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HEALTH OUTCOMES OF WOMEN WITH BREAST CANCER

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TITLE OF THESIS

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“Reality is not always probable, or likely”

— *Jorge Luis Borges*

ABSTRACT

The overall survival of breast cancer patients has increased quite remarkably in the past decades in the developed countries due to substantial improvements in diagnosis and treatment. As a consequence, the proportion of women alive after a breast cancer diagnosis is currently increasing. It is therefore becoming of outmost importance to also focus on medium- and long-term health outcomes of women with breast cancer.

Swedish population registers were used to study time-dependent survival of breast cancer patients according to age and tumor characteristics for the following health outcomes: causes of death, distant metastasis, risk of hospitalization due to a bone fracture, and risk of hospitalization due to an infection. Different survival analysis methodologies were applied including Cox regression models, Poisson regression models and flexible parametric survival models. Several measures were used to assess the outcomes of interest: rates, ratios and cumulative incidences. Comparisons with the general population using standardized incidence and mortality ratios were also performed.

The risk of dying from breast cancer varied by age, and by tumor characteristics in a time-dependent fashion. Circulatory system disorders were an important cause of death in our study population, in particular among women diagnosed with breast cancer after the age of 60 years. The risk of distant metastasis was still non-negligible after five years from breast cancer diagnosis in most subgroups of patients. Women with breast cancer were at increased risk of being hospitalized with a bone fracture or with an infection for at least ten years since diagnosis. The risk of hospitalization due to an infection was particularly increased for skin infections and sepsis. Women with breast cancer were also at significantly increased risk of dying after being hospitalized with a bone fracture or an infection.

Lymph node status at breast cancer diagnosis was not only found to be an important long-term predictor of overall and disease-free survival, but also of risk of hospitalization due to bone fracture or infection. Women with estrogen receptors-negative breast tumors showed a worse overall prognosis as compared with patients with estrogen receptors-positive breast tumors only in the first five years after diagnosis. Estrogen-receptor positive tumors carried a low but persistent risk of distant recurrence and death. A breast tumor size of more than 20mm at diagnosis was mainly associated with a worse short-term prognosis, however a mild significant association was detectable for more than five years from diagnosis.

In conclusion, there is no evidence to support discontinuation of clinical follow-up in breast cancer patients. Further investigation on more targeted approaches for different subgroups should be considered and more attention to medical conditions not directly related to breast cancer would be probably beneficial for these women. Preventive measures for bone fractures and infections could be taken into more consideration for all breast cancer patients at increased risk.

LIST OF SCIENTIFIC PAPERS

- I. Colzani E, Liljegren A, Johansson AL, Adolfsson J, Hellborg H, Hall PF, Czene K. **Prognosis of patients with breast cancer: causes of death and effects of time since diagnosis, age, and tumor characteristics.** *J Clin Oncol* 2011; **29**(30):4014-21.
- II. Colzani E, Johansson AL, Liljegren A, Foukakis T, Clements M, Adolfsson J, Hall P, Czene K. **Time-dependent risk of developing distant metastasis in breast cancer patients according to treatment, age and tumour characteristics.** *Br J Cancer* 2014; **110**(5):1378-84.
- III. Colzani E, Clements M, Johansson AL, Liljegren A, Adolfsson J, Hall P, Czene K. **Risk of hospitalization due to bone and femur fracture in women after breast cancer diagnosis.** *Manuscript*.
- IV. Brand J, Colzani E, Johansson AL, Hall P, Czene K. **Long term infection patterns in breast cancer patients.** *Submitted*.

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LIST OF ABBREVIATIONS

ADCC	Antibody-Dependent Cell-mediated Cytotoxicity
ASCO	American Society of Clinical Oncology
ASR	Age-Standardized Rate
BMI	Body Mass Index
CAHRES	Cancer And HorMonEs in Sweden
CDR	Swedish Cause of Death Register
CI	Confidence interval
CIF	Cumulative Incidence Function
CMF	Cyclophosphamide Methotrexate Fluorouracil
CNS	Central Nervous System
DCIS	Ductal Carcinoma In Situ
DNA	Deoxyribonucleic acid
ER	Estrogen Receptor
GWA	Genome-Wide Association
Gy	Gray
HER2	Human Epidermal growth factor Receptor 2
HR	Hazard Ratio
HRT	Hormone Replacement Therapy
IARC	International Agency for Research on Cancer
ICD	International Classification of Diseases
ID	Personal Identification
IMRT	Intensity Modulated Radiation Therapy
INCA	Information Network of Cancer treatment
IRR	Incidence Rate Ratio
LCIS	Lobular Carcinoma In Situ
LIBRO-1	Linné-bröst 1
NCI	National Cancer Institute
NCR	Swedish National Cancer Register
PR	Progesterone Receptor
RCC	Regional Cancer Center

SBCR	Stockholm Breast Cancer Register
SEER	Surveillance, Epidemiology and End Results program
SERMS	Selective Estrogen Receptor Modulators
SIR	Standardized Incidence Ratio
SMR	Standardized Mortality Ratio
SNP	Single Nucleotide Polymorphism
TNM	Tumor Nodes Metastasis
VTE	Venous thromboembolism
WTCCC	Wellcome Trust Case Control Consortium

1 INTRODUCTION

Breast cancer is the most common malignancy in the female population worldwide and its prevalence is increasing in most countries. In the last decades there has been however a significant decrease in breast cancer mortality in many developed countries. This success is probably due to the effect of early diagnosis and particularly to the remarkable improvements in treatment. Incidence is instead still steadily increasing, possibly due to lifestyle and historical trends but probably also because of improved sensitivity of diagnostic approaches and procedures. These epidemiological trends translate into a general relevant increase of the number of women living with a previous breast cancer diagnosis. The better survival currently observed in many developed countries is now allowing the investigation of more long-term health outcomes in breast cancer patients, including those health outcomes that are not directly caused by or related to the disease. In particular, it would be interesting to shed more light on the type and timing of causes of death, distant recurrences and cause-specific hospitalizations according to different patient and tumor characteristics in order to assess the long-term behavior of some of the known prognosticators. This might also have implications on the way breast cancer patients are followed and monitored after the initial treatment period and it could also help clarify whether there is any evidence that the clinical follow-up can be modified or interrupted after some years in certain groups of patients. Another important question to address is whether certain subgroups of women with breast cancer are at increased long-term risk for bone fractures or infections and whether there is any need to consider more preventive care aimed at these two possible comorbidities.

Finally, it is of potential interest to see whether some of these health outcomes occur more often as compared to the general female population from the same age, region and calendar period. Differences with the general population might underlie some inherent or acquired (e.g., treatment) characteristics of women who have been diagnosed with breast cancer influencing their overall survival and quality of life. We tried to give an overall picture of some of the relevant health outcomes in Swedish women with a previous diagnosis of breast cancer, and to study the time-dependent long-term effects of age and tumor characteristics.

2 BACKGROUND

2.1 EPIDEMIOLOGY

Breast cancer is the most common malignancy in the world and the second most common cancer overall in the female population, accounting for about 25% of all new cancer diagnoses. Breast cancer can also rarely affect males, but it is generally considered a malignancy mainly concerning the female population. In 2012, the global estimated amount of new breast cancer cases was 1,677,000, of which 794,000 occurring in the developed regions (of these, 367,000 in the European Union), and 883,000 occurring in the developing regions (Figure 1). In the period 2008-2012, there were about 6,255,000 breast cancer incident cases worldwide and 1,467,000 in the European Union. The incidence rates varied from the lowest estimate of 27 per 100,000 in Central Africa and Eastern Asia to the highest of 96 per 100,000 in Western Europe [1].

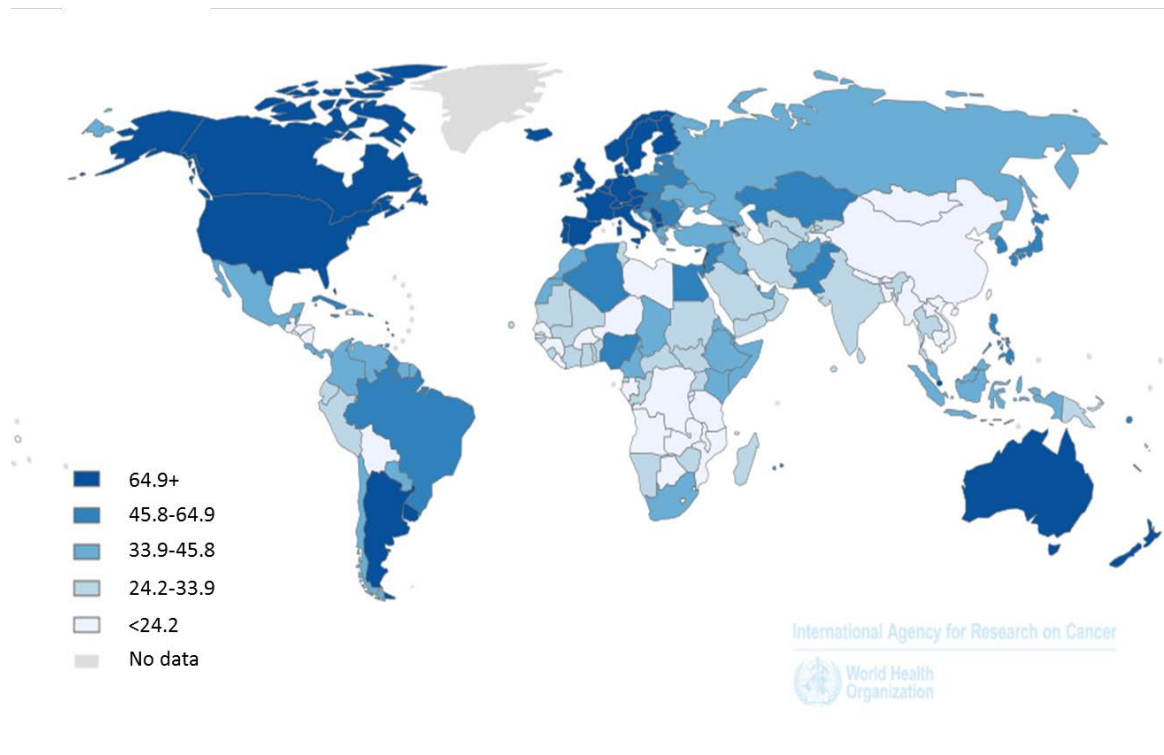


Figure 1. Age-standardized incidence rates of breast cancer per 100,000 (adapted from GLOBOCAN 2012, IARC).

Breast cancer is the world's fifth cause of death from cancer overall with 522,000 deaths occurring in 2012. In developed countries it is the second most common cause of death due to a malignancy after lung cancer with 198,000 deaths (15.4% of all cancer deaths) in 2012, of these, 92,000 in the European Union alone. In developing countries breast cancer caused about 324,000 deaths (14.3% of all cancer deaths) in 2012. The mortality rates were ranging from 6 per 100,000 in Eastern Asia to 20 per 100,000 in Western Africa [1] (Figure 2).

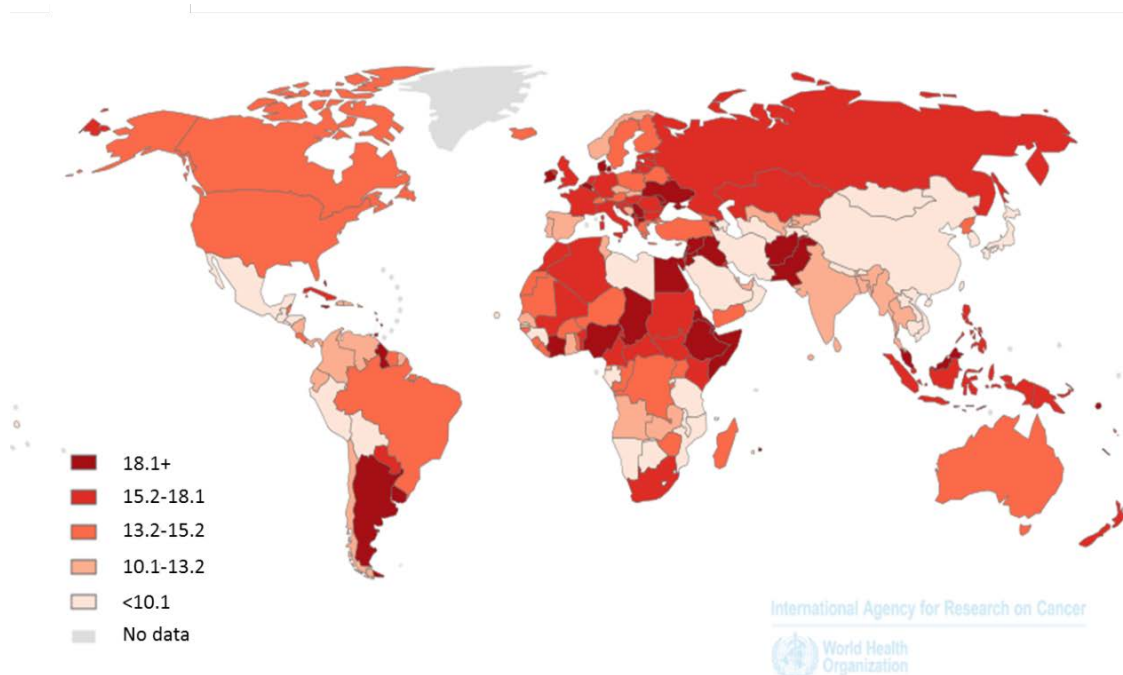
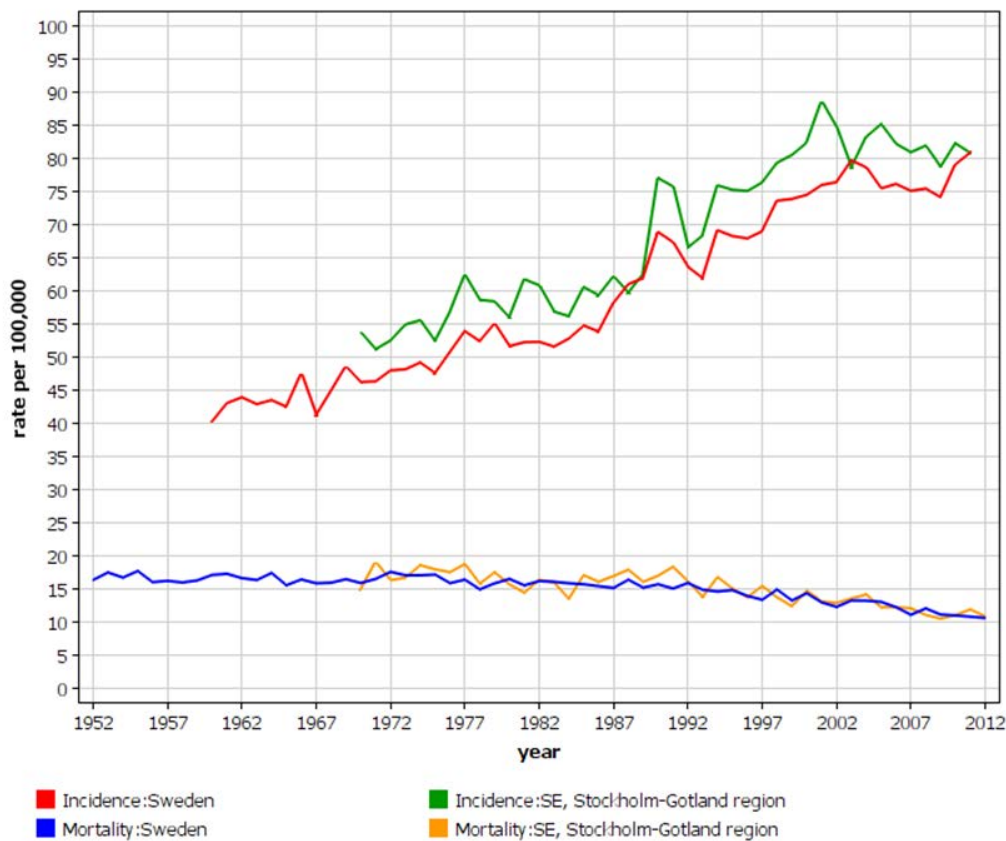


Figure 2. Age-standardized mortality rates of breast cancer per 100,000 (adapted from GLOBOCAN 2012, IARC).

In Sweden breast cancer accounts for almost 30% of all cancers and for 13.5% of all cancer deaths, with a risk of dying from the disease by the age of 75 years of 1.5%. About 8,000 women develop breast cancer every year. The age-standardized incidence has almost doubled between 1960 and 2010 (Figure 3) while there has been a parallel decrease in mortality over calendar time. The incidence has been steadily increasing by 1.3% in the last 20 years, but this increase has been weaker (0.9%) in the last 10 years maybe due to the decrease in the use of hormone replacement therapy (HRT) or to saturation in mammographic screening. The current risk of developing breast cancer by the age of 75 years for Swedish women is 9.1%. The 5-year survival of a breast cancer patient is estimated to be around 92% [2].

Breast
ASR (World), Female age 0-74



NORDCAN © Association of the Nordic Cancer Registries (10.11.2014)

Figure 3. Age-standardized incidence and mortality rates of breast cancer per 100,000 in the Swedish female population (age 0-74 years) over calendar time (from NORDCAN 2014).

Breast cancer survival has improved dramatically in the last few decades. The 5-year survival for a breast cancer patient went from less than 70-80% in the 1970s to about 90% in recent years with some differences according to age at diagnosis and tumor subtype [3, 4] (Figure 4). This remarkable improvement is due to several factors, and in particular to adjuvant treatment (treatment given following surgery) and early diagnosis. Early diagnosis allowed more conservative treatment approaches, thus improving the quality of life of breast cancer patients. New polychemotherapy regimens have contributed to about 25% decrease in annual death rates from 1980s and, in combination with 5-year hormonal treatment, have approximately halved death rates among middle-aged women with estrogen receptor (ER)-positive tumors [5].

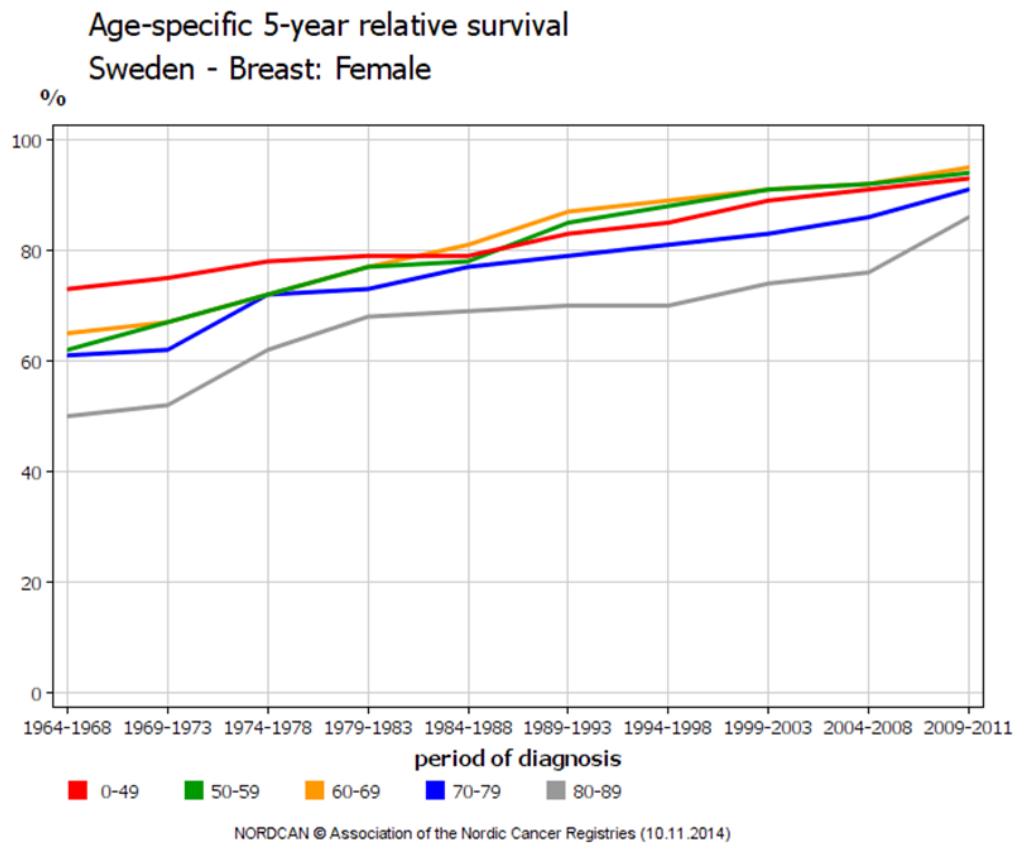


Figure 4. Secular trends in age-specific survival of Swedish breast cancer patients by calendar time and by age (from NORDCAN 2014).

2.2 NATURAL HISTORY

2.2.1 Risk factors

2.2.1.1 Hereditary and genetic

Breast cancer is a malignancy with an important hereditary component. A family history of breast cancer in a first-degree relative implies a two-fold life-time increased risk of developing breast cancer. If more than one first-degree relative have been diagnosed with breast cancer, the risk can be even higher. Two high penetrance susceptibility genes are known to be very strongly associated with breast cancer: *BRCA-1* and *BRCA-2* [6]. These genes are transmitted in a dominant fashion from parent to children and they carry a 45-65% life-time risk of developing the disease. About 16% of breast cancer is attributable to any of these two genes that are also responsible for an increased risk of ovarian cancer and other cancers also in males [7]. Other high penetrance genes have been more recently identified but they are considered rather rare: *TP53*, *PTEN*, *STK11* and *CDH1*. Also quite rare are intermediate penetrance genes like *CHEK2*, *ATM*, *BRIP1* and *PALB2* [8]. Single gene inheritance is considered to contribute to about 20% of all familiar risk of breast cancer [7], all remaining cases are believed to have a polygenic component. This means that specific sets

of genes variants, that individually would only slightly increase the risk, can actually cause breast cancer. In order to identify such sets of genetic variants, genome-wide association (GWAS) studies are used in order to compare single nucleotide polymorphisms (SNPs) across different genes between breast cancer cases and controls. SNPs are variations across individuals at a single position in the DNA sequence that are studied for their possible association with specific phenotypes (e.g., risk of developing diseases, drug resistance, survival). The Breast Cancer Association Consortium has recently assessed 41 common non-synonymous SNPs previously indicated by the Wellcome Trust Case Control Consortium (WTCCC) as associated with breast cancer [9]. Recent GWAS have identified up to 77 SNPs in breast cancer susceptibility loci [10] and some SNPs have also been reported to be associated with breast cancer prognosis [11]. The GWAS need to use large number of subjects in order to obtain enough power to detect signals and therefore are often performed by big consortia and require a lot of genetic data available. The GWAS approach only allows for generation of hypotheses as they are not aimed at testing *a priori* hypotheses [12].

2.2.1.2 *Hormone-related*

Breast tissue is responsive to sex hormones, and to estrogen in particular. Sex hormones regulate the growth and the function of the breast gland, though only a minority of normal breast cells expresses receptors for estrogen (and progesterone) [13]. The overexpression of estrogen receptors, particularly in postmenopausal women, is associated with an increased risk of breast cancer [14]. Moreover, an association between risk of breast cancer and persistently high levels of blood estrogen has been found in many studies [15]. Most non-hereditary breast cancer risk factors seem to be actually related to endocrine or exogenous estrogen exposure through different mechanisms [13].

The duration and estrogen-exposure variations in the reproductive life of a woman can modify the life-time risk of developing breast cancer. Young age at menarche and older age at menopause (i.e., longer reproductive life, more menstrual cycles and more exposure to cyclic sex hormones increment by the ovaries) increase the risk of breast cancer [16, 17]. Breastfeeding, younger age at first full-term pregnancy and multiple full-term pregnancies independently decrease the risk of breast cancer [18, 19]. It seems in fact that the number of undifferentiated cells in the breast decreases at each pregnancy, thus lowering the long-term risk of potential malignant transformations [20]. Nevertheless, intrauterine life and pregnancy have also been discussed as possible moments when the exposure to sex hormones can increase the risk of developing breast cancer [20]. Also a high body mass index (BMI) may increase the risk of developing breast cancer in postmenopausal women [21]. The conversion of androgens to estrogens in the adipose tissue is actually the main source of endogenous estrogen exposure in postmenopausal women [22] and may be the reason of the increased risk in women with a high BMI.

Exogenous hormone exposure is also associated with the development of breast cancer. In the 1990s many women underwent hormone replacement therapy (HRT) in order to reduce the side effects of menopause (e.g., hot flashes, bone loss, hypertension). They were given HRT

either through estrogen alone or in a combination of progesterone and estrogen. After some years, an increased incidence of breast cancer was detected in these women suggesting a direct link with HRT and with postmenopausal exposure to sex hormones [23]. The risk increased with the duration of use and was estimated to be about 7.6% per year when using estrogen and progesterone in combination [24]. Oral contraceptives have also been found to be associated with an elevated risk of breast cancer in current or recent users [20].

2.2.1.3 Breast density

Mammographic density is another risk factor for breast cancer and is thought to be associated with heritability and cumulative exposure to estrogens. It is not clear whether it is also a predictor of aggressiveness of the tumor since it does not seem to be associated with the risk of distant recurrence, with tumor characteristics, or with known molecular breast cancer subtypes [25, 26]. Some recent studies suggest that changes in breast density during hormonal treatment can serve as long-term predictors of prognosis [27].

2.2.1.4 Age

As for most solid cancers, the risk of developing breast cancer increases with age. In particular there is a steep natural increase in the incidence of the disease at around the age of 50 years independent of screening, suggesting an association with menopause and with the related change in hormone secretion. However, breast cancers that are developed at a younger age are usually more likely to be caused by genetic predisposition, to be more aggressive and to carry a worse prognosis.

2.2.1.5 Other risk factors

Studies carried out on atomic bomb survivors in Japan have shown a significantly increased risk of cancer even years after exposure to ionizing radiation. An apparent dose-response relationship with breast cancer was found among survivors. The risk of breast cancer was higher for exposures occurring at a younger age as compared with exposures occurring at an older age [28]. Alcohol intake was found to be associated with a moderate increase in risk of breast cancer in some studies [29]. A weekly moderate physical activity and an exposure to some vitamins in the diet have been reported to have some degree of protective effect but the studies are quite inconsistent [20, 30]. There is no current evidence that smoking is associated with the risk of breast cancer [31]. Finally, as for many other cancers, a previous diagnosis of breast cancer significantly increases the risk of other future primary breast cancers in the same person.

2.2.2 Cancer growth and spread

2.2.2.1 Carcinogenesis

Carcinogenesis describes the process of cancer initiation and evolution through a multistep process. It is still a controversial topic and several theories have accumulated over the years. For the cancers not directly inherited through the germline of the carrier (the so-called

sporadic cancers) there are two main prevailing theories. The somatic mutation theory has been prevailing for the last 50 years and considers that cancer originates from a primary disruption of the cell cycle in a single cell (monoclonality). Basically a single normal cell undergoes a series of consecutive mutations of its DNA in genes that control cell proliferation and cell cycle. These events cause an uncontrolled and increasingly undifferentiated proliferation that will alter the normal tissue's architecture and function, and will eventually lead to cancer growth, invasion and spread. During following subsequent mutations, cancer cells may acquire also the capacity to invade tissues and to undergo metastatic (i.e., distant) spread [32].

An alternative and more recent theory (the tissue organizational field theory) considers as the main driver of the carcinogenetic process an alteration of the microenvironment that controls the functional architecture of the normal tissue [33, 34]. The role of the microenvironment in the tumor development has been recently studied with increasing interest. It was found that stromal cells like fibroblasts, that are not parenchymal cells of a given organ from which a *carcinoma* usually originates (e.g., breast epithelial cells), are potentially able to modulate the carcinogenic process and to create the ideal conditions for cancer growth and spread [35].

2.2.2.2 *Cancer invasion*

Cancer invasion is the process by which a *carcinoma* (i.e., cancer) develops the capacity to go beyond the basal membrane of an epithelium and invade the *stroma* (i.e., mesenchymal tissue) where most blood vessels and lymphatic vessels are located, therefore acquiring the potential for distant spread. This step is the first event in the so-called "metastatic cascade" and it involves an initial loss of the cell-to-cell adhesion thus enabling cells to detach from the primary tumor. These cells then need to acquire a motile phenotype through changes in the cell-matrix interaction in order to perform the actual stromal invasion. The interaction between the primary tumor and the *stroma* are also very important for the angiogenesis (i.e., generation of new blood vessels) that the tumor needs for its development when it exceeds a certain size (about 2mm) [36].

2.2.2.3 *Distant spread*

Metastasis is the result of the spread of a cancer outside of the organ and of the tissue where it originated. It is a complex and still not completely understood process that can negatively impact the overall prognosis of a cancer patient.

Distant spread classically can follow two main pathways: cancer cells can either migrate through the blood or they can spread via the lymphatic system and the lymph nodes. For distant metastasis to develop, cancer cells need first, to acquire the capacity to invade the surrounding connective tissue (i.e., *stroma*) and to enter the blood vessels or the lymphatic channels (intravasation); then, to be able to migrate and survive against mechanical strain and immune cells in the blood stream or in the lymph; and eventually, to exit the blood vessels or the lymphatic channels (extravasation). Finally, in order to grow a secondary tumor mass in the new tissue, the cancer cells need to be able to survive in the new microenvironment and to

create the conditions for their proliferation and growth by developing a suitable local vascular system (i.e., angiogenesis) [37-39].

Breast cancer metastatic dissemination commonly privileges an initial lymphatic spread to the loco-regional lymph nodes, more often of the ipsilateral axilla. Alternatively, depending on the location of the tumor, the loco-regional metastatic spread can initially affect the internal mammary nodes close to the sternum, or the infraclavicular and supraclavicular nodes, but these locations are much less frequent. The lymphatic spread to the loco-regional lymph nodes has many relevant implications in the management of breast cancer and may influence the decision to perform an axillary node dissection or even decisions concerning the adjuvant treatment to administer after the surgery.

Distant metastasis can develop and grow in any organ and tissue of the body reaching its destination through the bloodstream (Figure 5). Each cancer, and specific subtypes of cancer as well, has preferential tissues where to spread. Breast cancer typically metastatizes to the skeleton, the liver, the central nervous system and the lungs: these are the organs and tissues where breast cancer metastasis tends to occur more often. Even metastatic cancer cells can show specific tissue tropism (i.e., predilection for specific organs/tissues) and the effect of the metastasis on the host tissue can be different according to the cancer of origin: bone metastasis in breast cancer patients tends to be preferentially osteolytic (i.e., with bone resorption capacity), while bone metastasis in prostate cancer for instance tends to be more often osteoblastic (i.e., with bone formation capacity) [36].

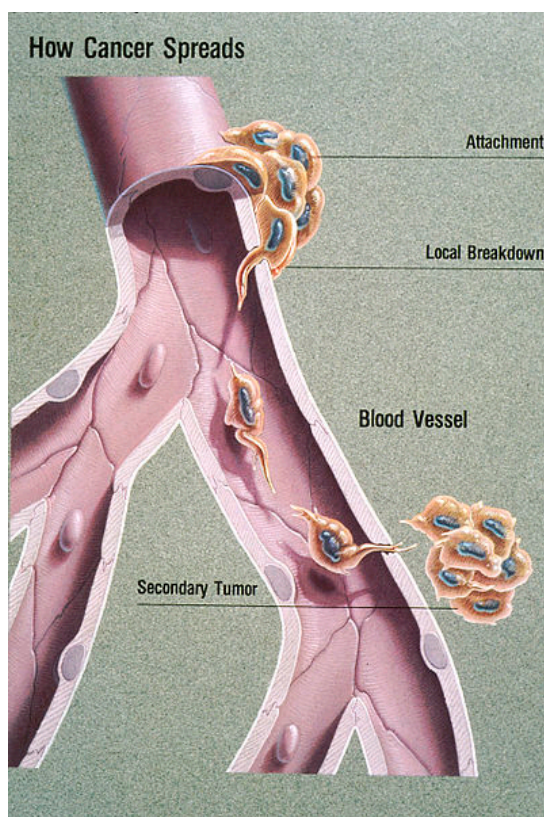


Figure 5. Metastatic spread of cancer through the blood vessels (from National Cancer Institute – NCI).

2.2.2.4 Recurrence

After an initially successful treatment, cancer can relapse locally, regionally or directly with a distant metastatic presentation. All these events are called recurrences, and in the case of breast cancer they can take place either at the surgical scar on the breast, in the chest wall or in the remaining breast tissue (local recurrence); at the regional lymph nodes (regional recurrence); or in a distant organ (distant recurrence). Distance recurrences often convey a non-favorable prognosis and can only undergo a palliative treatment. Local and regional recurrences can instead be treated with a good success rate [40].

2.3 BREAST CANCER CLASSIFICATIONS

Breast cancer can be classified according to different characteristics. The histopathological classification of breast tumors is the more traditional classification and is based on characteristics seen upon light microscopy of biopsy specimens. Current treatment, however, is mainly based on different aspects that relate to tumor aggressiveness, spread and molecular characteristics, therefore other classifications are also used.

2.3.1 Histopathological classification

The histopathological classification of invasive breast tumors identifies several types of breast *carcinomas*: ductal, lobular, mucinous (colloid), tubular, medullary, papillary, and other less common subtypes [41]. In addition to these, there are the mesenchymal tumors (including *sarcomas*) that do not originate from the breast tissue epithelium but from the connective and fat tissue surrounding the breast gland, and are rarer. The most frequent histological types of breast cancer are the ductal *carcinoma* and the lobular *carcinoma* that originate from the epithelium of the mammary ducts and lobules of the breast gland, respectively.

The *carcinoma in situ* of the breast, as opposed to invasive breast cancer, is a malignant cells proliferation within the epithelium of the breast that is not crossing the basal membrane of the breast epithelium. The *carcinoma in situ* of the breast can be either a ductal *carcinoma in situ* (DCIS, much more common) or a lobular *carcinoma in situ* (LCIS), depending on the respective origin within the breast. With improved early breast cancer diagnosis the incidence of DCIS in particular is rapidly increasing, suggesting that many of these lesions might not actually evolve into an invasive tumor [42].

2.3.2 Histologic grade

Another classification of breast cancer takes into account the grade of the tumor, which defines the degree of aggressiveness and the potential for proliferation of the cancer cells within the tumor tissue. The grade of the tumor is assessed by a pathologist after a biopsy of the tumor tissue and is generally classified as low, when the tumor looks less aggressive, or as high, when the tumor looks more aggressive with an increased potential for fast

proliferation, invasiveness and spread. Different scoring systems for tumor grade have been proposed and applied for different cancers. The Elston-Ellis modification of the Scarff-Bloom-Richardson grading system (Nottingham grading system) is the most often used for breast cancer. This grading system recognizes three levels, from low (grade I) to high (grade III) and the score is given based on three criteria: nuclear grade, tubule formation and mitotic rate [43].

The tumor grade strongly correlates with prognosis in breast cancer but it also strongly interacts with staging in predicting prognosis [44]. Some authors have argued that grade II (moderate) should not be taken into account in the assessment of indications for adjuvant therapy as it seems to be not as clearly correlated with prognosis as grade I and grade III [45]. Recent molecular profiling has identified protein signatures of histologic grading and suggested further subdividing grade II into two subcategories with significantly different prognostic value: grade IIa and grade IIb [46].

2.3.3 Biological/molecular classification

A more recent and evolving classification of breast cancer subtypes, which is more directly linked to prognosis and current treatment, is the biological or molecular classification. It is based on the expression in the cancer cells of specific molecular receptors that can affect cancer growth pattern and behavior: the estrogen receptor (ER), the progesterone receptor (PR), the human epidermal growth factor receptor 2 (HER2/neu), and the cancer proliferation marker, Ki-67. These proteins are all modulators of cells growth and their level of expression can modify the prognosis of breast cancer. They can in fact predict the behavior of the disease and some can be also used as targets for treatment.

2.3.3.1 Estrogen receptor status (ER status)

The estrogen receptor (ER) is an intracellular protein that is coded by two different genes in two forms, α and β , and that binds to the sex hormone estrogen. The ER- α is mostly expressed in breast cells, endometrium, ovarian stromal cells and hypothalamus. The ER- β is notably more expressed in the bone, kidney, brain, heart, lungs, intestinal mucosa, prostate and endothelial cells. ER is overexpressed in about 70% of breast cancers and these will be called ER-positive cancers (thus possibly susceptible to hormonal treatment) (Figure 6). ER status at breast cancer diagnosis is a very important prognosticator since ER-negative and ER-positive tumors show rather different natural histories and are subject to different treatments [47, 48]. The ER expression and the ER status of the tumor found at breast cancer diagnosis can however change over time after neo-adjuvant or adjuvant treatment in about one third of cancers, and discordance in ER status may be found between the original tumor mass and the metastatic tissue. This may have important prognostic and clinical implications [49-51]. There has been a certain degree of heterogeneity in the past in the accuracy, precision and consistency of interpretation of the ER status (and also of PR status, HER2/neu status and Ki67 measurement) but the introduction of recent guidelines is trying to make the assessment more homogeneous [52, 53]. ER-negative breast cancers carry a significantly

poorer prognosis as compared to ER-positive breast cancers. The difference in survival can in fact last up to 11 years after diagnosis and is particularly pronounced in the first 5 years [5, 54, 55].

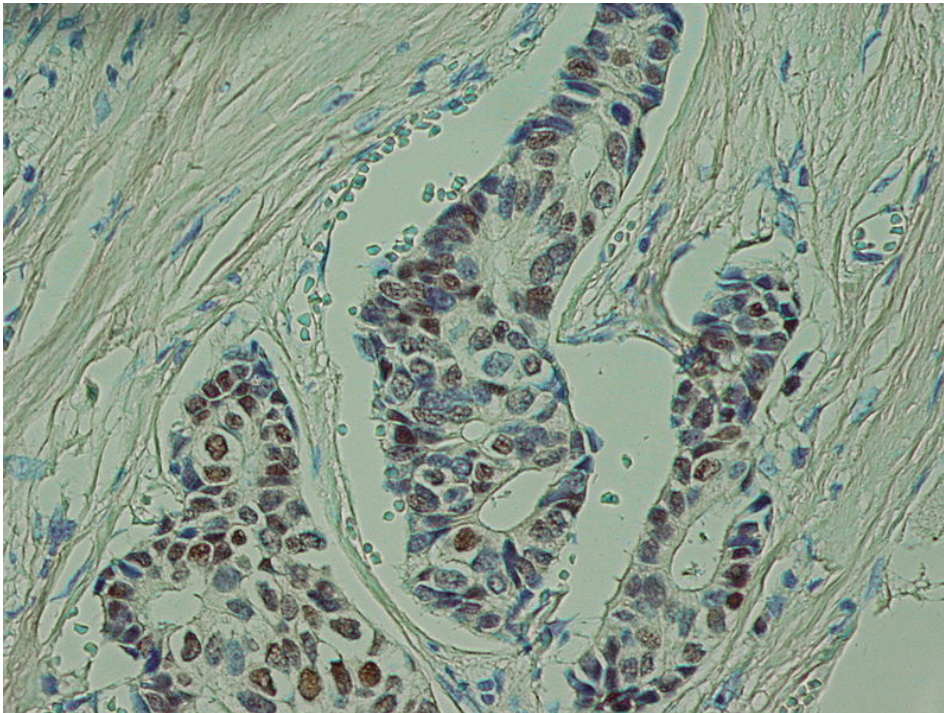


Figure 6. Estrogen receptor staining in breast cancer tissue (from Itayba / CC BY-NC-SA 3.0).

2.3.3.2 Progesterone receptor status (PR status)

The progesterone receptor (PR) is an intracellular protein that binds to the hormone progesterone and like the estrogen receptor it recognizes two forms, A and B. Although universally considered less important than ER status for breast cancer prognosis, there are some indications that low PR expression can possibly be associated with enhanced growth factor signaling and enhanced tumor aggressiveness [56]. PR status seems to also modify the responsiveness to specific hormone treatments [57, 58].

2.3.3.3 HER2/neu status

The Human Epidermal Growth Factor Receptor 2 (HER2/neu) is a protein that is normally regulating cell growth, differentiation and survival. In 10-30% of breast cancers HER2 is overexpressed and this translates into an increased aggressiveness of the disease [59]. HER2-positive status used also to imply a poorer prognosis until a new treatment, trastuzumab, was introduced in early 2000s. The introduction of trastuzumab dramatically improved the prognosis of HER2-positive breast cancer patients. The assessment of the HER2 status is considered of great clinical and prognostic relevance [60].

2.3.3.4 *Ki-67 proliferation index*

Ki-67 is a cellular protein that is strongly associated with cell proliferation. It is detectable in all active phases of the cell cycle (G1, S, G2, and mitosis) but it is absent in the resting phase (G0) [61]. The fraction of Ki-67 positive tumor cells is a reliable marker of cell proliferation and tumor growth and it is independently associated with survival. Ki-67 is routinely measured and used for prognosis and decision making in clinical practice.

2.3.3.5 *Biological/Molecular subtypes of breast cancer*

Despite the complexity of the genetic and molecular information that makes each breast cancer quite unique, the current molecular classification identifies four major subtypes that directly reflect into the disease behavior, prognosis and potential for treatment [62]:

- Luminal A (ER-positive and/or PR-positive, HER2-negative, with low Ki-67 expression): the most common molecular subtype of breast cancer accounting for about 40% of all invasive breast cancers; expected to be generally sensitive to hormonal treatment directed towards ER in particular;
- Luminal B (ER-positive and/or PR-positive, HER2-positive or HER2-negative with high Ki-67): the second most common molecular subtype of breast cancer; expected to be generally sensitive to hormonal therapy; usually of higher histological grade and worse prognosis as compared with luminal A;
- Triple negative/basal-like (ER-negative, PR-negative, HER2-negative): accounting for about 15-20% of all different subtypes and generally carrying the least favorable prognosis due to its aggressiveness and lack of receptors to be used as treatment targets [63];
- HER2 type (ER-negative, PR-negative, HER2-positive): the least common subtype (10-15%); currently treated with trastuzumab given its HER2 receptor positivity.

Other subtypes have been identified in some studies (e.g., luminal C, normal breast-like) however they do not seem to be as strongly characterized [62]. Molecular classification of breast tumors is a complex, controversial and rapidly evolving subject. Molecular subtypes are currently mostly used only in research setting. In the clinical setting, many decisions are often taken based on the tumor grade, the tumor stage and the independent expression of ER, PR, HER2/neu and Ki-67. A full integration of the use of molecular breast cancer subtypes into the routine clinical setting could be beneficial.

2.4 STAGING

Staging is used in clinical medicine to define the extent of the spread of a cancer. The most frequent staging classification, which is commonly used also for breast cancer, is the TNM staging: each letter define a criteria that is given a specific score. The letter “T” stands for “tumor size” and is categorized as follows:

- TX = primary tumor cannot be assessed;
- T0 = no evidence of primary tumor;
- Tis = *carcinoma in situ*;
- T1 = tumor size is less than 20mm;
- T2 = tumor size is 20-50mm;
- T3 = tumor size is larger than 50mm;
- T4 = tumor of any size growing into the chest or skin.

The letter “N” stands for “lymph node involvement” and is subdivided as follows:

- NX = nearby lymph nodes cannot be assessed;
- N0 = cancer has not spread to nearby lymph nodes;
- N1 = cancer has spread to 1-3 axillary lymph nodes and/or tiny amounts of cancer can be found in internal mammary lymph nodes;
- N2 = cancer has spread to 4-9 axillary lymph nodes or internal mammary lymph nodes are enlarged due to cancer;
- N3 = cancer has spread to at least one axillary lymph node and has enlarged the internal mammary lymph nodes, or cancer has spread to four or more axillary lymph nodes and small amounts of cancer are found in internal mammary lymph nodes, or cancer has spread to supraclavicular lymph nodes with at least one area of the cancer spread greater than 2mm.

The letter “M” stands for “distant metastasis”:

- MX = distant spread cannot be assessed;
- M0 = absence of distant metastasis on imaging procedures or on physical examination;
- M1 = presence of distant metastasis.

2.4.1 Tumor size

The size of the tumor is a measure of local tumor growth and is taken by the pathologist measuring the largest diameter of the tumor mass [64]. Early diagnosis and the introduction of mass screening programs have contributed to the observed gradual decrease in tumor size found at breast cancer diagnosis. Women diagnosed with breast tumors smaller than 2 cm have more than 90% 5-years survival on average, while tumors larger than 5 cm at diagnosis have the worst risk of recurrence [65, 66]. Tumor size and number of positive axillary lymph nodes at breast cancer diagnosis are highly correlated but they may differently affect prognosis [67]. This might be due to the different propensity to metastatize of some molecular subtypes of breast tumor at different stages of the local tumor growth: the size of tumor at diagnosis does not in fact tell much about the growth rate of the tumor. Triple negative tumors for instance show an attenuated association between tumor size, lymph node status and survival [63].

2.4.2 Lymph node status

The presence and the number of metastatic (positive) loco-regional lymph nodes at breast cancer diagnosis is considered a very important prognostic marker in breast cancer patients and it is often taken into high consideration for the clinical decision making [67]. Lymph nodes status at breast cancer diagnosis is also an independent marker of tumor aggressiveness [68]. The number of affected lymph nodes is negatively associated with prognosis and the highest risk of recurrence is for patients who have four or more positive lymph nodes at diagnosis [69]. The axillary lymph nodes (Figure 7) are the most common site of loco-regional metastasis and their surgical removal or irradiation is performed, when indicated, in order to avoid recurrences in the axilla, to assess prognosis and decide which type of additional treatment to perform.

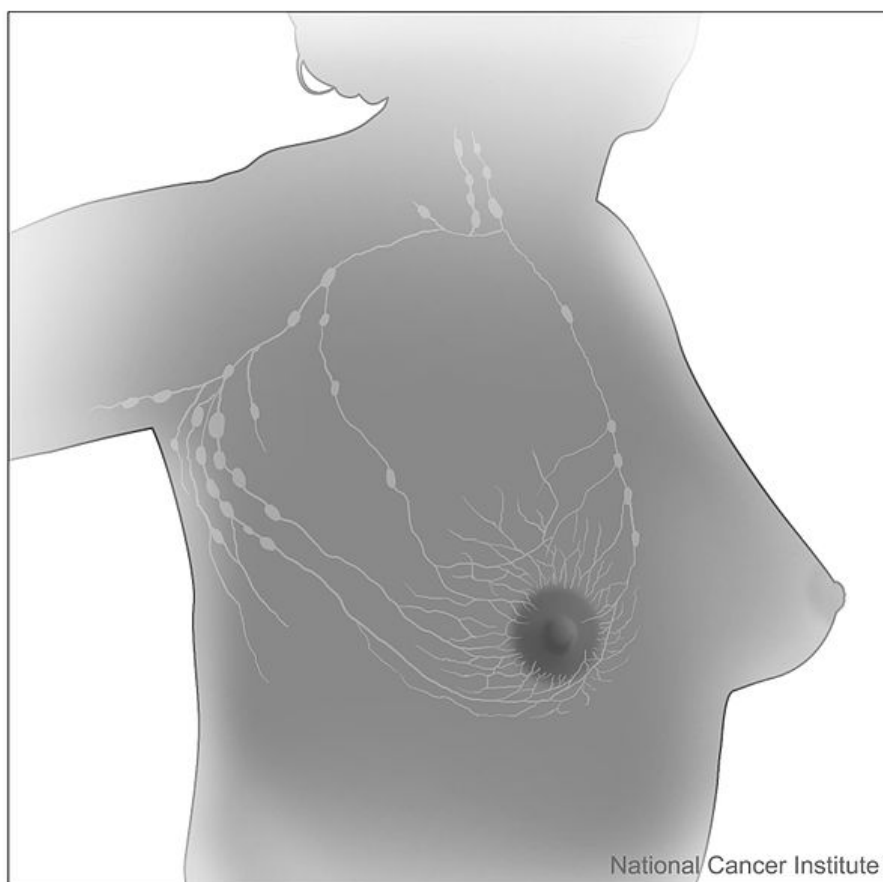


Figure 7. Anatomical disposition of lymph nodes in a normal breast ((from National Cancer Institute – NCI).

2.4.3 Metastasis

The presence of distant metastasis at breast cancer diagnosis is automatically considered stage IV, which is the stage with the worst prognosis according to the TNM staging system, and treatment is not considered curative. The development of distant metastasis is in fact still a predictor of negative prognosis in breast cancer patients (estimated 5-year survival: 22%), although a positive trend in survival has been observed in the last 15 years, at least in patients younger than 60 years at diagnosis [70]. Ongoing improvements in the understanding of the

metastatic process leading to a more targeted treatment will hopefully change the prognosis of metastatic patients in the next future.

2.4.4 TNM stages

The different stages of the TNM classification are derived based on the T, N and M scores.

Stage 0 basically corresponds to ductal *carcinoma in situ* (DCIS) or non-invasive Paget disease of the nipple, while lobular *carcinoma in situ* (LCIS) is generally not considered a true cancer or pre-cancer.

Stage I is defined by a tumor size less than 20mm with no macroscopic loco-regional or distant spread. Stage I is further subdivided into stage IA (T1 N0 M0) and stage IB (T0/T1 N1mi M0, where N1mi indicates presence of micrometastasis in 1-3 axillary lymph nodes).

Stage II is further classified as stage IIA and stage IIB: stage IIA can occur when the primary tumor is less than 20mm or undetectable in the concomitant presence of 1-3 positive axillary lymph nodes (T0 or T1, N1, but not N1mi, M0); or when the primary tumor is 20-50mm without any positive axillary lymph nodes distant spread (T2, N0, M0). Stage IIB occurs for instance when the primary tumor is 20-50mm and has 1-3 positive axillary lymph nodes (T2, N1, M0) or when it is larger than 50mm without any loco-regional or distant spread (T3, N0, M0).

Stage III represents cancers that have spread loco-regionally but not to other organs. Stage III is categorized as stage IIIA (T0 to T2, N2, M0 or T3, N1 or N2, M0), stage IIIB (T4, N0 to N2, M0) and stage IIIC (any T, N3, M0).

Stage IV corresponds to any cancer with distant spread to other organs independent of T and N scores (any T, any N, M=1) [64].

TNM staging strongly correlates with prognosis and is widely used for clinical decision making [67]. Its integration with molecular subtyping can improve its capacity to predict prognosis and to more accurately indicate appropriate treatment [71], particularly in triple negative cancers [72].

2.5 TREATMENT

2.5.1 Loco-regional treatment

2.5.1.1 Surgery

As for many other solid tumors, the treatment of breast cancer is primarily surgical. Until the 1970s breast cancer was considered essentially a local disease, and surgery was practiced in a very extensive way through radical mastectomy that consisted in the removal of all breast together with all pectoral and chest muscles in contiguity with the breast, and of all the axillary lymph nodes draining from the breast. It was a very disfiguring type of surgery with

some remarkable side effects, like lymphedema of the upper limb due to the removal of all the lymphatic drainage from the arm [73].

After the growing recognition of breast cancer as a systemic disease from the time of diagnosis, surgery started being considered a fundamental part of a more composite therapy, and not as the main treatment option. Different surgical procedures were gradually introduced: from the modified radical mastectomy (removal of the breast tissue and axillary lymph nodes, rarely performed nowadays) and the total mastectomy (removal of all breast tissue), to the breast conserving surgical approaches like quadrantectomies (removal of the affected quadrant of the breast) and lumpectomies (consisting in the removal of all the cancerous tissue and of some of the surrounding breast tissue). Breast conserving surgery was introduced because it showed similar effectiveness to more aggressive types of surgery when appropriately applied and followed by adjuvant treatment [74]. The idea behind breast conserving surgery comes from the lymph node sentinel approach: by injecting a dye into the breast close to the tumor during surgery, it is possible to see which lymph nodes are draining first from the cancer. These lymph nodes are then analysed for the presence of metastatic cells. If the lymph nodes are clear from cancerous cells, the tumor will be considered localized, and a breast conserving surgery will usually be carried out without any need of lymph node removal. If they are found to be affected, then an axillary lymph node removal will be considered as extensively as needed [75].

Breast conserving surgery alone however is not enough to effectively prevent local and distant recurrence, and it has to be combined with additional treatment, usually given within weeks or months from surgery. This treatment aimed at reducing the chance of future recurrences is called adjuvant treatment and can be administered through different combinations of radiation therapy, chemotherapy, hormone therapy, and other types of targeted therapy. The decision on which combination of adjuvant treatment to use is based on several factors, including tumor staging, molecular characteristics of the tumor, histological grading, general conditions of the patient (e.g., age, comorbidities) and preferences of the patient [76].

2.5.1.2 Radiation therapy

Radiation therapy after breast conserving surgery usually consists of an external beam irradiation of the remaining breast tissue, and of the surrounding area where surgery was performed, in order to reduce the chance of local recurrence if some cancerous tissue or cells were still left. Adjuvant radiation therapy has been shown to improve disease-free survival and breast cancer-specific survival. However, there have been some concerns about irradiation of the heart and possible consequent cardiovascular events, and also about irradiation of the lungs and increased long-term risk of second primary lung cancer [40, 77-79]. Other possible side effects of radiotherapy for breast cancer include redness of the irradiated skin, lymphedema of the arm in case of irradiation of the axilla, and pneumonitis. New radiation therapy techniques, such as intensity modulated radiotherapy (IMRT), tend to employ smaller fields compared to the past [80] and this may mean fewer acute adverse

effects, however this may also mean more scattered doses to the lung and to other organs. International guidelines made specific recommendations balancing risk and benefit of the breast irradiation treatment. In Sweden the treatment is usually given in a total dose of 50 Grays (Gy) with a daily dose of 2Gy for 5 days per week [81].

2.5.2 Systemic treatment

2.5.2.1 Chemotherapy

Chemotherapy is a systemic treatment often given after surgery with the aim of eliminating remaining cancerous cells from the body. Chemotherapy usually tends to interfere with rapid cell proliferation and therefore is not only acting on cancer cells but also on normal cells. This means that chemotherapy is associated with a certain amount of toxicity according to the agent and the dosage used. Some of the possible adverse effects of chemotherapy during treatment are neutropenia (infections), anemia, bleeding, hair loss, nausea and vomiting, fatigue, diarrhea, constipation, sexual and fertility changes, skin and nail changes, swelling, urination changes, pain, and nerve changes [82]. Several types of chemotherapeutic agents can be used and they are usually combined in different polychemotherapeutic regimens:

- cyclophosphamide, doxorubicin/Adriamycin, and 5-Fluorouracil;
- docetaxel/Taxotere, doxorubicin/Adriamycin, and cyclophosphamide;
- doxorubicin/Adriamycin and cyclophosphamide followed by paclitaxel/Taxol or docetaxel/Taxotere;
- 5-Fluorouracil, epirubicin, and cyclophosphamide followed by docetaxel/Taxotere or paclitaxel/Taxol;
- docetaxel/Taxotere and cyclophosphamide;
- docetaxel/Taxotere, carboplatin, and trastuzumab/Herceptin for HER2/neu positive tumors;
- cyclophosphamide/Cytosan[®], methotrexate, and 5-fluorouracil (CMF);
- doxorubicin/Adriamycin followed by CMF;
- epirubicin/Ellence and cyclophosphamide;
- doxorubicin/Adriamycin and cyclophosphamide.

In Sweden, lymph node negative patients requiring chemotherapy are offered an anthracycline combination of drugs or CMF, while lymph node positive patients are recommended a stronger treatment with polychemotherapy involving anthracyclines and taxanes [81]. Chemotherapy is generally indicated for the treatment of triple negative breast cancers and is also commonly given in HER2-positive tumors in combination with trastuzumab. ER-positive, HER2-negative patients are offered chemotherapy if the tumor size is larger than 5cm, if there are four or more positive lymph nodes, if the histological grade is 3, if there is a high proliferation rate, or if there is an extensive peritumoral vascular invasion [83]. However, some specific indications and therapeutic regimen choices may vary. Chemotherapy can also be given before surgery (neoadjuvant treatment), alone or in combination with other treatments (e.g., hormonal treatment). The aim of neoadjuvant

treatment is to reduce the mass of the primary tumor and the tumor loco-regional spread in order to enable or facilitate surgery and to increase chances of surgical success [84].

2.5.2.2 *Endocrine therapy*

Endocrine or hormone therapy is administered according to the molecular characteristics of the breast tumor. The selective estrogen receptor modulators (SERMS) can act as estrogen agonists or antagonists in different tissues and are the first and most common class of hormone treatment that has been utilized. Tamoxifen is the most widely used SERMS for breast cancer adjuvant treatment and is given to patients with tumors expressing the estrogen receptor (ER-positive). Tamoxifen is usually given for five years since breast cancer diagnosis in ER-positive patients [85-87], however new international guidelines suggest a prolonged use possibly up to ten years [88]. Unfortunately some relevant adverse effects might undermine its use for long periods: tamoxifen is in fact associated with an increased risk of vaginal bleeding, uterine malignancies, venous thrombosis, pulmonary embolism, hot flashes and night sweats [89-91]. The use of tamoxifen in small doses for the prevention of breast cancer has also been suggested [92].

Aromatase inhibitors are another class of hormonal treatment that has been introduced in the late 1990s. This class of drugs is able to interfere with the peripheral formation of estradiol (i.e., estrogen) by inhibiting the aromatase enzyme in the fatty tissue. Aromatase inhibitors do not affect the ovary incretion of estrogen, therefore they are effective in decreasing the level of circulating estrogen only in postmenopausal women, when the ovary has already ceased its activity. Aromatase inhibitors were shown to improve the disease-free survival but not the overall survival of breast cancer patients, probably due to some of their side effects on the heart and on the bone [5, 89-91, 93]. Aromatase inhibitors have been so far mainly recommended after 2-3 years of tamoxifen in postmenopausal breast cancer patients, so that hormonal adjuvant treatment can be performed without significantly increasing the risk of uterine cancer or of the other side effects related to the use of tamoxifen [5, 88-91, 93-96]. New guidelines are now recommending a prolonged use of hormonal treatment up to ten years since breast cancer diagnosis with different serial combinations of tamoxifen and aromatase inhibitors [88].

2.5.2.3 *Targeted therapies other than hormonal therapy*

New molecules within cancer cells and on cancer cells surface are being identified and can be potentially used as targets for new specific drugs. Just like hormone therapy, these targeted drugs do not affect the healthy tissue and specifically affect the tumor. The most famous non-hormonal targeted therapy in breast cancer, that has successfully been in use for quite some years now, is the already mentioned trastuzumab, a monoclonal antibody acting against the HER2/neu through an antibody-dependent cell-mediated cytotoxicity (ADCC) mechanism and therefore effective on HER2/neu overexpressing tumors (Figure 8). It is administered to HER2-positive patients following chemotherapy and has dramatically improved the prognosis of these patients [81]. Other similar drugs have been recently approved (e.g.,

lapatinib, pertuzumab) and others are currently under development and hopefully in the future there will be more therapies allowing for instance the targeted treatment of triple negative breast tumors.

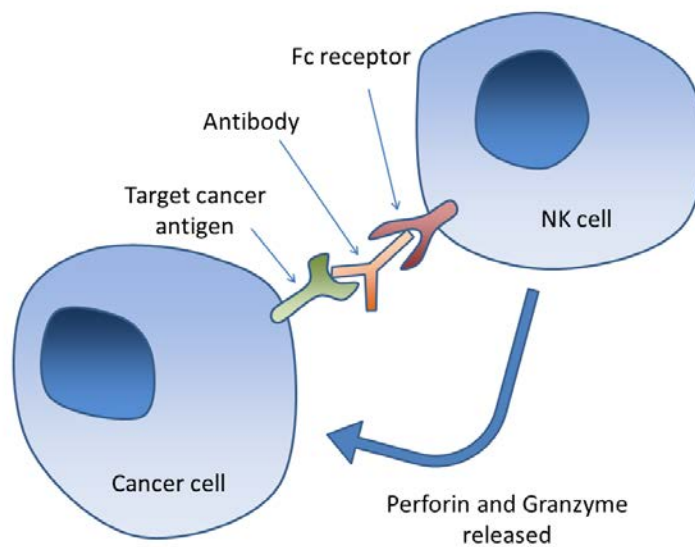


Figure 8. Antibody-dependent cell-mediated cytotoxicity (ADCC) mechanism of attack of breast cancer cells by the immune system (from Simon Caulton / CC BY-NC-SA 3.0).

Some alternative types of targeted cancer treatment other than hormonal treatment and immunotherapies are apoptosis inducers, angiogenesis inhibitors, gene expression modulators, signal transduction inhibitors and monoclonal antibodies delivering toxic molecules [97].

2.6 CLINICAL FOLLOW-UP

After the end of the treatment period regular medical examinations of breast cancer patients are periodically performed in order to detect possible recurrences at an early stage. The American Society of Clinical Oncology (ASCO) currently recommends performing a physical examination every three to six months for the first three years, then every six to twelve months until five years from diagnosis, and annually thereafter. Mammographic examinations should instead be performed annually. Other examinations are not recommended for routine follow-up [98].

2.7 HEALTH OUTCOMES

2.7.1 Causes of death and disease-free survival

Despite the aforementioned improvements, the survival of breast cancer patients is still lower than the one of the general population from the same age groups up to twenty years since

diagnosis [99, 100]. The risk of dying from breast cancer decreases with time since diagnosis and is usually higher in younger patients than in older patients. Conversely, the risk of dying from cardiovascular disorders and other cancers increases with age and consequently with time since breast cancer diagnosis [101, 102]. The impact of causes of death other than breast cancer is a topic of interest when looking at long-term outcomes in these patients. Moreover, few studies have tried to comprehensively take into account time trends, age, tumor characteristics, and treatment when studying the risk of dying from breast cancer [103, 104]: in particular, time-dependent variations by age at diagnosis and tumor characteristics of the survival patterns have not been thoroughly investigated.

Understanding in detail the disease-free survival patterns of patients with different breast cancer subtypes is also of great clinical relevance. The development of distant metastasis still unfortunately means that the patient is beyond cure [40, 105, 106]. We know that triple negative cancers tend to metastasize earlier and more frequently than other breast cancer molecular subtypes, however this different behavior seems to disappear after five years since diagnosis among the survivors [107, 108]. The risk of developing distant metastasis may in fact vary over time since diagnosis and across different subgroups of patients [63, 109-111]. Its thorough understanding would enable to consider a more tailored clinical follow-up of breast cancer patients in the future. The prognosis after the development of distant metastasis has already been analyzed and studied in more detail. Following the development of distant metastasis, age at breast cancer diagnosis, hormonal receptor status and site of metastasis are the most relevant factors for predicting survival [70, 112, 113].

Women with a previous breast cancer diagnosis may also be more exposed to other health-related conditions that can possibly affect their quality of life and their overall prognosis [114, 115]. This is an evolving area of relatively recent research thanks to the remarkable improvements in breast cancer survival. It is currently not quite clear what is the actual long-term risk of developing comorbidities in breast cancer patients since most of the adverse events have been studied close to the administration of the treatment. Some recent studies have shown that comorbidities like diabetes and stroke are significantly associated with the management and the survival of women with breast cancer [116, 117].

2.7.2 Bone fractures

Some evidence of an overall increased risk of bone fractures in women diagnosed with breast cancer as compared with the general female population has already been found, however the determinants and the magnitude have not been completely clarified [118]. Bone cells can express estrogen receptors and bone tissue is quite sensitive to variations in estrogen levels. Due to the drop in estrogen levels after menopause, postmenopausal women are in fact at increased risk of osteoporosis and bone fractures, namely hip fractures (Figure 9), vertebral fractures and fractures of the wrist [119]. Hormone treatment can therefore also affect calcium and bone metabolism through different mechanisms and thus influence the risk of bone fractures [120]. For instance, aromatase inhibitors, through their peripheral antagonist action in postmenopausal women against estrogen formation, can directly induce bone loss

and cause bone fractures [121]. Tamoxifen has instead a protective effect on the bone thanks to its estrogen-modulator characteristics that make it act as an estrogen antagonist in the breast tissue and as an estrogen agonist in the bone tissue [85-87, 89, 122, 123]. Other types of oncologic adjuvant treatment can have a negative effect on the skeleton independent of sex hormonal metabolism [124, 125]. Increasing evidence suggests an involvement of the bone marrow microenvironment in the metastatic process, and a possible effect of bone-targeted drugs in reducing bone metastasis and improving survival [126, 127]. For all these reasons other drugs like bisphosphonates are currently administered in parallel to the aromatase inhibitors in order to reduce the risk of bone metastasis and to strengthen the bone tissue [128, 129].



Figure 9. X-ray of a hip fracture (from © Nevit Dilmen / CC BY-NC-SA 3.0).

2.7.3 Infections

Infections are known complications that can be observed in cancer patients and may be due to cancer treatment, through immunosuppression, or to the cancer itself [130, 131]. Previous clinical trials have shown typical side effects of breast cancer treatment. For instance, it is well known that most chemotherapy agents and regimens can cause a decrease in blood cell counts, in particular neutrophils, thus leading to an increased risk of infections during the treatment period. Radiation therapy is administered locally and can cause some skin inflammation that sometimes can be subject to infection. Surgery itself, particularly when

removal of axillary lymph nodes is involved, can possibly lead to an increased risk of lymphedema of the upper limb with potential swelling and movement impairment, and also to subsequent skin infections (Figure 10).



Figure 10. Skin infection (*erysipela*) of the upper limb (from Poupou l'quourouce / CC BY-NC-SA 3.0).

Cancer patients with infections can experience prolonged hospitalizations and treatment delay [132, 133] as well as a worse long-term prognosis [130, 134]. Research has so far mainly focused on infections associated with neutropenia during periods of adjuvant treatment [134, 135] and little is known about the incidence of infections in breast cancer patients beyond the treatment period. Specific tumor characteristics and adjuvant therapies may predispose to certain types of infections even following the end of the treatment, but not much information is currently available on this topic.

2.7.4 Other potential health outcomes

There is also evidence that women with breast cancer may be at increased risk of other health-related conditions. These additional health outcomes should be taken into account as well in the management of breast cancer patients.

2.7.4.1 Second primary cancers

It is well-known that women with a breast cancer diagnosis are at significantly increased risk of developing other primary cancers as compared to women without a history of breast cancer. *BRCA-1* gene carriers have for instance a cumulative risk of developing a contralateral breast cancer of 87% by the age of 70 years and a risk of 44% of developing ovarian cancer by that time; they are also at 4-fold increased risk of developing a primary colon cancer [136]. Also, in all breast cancer patients increased percentage risks were found for the following cancers: soft tissue sarcoma (125%), thyroid cancer (62%), non-melanoma skin cancer (58%), leukemia (52%), endometrial cancer (52%), stomach cancer (35%), melanoma (29%), kidney cancer (27%), lung cancer (24%), colon cancer (22%) [137].

Moreover, as already discussed, the prolonged use of tamoxifen contributes to the increased risk of uterine cancer in breast cancer patients [90] and the exposure to irradiation may increase the long-term risk of second primary lung cancer [78] in particular among cigarette smokers [77].

2.7.4.2 Endocrine disorders

An association between diabetes mellitus and cancer has been found by several studies [138, 139]; there may be several reasons for this association but the real causes are currently not very well understood [140]. Diabetes mellitus however seems to be more of a risk factor for breast cancer and subsequent death [141-143].

2.7.4.3 Circulatory system disorders

Women with a previous breast cancer diagnosis can also be at increased risk of circulatory system disorders. Exposure to radiation therapy to the breast can affect the heart and may cause an increased risk of myocardial infarction from few years after irradiation up to at least 20 years since breast cancer diagnosis, particularly in women at previously increased risk of ischemic heart disease [79]. Other adjuvant treatment, like for instance anthracyclines agents used in chemotherapy and aromatase inhibitors, were found to have some toxic effect on the heart [89, 144].

Venous thromboembolism (VTE) is a common known complication of malignant diseases but its pathophysiology still remains poorly understood. VTE can also cause pulmonary embolism. The risk of deep vein thrombosis has been found higher in patients undergoing surgery for a malignancy, and the risk of recurrence after a first episode of VTE is higher in cancer patients than in other patients [145-147]. Additionally, patients with cancer who developed a VTE have a lower survival compared to cancer patients who did not develop a VTE [148]. As already mentioned, women treated with tamoxifen face an increased risk of blood clots and pulmonary embolism.

2.7.4.4 Neurological disorders

Recent studies have also highlighted a significant association between Parkinson's disease and breast cancer. Parkinson's disease is usually associated with a decreased overall risk of cancer, with breast cancer and skin cancers as notable exceptions. The mechanism of this association are not clear, however common risk factors including, but not exclusively, genetic predisposition are considered among the most likely culprits [149-153].

Chemotherapy has been also suspected to cause frontal dementia, the so-called "chemo-brain", and other neurological disorders in breast cancer patients, however little evidence emerged from previous studies on this matter [154].

2.7.4.5 Psychiatric disorders

Lastly, breast cancer can take an important psychological toll that can often go under-recognized or undertreated. Although major depression does not seem to occur in the majority of breast cancer cases, women with a previous breast cancer diagnosis have shown an increased risk for this condition. Breast cancer patients can also experience treatment-related distress, post-traumatic stress disorder, other mood disorders, functional impairments, fear of recurrences, changes in body image and in sexuality in up to one third of cases, and some of these symptoms may last up to twenty years since breast cancer diagnosis [155-157]. Taking care of the psychological side effects of a breast cancer diagnosis and of the related treatment should be considered part of the routine clinical management of breast cancer [158, 159].

3 AIMS OF THE THESIS

The aim of this thesis was to investigate and describe the medium- and long-term health outcomes of women with breast cancer over time since diagnosis by looking at causes of death, first distant metastasis and causes of hospitalization, and to study potential associations with age at diagnosis, tumor characteristics and treatment.

In order to reach these goals, four studies on women with a diagnosis of invasive breast cancer in Sweden were performed using population-based registries:

Study I: to investigate causes of death and time-dependent effects of age and tumor characteristics on risk of dying from breast cancer;

Study II: to investigate the sites of first distant metastasis and the time-dependent effects of age and tumor characteristics on risk of developing distant metastasis;

Study III: to investigate the risk of hospitalization due to bone fracture in women with breast cancer;

Study IV: to investigate the risk of hospitalization due to infection in women with breast cancer.

4 MATERIALS AND METHODS

4.1 DATA SOURCES

Data were collected through Swedish population registers. In Sweden a unique identification number (ID) is available for all citizens [160]. The Swedish id number is a 10-digit numerical identifier that has been in use in Sweden since the 1940s. The first six digits refer to the date of birth, the following two used to refer to the place of birth, the second last digit refers to the gender and the last digit is a control digit that is calculated based on the other nine digits, so that it would give an error message in case of mistakes in entering the previous nine digits.

4.1.1 Swedish population register

It comprises information on demographic individual data of all Swedish citizens (e.g., unique ID, name, date of birth, place of birth, civil status, address) that can be linked through the unique ID to the other population registers [161]. Demographic information about the Swedish population can be traced back since the 17th century and was initially collected by the Church before becoming a population register.

4.1.2 Swedish cancer register

Established in 1958, the Swedish National Cancer Register (NCR) contains information on all primary incident cancers diagnosed in Sweden [162]. Given the mandatory independent reporting of physicians and pathologists, the register has reached a high level of accuracy and 99% completeness [163]. All cancers are classified according to the International Classification of Diseases (ICD) and 99% have also been morphologically verified [164, 165].

4.1.3 Stockholm-Gotland breast cancer register

The Stockholm-Gotland breast cancer register (SBCR) registered all primary breast cancer cases in the counties of Stockholm and Gotland since 1976 and has 99% completeness for women less than 75 years at diagnosis. The register contains detailed information about breast cancer tumor characteristics and treatment and is also used to verify the quality and equity of breast cancer care across different healthcare centers [166].

4.1.4 Cause of death register

All causes of death are mandatorily reported and the computerized version of the register is in place since 1952. Information is considered reliable since 1961, with basically no missing death, and is reported according to the ICD as underlying cause of death with up to 10 contributing causes of death. In particular, it is able to correctly classify up to 98% of breast cancer deaths [167, 168].

4.1.5 Inpatients register

The Swedish National Inpatient Register includes information about all hospitalizations occurring in Sweden and reports data on the main cause of hospitalization. It was established in the 1960s and since 1987 it includes all information about inpatient care in the whole of Sweden. The information available in the national register is divided into patient data, geographical data, administrative data and medical data and is updated once a year. Quality control is routinely carried out and the drop-out rate was estimated to be less than one per cent in 2007 [169, 170].

4.2 STUDY POPULATIONS

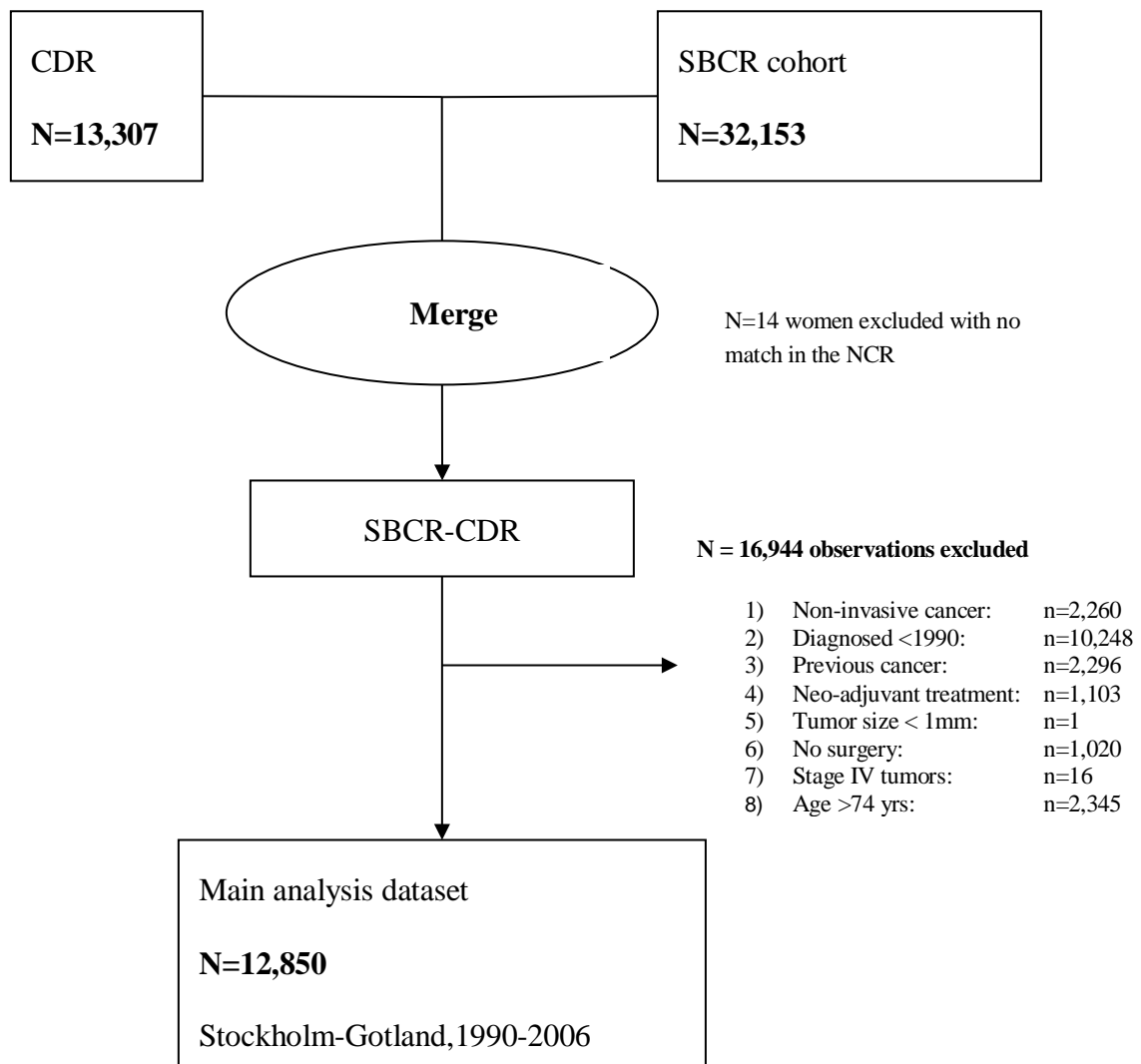
4.2.1 The Stockholm-Gotland regional cohort

In studies I-III, we used a study population of Swedish breast cancer patients diagnosed in the period between 1 January 1990 and 31 December 2006 in the Stockholm and Gotland counties. Women with a first diagnosis of breast cancer, younger than 75 years and with no previous cancer diagnosis, were extracted from the SBCR. In all three studies some additional exclusions were made:

- women who did not undergo any surgery: these women do not have pathologic information available from surgery and might not be operated for their breast cancer due to some specific reason unrelated to the disease;
- women who underwent neoadjuvant treatment: the pathologic information available may have been affected by the treatment carried out before surgery;
- women with a stage IV breast cancer: these patients would undergo palliative treatment;
- women who had a reported tumor size less than 1mm: reporting a size less than 1mm for an invasive cancer may significantly increase the chance of considering a *carcinoma in situ*, or even a non-malignant lesion, an invasive breast cancer.

After all exclusions, in study I and study III the cohort comprised 12,850 women with a previous breast cancer diagnosis (Figure 11). In study II (N=12,322) women diagnosed with distant metastasis within the first three months since breast cancer diagnosis and women with breast cancer as underlying cause of death but no distant metastasis reported in the SBCR were also excluded from the analysis.

Information on date of breast cancer diagnosis and treatment was complete for all women. Information on number of positive lymph nodes and tumor size was available for 94.6% and 98.4% of women, respectively, while information on ER status was available for 80.3%.



CDR: Cause of Death Register

SBCR: Stockholm-Gotland Breast Cancer Register

Figure 11. Flowchart of the Stockholm-Gotland regional cohort (Study I).

In studies I-III, this cohort of breast cancer patients was followed until event occurrence, censoring date, or 31 December 2006 (end of follow-up), for maximum 10 years since diagnosis.

4.2.2 The Swedish national cohort

In studies III and IV, part of the analysis was performed on a national cohort of breast cancer patients selected from the NCR, and linked to the Total Population Register, the CDR and the Inpatients Register. This Swedish nationwide cohort of women diagnosed with breast cancer between 1 January 1990 and 31 December 2009 (end of follow-up) included 77,174 individuals. Breast cancer patients were followed for hospitalization or death from bone fractures and infections and were then compared with the rates of hospitalization due to bone fracture and infection in the general Swedish population.

4.2.3 The LIBRO-1 cohort

The Linné-bröst 1 (LIBRO-1) cohort is a regional cohort of 9,328 women diagnosed with primary invasive breast cancer in the period 2001-2008 in the Swedish counties of Stockholm and Gotland. The cohort is linked to the Information Network for Cancer care (INCA) register of all Swedish quality registers [171] and to the Regional Cancer Centers (RCC) [172]. INCA was initiated in 2007 and includes records of all women diagnosed with breast cancer in Sweden. It contains detailed information about tumor characteristics (e.g., histological grade, tumor size, estrogen receptor status, number of positive lymph nodes) and treatment, including type of surgery performed.

In study IV, the LIBRO-1 cohort was used in order to investigate the risk of being hospitalized with an infection over time since breast cancer diagnosis according to age, tumor characteristics and treatment. After applying the same exclusion criteria as in the Stockholm-Gotland regional cohort (see 4.2.1), 8,111 breast cancer patients were included in the analysis.

4.2.4 The CAHRES study

In study IV, a sensitivity analysis of the main findings from the LIBRO-1 cohort was performed using a population-based case-control study on postmenopausal women, the Cancer And HorMonEs in Sweden (CAHRES) study, to assess potential unmeasured confounding. The CAHRES study comprises women aged 50-74 years with no previous breast cancer diagnosis, resident in Sweden between 1 October 1993 and 31 March 1995 [173]. Cases were identified from the six Swedish regional cancer registers where 98% of all cancer diagnoses in Sweden are reported, and controls were randomly chosen from the Total Population Register. In total, 2,802 breast cancer cases and 3,113 population-based age-matched controls were included. All cases and controls had detailed information on background risk factors (i.e., body mass index, lifestyle factors and disease history) and had complete follow-up till death, emigration and inpatient hospitalizations or until 31 December 2008 [174].

4.3 STATISTICAL ANALYSIS

4.3.1 About survival analysis

Survival analysis comprises all the methodologies studying the effect of an exposure on time to an event occurrence, such as death, hospitalization or disease recurrence. It focuses on time to event data and enables the study of the likelihood of a certain event at a certain time point, given that the subject (observation) has “survived” till that time point [175]. In our studies we used survival analysis in order to investigate the risk of developing different types of outcomes over time since diagnosis in breast cancer patients.

In survival analysis the dependent variable generally has two dimensions: the event indicator (usually a binary indicator) and the time at risk, and it can therefore be considered a rate in mathematical terms. The hazard (or event rate) can be also seen as the “speed” (rate) of events occurring over time.

Survival analysis is based on a density function $f(t)$, on a survivor function $S(t)$ and on a hazard function $h(t)$. The density function $f(t)$ corresponds to the probability distribution of failure times in a subject, the $F(t)$ denotes the cumulative probability distribution of failure times or failure proportion (cumulative density function), while the survivor function is the probability of surviving (i.e., not failing) at least until time t : $S(t)=Pr(T\geq t)$ (Figure 12). The relationship between the survivor function and the cumulative density function is the following: $S(t)=1-F(t)$.

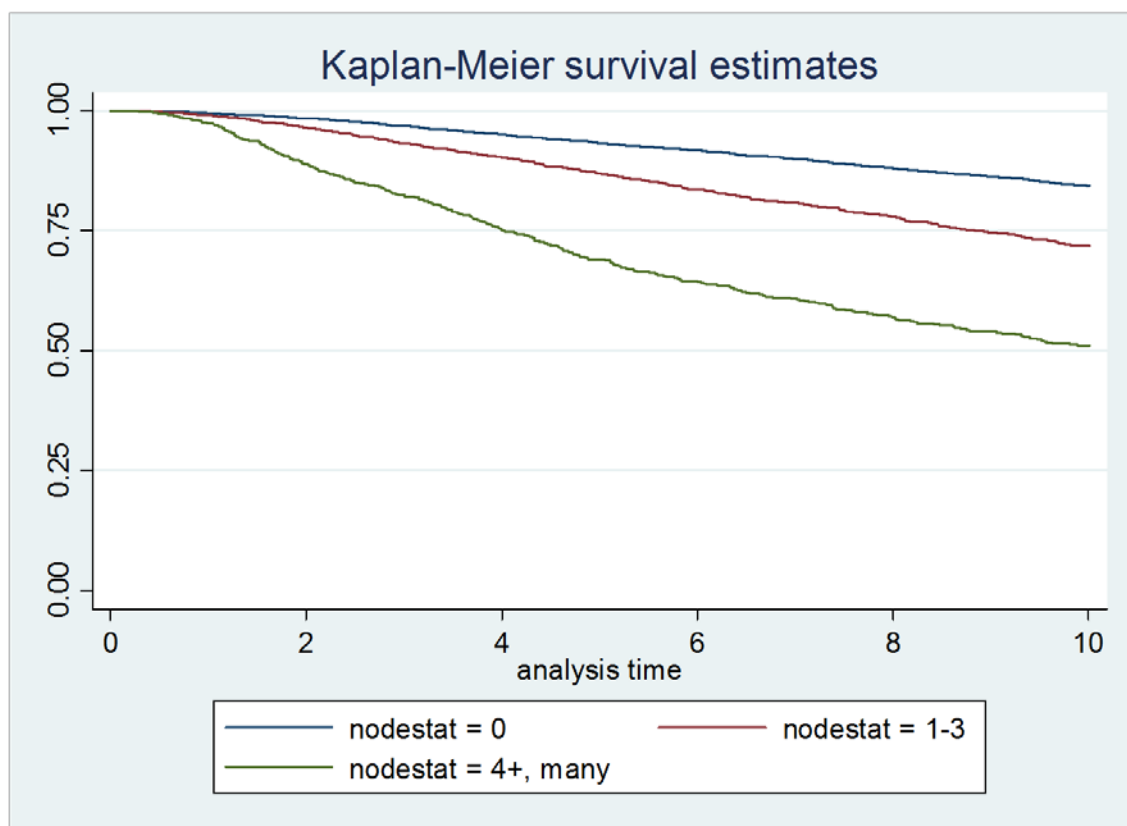


Figure 12. Example of a survival curve of breast cancer patients (event=death) by lymph node status.

The rate in decline of the survival proportion (i.e., the proportion of “alive” subjects at time t over the total number of subjects “alive” at time zero) is instead called hazard function. The hazard function is defined as the instantaneous event rate (i.e., hazard) at time t conditional on survival till time t . The hazard function can alternatively be presented as cumulative hazard function, conventionally denoted as $\Lambda(t)$. The formula linking the survivor function to the cumulative hazard function is the following: $S(t)=\exp(-\Lambda(t))$

4.3.1.1 *Estimators commonly used in survival analysis*

The survivor and the hazard functions can be estimated through specific parametric distributions based on certain density functions. Some of the most commonly used parametric survival distributions in medical survival studies are the exponential distribution, the Weibull distribution, the Gompertz distribution and the log-normal/log-logistic distribution [176]. If parametric distributions are appropriate, they will result in more efficient estimates (i.e., narrower confidence intervals) of the parameters of interest. Unfortunately the assumption of an inappropriate distribution for survival time will result in wrong estimates.

An empirical estimate of the survival function may also be developed using non-parametric estimators. Non-parametric methods for estimating the survivor function involve estimating the survival proportion at discrete values of time t and then interpolating them. The most common non-parametric estimation of the survival function is the Kaplan-Meier method which is defined as the probability of surviving in a given length of time while considering time in many small intervals. Starting with the shortest survival time the interval of time is tabulated in ascending order for which probabilities of occurrence of event are evaluated; and these successive probabilities are multiplied by any earlier computed probabilities to get the final estimate. The Kaplan-Meier estimator is also called “product limit estimate”.

The Kaplan Meier estimator is quite similar to life tables (actuarial estimator), another common non-parametric method for the estimation of the survival function. The non-parametric estimators are often used since they are fairly robust, efficient as compared to parametric tests, and rather easier and intuitive to understand.

4.3.1.2 *Testing differences in survival*

Median survival can be used as a measure to summarize and compare the survivals in different cohorts or subgroups and it represents the time t at which the survivor function $S(t)$ falls below 0.5 (50%). The cumulative survival can be used as well for this purpose and it simply consists in the proportion of subjects still “alive” at a certain time t (i.e., 5-year survival in cancer epidemiology). There are several parametric and non-parametric methods to statistically test the differences between survival curves in different groups. The most commonly used is the non-parametric log-rank test. The log-rank test can be roughly compared to a chi-square test incorporating follow-up time and is typically used to test the difference of univariate Kaplan-Meier survival curves across different subgroups of patients. Kaplan-Meier curves are also widely used even for simple graphical comparisons (Figure 12). However, it is always preferable whenever possible to use modelling because it also allows the study of confounding and effect modification, and because it provides with estimations (e.g., hazard ratios) rather than just visual comparisons or testing of a null hypothesis.

4.3.1.3 *Proportional hazards and time-dependent effects*

Some of the most common models used in survival analysis (e.g., Cox regression) assume proportional hazards over time across different levels of exposures. The proportional hazards assumption in fact implies that the survival curves within two strata are proportional over time (i.e., the hazard ratio does not vary over time). In real life, the hazard ratio comparing specific covariate patterns can actually vary over time. When appropriate, it may be thus more correct to allow for time-dependent effects in the models in order to be able to study time-varying hazard ratios. This is particularly useful as follow-up time gets longer and as the effect of a covariate on the event of interest can be modified by follow-up time, as when studying long-term effects of an exposure.

4.3.1.4 *Statistical models used in survival analysis*

Hazard functions in survival analysis can be modelled in different ways by using non-parametric, semi-parametric and parametric models. Parametric models typically assume a known underlying distribution of the parameters considered, as for instance a Weibull or a lognormal distribution. However it is sometimes difficult to assume the underlying distribution of such parameters and an inaccurate assumption may lead to misleading results. Some parametric distributions like the Weibull are often criticized as they assume a monotonic increase or decrease of the baseline function that do not allow for more complex patterns. The use of mathematical piecewise polynomials, also known as splines, allows instead for more flexibility and complexity. The points at which each polynomial joins are called knots. In our studies we used in particular three types of survival models: the Cox regression model, the Poisson regression model and the flexible survival models.

4.3.1.4.1 Cox regression model

The Cox regression model is a widely used semi-parametric model that allows for comparison of effects of different exposures on a time to event outcome [177]. The model links the hazard for an individual subject at time t (i.e., $\lambda(t)$) to the baseline hazard $\lambda_0(t)$ by the following equation:

$$\ln[\lambda(t|X)] = \ln[\lambda_0(t)] + \beta X$$

The Cox regression does not make assumptions about the baseline hazard function, thus considered semi-parametric, and it assumes non-informative censoring (see 4.3.1.5). The Cox regression model can be adjusted for different covariates and it usually assumes proportional hazards, so that hazard ratios are expected to be constant over time (i.e., not time-dependent). In other words, this means that the hazards of each exposure level are assumed to be parallel over time on the log scale. The Cox regression model allows for comparison across different levels of exposures by estimating hazard ratios (HR). The proportional hazards assumption is a strong assumption and should always be tested. We used Cox regression in study III and study IV when studying the association of age and tumor characteristics with risk of hospitalization among breast cancer patients.

4.3.1.4.2 Poisson regression model

Poisson regression belongs to the family of generalized linear models and allows modelling of event rates. It assumes that the logarithm of the event rate variable is linearly related to the exposure variable (log-linear regression) according to the following formula:

$$\ln(\lambda) = \beta_0 + \beta_1 X$$

The parameter β_1 is the effect per unit increase of X as a change in the log rate. Poisson regression model estimates the baseline hazard rate and incidence rate ratios (IRR) for different exposure levels and therefore allows for comparisons across exposure levels. It also enables the study of time-dependent effects by splitting the data on time in order to have the hazards piecewise constant within predefined time period bands. We used Poisson regression in study III and in study IV when looking at IRRs of hospitalizations in breast cancer patients as compared with non-breast cancer patients.

4.3.1.4.3 Flexible parametric models

Flexible parametric models use restricted cubic splines as the baseline log cumulative hazard function. Cubic splines are called restricted because they are forced to be linear before the first knot and after the last knot. These parametric models allow for more flexibility as compared to other traditional parametric distribution used in survival analysis. By modeling the underlying baseline rate parametrically, it is possible to estimate various fitted curves from the model, such as event rates over time. Flexible parametric models tend to correlate very well with the Cox regression estimates when assuming proportional hazards, but they are also particularly effective in modelling time-dependent effects by breaking the proportional hazard assumption. Within the flexible parametric modeling framework, it is in fact possible to allow covariates to have time-dependent effects by fitting interactions between the covariate and time using a second spline function. These interaction splines typically use fewer degrees of freedom and are deviation effects from the baseline spline. Differences between groups are reported as HRs, but the HRs are now functions of time rather than constants [178, 179]. We used flexible parametric models in study I and study II when investigating the time-dependent effects of age and tumor characteristics on the risk of death and distant recurrence in women with breast cancer.

4.3.1.5 *Censoring*

Quite often in survival analysis information is not available for all individuals for all the study period. Some individuals may start being observed at different time points (entry times) and some may drop out or stop being followed (censored) before the end of the study period. Censoring can occasionally occur in individuals who enter the study at a later stage and who may have already developed the event of interest in the unobserved time before their study entry (left censoring). More commonly, individuals (observations) stop being followed before the end of the study period: they will be considered as censored because the unobserved event may occur later than the observed time for that given individual (right censoring). There is

also the possibility to have interval censoring when the true unobserved event for an individual lies between two observed time windows, but it is a quite rare situation.

Censoring is called independent or non-informative when an individual censored at time t is representative of all individuals followed till that time. This means that, given certain common characteristics measured at baseline (i.e., belonging to the same subgroup), each individual observation will have the same chance of being censored up until time t . Informative censoring occurs when the mechanisms giving rise to censoring of individual subjects are related to the probability of the outcome occurring, therefore biasing the analysis. Informative censoring can be partly dealt with by using drop-out events as study termination, through imputing techniques, or by the use of sensitivity analyses mimicking best-case and worst-case scenarios [180].

4.3.1.6 *Competing risks*

Another common issue in survival analysis is caused by competing risks. When the outcome is defined as cause-specific, like cause-specific death, there will be the need to take into account the fact that a person can also die from causes that are not the event of interest. One way to deal with this issue is to censor observations at time of death due to causes that are not the cause of interest. Assuming that the censoring is non-informative, this is fine when the aim is to compare hazard ratios across different covariates in order to detect groups at a higher or lower risk of cause-specific death. However, if the aim is to study the real life probability that a patient would die from a certain cause by a certain time point, then the risk will be probably overestimated. A possible solution to this problem implies the use of so-called sub-distribution hazards which means to add to each risk set also those observations that have already been censored (e.g., individuals who have already died due to other causes). In other words, the time in the study of the censored individuals would be considered to be larger than all event times in order to modify all risk sets accordingly. This way it would be possible to calculate the real life cumulative incidence function (CIF) and better estimate the real probability that a person dies from a certain cause or develops a certain condition by time t or within a certain time interval [181].

4.3.2 **About standardized incidence/mortality ratios**

Standardized ratios are indirect standardization methods and are used to determine whether the occurrence of a certain event (e.g., mortality, incidence) in a specific study population is higher or lower as compared with a reference population after adjusting (i.e. standardizing) for a chosen variable. In order to calculate the standardized mortality ratios (SMRs) or the standardized incidence ratios (SIRs), the observed number of events in the study population needs to be divided by the expected number of events taken from the reference population (i.e., background number of events). By expected number of events, it is meant the number of events that would occur in the study population if the same event rate of the reference population occurred in the study population. For its calculation, each variable's stratum-specific incidence or mortality rate from the reference population is multiplied by each

variable's stratum of the study population, and then the results are added up. Since age is the strongest predictor of incidence and mortality for most health-related outcomes (e.g., breast cancer, hospitalizations, bone fractures, infection), it is commonly used as standardization variable in the calculation of the SMRs and SIRs, however any other variable can be additionally or alternatively used.

The SIR/SMR will be then calculated by doing a simple ratio between observed and expected cases in the study population. Confidence intervals can also be calculated around SMRs and SIRs and their statistical significance can be interpreted as for all other measures of associations commonly used in epidemiology (e.g., risk ratios, incidence rate ratios and odds ratios). If the ratio is more than one, it will mean that the observed cases are more than the expected, so in our study population the mortality or the incidence will be considered higher than expected. If the ratio is less than one, then it will mean that in the study population the mortality or the incidence will be considered lower than expected. And in case the ratio is not different from one, the study population will have the expected mortality or incidence.

Standardized mortality ratios and standardized incidence ratios were used in study IV.

4.3.3 Study I

Women were followed from the date of breast cancer diagnosis until date of death, end of study (December 31, 2006), or up until 10 years since diagnosis. When considering specific causes of death, the time at risk was calculated from the date of breast cancer diagnosis to the date of cause-specific death, and women who died as a result of other causes were considered censored at date of death.

Death rates were modeled using flexible parametric models with a restricted cubic spline function as baseline death rate [178, 179]. A spline with five degrees of freedom (four intermediate knots and two knots at each boundary, placed at quintiles of distribution of events) was used for the baseline rate. In all analyses time since diagnosis of breast cancer was used as underlying time scale.

The following clinically relevant variables were included in the models: age group at breast cancer diagnosis (<45, 45 to 54, 55 to 64, and 65 to 74 years), calendar period at breast cancer diagnosis (1990 to 1994, 1995 to 1999, and 2000 to 2006), tumor size (1 to 20 and >20 mm), lymph node status (number of positive lymph nodes: none, one to three, four or more), ER status (ER-positive/ER-negative), and treatment. Treatment was categorized according to different combinations of surgery with adjuvant therapy as follows: surgery only; surgery with radiotherapy; surgery with radiotherapy and chemotherapy; surgery with radiotherapy and hormone therapy; surgery with radiotherapy, chemotherapy, and hormone therapy; surgery with chemotherapy; surgery with hormone therapy; and surgery with chemotherapy and hormone therapy.

The overall effect of each variable was initially modeled using proportional hazards models. Then each tumor characteristic was modeled separately as a time-dependent covariate while

adjusting for other variables as non–time-dependent covariates (using three degrees of freedom for the interactions). We formally tested the potential three-ways interaction of age and tumor characteristics over time since diagnosis using likelihood ratio tests by comparing models with and without interaction with age, tumor characteristic, and time. All tests were two sided, with significance level of 5%.

4.3.4 Study II

Women were observed and contributed to the time at risk from the date of breast cancer diagnosis until the date of first distant metastasis (outcome of interest), date of death, diagnosis of a second primary cancer, end of study (31 December 2006) or up until 10 years since diagnosis.

The rates of first distant metastasis were modeled using flexible parametric survival models with a restricted cubic spline function for the cumulative baseline hazard rate [178, 179]. We used a spline with five degrees of freedom (two knots at each boundary and four intermediate knots placed at quintiles of the distribution of events) for the baseline rate. Time since breast cancer diagnosis was the underlying timescale in all analyses.

Flexible parametric models allow to post estimate the cumulative risk of developing metastasis in the presence of competing risks [182]. The 5-year cumulative risk of first distant metastasis since breast cancer diagnosis was estimated for different covariate patterns taking into account the competing event “death due to causes other than breast cancer”. The cumulative risk of first distant metastasis was also estimated for the period between 5 and 10 years from diagnosis, conditional upon surviving metastasis-free up to 5 years.

The following covariates were used in the models: age at breast cancer diagnosis (≤ 50 , 51–60, 61–74 years), calendar period at breast cancer diagnosis (1990–1994, 1995–1999, 2000–2006), tumor size (1–20 mm, >20 mm), lymph node status (positive/negative), ER status (ER-positive/ER-negative) and treatment. Treatment was categorized as local treatment (surgery without adjuvant treatment; surgery with radiotherapy only) and systemic treatment (any combination of surgery with either chemotherapy or hormone therapy).

First, time-dependent hazard ratios for the risk of developing first distant metastasis were estimated through flexible parametric models by age and tumor characteristics. Then, the 0–5 years and 5–10 years cumulative risks of first distant metastasis were estimated in the presence of competing risk by different sets of covariate patterns.

4.3.5 Study III

Different analyses were performed using the Swedish national cohort (see 4.2.2) and the Stockholm-Gotland regional cohort (see 4.2.1).

4.3.5.1 *Swedish national cohort analysis*

In the Swedish national cohort, women were considered at risk until hospitalization due to any bone fracture or due to femur fracture (outcomes of interest), date of death, date of emigration or 31 December 2009, whichever came first. The analysis was divided by time before and after first recorded hospitalization, and by time before and after a breast cancer diagnosis, treating breast cancer status as time-varying exposure. Time was split by attained age, attained calendar period, and by time since cancer diagnosis only for breast cancer patients. Poisson regression was used for modeling hospitalization rates using attained age, attained calendar period and breast cancer status in order to compare hospitalization rates between women with a breast cancer diagnosis and women without a breast cancer diagnosis.

4.3.5.2 *Stockholm-Gotland regional cohort analysis*

In the Stockholm-Gotland regional cohort, breast cancer patients (study population) were followed from date of diagnosis until date of first hospitalization due to any-bone fracture or due to femur fracture (outcomes of interest), date of death, date of first distant metastasis, 31 December 2006 or up until 10 years since breast cancer diagnosis.

The covariates used in the models were age at breast cancer diagnosis (≤ 50 , 51–60, 61–74 years), calendar period at breast cancer diagnosis (1990–1994, 1995–1999, 2000–2006), tumor size (1–20 mm, >20 mm), lymph node status (positive/negative), ER status (ER-positive/ER-negative), type of surgery (total mastectomy, partial mastectomy, other) and adjuvant treatment. Adjuvant treatment was categorized as any combination of chemotherapy without hormone therapy, any combination of hormone therapy without chemotherapy, chemotherapy and hormone therapy in any combination, and other.

Cox regression models assuming proportional hazards were used to check for the possible association of different tumor characteristics with the risk of being hospitalized with bone fracture.

The risk of dying after being hospitalized with a bone fracture was also analyzed using Cox regression in the same regional cohort. The person-time was divided by time before and after first recorded hospitalization due to a fracture. For this analysis the outcomes of interest were: death due to any cause, death due to breast cancer and death due to causes other than breast cancer. When looking at cause-specific death we censored the analysis for the other causes of death.

4.3.6 Study IV

We used the Swedish national cohort (see 4.2.2), the LIBRO-1 cohort (see 4.2.3) and the CAHRES study population (see 4.2.4) to perform separate analyses.

4.3.6.1 *Swedish national cohort analysis*

In the Swedish national cohort we investigated the rates of hospitalizations due to infection and the subsequent death rates comparing breast cancer patients with the general population.

Number of person-years at risk for each patient was calculated from the date of breast cancer diagnosis until the date of first hospitalization due to an infectious disease, date of death, date of emigration or 31 December 2009, whichever came first. We calculated standardized incidence ratios (SIRs) and standardized mortality ratios (SMRs) by dividing the observed number of hospitalizations due to an infection, and of related deaths, by the expected numbers based on the general Swedish female population, with 95% confidence intervals (CIs) based on Poisson distribution [183]. The expected numbers of events were calculated by multiplying age-specific (5-year interval) and calendar year-specific (5-year interval) incidence and mortality rates by corresponding person-years from the general female population. Standardized incidence ratios were calculated overall and by time since diagnosis modeled as a categorical variable (0-1, 1-2, 2-5, 5-10, >10 years) in order to detect changes over time. The analysis was repeated by looking at specific sites of infection as outcomes of interest.

4.3.6.2 *CAHRES analysis*

The CAHRES study population was used to check for robustness of our findings in a smaller sample with information about additional potential confounders. Incidence rate ratios (IRRs) for hospitalizations due to an infection were estimated through Poisson regression with time since study entry (i.e. diagnosis date for cases and sampling date for controls) as underlying time scale. Age-adjusted IRRs were compared with IRRs from models adjusting for additional background risk factors (i.e., BMI, physical activity, smoking, history of hypertension and diabetes).

4.3.6.3 *LIBRO-1 analysis*

The LIBRO-1 regional cohort was used to study the association of tumor characteristics and treatment with the risk of being hospitalized with an infection. Poisson regression was used to model rates of hospitalization due to an infection with time since diagnosis as the underlying time scale. The covariates used in the analysis were tumor size (<10 mm, 10-20 mm, >20mm), histological grade (low, intermediate, high), ER status (ER-negative, ER-positive), number of positive lymph nodes (0, 1-5, >5); number of dissected lymph nodes (<5, 5-10, >10), endocrine and chemotherapy (none, endocrine only, chemo only, endocrine plus chemotherapy), radiation therapy (none, breast only, breast plus nodes, nodes only, organ unspecified), surgery (partial mastectomy, total mastectomy). Adjustment for age at diagnosis (years) with mutual adjustment for other tumor characteristic and treatment variables was performed in all analyses.

5 MAIN RESULTS

5.1 STUDY I

Breast cancer was the underlying cause in 1,188 (64.2%) of the 1,849 deaths occurred in the Stockholm-Gotland regional cohort of 12,850 women diagnosed with breast cancer in the period 1990-2006 and followed up for a maximum of 10 years. Breast cancer accounted for 226 (95%) of the 238 deaths among women younger than 45 years at diagnosis while, among women 65-74 years at diagnosis, an underlying cause other than breast cancer accounted for the majority (n=390, 55%) of the 703 deaths. The proportion of circulatory system disorders and of other cancers as underlying cause of death increased with age at diagnosis (from 0.4% and 2.1% in less than 45 years, to 24.0% and 14.9% in 65-74 years, respectively).

Breast cancer-specific death rates peaked within 5 years from diagnosis. They were higher overall in women younger than 45 years at diagnosis, however they converged with the ones observed in the older age groups at 10 years of follow-up (15 per 1,000 person-years). The rates of death due to circulatory system disorders and due to cancers other than breast cancer increased with age and time since diagnosis.

The risk of dying due to any cause increased with age at diagnosis, while the risk of breast cancer-specific death was higher in women younger than 45 years at diagnosis as compared with women 45-54 years at diagnosis (HR=1.2 95% CI: 1.0-1.4). Women with positive lymph nodes at breast cancer diagnosis were at significantly increased risk of breast cancer-specific (and overall) death within 10 years from diagnosis (HR=2.4; 95% CI: 2.0-2.9 for patients with 1-3 positive lymph nodes at diagnosis; HR=5.9; 95% CI: 4.9-7.1 for patients with 4 or more positive lymph nodes at diagnosis) (Table 1). Women with tumor size larger than 20mm or with ER-negative tumor were also at increased risk of breast cancer-specific and overall death within 10 years from diagnosis (Table 1), however not for the full period as shown in the time-dependent effects analysis (Figure 13b).

The risk of dying from a circulatory system disorder was higher in older as compared with younger patients. The risk of dying due to a circulatory system disorder was significantly higher in women with positive lymph nodes at diagnosis (HR=2.0; 95% CI: 1.4-2.9 for women with 1-3 positive nodes; HR=1.9; 95% CI: 1.0-3.4 for women with 4 or more positive nodes). The risk of dying from a circulatory system disorder was higher also for women with a tumor size larger than 20mm (HR=1.5; 95% CI: 1.1-2.1) but it was not affected by ER status (HR=0.8; 95% CI: 0.5-1.3) (Table 1).

Tumor characteristics at breast cancer diagnosis did not affect the risk of dying from cancers other than breast (Table 1).

Table 1. Hazard ratios (HRs) for different causes of death by tumor characteristics among breast cancer patients in the Stockholm-Gotland Breast Cancer Register, 1990-2006.

	Breast cancer		Circulatory system disease		Other cancer ^b		All-cause	
	HR ^b	95% CI	HR ^b	95% CI	HR ^b	95% CI	HR ^b	95% CI
Positive nodes								
0	1.0	-	1.0	-	1.0	-	1.0	-
1-3	2.4	2.0-2.9	2.0	1.4-2.9	1.3	0.8-1.9	2.1	1.8-2.4
≥ 4	5.9	4.9-7.1	1.9	1.0-3.4	1.6	0.9-3.0	4.3	3.7-5.0
Estrogen receptor								
ER+	1.0	-	1.0	-	1.0	-	1.0	-
ER-	2.1	1.8-2.4	0.8	0.5-1.3	1.5	0.9-2.4	1.8	1.6-2.1
Tumor size, mm								
1-20	1.0	-	1.0	-	1.0	-	1.0	-
> 20	1.9	1.7-2.2	1.5	1.1-2.1	1.0	0.7-1.4	1.7	1.5-1.9

a - estimated from a flexible parametric survival model assuming proportional hazards, i.e. constant HRs for all variables throughout the 10-year follow-up. The HRs were adjusted for all variables in the column and time-since-diagnosis (the underlying time scale)

b - "Other cancer" include all cancers other than breast cancer

When looking at time-dependent effects of tumor characteristics on the risk of dying from breast cancer, women with positive lymph nodes at breast cancer diagnosis remained at significantly increased risk of breast cancer-specific death for 10 years since diagnosis (Figure 13a). ER-negative patients were at significantly increased risk of dying in the first 5 years since breast cancer diagnosis as compared with ER-positive patients. After 5 years of follow-up this difference was not any more significant among the survivors (HR=1.3; 95% CI: 0.9-1.7 at 5 years from diagnosis) (Figure 13b). Patients with a tumor larger than 20mm were at increased risk of dying compared to patients with tumor size less than 20mm up to about 8 years since breast cancer diagnosis. Age did not significantly affect time dependent effects of tumor characteristics on risk of death.

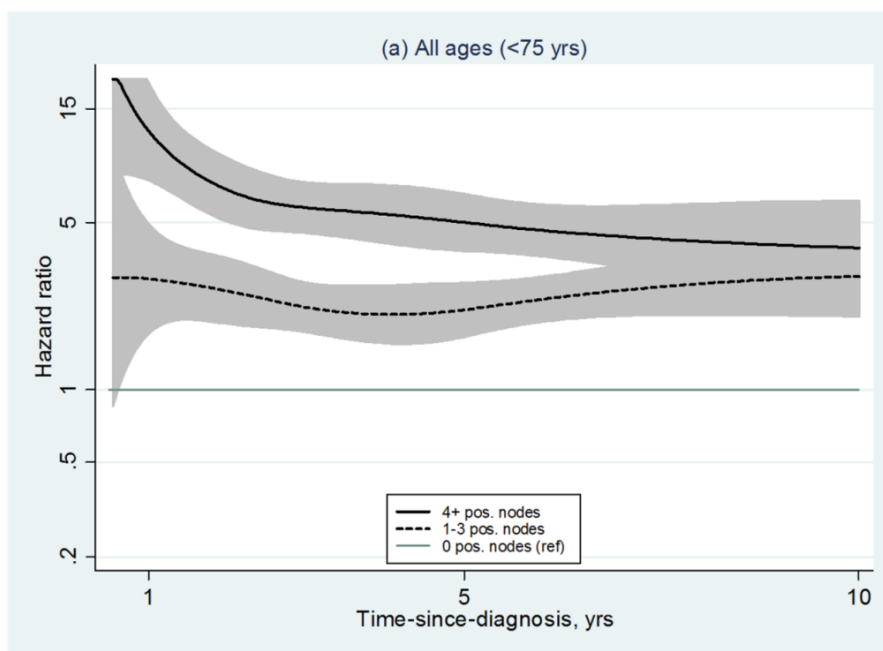


Figure 13a. Time-dependent hazard ratios (HRs) for dying from breast cancer by lymph node status in the study population.

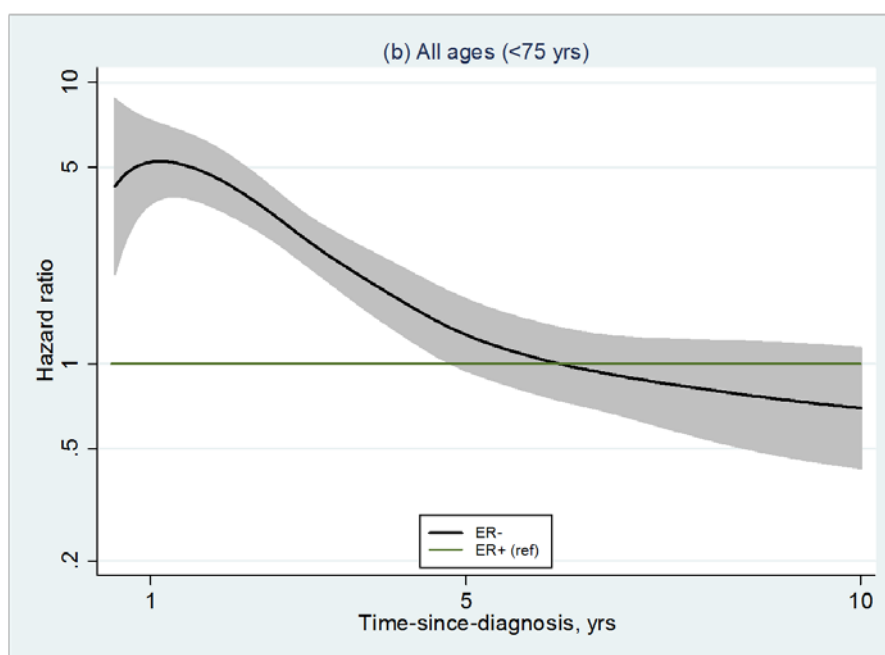


Figure 13b. Time-dependent hazard ratios (HRs) for dying from breast cancer by ER status in the study population.

5.2 STUDY II

In the Stockholm-Gotland regional cohort, 995 (10.4%) women developed distant metastasis during the study period. Overall, metastasis to the skeleton (32.5%) and multiple sites of metastasis (28.3%) were the most frequent presentations of distant metastasis within 10 years.

The proportion of first distant metastasis to the skeleton significantly increased over time since breast cancer diagnosis, while the proportion of central nervous system (CNS) and liver metastasis significantly decreased (Figure 14).

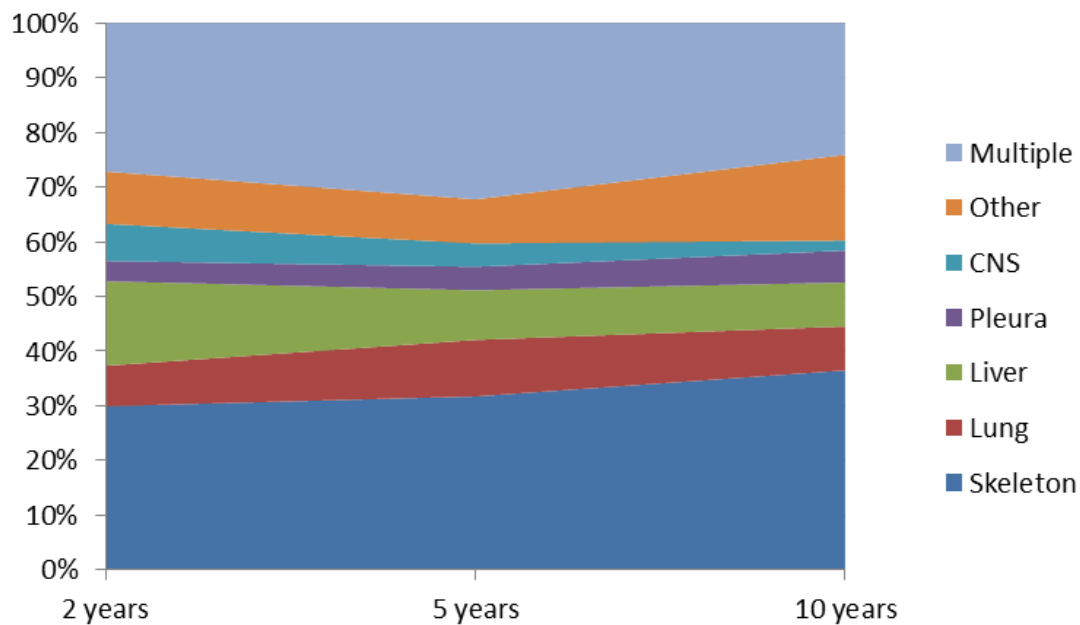


Figure 14. Distribution of sites of first distant metastasis by time since breast cancer diagnosis.

Women with positive lymph nodes at breast cancer diagnosis were still at increased risk of developing distant metastasis at 10 years from diagnosis (HR=2.6; 95% CI: 1.9–3.5). Women with ER-negative tumors were at increased risk of distant metastasis compared to women with ER-positive tumors only in the first 5 years since breast cancer diagnosis (HR=1.4; 95% CI: 1.1-1.7, at 5 years; HR=0.9; 95% CI: 0.6-1.4, at 10 years). Patients with a tumor larger than 20mm at diagnosis were still at increased risk of metastasis at 10 years since breast cancer diagnosis (HR=1.5; 95% CI: 1.1–2.0).

The cumulative incidence of distant metastasis varied across different ages and tumor characteristics and over time since diagnosis. After taking into account competing risk, women younger than 45 years, lymph node positive, ER-negative, and with tumor larger than 20mm at breast cancer diagnosis had more than 60% risk of developing distant metastasis within 10 years, while women aged 45-54 years with lymph node positive, ER-positive, tumor size less than 20mm at diagnosis had about a 20% risk of developing metastasis within 10 years (Figure 15). In the period 5–10 years of follow-up, women with ER-positive, lymph node-positive and ≤ 20 mm tumors at breast cancer diagnosis showed the highest risk of distant recurrence.

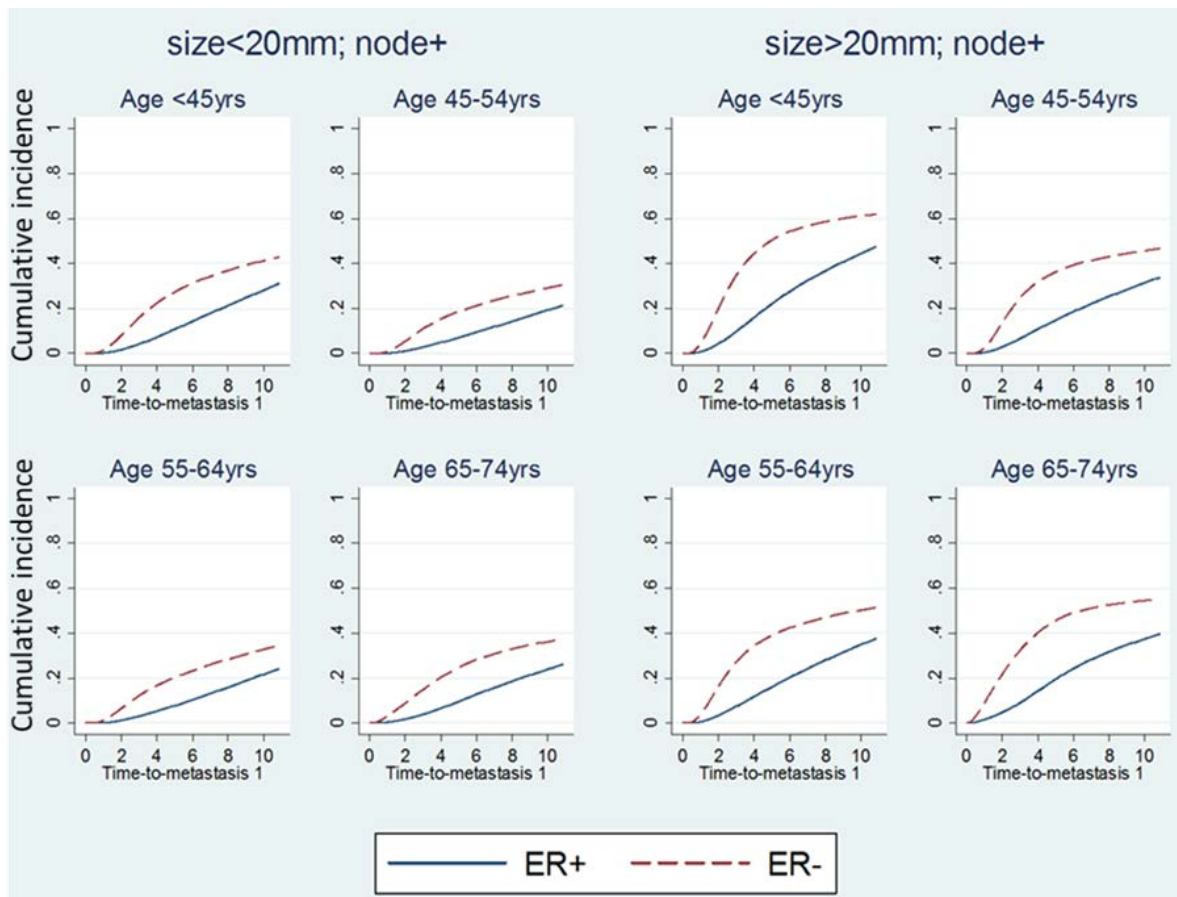


Figure 15. Cumulative incidence function (CIF) for the development of first distant metastasis over time since diagnosis in lymph node-positive breast cancer patients taking competing risk into account.

5.3 STUDY III

Of the 6,939 hospitalizations due to bone fracture diagnosed among breast cancer patients in Sweden in the period 1990-2009, 2,701 (38.9%) were reported as femur fractures, 1,474 (21.3%) as other lower limb fractures, and 1,702 (24.5%) as upper limb fractures. The proportion of hospitalizations due to femur fracture over the total amount of hospitalizations due to any-bone fracture increased with age and consequently with time since breast cancer diagnosis. The overall rate ratios of hospitalizations due to any bone fracture, and due to femur fracture alone, were 1.25 (95% CI: 1.23-1.28) and 1.21 (95% CI: 1.17-1.25), respectively. These rate ratios were gradually decreasing over time since breast cancer diagnosis remaining significant for over 10 years (Figure 16), and were significantly increased in all age groups.

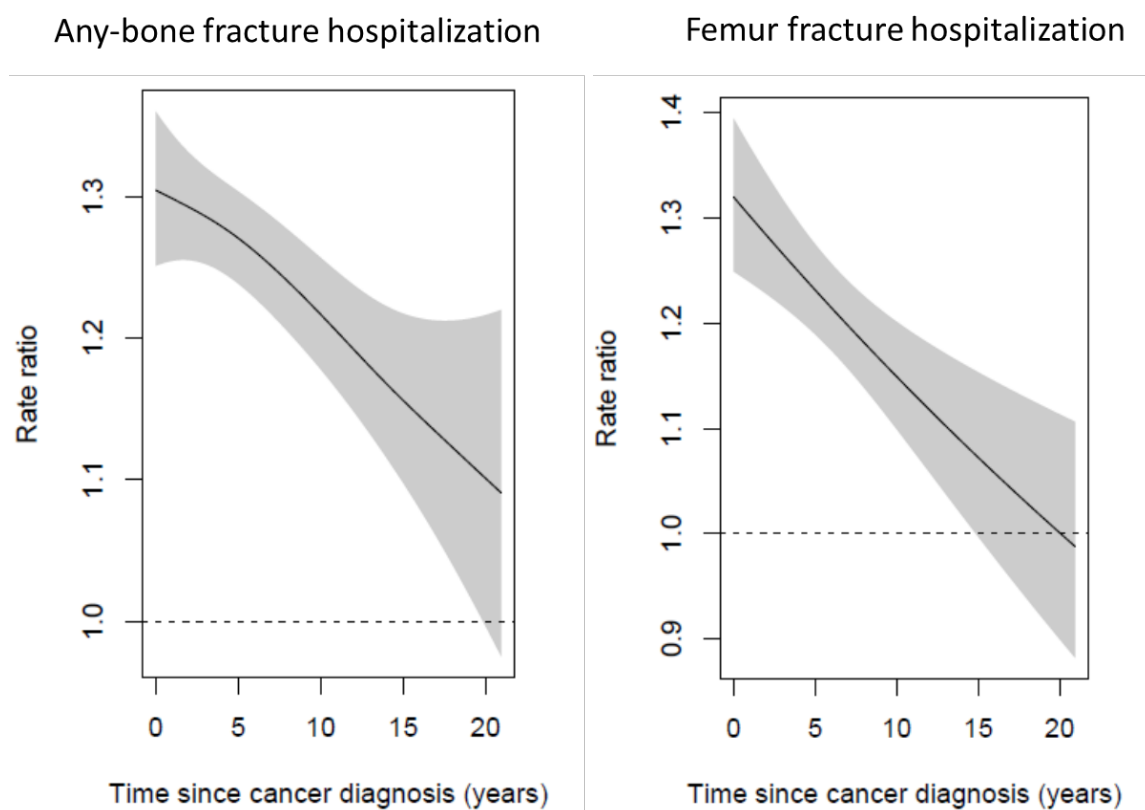


Figure 16. Rate ratios for hospitalizations due to bone fracture after breast cancer by time since diagnosis.

Tumor characteristics did not differentially affect the risk of being hospitalized with a bone fracture among breast cancer patients. The risk of being hospitalized with a bone fracture was significantly increased in women older than 60 years at breast cancer diagnosis as compared with younger breast cancer patients.

Breast cancer patients were at increased overall risk of dying after a being hospitalized with a bone fracture (HR=1.81; 95% CI: 1.46-2.25), particularly if diagnosed at an older age. A hospitalization due to a bone fracture following breast cancer diagnosis did not significantly affect the risk of dying from breast cancer (HR=0.92; 95% CI: 0.63-1.35), while it significantly increased the risk of dying due to causes other than breast cancer (HR=3.14; 95% CI: 2.39-4.14). The risk of dying after being hospitalized with a femur fracture was even more increased (HR=2.60; 95% CI: 1.94-3.48), in particular when looking at the risk of dying from causes other than breast cancer (HR=4.00; 95% CI: 2.75-5.72) (Table 2).

Table 2. Risk of dying after any-bone and femur fracture hospitalization in breast cancer patients.

Causes of death	Any-bone fracture	Femur fracture
	HR (95% CI)	HR (95% CI)
Overall death	1.81 (1.46-2.25)	2.60 (1.94-3.48)
Breast cancer-specific death	0.92 (0.63-1.35)	1.43 (0.85-2.39)
Other cause of death	3.14 (2.39-4.14)	4.00 (2.75-5.72)

5.4 STUDY IV

In the nationwide cohort we found an increased risk of being hospitalized with an infection for breast cancer patients as compared with the general population (SIR=1.74; 95% CI: 1.70-1.78). In particular, SIRs were highest in the first year after diagnosis and remained significantly increased after 10 years since diagnosis (SIR=1.30; 95% CI: 1.24-1.36) (Figure 17). This increased risk over 10 years since breast cancer diagnosis was particularly high for infections of the skin (SIR=1.68; 95% CI: 1.50-1.88).

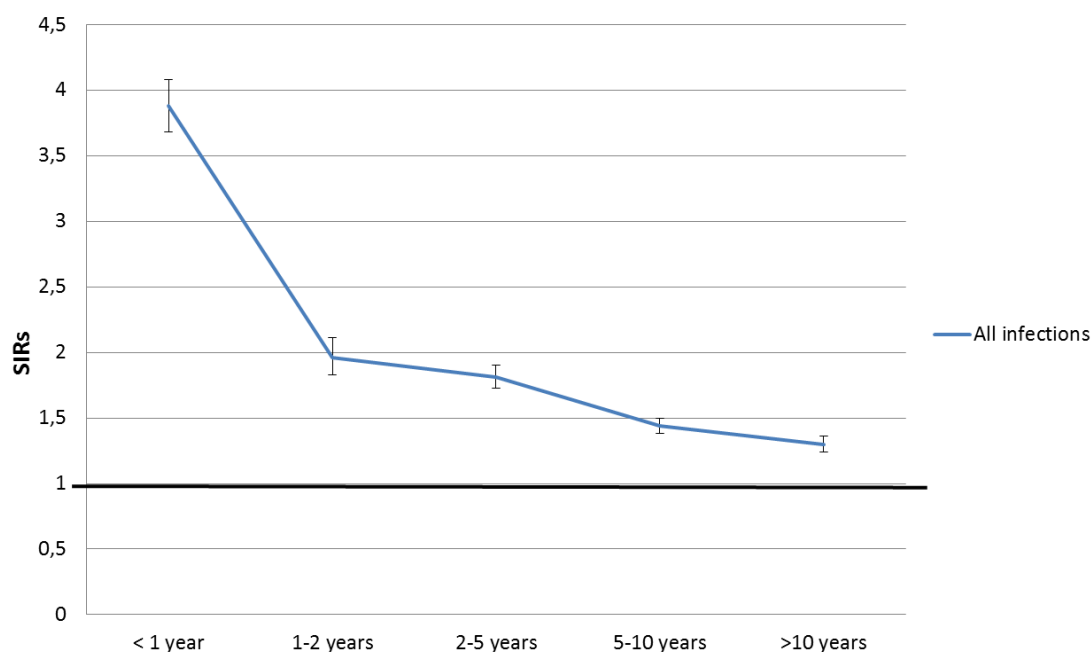


Figure 17. SIRs for hospitalizations due to an infection over time since breast cancer diagnosis comparing breast cancer patients with the general population.

Breast cancer patients had a higher infectious disease-specific mortality compared with the general female population. In the nationwide cohort, 328 deaths due to infection were reported, a number that is higher than the expected ($n=290$) calculated in the general Swedish female population (SMR=1.17; 95% CI: 1.02-1.26).

In the LIBRO-1 regional cohort of breast cancer patients, the risk of being hospitalized with an infection was higher for patients with a more aggressive pattern of tumor characteristics at breast cancer diagnosis (high histological grade, large tumor size, positive lymph nodes, ER-negative status) (Table 3). The risk of sepsis was higher in patients with ER-negative, positive lymph nodes and high histological grade tumors, while risk of skin infections was higher in patients with tumor size larger than 20mm (IRR=2.04; 95% CI: 1.14-3.62) and with more than 5 positive lymph nodes at diagnosis as compared to none (IRR=2.38; 95% CI: 1.26-4.51). Undergoing chemotherapy was significantly associated with risk of sepsis (IRR=4.35; 95% CI: 1.84-10.30), while receiving loco-regional radiation therapy was significantly associated with infections of the skin (IRR=2.81; 95% CI: 1.19-6.63).

Table 3. Association of tumor characteristics with the risk of being hospitalized with an infection in breast cancer patients (regional cohort, N = 8,111).

Regional cohort		IRR (95% CI)		
	N	Any infection	Skin infection	Sepsis
Tumor characteristic¹				
Size in mm				
<10	2042	REF (1.00)	REF (1.00)	REF (1.00)
10-20	3685	1.08 (0.90-1.31)	1.54 (0.90-2.64)	1.23 (0.83-1.82)
>20	2384	1.26 (1.03-1.55)	2.04 (1.14-3.62)	1.35 (0.88-2.07)
Histological grade (Elston)				
Low	989	REF (1.00)	REF (1.00)	REF (1.00)
Moderate	2582	1.27 (0.96-1.69)	1.11 (0.59-2.08)	1.63 (0.82-3.23)
High	1510	1.47 (1.08-2.00)	0.83 (0.40-1.73)	2.12 (1.03-4.33)
ER status				
Positive	5476	REF (1.00)	REF (1.00)	REF (1.00)
Negative	937	1.34 (1.07-1.66)	1.08 (0.59-2.00)	1.92 (1.30-2.82)
No. positive lymph nodes				
0	5117	REF (1.00)	REF (1.00)	REF (1.00)
1-5	2267	1.26 (1.06-1.49)	1.18 (0.75-1.85)	1.41 (1.01-1.96)
>5	454	2.14 (1.65-2.78)	2.38 (1.26-4.51)	1.87 (1.12-3.14)

Abbreviations: IRR = incidence rate ratio; CI = confidence interval. All analyses are adjusted for age at diagnosis (years) and mutually adjusted for the variables listed (tumor characteristics and treatment separately).

¹ Missingness on individual variables < 4%, except for histological grade (37.4%, N = 3030) and ER status (20.9%, N = 1698).

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

6.1.1 Strengths

The use of Swedish population-based registers linked by personal identification number has virtually eliminated loss to follow-up in our studies and minimized the possibility of related bias. The information available in the registers is quite exhaustive with detailed information on tumor characteristics and treatment allowing comprehensive and powerful analyses on risk stratification. The large breast cancer cohorts used were followed up to at least 10 years with accurate and complete information, enabling to apply a comprehensive design and methodology to check for differences in risk within different subtypes of breast cancer patients and between breast cancer patients and the general population.

We studied relevant health outcomes of women with breast cancer over time since diagnosis by using different measures and by providing an overall picture of risks over time: we used rates (e.g., rates of death, rates of first distant metastasis, rates of hospitalization), ratios (e.g., hazard ratios, incidence rate ratios, standardized incidence ratios, standardized mortality ratios) and absolute risks (e.g., cumulative incidence). Not assuming proportional hazards in some analyses allowed for the study of the interaction of tumor and patient characteristics with time since diagnosis, providing a more accurate picture of the follow-up of these patients. We were also able to take competing risk into account when looking at the absolute risk of distant metastasis in different subtypes of patients. Swedish national and regional practices for breast cancer diagnosis and treatment during the study period have remained rather consistent according to updated international and national guidelines and this may have contributed to more homogeneity in the study populations for some of the additional factors we were not able to take into account in the analyses.

6.1.2 Random error

The random error can always influence the validity of the conclusions of a study and in particular it can affect the precision, especially when a study population is rather small. As opposed to systematic errors, where the measurement is systematically inaccurate due to an inherent bias, the estimates affected by random errors are distributed around the true value. In statistics, the hypothesis of an association of two variables observed in the study sample is usually tested against the null hypothesis of no association. The output of a statistical test is usually a p-value which is the probability, under the assumption that the null hypothesis of no association is true, of obtaining a result equal or more extreme to the one observed. The lower the p-value, the less likely is that what we observed is true under the null hypothesis (i.e., there is no association). A threshold under which the null hypothesis is rejected is often chosen and it is commonly set at $\alpha=0.05$ for single comparisons. The random error may cause two types of mistakes in statistical testing: type I error (rejecting the null hypothesis when it is true) and type II error (accepting the null hypothesis when it is false). The probability of committing a type I error is usually called α , while the probability of committing a type II

error is usually called β . Since we used large population-based registers in all our studies we do not think that the random error is a particular issue of concern in our four studies.

6.1.3 Selection bias

Selection bias in epidemiology is a systematic error that often occurs when the subjects (observations) included in a study (i.e., study population) are not representative of the population the researchers want to infer about (i.e., target population). Selection bias can affect both the internal and the external validity of a study. More specifically, selection bias occurs when the selection/participation probabilities are influenced by exposure or disease status: for instance, there will be selection bias if women diagnosed with cancer who have a worse prognosis are more likely than women with a better prognosis to be recruited in a study on the risk of death from cancer. Selection bias in prospective studies can also occur after the recruitment, or selection phase, if participants drop out from the study not randomly: a typical example can occur with loss to follow-up. Here below, some examples of selection biases that could potentially concern some of our studies.

6.1.3.1 Informative censoring

Informative censoring occurs when participants are censored due to reasons related to the study [184]. In our studies we performed censoring for death due to causes other than breast cancer (study I and study III), or due to all causes of death (study II-IV). This censoring would be informative if subjects who died during the follow-up (i.e. censored observations) were more or less likely to develop the outcome of interest (e.g., develop distant metastasis) than those who did not die before the occurrence of the event (i.e. not censored observations). The risk of informative censoring increases with length of follow-up time [185].

In study I, we found that tumor characteristics are not good predictors of causes of death other than breast cancer, therefore we consider the censoring performed largely non-informative for the main analysis carried out on risk of breast cancer-specific death. In study II, we did not consider in the analysis the women who were reported to die from breast cancer without developing any distant metastasis, as it was considered unreliable. Thus, informative censoring in this case could have only occurred if dying from causes other than breast cancer were somehow associated with the risk of distant metastasis, which is difficult to imagine unless we assumed that a more aggressive disease would be treated more aggressively and many women would die from the side effects of the treatment within ten years. In study III and IV we cannot completely rule out the risk of some informative censoring, as patients who died (or who developed metastasis in study III) before being hospitalized for bone fracture or infection may have also been at significantly increased (or decreased) risk of developing the outcome, as compared to the breast cancer patients who were not censored.

6.1.3.2 Competing risks

When predicting the probability or the risk of an event by a certain time t , one has also to take into account the possible occurrence of other competing events that may prevent the event of

interest from happening. This issue was described in the methods section as a typical problem in survival analysis when trying to estimate the absolute risk of an event by a certain time (see 4.3.1.6). In study II we tried to predict the cumulative incidence function (CIF) of distant metastasis for different subgroups of breast cancer patients according to different exposure levels of age, treatment and tumor characteristics at diagnosis. Being aware of the risk of overestimating the real-world cumulative incidence of distant recurrence over time, we took into consideration in the analysis the competing risk of dying during the study period before developing distant metastasis. Figure 18 shows the cumulative incidence functions for the outcome of interest (i.e., distant recurrence) and the competing risk (i.e., death from cause other than breast cancer) stacked on top of each other over time since diagnosis, enabling to see the relative contribution of the two events over time (Figure 18).

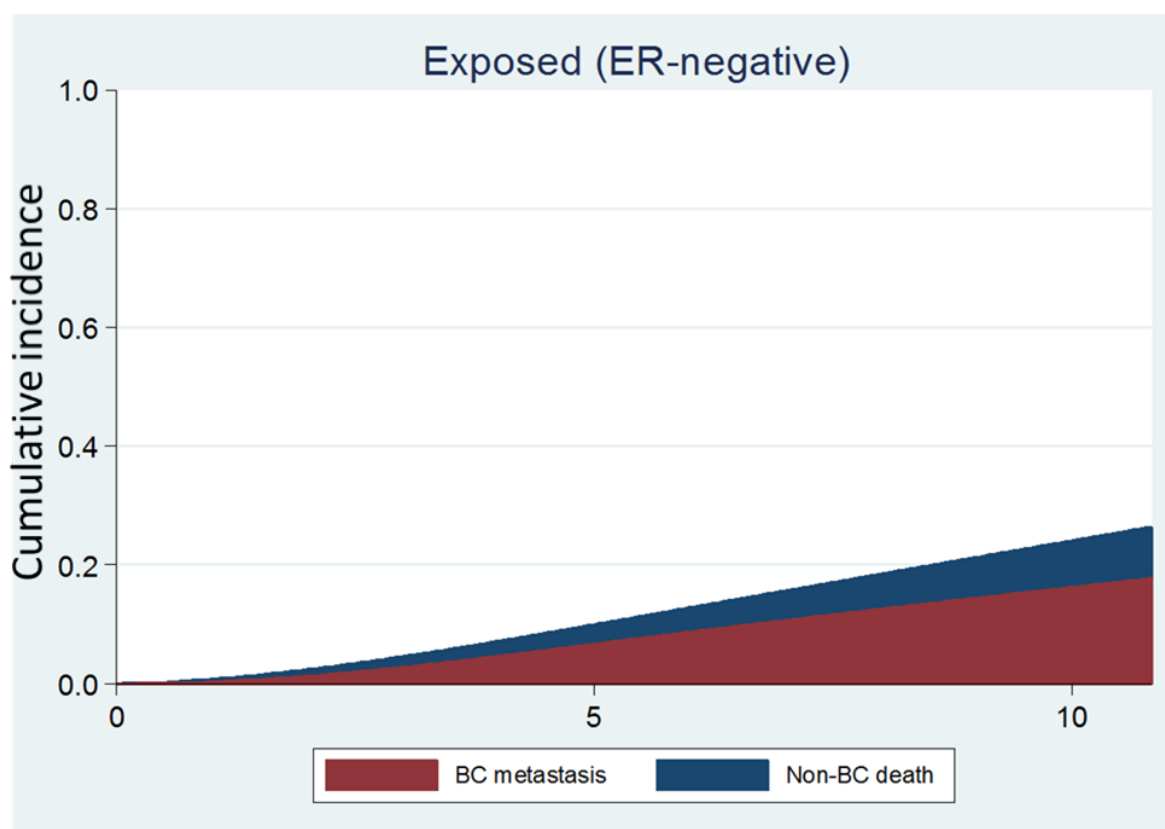


Figure 18. Stacked graph of a cumulative incidence function (CIF) showing the relative contribution of competing risk of death when looking at distant recurrence in breast cancer (BC) patients as an event.

6.1.3.3 Choice of reference population

When using the general population as the reference population in order to obtain the expected number of deaths for the standardized mortality ratios calculation or the expected incidence for the standardized incidence ratios calculation, it is important to consider that this may be a source of bias if the general population were not representative of the unexposed population. This is due to the fact that the exposed population (e.g., women with breast cancer) is also part of the general population. In order for a bias to occur, there either should be a high prevalence of the exposure in the population, or high values of standardized mortality ratios

or standardized incidence ratios [186]. The direction of this bias is towards the null, so any value can either be equal or more extreme than the one found. In study IV we used standardized incidence and mortality ratios and the Swedish general female population as a reference for the expected number of hospitalizations. We cannot therefore rule out the conservative effect of this bias on our estimates that however may be even larger than those we have shown.

6.1.4 Information bias

Information bias is another type of systematic error and occurs when a measurement of exposure or of an outcome is systematically inaccurate. It is also commonly known as misclassification due to the consequent incorrect classification of exposure status and/or cases-control (i.e., outcome) status. Misclassification can typically be differential (i.e., affecting exposed and unexposed, or cases and controls, in an uneven way) and lead to a bias that will have a clear direction, or non-differential (i.e., similarly affecting exposed and unexposed, or cases and controls) and bias the measure of association towards the null (no association).

6.1.4.1 Misclassification of tumor characteristics at baseline

If the accuracy in the measurement of the exposure status is carried out differentially between those who will develop the outcome of interest and those who will not, there will be a bias. The data sources we used have a very good level of completeness of information (see 4.1). In particular, in the Stockholm Gotland regional cohort (study I-III), the information about age at diagnosis, date of diagnosis and treatment combination was available for virtually every individual, information on number of positive lymph nodes at diagnosis and tumor size was available for about 95% and 98% of patients, respectively and information on ER status was available for about 80%. The information contained in our data sources has been validated and has shown a very good level of accuracy for most of the variables (see 4.1). The measurement of tumor characteristics is routinely performed and reported by trained professionals, and despite some heterogeneity in some specific measurement in the past, we believe that any such heterogeneity will not be differentially distributed across different levels of exposure.

6.1.4.2 Lead-time bias

Lead time bias may typically occur when assessing the survival of a screened population versus an unscreened population. Screening allows for an earlier diagnosis of a disease in order to anticipate the treatment and achieve better results with a less aggressive therapy. Patients who undergo screening are thus diagnosed earlier as compared to patients who are diagnosed with the disease only after the onset of the symptoms. This may cause an overestimation of the survival time of the screened patients due to the anticipation of diagnosis. The time between early diagnosis with screening and time where diagnosis would have occurred without screening is called “lead time”. Lead time should not be considered increased survival due to screening, but just a simple artifact due to the anticipation of

diagnosis among screened patients (Figure 19). Lead time bias is particularly important when population screening campaigns, such as those for some solid cancers (e.g., breast cancer), are in place.

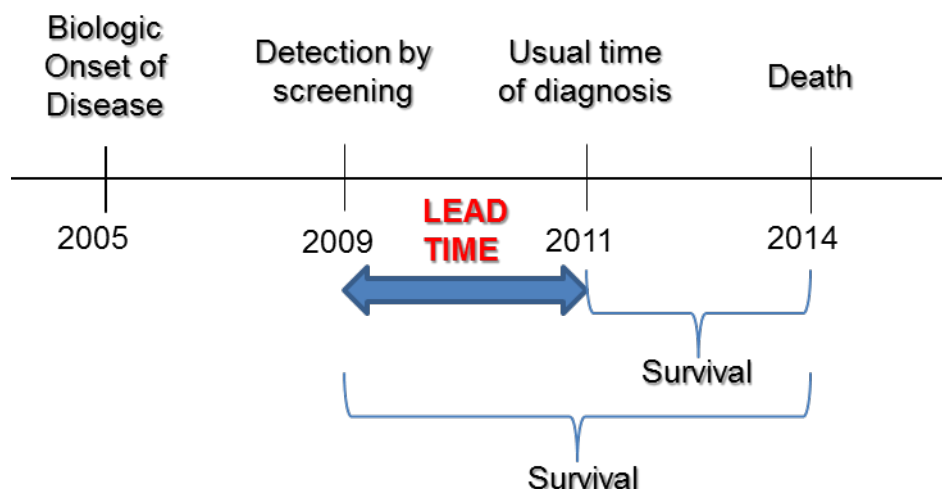


Figure 19. Lead-time bias in screening-detected chronic diseases.

The effect of mode of detection was not assessed in our studies, therefore lead-time bias cannot be ruled out. The Swedish Board of Health and Welfare recommended mammographic screening implementation in 1985. By 1990 up to 93% of women in target age groups had been invited to screening, and by 1997 nationwide coverage was obtained. The mammographic screening officially started in Stockholm in 1989 for women aged 50-69 years and was recommended to be performed every second year. Screening is currently offered to all women aged 40-74 years in 10 out of 21 counties in Sweden [187]. The participation rate to mammographic screening in Sweden is the highest recorded in any country, between 75-85% currently; in particular, in the area of Stockholm the participation rate was 72% as early as 1995-96 [188]. Given the high participation rate and the good level of accuracy in early diagnosis of the Swedish health care system, we believe that lead-time bias may not have differentially affected our study population during the study period. Although unable to rule out some effect of this bias on our estimates, we do not believe this effect to be particularly pronounced.

6.1.4.3 *Misclassification of the outcome*

When dealing with specific health outcomes like underlying cause of death (e.g., death due to a circulatory system disorder) or main diagnosis of hospitalization (e.g., femur fracture), there might be some degree of inaccuracy in the ICD coding, that may bias the results if misclassification has occurred. While acknowledging the good quality of the data sources used in our studies (see 4.1), we additionally tried to reduce the impact of this potential source of information bias by being more inclusive and using larger disease categories when selecting the ICD codes.

In study I the analysis of cause-specific mortality statistics may be affected by misclassification of underlying main causes of death. We used cause-specific outcomes like “death due to diseases of the circulatory system”, in which ischemic heart disease accounted for approximately 45% of cause-specific deaths and cerebrovascular diseases for approximately 20%. We also used “death due to cancers other than breast cancer”. Previous studies have already shown that the use of underlying cause of death from the Swedish Causes of Death Register is overall reliable [168, 189], although one can never exclude some residual misclassification. In study II, the date of diagnosis of distant metastasis might be subject to timing of clinical work-ups and type of follow-up. In addition, the site of first distant metastasis could be affected by detection bias as some locations might cause earlier symptoms than other or may be detected earlier; we therefore decided not to focus on specific site of metastasis in the main analysis. In study III we considered that hip fractures, and more in general femur fractures, usually require hospitalization, therefore we believe that our analysis captured most such cases; however when looking at all fractures, there may be some underestimation in the number of other fractures associated with bone loss, like wrist or vertebral fractures that can also be treated in an outpatients setting. There are some potential limitations related to the difficulty to discern between pathological fractures (due to bone metastasis from the primary breast cancer) and other bone fractures, in case bone metastases were not correctly and timely reported into the register. However, the diagnostic validity of at least femur fractures is considered very high in Swedish Inpatient Register with an estimated specificity quite close to 100% [190]. Also in study IV there was some concern about potential misclassification of the outcome. A recent evaluation of the Swedish National Inpatient register indicated high coverage and validity for most diagnoses [169], however infectious diseases have not extensively been validated in this particular setting. Previous studies in selected (patient) populations show that using main inpatient diagnosis is suitable for monitoring hospitalizations due to infection [191, 192], but the sensitivity seems to vary by organ site and appears to be the lowest for sepsis (about 50%) [193]. Furthermore, there are some indications that the sensitivity might depend on age, comorbidities and disease severity [194], and lower sensitivity estimates have been reported with older age, severe comorbidity and presence of immunosuppression. This, however, should result in more conservative risk estimates rather than producing spurious ones.

6.1.4.4 Referral bias and health seeking behavior

Having been diagnosed with cancer might make breast cancer patients more likely to be hospitalized than other individuals from the same area, period and age group, because of increased cautiousness of the patient or of the physicians. Compared with the general population, breast cancer patients might therefore be more likely to be hospitalized because of health-seeking behavior and/or referral bias. This could explain the initial increase observed in study III and study IV in rate ratios for hospitalizations due to bone fracture and in SIRs hospitalizations due to infection. However, it hardly explains the increased risk of hospitalization observed also beyond 5 years since diagnosis, nor it explains the observed increase in mortality which is less subject to this type of bias. Also, the associations did not

significantly differ when using inpatient individuals as reference. Hence, it seems unlikely that this type of bias fully explains the observed associations in study III and study IV.

6.1.5 Confounding

Confounding is a spurious association between an exposure and an outcome of interest due to a third variable. A confounder is defined as a variable that is causally associated with the outcome of interest in the unexposed and that is also associated with the exposure in the source population (Figure 20). Another fundamental characteristic of a confounder is that it should not be an intermediate cause between the exposure and the outcome of interest, in other words it does not have to be a factor along the causal pathway between the exposure and the outcome. Confounding is a typical problem in observational studies and is the main reason why they are generally considered inferior to experimental study designs where randomization can be applied in order to get rid also of residual confounding. Confounding can be adjusted in the analysis given that information on potential confounders was previously collected, as opposed to bias where one can just tell the direction of the systematic error without being able to adjust for it in the analysis. In observational studies, confounding is usually dealt with adjustment carried out by stratification, standardization or modelling. If measurement of potential confounders is not possible, this will become residual or unmeasured confounding and will potentially bias the findings.

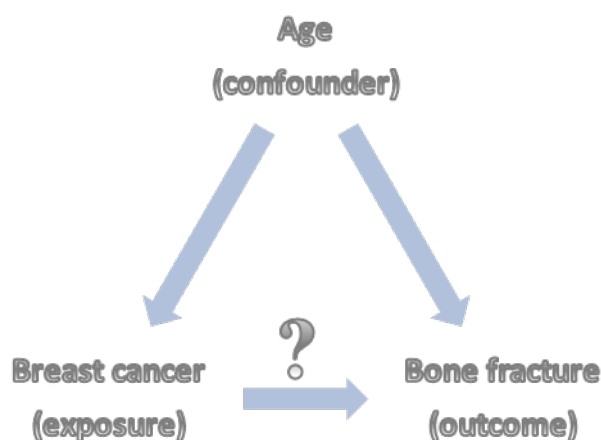


Figure 20. Example of a relationship between confounder, exposure and outcome variables.

In our studies we considered some of the measurable factors that can be causally linked to the main outcomes of interest (death, distant metastasis, bone fracture-related hospitalization, infection-related hospitalization). We included in most of our models age at breast cancer diagnosis, calendar period, tumor size, lymph node status, ER status and treatment combination. We did not take into account other factors like comorbidities (e.g., concomitant severe diseases for mortality, osteoporosis for bone fractures, diabetes for infections); specific medications or treatments (e.g., bisphosphonates and aromatase inhibitors for bone fractures, corticosteroids for infections and bone fractures); diagnostic and invasive procedures (e.g., venous catheter use and blood transfusions); personal behaviors (e.g., smoking and alcohol consumption) due to the lack of available information in the data sources used. Moreover we

could not take into account specific agents introduced in the management of breast cancer patients during the study period, like aromatase inhibitors and bisphosphonates or like trastuzumab in HER2-positive cancers, and also changes in the prescription and use of specific chemotherapeutic agents. Some of these factors can potentially affect the risk of bone fracture and infection, and also the overall survival especially at an older age [195-197]. We partly evaluated the impact of potential residual confounding only in study IV, by running additional analyses in a case-control study population (CAHRES study, see 4.2.4) with more detailed information on background risks, and we found that the estimates were not affected by adding these additional factors in the model (i.e., BMI, physical activity, smoking, history of hypertension and diabetes).

One should always be careful when choosing what to adjust for confounding, since there may be instances when this is not appropriate as when one factor is on the causal pathway between exposure and outcome. In our studies, the high degree of correlation between tumor characteristics and treatment has made it quite challenging to disentangle and isolate each respective effect. The type of adjuvant treatment is generally chosen based on type of surgery, tumor grading and staging and on receptors status, together with other clinical and general considerations, like for instance the age or the general conditions of the patient. Treatment can therefore be considered partially on the causal pathway between tumor characteristics and outcome. In all studies, we noticed a consistent robustness of the estimates when adjusting or not adjusting by adjuvant treatment. We also need to stress that the effect of treatment is generally better studied within an experimental study design where randomization minimizes the risk of confounding by indication.

6.1.6 Generalizability

As in all studies requiring a long follow-up, the estimated long-term risk of a specific health outcome might not reflect the current risk for newly diagnosed patients: in particular, adjuvant treatment has been changing over time and recently diagnosed patients may not show the same risk patterns as the ones observed in our studies.

6.2 FINDINGS AND INTERPRETATION

6.2.1 Study I

The effects of ER status, lymph node status, and tumor size on the risk of dying from breast cancer were all time-dependent. Previous studies based on the Surveillance, Epidemiology, and End Results (SEER) database reported that the difference in survival associated with hormonal receptor status may persist up to 11 years after diagnosis [54], and that women with ER-negative tumors were consistently more at risk of dying regardless of other tumor characteristics [55]. In our study we were able to assess the effect of ER status on the risk of dying from breast cancer while taking into account hormone treatment. We found that, after 5 years since diagnosis, ER status was not significantly associated with the risk of dying from

breast cancer. Our results are also in agreement with clinical trials showing a low but continuous risk of relapse from 5 to 15 years after primary diagnosis in ER-positive patients, and most relapses occurring from 2 to 3 years since diagnosis in ER-negative patients [5, 198]. One possible explanation of our findings could be that ER-negative patients, who survive more than 5 years, are the fittest independent of other known prognostic factors.

The effect of lymph node status on survival remained significant for up to 10 years after breast cancer diagnosis and accordingly we can infer that lymph node status at diagnosis is a long-term prognosticator of survival as previously reported [199]. Previous research reported that, for each additional positive lymph node, the risk of breast cancer-specific death increases by 6%, and each millimeter increase in tumor size increases the risk by approximately 1% [200]. Recent findings showed that even micrometastasis to the lymph nodes predicts prognosis [201-203]. In our study population, having 1-3 versus 4 or more positive lymph nodes at diagnosis had a positive effect on survival in the first 5 years since diagnosis, while after 5 years this effect gradually disappeared. This could again be explained by survival of the fittest in the group with poorer prognosis (i.e., 4 or more positive lymph nodes at diagnosis) and by delayed mortality within the group initially considered to have a better prognosis (i.e., 1-3 positive lymph nodes at diagnosis). A similar pattern was seen for tumor size, although the magnitude of the effect was less pronounced.

Circulatory system disorders were an important cause of death in our study population: about 45% were caused by ischemic heart disease and about 20% by cerebrovascular disease. Deaths resulting from circulatory system disorders might partly reflect the toll of stress on elderly women diagnosed with a severe disorder [204]. In fact, women diagnosed with less favorable tumor characteristics (large tumor size or positive nodes) showed a borderline increased risk of dying from circulatory system disorders, and this effect was more pronounced within the first year since diagnosis. Finally, the risk of dying from cancers other than breast cancer was not significantly associated with tumor characteristics.

6.2.2 Study II

In our cohort, up to one-third of distant metastasis was diagnosed in the skeleton. This proportion significantly increased over time since diagnosis, whereas the proportion of metastasis to the liver and central nervous system (CNS) significantly decreased. This seems to reflect the natural history of distant recurrences, as women with ER-positive tumors more often develop metastasis later during follow-up (and more preferably to the skeleton), whereas women with ER-negative tumors more often tend to develop liver and CNS metastasis earlier [205].

Lymph node status at breast cancer diagnosis was significantly associated with the risk of distant recurrence for at least 10 years, whereas ER status at diagnosis was significantly associated with risk of distant recurrence only in the first 5 years [206]. The similarity with the pattern observed in the same cohort when looking at the risk of dying from breast cancer

(study I) suggests that the development of distant metastasis is still a predictor of poor prognosis in breast cancer patients [207, 208].

The cumulative risk of first distant metastasis was still relevant for most patients from 5 years since breast cancer diagnosis onwards. Patients with ER-positive and lymph node-negative tumors were at rather low but similar cumulative risk of distant recurrence over the different periods of follow-up 0-5 years and 5-10 years. More clinical attention should however be given to other subgroups of patients that were found to be at a higher risk. In particular, ER- and lymph node-positive patients with tumors larger than 20mm still had a risk higher than 10% to develop distant metastasis in the period between 5 and 10 years since breast cancer diagnosis. Although evidence supporting the change of current practice is rather weak [209], following future improvements in prevention and treatment of metastatic breast cancer, a differential follow-up of subgroups of breast cancer patients could be considered, given remarkably different risks of spreading, natural histories and treatment options available.

6.2.3 Study III

In the Swedish national cohort, breast cancer patients were at long-term increased risk of being hospitalized with a bone fracture. This risk remained significantly increased for more than 10 years since diagnosis. Treatment and severity of the disease may explain this difference for the period shortly after breast cancer diagnosis, but it can hardly explain it at 10 years or further. Bone tissue is sensitive to sex hormones [210] and previous studies have clarified the important role of hormonal treatment in affecting bone metabolism [85-87, 89, 120, 122, 123, 211]. While tamoxifen has a protective effect against osteoporosis and subsequent bone fractures [122, 211], other hormonal treatments, like aromatase inhibitors, can lead to a negative impact on bone metabolism and to a higher risk of fractures in women with breast cancer, especially in combination with menopause and older age [211-214]. Prostate cancer patients showed an increased risk of fractures compared to the general population, especially if treated with an androgen deprivation therapy [215-217]. There were also some studies unable to show a significantly increased risk of bone fracture in women with a previous breast cancer diagnosis [216, 218].

In our regional cohort of breast cancer patients, no differential effect of tumor characteristics and adjuvant treatment combination on the risk of being hospitalized for a bone fracture was found. The correlation between tumor characteristics and adjuvant treatment combination, the lack of information about specific administered agents, duration of treatment and dose, and the combination of agents with different effects on bone metabolism may have also hidden any existing differential association.

We finally found an increased overall risk of death after a bone fracture hospitalization (not only after a hip fracture hospitalization) in women with a previous breast cancer diagnosis. This increased risk seems completely driven by an increased risk of dying from causes other than breast cancer, in particular among women 61-74 years at breast cancer diagnosis. Previous studies showed that osteoporotic fractures are associated with a 1.4-2.4-fold

increased risk of death for at least 5 years and this risk remains significantly increased for at least 10 years after a hip fracture [119]. In older people, hip fractures are associated with a 5- to 8-fold increase in mortality within 3 months, and a 2-fold increase within a year [219, 220]. Hip fractures can be associated with complications like pressure ulcers, deep vein thrombosis and pulmonary embolism, while vertebral fractures can affect pulmonary function and cause chronic back pain [221-225]. These findings further stress the importance of preventing bone fractures in breast cancer patients in order to improve the overall prognosis.

6.2.4 Study IV

We found breast cancer patients to be at increased risk of being hospitalized with an infection for up to 10 years since diagnosis, as compared with the general population. Two possible explanations of this finding are cancer-induced immunosuppression and treatment-related adverse effects as they both seem to play a role in infectious disease susceptibility. Recent molecular studies showed that cancer cells can disrupt the host immune surveillance and that the interplay between immune components and cancer cells is crucial for disease progression [226, 227]. As for treatment, short-term suppression of neutrophil production is a well-known side effect of chemotherapy; also, chemotherapy and radiotherapy can impair mucosal immunity, and local flora may invade once the physical barrier of epithelial lining is damaged [130, 228].

The increased risk of being hospitalized with an infection was particularly referred to skin infections and sepsis, and this is in accordance with two related complications commonly observed in breast cancer patients: lymphedema and neutropenia [229, 230]. Approximately 30% of breast cancer patients develop lymphedema within 18 months after diagnosis [229]. Lymphedema can actually cause skin infections [231] and this explains the excess risk found for this specific type of infection. The observed overlap in risk factor profile (in terms of tumor characteristics, chemotherapy and axillary radiation) reflects the close correlation between lymphedema and skin infections [232]. Our results also complement previous work showing that *erysipela* is the most common clinical presentation of skin infections in breast cancer patients [233]. *Erysipela* is an inflammatory cellulitis of the upper dermis usually caused by streptococcal infections [234]. If left untreated, it can spread through lymphatic vessels and cause severe vessel injury (i.e. lymphangitis), and possibly sepsis as well [233, 234]. Neutropenia is also closely associated with the risk of sepsis as a common complication of chemotherapy [130].

Several markers of disease severity (including tumor size, grade, lymph node and ER status) were associated with the risk of being hospitalized with an infection, and in particular with the risk of sepsis. A significant association with lymph node status was previously reported in concordance with what we found [134]. The risk of being hospitalized with an infection was higher in women diagnosed at age less than 50 years and this could be explained by the fact that young breast cancer patients are often diagnosed with a more advanced disease and tend to receive more aggressive treatment.

Breast cancer patients from our study population were also at increased risk of dying after a being hospitalized with an infection as compared with the general population. Therefore, averting infections may also improve overall survival of women with breast cancer.

6.3 CONCLUSIONS

In this thesis we looked at some of the main health outcomes in women with breast cancer with a particular focus on the long-term outlook. We investigated the time-dependent effect of tumor characteristics and age on overall survival and on specific causes of death. We also studied the risk of developing distant recurrences according to different tumor characteristics and looked at specific sites of metastasis as well. We finally analyzed the occurrence of two common causes of hospitalization, bone fractures and infections, in breast cancer patients, and investigated their association with age and tumor characteristics among breast cancer patients. After performing the four studies these are our main conclusions:

- The effects of different tumor characteristics on the risk of distant recurrence and death in women with breast cancer significantly change over time since diagnosis.
- Women with positive lymph nodes at diagnosis carry a worse long-term overall prognosis compared with lymph node negative patients; this is not only true in terms of overall survival and risk of distant recurrence, but also in terms of hospitalizations for common complications like bone fractures and infections.
- ER status is a strong independent predictor of distant recurrence-free survival and overall survival in the first 5 years after breast cancer diagnosis but not any further.
- ER-positive patients seem to have a low but persistent risk of distant recurrences and death and should therefore be appropriately treated and monitored.
- The risk of distant metastasis is non negligible even after 5 years since breast cancer diagnosis in most subgroups of patients.
- Tumors larger than 20mm at breast cancer diagnosis significantly increase the risk of dying, in particular shortly after diagnosis, and of distant recurrences within 10 years from diagnosis.
- Causes of death other than breast cancer are reported in more than 50% of breast cancer patients, diagnosed after the age of 65 years, within 10 years since diagnosis.
- Women with breast cancer are at significantly increased risk of being hospitalized with a bone fracture or with an infection for at least 10 years since diagnosis.
- Tumor characteristics do not differentially affect the risk of being hospitalized with a bone fracture.
- Women with breast cancer are at increased risk of dying after being hospitalized with a bone fracture or with an infection.
- Sepsis and skin infection are the most commonly reported causes of hospitalization due to an infection in our study population of women with breast cancer.
- The risk of being hospitalized with an infection is positively associated with more aggressive/advanced tumor characteristics at diagnosis in breast cancer patients.

- Preventive measures for bone fractures and infections could be taken into considerations for all patients at increased risk.
- Thanks to remarkable improvements in prognosis, overall survival should not be the only main indicator for the management of breast cancer patients.
- There is no evidence for discontinuation of clinical follow-up of breast cancer patients.

7 FUTURE PERSPECTIVES

The increasing understanding of the molecular mechanisms of breast cancer is going to lead to more targeted treatment and may even allow potential individualized approaches in the future. The growing availability of molecular information can also contribute to shed additional light on the prognosis of different breast cancer subtypes, so that also clinical follow-up may become tailored according to the personal outlook of the patient [235-238]. Attempts to develop composite prognostic scores for breast cancer are in fact already undergoing [239].

As much as molecular profiles of breast cancer influence prognosis and likelihood of distant recurrence [54, 55, 240, 241], also gene expression profiles of breast tumors could be used in the next future for the prediction of local and distant patterns of spreading, and as potential candidate targets for the development of new drugs [242-246]. Three areas of primary research interest are the full identification of comprehensive molecular portraits of breast tumors, the influence of the microenvironment and of the stromal tissue on local and distant cancer development, and the interaction between cancer and the immune system of the host.

In particular, the understanding through genome-wide association studies of how the genetic predisposition can affect late relapses, non-breast cancer related health outcomes, and even potential markers of breast cancer prognosis, would be of considerable clinical relevance. A recent study found that a 20% decrease in mammographic breast density in women treated with tamoxifen is associated with an improved 15-year survival [27]. Moreover, some studies reported that bone mineral density, which is known to be inversely associated with the risk of breast cancer [247, 248], may be also associated with prognosis [249]. Finally, changes in breast density during breast cancer treatment might be possibly related to changes in bone mineral density [250]. In this case, bone mineral density could be evaluated as a potential additional marker of late relapses, and breast density as a potential indicator of the risk of bone fractures in women with breast cancer.

Improving the capacity to correctly identify subgroups of breast cancer patients at prolonged low risk of recurrence could also spare women from part of the cancer treatment and thus decrease their risk of long-term adverse events from the therapy. Furthermore, this could also potentially save some of the ever increasing cost associated with treatment of breast cancer. The cost of oncologic drugs is remarkably increasing over time going from less than 10,000 dollars per patient in the early 2000s up to 30,000-50,000 dollars per patient in 2011, amounting to at least 5,000 dollars per month or per cycle [251]. On the other hand, new targeted drugs, when truly effective, may save a lot of resources by effectively curing patients and preventing complications of breast cancer and of adverse effects of current adjuvant treatment. Regardless of the national policies in place, the fast increasing cost of oncologic technologies for diagnosis and treatment cannot be ignored and need to be incorporated in cost-benefit analyses performed with sound health technology assessment's methodology. This evaluation should be however carried out after correctly assessing drug effectiveness by

performing well-designed comparative trials with the existing drugs (and not just studies of non-inferiority) and by using appropriate end points (and not just surrogate ones).

The remarkable improvements in survival and the future further achievements should also draw more research and attention to the quality of life of breast cancer patients. In particular, endocrine, circulatory, psychological and neurological disorders are areas of major clinical interest. The treatment of an oncologic patient should never be considered the exclusive pertinence of a specialist but a team effort of different professionals putting the patient, and not the disease alone, at the center of the clinical care. This implies an organizational structure allowing for an oncologic treatment tailored for each specific type of patient, and not separately suiting different clinical specializations. Finally, patients should be more involved in the decision making concerning breast cancer treatment, and the development of new guidelines should always take cost-benefit and patient perspective into consideration.

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