrease PD (%-units)	**95% CI** for mean decrease of PD	P-value
Total	422	-3.94	-4.89 , - 3.00	-
CBC Case/control status				0.09
Cases	211	-3.13	-4.39 , - 1.87	
Controls	211	-4.75	-6.16 , - 3.34	
Age at time of (first) cancer :				<0.01
< 45 years	74	-5.30	-8.39 , - 2.22	
45-54 years	136	-5.85	-7.61 , - 4.10	
55-64 years	112	-4.13	-5.60 , - 2.65	
≥ 65 years	100	**-0.13**	**-1.50 , - 1.25**	
Calendar period of (first) cancer:				0.25
1976-1980	62	-4.11	-7.57 , - 0.66	
1981-1985	80	-3.78	-6.24 , - 1.32	
1986-1990	90	-2.27	-4.09 , - 0.46	
1991-1995	102	-3.91	-5.70 , -2.12	
1996-2005	88	-5.71	-7.26 , -4.16	
Total breast area at baseline:				0.27
Smallest quartile	103	-2.54	-4.66 , -0.41	
2nd quartile	105	-4.68	-6.71 , -2.66	
3rd quartile	110	-4.91	-6.75 , -3.07	
Largest quartile	104	-3.56	-5.16 , -1.96	
Adjuvant therapy of (first) cancer:				<0.01
No adjuvant therapy	78	-1.09	-3.09 , 0.92	
Radiotherapy only	114	-3.22	-5.14 , -1.30	
Hormone therapy (with/without radiotherapy)	174	-4.1 5	-5.37 , -2.93	
Chemotherapy (with/without other adjuvant therapy)	56	-8.74	-5.24 , -12.24	
Menopause status at (first) cancer:*				<0.01
Premenopausal	173	**-5.90**	**-7.70 , -4.10**	
Postmenopausal	243	**-2.56**	**-3.54 , -1.58**	
Postmenopausal HRT use:*				<0.01
with **Current use** of **HRT** at diagnosis	51	**-6.55**	**-8.90 , -4.19**	
with **No current use** of **HRT** at diagnosis	127	**-1.66**	**-2.90 , -0.43**	
ER-status of (first) cancer:				0.63
ER-positive	295	-4.15	-5.29 , -3.01	
ER-negative	53	-3.46	-5.92 , -0.99	
TNM-stage of (first) cancer:*				0.67
1	244	-4.06	-5.27 , -2.85	
2	157	-3.48	-5.10 , -1.86	
3	16	-5.36	-11.19 , 0.47	

* 6 (1%) women with unknown menopause, 65 (27%) postmenopausal women with unknown HRT. 5 women with unknown TNM-stage

variable has recently been shown to be preferential to adjusting for BMI[22]. The risk of CBC as a function of baseline mammographic density was analyzed using conditional logistic regression, contrasting CBC patients as cases to unilateral breast cancer patients as controls. Percentage density at baseline was categorized into ≤5% (reference level), >5-25% , >25-50% and >50%, these cutoffs have been used extensively[1]. Dense area at baseline was categorized into: ≤20 cm^2, >20-40 cm^2, >40-60 cm^2 and >60 cm^2, with these categories corresponding approximately to quartiles of the baseline dense area distribution. All analyses were adjusted through matching for age and calendar period of diagnosis, adjuvant therapy and follow-up time. In an additional model we further adjusted the estimates for non-dense area at first diagnosis, categorized in quartiles.

Conditional logistic regression was also used for analyzing risk of CBC as a function of *change* of mammographic density from baseline to first follow-up mammogram, categorized in three levels: absolute decrease ≥10%, stable (-10% to +10%, reference level) and absolute increase ≥10%, in agreement with previous litterature[4]. Further, we investigated change of density in terms of absolute dense area, also categorized in three levels: ≥ 10 cm^2 reduction, stable (-10 cm^2 to +10 cm^2, reference level) and ≥ 10cm^2 increase in dense area. Both analyses were adjusted through matching for age and calendar period of first diagnosis, first adjuvant therapy and follow-up time. In an additional model, we made further adjustments for baseline non-dense area and baseline mammographic density (percent density when using change in percent density and area density when using change in area density), both categorized in quartiles. In all analyses examining the impact of changes in density, patients with <10% or >90% percent mammographic density at baseline, or those with <10 cm^2 or >70 cm^2 dense area, were excluded, since they cannot possibly undergo changes in percent mammographic density, or dense area, of the defined magnitude. A similar strategy has previously been used by others when studying changes in mammographic density[2].

All data preparation and analyses were carried out using SAS Statistical Package 9.2. The Ethical Review Board at Karolinska Institutet, Stockholm, Sweden, approved the study.

Results

422 subjects (211 cases and 211 controls) were included in the analysis (Table 1). The mean time from diagnosis to follow-up mammogram was 1.5 years, 90% of the follow-up mammograms are taken between 1 and 2.2 years after diagnosis of first breast cancer and there was no difference between cases and controls. The mean breast density at baseline was 28%.

Table 2 describes the mean change of mammographic density, measured in absolute percent density, from baseline to follow-up mammogram. The change is similar over calendar period and over different categories of total breast area. As expected the mammographic density decreases significantly more in the women diagnosed before menopause, compared to women diagnosed after menopause (P-value <0.01). Among postmenopausal women the decline of mammographic density was more marked for women who were current users of hormone replacement therapy at time of their first diagnosis (P-value <0.01).

We found no association between mammographic density at baseline and risk of CBC using either percent mammographic density or dense area (Table 3). We further compared the baseline density between unilateral breast cancer patients and healthy controls; as expected we found a statistically significant increasing risk of breast cancer with increasing mammographic density (p-value for trend <0.01).

Using conditional logistic regression we observed a 55% lower risk of CBC for women with an absolute decrease in mammographic density of ≥10% from baseline to follow-up mammogram, compared to women with stable mammographic density (OR=0.45 [95% CI: 0.24-0.84]) (Table 4).

We found no statistically significant effect of increasing absolute mammographic density compared to stable density (OR=0.83 [95% CI: 0.4-2.87]). Through the matched case-control design, these findings are independent of age and calendar period of first diagnosis, first adjuvant therapy and follow-up time. The adjustments for non-dense area at baseline and percent density at baseline affected the estimates only marginally, but are included in Model 2 since they are potential confounders. Using absolute dense area as a measure of mammographic density we found a similar effect of 46% risk decrease for women with ≥10 cm^2 decrease from baseline to follow-up mammogram, compared to women with stable density (OR=0.54; 95% CI: 0.30-0.99) (Table 4). No statistically significant effect was seen for ≥10 cm^2 increase of density compared to women with stable density (OR=0.71 [95% CI: 0.30–1.69]). Further, adjustment for hormone replacement therapy affected the estimates only marginally (OR for ≥10% decrease= 0.41 [95% CI: 0.21-0.78], OR for ≥10% increase = 0.87 0.41 [95% CI: 0.25-3.07]) and is not included in the models.

As an exploratory analysis we stratified our population on menopause status at baseline mammogram and investigated the effect of change of mammographic density on the risk of CBC in the two subgroups, adjusting for non-dense area at baseline and percent density at baseline. Among the premenopausal women an absolute decrease of

Table 3: Odds ratio of CBC and unilateral breast cancer in relation to levels of mammographic density at the time of the first breast cancer diagnosis

Density at diagnosis	**CBC-patients vs. unilateral breast cancer patients**					**Unilateral breast cancer patients vs. healthy women**				
Percent density*	**N**	**OR***	**95% CI**	**OR*****	**95% CI**	**N**	**OR****	**95% CI**	**OR*****	**95% CI**
≤ 5%	24	1.00	Ref.	1.00	Ref.	19	1.00	Ref.	1.00	Ref.
> 5-25%	174	0.80	0.33 - 1.94	0.78	0.32 - 1.92	125	1.72	0.60 - 4.93	1.72	0.59 - 5.00
> 25-50%	184	0.88	0.34 - 2.25	0.89	0.34 - 2.33	111	4.47	1.46 - 13.65	3.91	1.20 - 12.77
> 50%	40	0.31	0.09 - 1.07	0.36	0.10 - 1.31	32	6.78	1.80 - 25.52	4.97	1.19 - 20.81
P-value for trend		0.22		0.40			<0.01		<0.01	
Area density*	**N**	**OR***	**95% CI**	**OR*** **	**95% CI**		**OR** **	**95% CI**	**OR*** **	**95% CI**
≤ 20cm^2	106	1.00	Ref.	1.00	Ref.	85	1.00	Ref.	1.00	Ref.
>20-40cm^2	144	0.80	0.47 - 1.35	0.74	0.43 - 1.27	101	1.55	0.87 - 2.77	1.55	0.85 - 2.81
>40-60cm^2	96	1.02	0.56 - 1.84	0.93	0.50 - 1.71	52	2.44	1.15 - 5.16	2.17	1.00 - 4.69
> 60cm^2	76	0.98	0.52 - 1.83	0.94	0.49 - 1.79	49	2.85	1.31 - 6.16	2.44	1.10 - 5.44
P-value for trend		0.84		0.96			<0.01		0.02	

*Analysis adjusted by CBC-patients being matched to unilateral breast cancer patients by age at diagnosis, calendar period of diagnosis, follow-up time and adjuvant therapy.

** Analysis adjusted by unilateral breast cancer patients being matched to healthy women by age at mammogram and calendar period of mammogram.

*** In addition to matching the analysis is also adjusted for non-dense area at baseline mammogram.

mammographic density of 10% or more from baseline to follow-up mammogram was associated with an OR of CBC of 0.29 (95% CI:0.09-0.92 (N=164)) compared to the reference level of stable density. The corresponding OR for post menopausal women was 0.49 (95% CI: 0.16-1.45) (N=188).

Discussion

To our knowledge, this is the first study to have ever investigated the risk of CBC as an effect of mammographic density at the time of diagnosis of the first cancer and of its subsequent changes. In a population-based setting we estimated that an absolute decrease of mammographic density from diagnosis to first follow-up mammogram of at least 10% confers a significant 55% decrease in risk of CBC. In contrast, mammographic density at diagnosis does not seem to predict risk of CBC.

Strengths of the study include its population-based setting, its ability to investigate both baseline density and its changes after first diagnosis, and the use of absolute (dense area) and relative (percent density) measures of mammographic density. The study is limited mainly by its small size, resulting mainly from the large proportion of cases that had to be excluded from the analysis because the required mammograms could not be traced. Exclusion of patients due to unavailability of mammograms is a potential source of bias if the missingness is differential; however, access to the patient's mammograms is by necessity a requirement for studies of mammographic density. Reassuringly, the excluded patients did not differ from those included in the analysis in relation to important confounding factors such as age at first diagnosis, calendar period of first diagnosis or type of adjuvant treatment regimen administered.

In contrast to the effect of mammographic density on the risk of unilateral breast cancer among healthy women, mammographic density at baseline did not seem to influence the risk of CBC in breast cancer patients (Table 3). This is a somewhat unexpected finding but mimics the effect of established hormonal/reproductive risk factors for breast cancer[12-14], which increases the risk of breast cancer[23,24] but not the risk of CBC[12-14]. An alternative explanation for the lack of association between mammographic density and risk of CBC could be that there was a systematic difference in mammographic density of the unilateral breast cancer patients selected as controls for the current study, compared to unilateral breast cancer patients in general. To investigate this concern we studied the effect of mammographic density on the risk of breast cancer in unilateral breast cancer patients compared to healthy women and reassuringly found the expected strong association between mammographic density and the risk of breast cancer.

Table 4: Odds ratio of CBC in relation to changes in mammographic density after the first breast cancer diagnosis

Percent density*	**N**	**OR***	**95% CI**	**OR****	**95% CI**
Absolute decrease ≥10%	96	**0.49**	**0.28 - 0.85**	**0.45**	**0.24 - 0.84**
Stable	243	1.00	Ref.	1.00	Ref.
Absolute increase ≥10%	17	0.74	0.23 - 2.40	0.83	0.24 - 2.87
P-value		0.04		0.04	
Area density***	**N**	**OR***	**95% CI**	**OR****	**95% CI**
Absolute decrease ≥10cm^2	108	0.67	0.38 - 1.16	**0.54**	**0.30 - 0.99**
Stable	197	1.00	Ref.	1.00	Ref.
Absolute increase ≥10cm^2	33	0.79	0.35 - 1.78	0.71	0.30 - 1.69
P-value		0.35		0.13	

* Adjusted through matching for age at diagnosis, calendar period of diagnosis, adjuvant therapy and follow-up time.
** Adjusted through matching for age at diagnosis, calendar period of diagnosis, adjuvant therapy and follow-up time, and additionally in the analyses for percent density and non-dense area at first diagnosis.
*** Patients with percent density≤10% or ≥90% at (first) diagnosis were excluded from analyses examining the effect of changes in percent density; similarly, patients with absolute dense area≤10 cm^2 or ≥70 cm^2 at (first) diagnosis were excluded from analyses investigating the effect of changes in absolute dense area.

Our study showed that women who experienced at least 10% decrease in mammographic density after the first breast diagnosis were at a substantial lower risk of developing CBC compared to those whose mammographic density remained stable (Table 4). Two previous studies had investigated the relation between change of mammographic density and risk of unilateral breast cancer and shown that decreasing density was associated with a decreasing risk of developing breast cancer[5,25]. However, no previous study has examined changes in density after a first diagnosis of breast cancer in relation to risk of CBC. Not only are CBC patients a selected subgroup of women with high susceptibility of breast cancer, they are on average younger and have a higher prevalence of family history of the disease. Furthermore, a large proportion of CBC patients are treated with adjuvant therapy for their first breast cancer. In a primary prevention study women with a high risk of breast cancer, primarily selected on the basis of a positive family history, were treated with tamoxifen or placebo and showed a decreased risk of breast cancer following a decrease in mammographic density[4]. This effect was more pronounced among the tamoxifen-treated patients, though the difference was not statistically significant. In the present study, we found that the decrease in the risk of CBC associated with a decline in mammographic density after the first diagnosis was independent of the type of adjuvant treatment administered but we believe that further research of the effect after specific adjuvant treatment regimens is warranted.

Kerlikowske et al[5] suggested that the effect of change in mammographic density on the risk of unilateral breast cancer might be more pronounced in premenopausal women. We stratified our analysis on menopausal status at first breast cancer diagnosis and indeed found a suggestion of a stronger effect of decreased density on risk of CBC in the premenopausal women. The majority of the premenopausal women diagnosed with breast cancer will go through menopause relatively soon, either naturally, due to ageing or artificially, due to adjuvant chemotherapy[26]. The change in mammographic density resulting from menopause is relatively large[27] and may result in a subsequent decrease of CBC risk. It might be that the change of density occurring during menopause is more important than changes in density due to other factors or during other periods of life. It is of note that very few studies to date have been able to study healthy premenopausal women, as most are conducted among women of the ages targeted by screening mammography.

Breast cancer patients are, during the remainder of their life, at a constant high risk of developing CBC; a cancer form of worse survival than the original cancer[16]. In contrast to the effect of mammographic density on the risk of breast cancer in healthy women, this study shows that mammographic density at diagnosis of the first cancer is not a useful predictor of CBC risk. On the other hand, our findings indicate that women who experience ≥10% absolute decrease in mammographic density from the first diagnosis until the first follow-up mammogram (approx. 1.6 years later) decrease their risk of CBC to about half. Furthermore, the effect of decreasing mammographic density on risk of CBC was independent of therapy given for the first cancer. The 10% cutoff has been previously shown as the minimum change that could be reproducibly detected visually[4] and might therefore be clinically useful, in the present study 23% of the participating women

experienced such a decrease. If confirmed, change of mammographic density can be used to predict the risk of CBC, and can thus contribute to decision making in follow-up routines and adjuvant treatment regimens as well as provide reassurance to the patients at decreased risk.

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