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**HORMONE-RELATED FACTORS
AND BREAST CANCER
- STUDIES OF RISK AND PROGNOSIS**

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Abstract

The main purpose of this thesis was to explore the influences of risk factors for breast cancer on breast cancer characteristics and survival.

We evaluated the associations between number and timing of births and breast cancer-specific survival using data from the Swedish Cancer Register, the Swedish Cause of Death Register, and the Multi-Generation Register. We identified more than 27,000 women born in Sweden and diagnosed with breast cancer in 1958-1997. We found a successively worse prognosis for women with a shorter delay between last birth and breast cancer diagnosis. Compared to women with their last birth more than 10 years before diagnosis, the risk of breast cancer death was increased by 72% for those with breast cancer diagnosis in the first year after last birth, and 39% for those diagnosed in the 3rd year after last birth. Women with a first birth before 20 years had a slightly worse prognosis than parous women with their first birth between 20-24 years. These findings suggest that a recent birth and possibly an early first birth influence tumor biology.

For the three following studies we used a Swedish population-based case-control study including more than 3,000 women aged 50-74 diagnosed with breast cancer in 1993-1995, and more than 3,000 age-frequency matched controls. The study holds information from questionnaires, patient records, and the Swedish Cause of Death Register.

We assessed the influence of menopausal hormone therapy, anthropometric factors, reproductive factors, recent smoking, recent alcohol intake, family history of breast cancer, and previous benign breast disease on the risk of ductal, lobular, or tubular breast cancer. Menopausal estrogen-progestin therapy was associated with increased risks for all subtypes, but the estimates for lobular and tubular cancer were stronger compared to ductal cancer. Menopausal estrogen alone therapy was similarly associated with ductal and lobular breast cancer. Recent alcohol intake was significantly associated with tubular cancer, and seemed non-significantly related to lobular but not ductal breast cancer. Increasing age at first birth was borderline significantly more strongly related to lobular compared to ductal breast cancer. Other reproductive factors, anthropometric factors, and smoking seemed similarly related to ductal and lobular breast cancer.

The same risk factors were also evaluated by joint estrogen (ER) and progesterone receptor (PR) status of the tumors. We found menopausal estrogen-progestin therapy to increase the risk for all receptor groups except the ER-PR- group. The risks were borderline significantly stronger for ER+PR+ tumors compared to ER-PR- tumors. Menopausal estrogen alone therapy was similarly associated with all receptor-defined groups. A high age at first birth seemed to increase the risk only for the ER+ groups, and substantial adult weight gain was associated only with the PR+ tumor groups. Most other risk factors seemed similarly associated with all receptor-defined tumor groups.

Current, but not past, compared to never use of menopausal hormone therapy was associated with tumors of low grade, low S-phase fraction, and improved breast cancer specific survival. The associations were stronger with longer duration, and breast cancer survival seemed more favorable with estrogen-progestin than estrogen alone therapy. The improved survival among current users of menopausal hormone therapy was only partly explained by differences in available tumor characteristics and mammography surveillance.

Key words: Epidemiology, breast neoplasms, body mass index, hormone replacement therapy, reproductive history, ductal carcinoma, lobular carcinoma, tubular carcinoma, estrogen receptor, progesterone receptor, risk assessment, survival

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List of papers

The thesis is based on the following papers;

- 1 Rosenberg L, Thalib L, Adami H-O, Hall P.
Childbirth and breast cancer prognosis
Int J Cancer. 2004 Sep 20;111(5):772-6.

- 2 Rosenberg LU, Magnusson C, Lindström E, Wedrén S, Hall P, Dickman PW.
Menopausal hormone therapy and other breast cancer risk factors in relation to the risk of different histological subtypes of breast cancer: a case-control study
Breast Cancer Res. 2006;8(1):R11.Epub 2006 Feb 17.

- 3 Rosenberg LU, Einarsdóttir K, Isaksson Friman E, Wedrén S, Dickman PW, Hall P, Magnusson C.
Risk factors for hormone-receptor defined breast cancer in postmenopausal women
Submitted

- 4 Rosenberg LU, Dickman PW, Einarsdóttir K, Persson I, Wedrén S, Granath F, Hall P.
Menopausal hormone therapy in relation to breast cancer characteristics and prognosis
Submitted

Abbreviations

BMI	Body Mass Index
CI	Confidence Interval
DNA	DeoxyriboNucleic Acid
ER	Estrogen Receptor
HR	Hazard Ratio
ICD	International Classification of Diseases
IGF	Insulin like growth factor
OR	Odds Ratio
PR	Progesterone Receptor

Introduction

Among Swedish women, and elsewhere in the Western world, breast cancer is the most common cancer, and the commonest cause of cancer death. One Swedish woman in ten will be diagnosed with breast cancer before the age of 75.

Breast cancer incidence has increased steadily during the last 50 years. Both mammographic screening and an increasing proportion of elderly people have contributed to the increased incidence, but some of the increase must be explained by changes in environmental factors in the population. Breast cancer is a heterogeneous disease that can be classified according to different tumor characteristics, such as morphology, receptor status, and grade. There are risk factors that seem to increase the risk particularly for some subtypes of breast cancer, like the strong association between menopausal hormone therapy and lobular breast cancer; this implies that tumor biology, at least to some extent, may depend on risk factor exposure.

The prognosis in breast cancer has improved dramatically in the last 50 years, so despite the increased incidence, breast cancer mortality in the Swedish population has declined slightly. The introduction of mammographic screening, increased awareness, and improved therapy are explanations to the improved prognosis. In addition, if risk factors influence the tumor biology they may also influence the prognosis. Population changes in reproductive behavior and the introduction of menopausal hormone therapy may thus have contributed to the improved prognosis.

The aim of this work was to enhance the understanding of how breast cancer risk factors influence the risks of subgroups of breast cancer as well as breast cancer prognosis. Evaluating risk factors in relation to different subgroups of breast cancer may improve the understanding of carcinogenic pathways. In the clinical setting, risk factor assessment may also add to the prognostic information derived from traditional tumor characteristics.

Background

Descriptive epidemiology

Incidence and mortality

Breast cancer is the most common cancer among women worldwide (Parkin 1998). The reported incidence is around 3-6 times higher in Western Europe, North America, and Australia, as compared to south-east Asia and Africa (Shibuya 2002). The Swedish Cancer Register reported 6,925 women diagnosed with breast cancer in 2003, accounting for 29% of all new cancers in women (Socialstyrelsen 2005). In Sweden, one woman in ten will develop breast cancer before the age of 75 (Socialstyrelsen 2005). The mortality varies much less worldwide, around 2-3 fold (Shibuya 2002). In Sweden, 1500 women died from breast cancer in 2003, making up 3% of all female deaths (Socialstyrelsen 2003).

Trends

The incidence in Sweden has been increasing steadily since the Cancer Register was initiated in 1958 (Fig. 1). Despite the increasing incidence, the mortality decreased slightly in Sweden between 1970-2001 (Fig 1). The decreasing mortality is an effect of a dramatically improved survival. The 15-year relative survival is now estimated to be around 85%, and around 78,000 women in Sweden are living after a breast cancer diagnosis (Socialstyrelsen 2005).

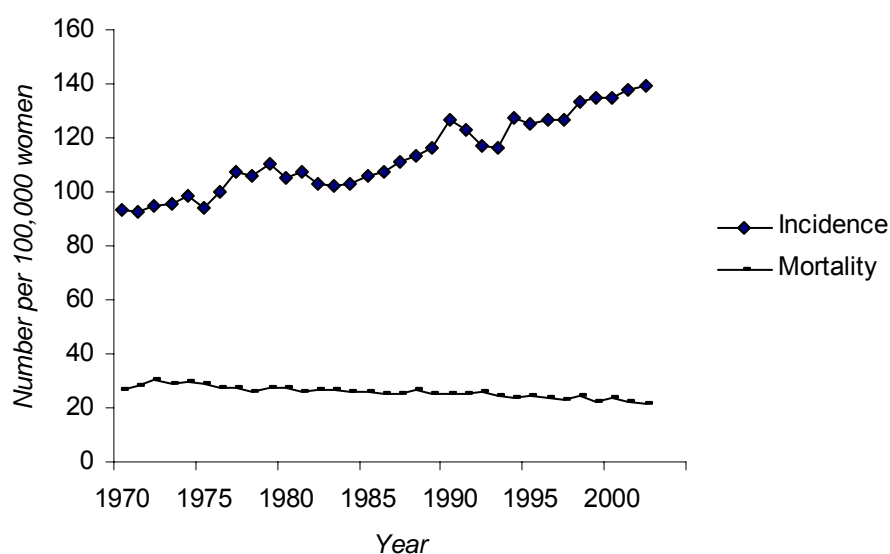


Fig. 1. Age-standardized breast cancer incidence and mortality per 100,000 women in Sweden 1970-2001. Adapted from the National Board of Health and Welfare.

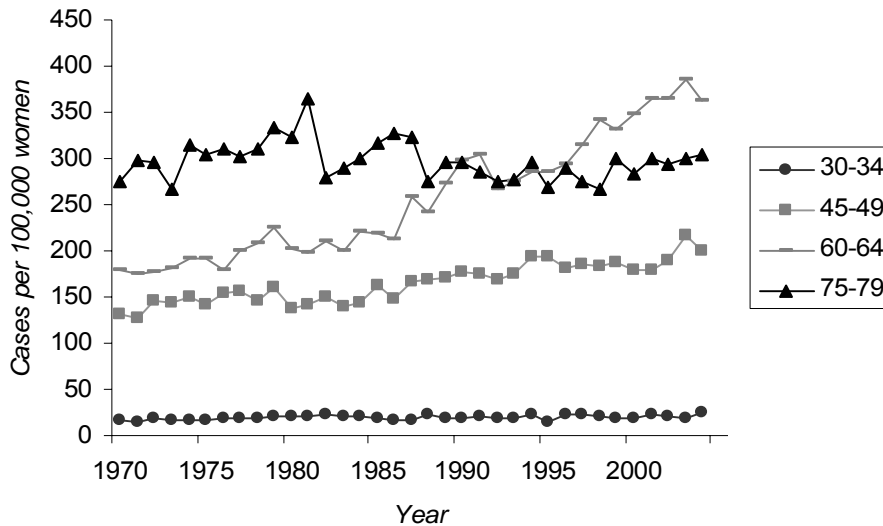


Fig. 2. Age-standardized breast cancer incidence per 100,000 women in Sweden 1970-2004 in selected age-groups. Adapted from the National Board of Health and Welfare.

The increased incidence is largely confined to postmenopausal women below the age of 75 years (Fig 2). Consequently, the age-specific incidence has changed in Sweden in the last 20 years (Fig 3).

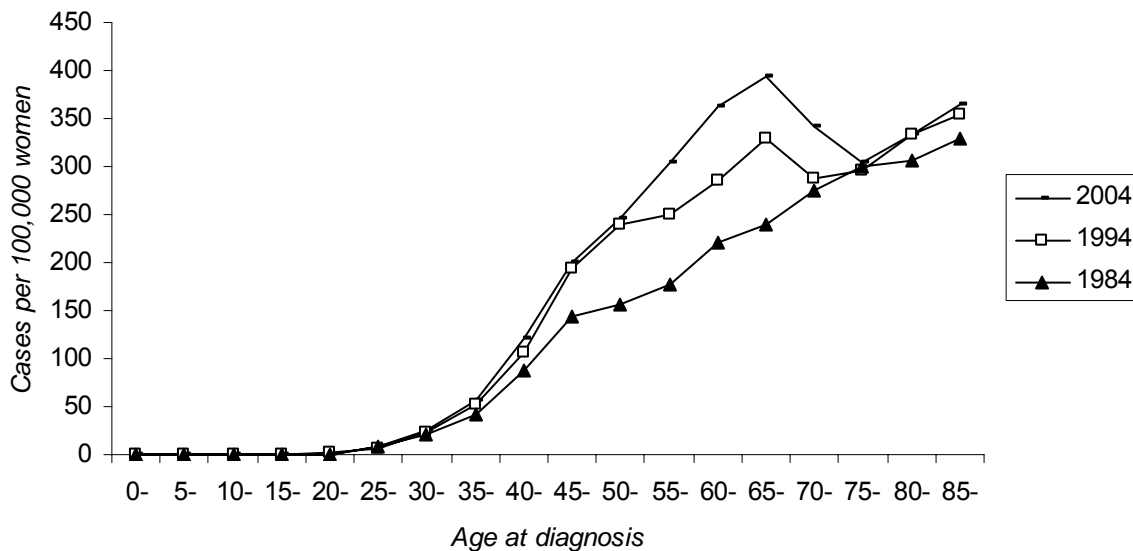


Fig 3. Age-specific incidence rates of breast cancer in Sweden in 1984, 1994, and 2004. Adapted from the National Board of Health and Welfare

In 1984, the incidence increase declined at 50 years, but in 2004, the incidence increase was stable until the age of 70. Parallel with the economic development and changes in life-style including reproductive pattern in south-east Asia, the breast cancer incidence is rapidly increasing (Chia 2005), so the differences compared to Western Europe and North America are currently decreasing.

Clinical aspects

Diagnostics

All suspected breast lesions should be examined with the triple diagnostics (palpation, mammography and cytology), as proposed by Swedish researchers in 1974 and golden standard in Sweden and many other countries nowadays (Bjurstam 1974, SBCG 2005). Before the introduction of mammography in the 1960s and especially mammographic screening in the 1980s, breast cancers were detected as palpable tumors, rarely <2 cm in diameter. Nowadays, most tumors are diagnosed through mammographic screening, and a majority of tumors are <2 cm in diameter. Starting in the 1960s, several randomized trials of mammographic screening were performed in Sweden and some other Western countries. They showed a reduced breast cancer mortality of about 15-25% among screened women, with the higher numbers among women >50 years (Harris 2005). There is an ongoing debate on the efficiency (in the randomized trials) and the effect (under usual conditions in the society) of mammography screening. One argument has been that mammographic screening will lead to over-diagnosis of cancers that otherwise would not have been discovered. In the Malmö mammographic screening trial, the over-diagnosis was 10% 15 years after the study stopped (Zackrisson 2006). As women in the control group have performed screening mammographies during and after the study, this is likely an underestimation. WHO states that in countries which are able to reach at least 70% of women in the target population, mammographic screening is recommended every 2-3 years after the age of 50 (Smith 2006). In 1986, the Swedish National Board of Health and Welfare recommended screening every second year among women, starting from age 40 or 50 to at least 69 years. Most counties in Sweden had introduced screening programs by 1990, and from 1996 all counties offer mammography screening.

Treatment

Breast cancer treatment involves several modalities. Detailed examination of the tumor through clinical, radiological, and pathological examination forms the basis for decision of treatment. Surgery is the primary treatment, and nowadays, to improve survival, other treatments are added before or after the surgery, often named adjuvant treatments.

Surgery

Breast conserving surgery followed by breast irradiation is as good as total removal of the mammary gland (mastectomy) for women with unifocal breast cancer, provided the tumor is not larger than 4 cm in diameter, or centrally placed (Clarke 2005). Currently, breast conserving therapy is performed in around 70% of breast cancer cases (Hiotis 2005). For larger, multifocal, or inflammatory tumors, and when women with early breast cancer do not accept radiotherapy, mastectomy should be performed.

Axillary lymph node dissection has until recently been the golden standard for all invasive breast cancers. Surgeons should aim at removing at least ten lymph nodes. Lymph node dissection is important for the staging of the tumor, but is associated with frequent lymphedema of the arm (Duff 2001). As the introduction of mammographic screening has led to an increased proportion of tumors without lymph node involvement, sentinel node biopsy has been introduced (Veronesi 2003). Via injection of radioactive tracer and/or dye, the first "sentinel" node is detected and excised. If this lymph node is free of tumor, no lymph node dissection is performed. Sentinel node biopsy has been shown not to increase the number of recurrences in the axilla (Veronesi 2003), and was recommended in 2004 in Sweden for unifocal tumors <3 cm in diameter (SBCG 2005).

Radiotherapy

Patients treated with breast conserving surgery should be given postoperative radiotherapy to the remaining breast. Women with high risk of recurrences should, regardless of type of surgery, be recommended loco-regional radiotherapy. Radiotherapy in fractions of 2 Gray per day, 5 days a week up to 50 Gray is the common procedure (SBCG 2005). Radiotherapy efficiently reduces loco-regional recurrences, but has adverse effects, in particular increased risk of cardiovascular events (Darby 2003). The net effect on mortality has therefore been debated. Recently, a large meta-analysis of randomized trials was able to show a reduction in overall mortality with radiotherapy (Clarke 2005). In women with small tumors with good prognostic characteristics, the absolute effect of radiotherapy on recurrences and mortality is small (SBCG 2005).

Chemotherapy

Postoperative treatment for 6 months with anthracycline-based combination chemotherapy reduces breast cancer annual mortality by around 20% for women aged 50-69 years, regardless of other tumor characteristics and treatment. In absolute terms, the largest reduction in mortality is thus among women with a poor prognosis (EBCTCG 2005). Preoperative chemotherapy can be an alternative for women with locally advanced or inflammatory breast cancer. Tumor shrinkage may make surgery possible, or lead to a better surgical result, and the responsiveness to chemotherapy can be assessed (SBCG 2005).

Endocrine therapy

The first endocrine therapy was introduced in 1896, when Beatson, a Scottish surgeon, treated women with metastasized breast cancer with ovarian ablation (Beatson 1896). In the 1940s, the first randomized trials of adjuvant ovarian ablation were performed (Clarke 1998). During the 60s, 70s and 80s, more randomized trials were performed, and they found an improved recurrence-free survival, but failed to find a significantly improved breast cancer survival. Therefore, ovarian ablation never became a wide-spread adjuvant therapy of premenopausal women. In 1992, a meta-analysis of randomized trials finally managed to show an improved survival among women <50 years at diagnosis (EBCTCG 1992). The effect of ovarian ablation inspired other attempts of hormone-modulating therapies. Diethylstilbestrol, a synthetic estrogen, was used to treat metastatic breast cancer from the 1940s and onward (Binnie 1944), and does paradoxically have an apoptotic effect on breast cancer cells (Lewis 2005).

In 1981, tamoxifen was found to be equally efficient as high-dose estrogen, with less adverse effects, and tamoxifen replaced high-dose estrogen treatment (Ingle 1981). Tamoxifen is a competitive estrogen receptor inhibitor in breast tissue but has estrogenic effects in other organs (Dhingra 1999). Adjuvant treatment of estrogen receptor (ER) positive tumors with tamoxifen for five years reduces the annual breast cancer mortality around 30% up to 15 years after diagnosis, regardless of progesterone receptor status, other tumor characteristics, and other treatments (EBCTCG 2005). Women with tumors without estrogen receptors do not benefit from tamoxifen treatment (EBCTCG 2005). In Sweden, five years of adjuvant tamoxifen treatment was shown to be superior to two years in 1996 (SBCG 1996). Aromatase inhibitors inhibit conversion of steroids to estrogens and one of them, anastrozole, has recently been shown to be superior to tamoxifen, both regarding effect and adverse effects, and will probably replace tamoxifen as the first choice for adjuvant therapy in the future (Howell 2005).

Antibody treatment

There is currently much interest in trastuzumab, an antibody treatment towards HER2/neu. Trastuzumab has been used for metastatic disease for a couple of years, and is now licensed as an adjuvant treatment for breast cancers over-expressing HER2/neu (Editorial 2005, Plosker 2006).

Primary prevention

Women with several family members diagnosed with breast cancer at young ages have a high life-time risk of breast cancer and primary prevention has attracted some interest. In Sweden, around 10% of these women seem to have mutations in BRCA1 or BRCA2 (Loman 2001).

Bilateral mastectomy seems to be the most efficient means of prevention, reducing the risk by 90-100% (Calderon-Margalit 2004), but is not accepted by all women. Prophylactic oophorectomy is another, quite invasive prevention that seems to reduce the risk by 50% (Calderon-Margalit 2004). Treatment with tamoxifen to high risk groups of women decreases the risk of ER+ breast cancer, but the side-effects (endometrial cancer, deep venous thrombosis, and stroke) are as common as the reduction in breast cancer cases, and a meta-analysis found that overall mortality was non-significantly increased among women treated with tamoxifen (Cuzick 2003, Fabian 2005). Raloxifene is another selective estrogen receptor modulator, with no signs of breast proliferation, initially designed to treat osteoporosis (Thiebaud 2001). Raloxifene is equally efficient as tamoxifen in reducing the risk of breast cancer, with fewer side effects like thrombosis, uterine bleedings, and endometrial cancer (Vogel 2006).

Tumor characteristics

Tumor characteristics are important in the clinical setting. They can be prognostic factors (indicating the risk of breast cancer death), predictive (indicating the benefit of endocrine or chemotherapy treatment), or both. Tumor characteristics reflect a combination of the underlying tumor biology and of how early the tumor was detected. The pathologist preferably assesses the tumor characteristics, as clinical or radiological information is less reliable. Ideally, the individual tumor would be completely characterized. New techniques in genetics might improve the characterization of tumors (Pawitan 2005). In addition, recent findings indicate the existence of a tumor stem cell for breast cancer in humans (Al-Hajj 2003), and recently, researchers have found a single breast stem cell in mice that gave rise to both luminal and myoepithelial cells (Shackleton 2006). This mouse stem cell was also overrepresented in premalignant tissue.

Carcinogenesis

At least six different genetic modifications in the same cell are required for the development of human malignancies (Hanahan 2000). The modifications must not be any random alterations; they must lead to independent growth, avoidance of apoptosis, sustained angiogenesis, and the ability of tissue invasion. According to this multi-step view of cancer development, it has been believed that ER+ breast tumors often become ER-, and low grade breast tumors develop into high grade tumors when they transit from localized to metastasized tumors (Allred 2004).

This concept is currently challenged by data from molecular, pathologic and genetic studies, where breast tumors seem to remain quite stable during progression from localized to metastatic tumors (Lacroix 2004). ER+ versus ER- tumors, and low grade versus high grade

tumors, have mutually exclusive mutations, making transition to ER- and high grade tumors respectively during late tumor progression unlikely (Buerger 1999, Lacroix 2004, Simpson 2005). Gene expression studies have found breast tumors clustering in a few groups related to ER status and grade, rather than a continuum of patterns (Lacroix 2004). In a screening study, tumors diagnosed in the first round were compared with tumors diagnosed in subsequent rounds. Tumors diagnosed in the first round have probably had a longer mean time to detection, and these tumors were larger and had more often lymph node involvement, but tumor grade was similar to tumors diagnosed in subsequent rounds (Webster 2005).

This does not contradict that tumors require several genetic alterations, but indicate that most of these genetic alterations arise before tumor detection. Tumor size and lymph node-involvement would thus be tumor markers influenced by when the tumor is detected, while histology, tumor grade and receptor status would reflect the stable biological phenotype of the tumor.

Tumor size and lymph node involvement

Tumor size is defined as the largest diameter of the tumor and is a prognostic factor for breast cancer death regardless of other tumor characteristics (Elston 1998, Mirza 2002, Cianfrocca 2004). Lymph node involvement is another important independent prognostic factor (Elston 1998, Cianfrocca 2004). Women with lymph node involvement have poorer prognosis compared to women without lymph node involvement, and increasing number of affected lymph nodes are associated with poorer prognosis. Tumor size and lymph node involvement are correlated, and none of them seems to be predictive of treatment effect (EBCTCG 2005).

Grade and proliferation

Tumor grade is a classification of the differentiation of the tumor into three groups, low, medium or high, where high grade are tumors most similar to normal tissue. It was introduced in 1925 by Greenough, who used seven criteria to define grade (Meyer 2005). In 1957, Bloom and Richardson modified it by only using three criteria for grading (Bloom 1957 169). The criteria were glandular (tubular) formation, cell size and shape, and proliferation. A modification of this classification, the Nottingham histological grade is currently the most commonly used (Elston 1991). Grade is moderately reproducible (Meyer 2005), but is nevertheless a prognostic factor after adjustment for tumor size and lymph node involvement (Elston 1991, Mirza 2002, Meyer 2005). Still, grade has not been widely used in the clinical setting, probably due to the relatively poor reproducibility.

Newer methods of assessing proliferation include S-phase fraction, mitotic index, and immunohistochemistry of proliferation-associated antigens such as Ki-67. S-phase is the proportion of cells in the synthetic phase of the cell cycle. It is measured by DNA flow cytometry. As proliferation is part of the grading classification, these factors are correlated to grade. They have, like grade, been shown to be prognostic factors after adjustment for tumor size and lymph node involvement, but they don't seem to add further information if grade is considered (Mirza 2002, Cianfrocca 2004).

DNA ploidy is the number of chromosomes in the cell nucleus, measured by DNA flow cytometry. Ploidy has been a prognostic factor in univariate analyses in some, but not all studies, and does not seem to be a prognostic factor after adjusting for tumor size or other tumor characteristics (Mirza 2002).

Morphology

Breast tumors are almost exclusively adenocarcinomas. Rarely, sarcomas or lymphomas develop, but these tumors are generally excluded when studying breast cancer. The morphology of the breast tumor has been in clinical use for a long time, and current classifications are modifications from the classification made by Fraser in 1927 (Fraser 1927). They are simply classified by their morphological appearance in the microscope. Still, the underlying carcinogenesis resulting in different histological types is largely unknown, and combinations between different types are common.

Non-invasive cancer used to be rather uncommon, but with the introduction of mammographic screening its proportion of all breast cancer cases is currently 10% in Sweden (Socialstyrelsen 2005). By definition, non-invasive cancer does not grow through the myoepithelial-basement membrane, and is not classified as cancer. Non-invasive breast cancer is a precursor of breast cancer, but also a marker of increased breast cancer risk in both breasts (Jones 2006, Li 2006a). Non-invasive breast cancers are divided into ductal or lobular, and further sub-divisions exist as well.

Historically, the two most common histological types of breast cancer were thought to be derived from the breast glandular ducts and lobules, respectively. This is not true; both types seem to derive from the terminal duct lobuloalveolar unit (Allred 2001). This is the functional unit of the breast, which produces milk during lactation. Ductal tumors make up the majority of breast cancers, and lobular cancers compose 5-15% of breast cancers (Li 2003a). Compared to ductal cancer, lobular cancer is more common among older women and is more often ER+, multifocal and bilateral (Chen 1999, Arpino 2004). The metastatic pattern is also somewhat different (Sastre-Garau 1996, Arpino 2004). Despite these differences, ductal and lobular breast cancer have similar prognosis (Li 2003b, Arpino 2004). There are also other rarer but well-defined histological types of breast cancer; mucinous, medullary, papillary and tubular cancers. Tubular cancers are by definition of low grade, and correctly classified they have an excellent prognosis (Li 2003b, Goldstein 2004). Some less common histological types, like papillary, mucinous and medullary tumors are also associated with better prognosis compared to ductal cancer (Li 2003b).

Estrogen and Progesterone Receptors

Estrogen and progesterone receptors (ER and PR) belong to the nuclear receptor super family. The classic mechanism of these receptors is to be activated by ligands (estrogen and progesterone) that bind to the receptor. The ligand-bound receptor then binds another ligand-receptor complex. Together with coactivators, corepressors, and other transcription factors in the cell nucleus this dimer binds to promoter regions of the DNA thereby influencing gene transcription (Yager 2006).

ER also binds to growth factors, for instance IGF-1, and activates gene transcription. There are two types of ER: ER α and ER β . They are derived from different genes, and are very similar in the DNA binding domain, but less similar in the ligand binding domain (Pettersson 2001). In addition to the intranuclear ER there is a membrane-bound receptor (similar to ER α) that activates the second-messenger system with rapid effects. Recent data indicates the existence also of mitochondrial estrogen receptors (both ER α and ER β) with largely unknown functions (Yager 2006).

In normal breast tissue, the concentrations of ER α are low, and expressed in cells in the tubulo-lobular alveolar unit of the breast (Allred 2004). The cells expressing ER α almost never simultaneously express proliferation markers. They are instead expressed in adjacent cells. In premalignant breast tissue, ER α is expressed at higher concentrations in a larger proportion of the cells, and often together with proliferation markers. In breast cancer, 60-80% of tumors express ER α , often at high levels (Allred 2004). The proportion of tumors expressing ER increases with increasing age (Anderson 2005).

Progesterone receptors exist in two variants, PRA and PRB. The two variants come from the same gene, but are regulated by two different estrogen-regulated promoters (Kastner 1990). In normal breast tissue, PRA and PRB are similarly expressed, while in atypical hyperplasia, non-invasive and invasive breast cancer, PRA and PRB are heterogeneously expressed in adjacent cells, and PRA is often much more expressed than PRB in noninvasive and invasive cancers (Mote 2002). This indicates that loss of control of PR expression is an early event in breast carcinogenesis.

ER and PR status were in the early 1990s usually measured with an enzyme immuno-assay, yielding a quantitative value of the receptor content. This was reported as amount of receptor per amount of DNA or protein in the specimen. The measurement of ER was ER α specific (Brouillet 2001). The cut-off value could differ between different laboratories, and the best definition of ER-positive tumors is probably “those with a statistically significant chance of responding favorably to hormonal therapy” (Allred 2004). Currently, receptor status is assessed with immunohistochemical methods, with at least 10% positive nuclei as a common cutoff (Chebil 2003).

ER and PR are correlated to each other. Absence of PR in ER+ tumors has been found to be correlated to tamoxifen resistance, and proposed to be an indication of nonfunctioning ER. However, recent data indicate that these tumors are not resistant to aromatase inhibitors, and that absence of PR instead indicates increased growth factor signaling (Cui 2005, Osborne 2005).

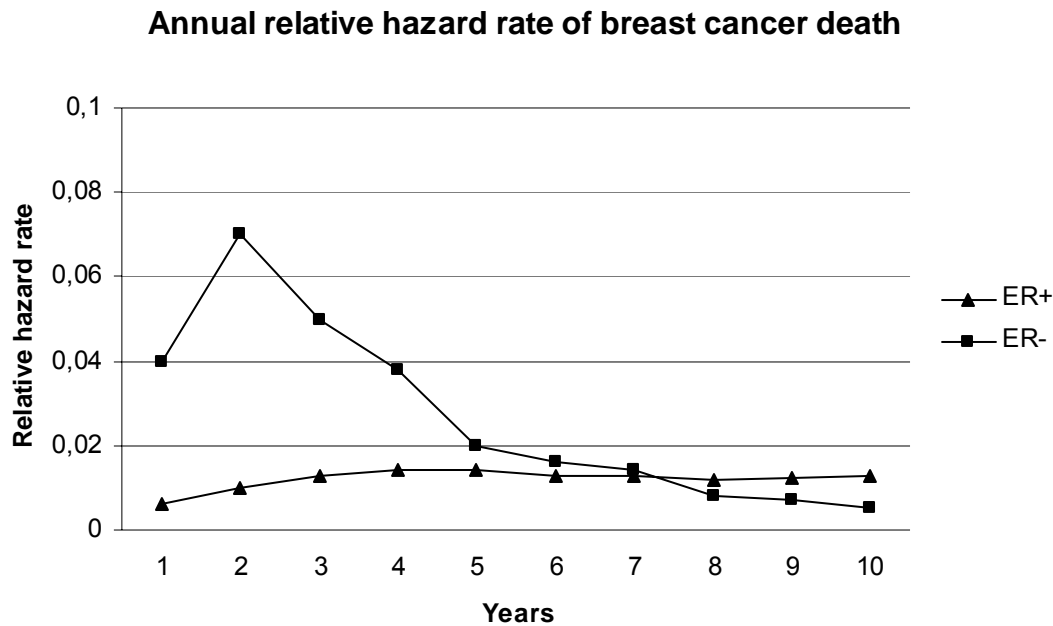


Fig 4. Annual relative hazard rate of breast cancer death in estrogen receptor positive and negative tumors. (Adapted from Anderson et al, Brest Cancer Res Treat 2005).

ER and PR are prognostic factors, in that the survival pattern differs between receptor positive and negative tumors (Anderson 2005). ER- and PR- tumors have a high mortality peaking around two years after diagnosis, then crossing the receptor positive curves to a much lower mortality rate (Fig 4.). On the other hand, ER+ tumors have a rather constant mortality. Consequently, ER+ tumors have a better survival in the first years after diagnosis, but 15 years after diagnosis, the breast cancer survival is unrelated to ER status (EBCTCG 2005, Anderson 2006). This pattern, with a rather constant mortality for low-risk tumors, and an early peak and then declining mortality for high risk tumors applies also for other tumor characteristics like grade, tumor size and lymph node involvement (Anderson 2005).

ER and PR are treatment predictive factors. A majority of tumors expressing ER and PR respond to anti-estrogenic therapy, both in the adjuvant and metastatic setting. ER+PR- tumors respond to tamoxifen, but not as good as ER+PR+ tumors (Bardou 2003), and recent data indicate that these tumors are more likely to respond to aromatase inhibitors (Cui 2005). ER- tumors do not respond to anti-estrogenic therapy (EBCTCG 2005)

HER2/neu

The c-erbB-2 (HER2/neu) proto-oncogene encodes a tyrosine kinase situated in the cell membrane. It is over-expressed in approximately 30% of breast cancers and associated with more aggressive tumor characteristics and poorer survival (Cianfrocca 2004).

Etiology

Genetic risk factors

A history of breast cancer among first or second-degree relatives is a well-established risk factor for breast cancer. Among women >50 years at diagnosis, around 15% report a family history of breast cancer (Magnusson 1998a). The relative risk of breast cancer is doubled for women with a first-degree relative with breast cancer, and the risk is even higher for women with relatives diagnosed before the age of 50, or with more than one affected relative (Pharoah 1997). Despite this, no more than 5% of breast cancer cases are due to highly penetrant inherited susceptibility genes (Antoniou 2003), and in Sweden the proportion might be even lower (Loman 2001, Margolin 2004). The most important genes are the tumor suppressors BRCA1 and BRCA2, which are linked both to breast and ovarian cancer. Carriers of mutations in these genes have a life-time risk of 45-65% for breast cancer (Antoniou 2003). Other rare conditions are germline mutations in the tumor suppressor genes p53 and PTEN (Turnbull 2005).

Environmental factors cannot be the only remaining explanation for the familial risk of breast cancer. This leads to the conclusion that there must be an influence from low-penetrant genes (Dapic 2005). Intense research efforts are currently made in this field. Until now, only one mutation, the CHEK2*1100delC has been linked to breast cancer (CHEK2 breast cancer case-control consortium 2004). Other findings have yet failed to be reproduced (Dapic 2005).

There are huge methodological problems related to this research. First, there is little prior knowledge on relevant polymorphisms. Second, the combination of a small effect of each polymorphism, an enormous amount of polymorphisms, and gene-gene or gene-environment interactions, make this area of research a challenge. Still, there is optimism on the possibilities in this field in the future. Increased knowledge of the function of genes and the architecture of human genetic variation in combination with new high throughput genotyping technologies used in large studies might lead to conclusive findings in the future (Houlston 2004, Pharoah 2004).

Hormones

The traditional definition of a hormone is a substance produced in one organ, and transported, usually through the blood, to another organ where it produces a metabolic effect. This definition does not fit the findings of paracrine and autocrine effects of hormones. A newer definition would be a product of living cells that circulates in body fluids and produces a specific effect on the activity of cells.

Steroid hormones are lipophilic molecules derived from cholesterol. In the blood the main fraction of different hormones are bound to albumin or serum hormone binding globulin, and thereby inactivated. The unbound fraction may diffuse through cell membranes due to its lipophilic properties, and bind to intranuclear, mitochondrial or other intracellular steroid receptors. Steroid hormones may also interact with other factors, like growth factors without receptor binding.

There is epidemiological evidence that ovarian hormones, i.e. estrogen and progesterone, are important in breast carcinogenesis. Male breast cancer exists, but is a rare event. Endogenous and exogenous hormones from prenatal to the postmenopausal period in life are associated with risk of breast cancer (Adami 2002). Before the era of mammographic screening and menopausal hormone therapy, the age-specific incidence curves flexed at the age of

menopause in Sweden and other Western countries (Fig 3). Among oophorectomized women, the curve flexes at the age of oophorectomy (Spicer 1993, Schairer 1997). This reduction in the increased incidence at menopause is likely to be due to the reduced endogenous levels of ovarian hormones (estrogen and progesterone) that could stimulate breast cancer development (Spicer 1993).

In a meta-analysis on postmenopausal serum hormone levels and subsequent breast cancer risk in 660 women with breast cancer and 1760 without breast cancer, associations with breast cancer was found for serum levels of total estradiol, free estradiol, estrone, estrone sulfate, androstendione, dehydroepiandrosterone, and testosterone (Key 2002). Women in the highest quartile compared to the lowest had doubled risks of breast cancer for most of the hormones. High levels of serum hormone binding globulin were associated with a reduced risk of breast cancer.

Estrogen

The most potent, natural estrogen is estradiol. Androstendione is the most important precursor, having two pathways to estradiol. The first is via testosterone, which is converted by aromatase to estradiol. Androstendione can also be converted by aromatase to estrone, a less potent estrogen, which can be converted to estradiol by 17 β -hydroxysteroid dehydrogenase. The aromatase activity is regulated by follicular stimulating hormone, produced by the pituitary gland. Another pathway, especially important in breast tissue, is conversion of estrone sulfate to estrone by estrone sulfatase (Pasqualini 1989).

During the fertile period of a woman's life, the follicular cells in the ovaries produce estradiol via estrone. During pregnancy, the maternal placenta produces estrone and estradiol to levels more than hundredfold the normal levels. The fetal placenta produces estriol to a level thousand-fold the normal levels.

Estrogens are also produced in fat tissue, muscles, and skin through conversion of androgens by aromatase into estrone and estradiol, or conversion of estrone sulphate into estrone. Estradiol production from estrone sulfate, estrone, or androgens requires 17 β -hydroxysteroid dehydrogenase, aromatase and/or sulfatase enzymes (Chetrite 2000). In postmenopausal women, conversion of androstendione to estrone in fat tissue is one important source of estrogen, supported by the finding that postmenopausal obese women have higher levels of estrone and estradiol (Key 2003).

Local production (paracrine or autocrine) of estrogens in adipose cells in breast tissue might be more important than estrogen in the blood for breast cancer development in postmenopausal women. Breast tissue concentration of estrogens has been found to be higher in women with breast cancer than in other women. In a study on women with breast cancer, both estrone sulfate and estradiol as well as sulfatase activity was much higher in tumor tissue compared to adjacent normal tissue (Chetrite 2000). It has been proposed that in postmenopausal women, most estrogens act locally and thereafter enters the circulation. This would indicate that circulating estrogen levels in postmenopausal women reflect rather than direct estrogen effects (Simpson 2003).

Estrogens are metabolized in the liver, but also in the kidneys and intestine. They are mainly excreted through the urine.

Conjugated estrogens are the most common compound used for menopausal hormone therapy in the US. They contain estrone and small amounts of estradiol, but also equilins which are not metabolized by the human steroid pathways, and therefore have a slow excretion.

Ethinyl estradiol is synthetic and commonly used in oral contraceptives, but rarely in menopausal hormone therapy.

Estrogen is believed to cause breast cancer by increasing cell proliferation via estrogen-receptor mediated transcription. This would lead to an increased number of DNA replications followed by an increased number of replication errors. Estrogen is also believed to have a direct mutagenic effect, by being metabolized to quinone derivatives which can react with DNA and remove purine bases (Yue 2003).

Progestin

Progesterone, the only endogenous progestin is synthesized in the corpus luteum in the ovary in the luteal (second) phase of the menstrual cycle. The adrenal glands produce small amounts of progesterone. Progesterone levels are consequently very low in the follicular (first) phase and among postmenopausal women.

There are many different synthetic progestins. They can be classified as testosterone-like or progesterone-like. All progestins stimulate estrogen-primed endometrium, but they differ with regard to absorption, metabolism, binding to serum proteins and the progesterone receptors PR-A and PR-B (Schindler 2003). Whether they also differ with regard to breast proliferation is not known. Studies on breast cancer cell lines indicate that testosterone-like, but not progesterone-like progestins may stimulate proliferation (Sitruk-Ware 2004).

Little is known about progestins and carcinogenesis. Studies on the progesterone receptor knockout mouse (mouse strain lacking the progesterone receptor) show that the progesterone proliferative signal is essential for breast development and breast cancer development (Ismail 2003). The PR expression in the adult mouse is mainly conferred to luminal epithelial cells, thought to be the precursor cells for breast cancer, and existing in adjacent but not in the same cell as proliferating cells (Clarke 1997, Ismail 2003). This indicates that paracrine mechanisms are involved. Breast cell proliferation peaks during the luteal phase, when progesterone levels are high (Pike 1993).

Androgen

Androgens, like testosterone, dehydroepiandrosterone, and androstendione are produced throughout life by the ovaries and the adrenal glands. The androgens can be converted to estrogens by aromatase and sulphatase activity in different organs. Androgen levels are actually higher than estrogen levels throughout life. Androgens stimulate bone and muscle tissue.

IGF-1

Insulin-like growth factor (IGF) I and II are two peptide hormones in the IGF family, and together with estrogen they stimulate cellular growth and differentiation. Circulating IGF-I levels seem to be modestly associated with breast cancer risk among premenopausal women, but not among postmenopausal women (Keinan-Boker 2003, Schernhammer 2005). Serum IGF binding protein 3 and IGF-II levels have been positively associated with postmenopausal ER-positive breast cancer risk in one study (Gronbaek 2004). Another study reported a possible synergistic effect of steroid hormone levels and IGF on breast cancer risk (Yu 2003).

Oral contraceptives

Oral contraceptives were introduced in the 1960s. Most combined oral contraceptives contain ethinyl estradiol. The estrogen dose has decreased from 100 µg in the early preparations to 20-30 µg today. Many different progestins have been used, in order to find regimens with low risk of venous thromboembolism (Girolami 2002). In 1996, a meta-analysis was performed on 54 epidemiologic studies (Collaborative Group on Hormonal Factors in Breast Cancer 1996). Current use was associated with a slightly increased risk (RR 1.24, 95% CI 1.15-1.33), which disappeared within 10 years after stopping. Current users who started before the age of 20 were at higher risk compared to those who started at an older age. Duration of use did not influence risk, indicating that oral contraceptives act as a late-stage promoter. There was no indication of different influences by different regimens of combined oral contraceptives. In a more recent study, though, there were indications of lower breast cancer risks with lower doses/newer regimes of oral contraceptives (Althuis 2003).

Menopausal hormone therapy

In the 1960s, estrogen was introduced not only for contraception, but also for alleviating menopausal symptoms. From the second half of the 1980s it became increasingly popular in Sweden and elsewhere in the Western world. Menopausal hormone therapy was believed to be the future life-long substitute to all women suffering from lack of hormones after menopause, and to reduce risks of cardiovascular disease, dementia and osteoporosis (Gambrell 1982). Estrogen was shown to increase the risk of endometrial carcinoma, but this risk was eliminated by adding progestin (Persson 1989, Voigt 1991, Weiderpass 1999). Since the beginning of the 1990s, women with a uterus have been recommended to use estrogen in combination with progestin for menopausal hormone therapy in Sweden (Odlind 2004).

The compounds of estrogen and progestin used have varied over the years and between countries. In Europe, 1-2 mg of estradiol is most commonly used, while in the US, 0.3-1.25 mg of conjugated estrogens is most common. Progestins can be added sequentially (often 7-16 days per 28 days) or continuously to estrogen, or be taken alone. Both testosterone-like (norethisterone, levonorgestrel, or lynestrenol) and progesterone-like (medroxy-progesterone acetate) progestins are used. More recently, selective estrogen substances, designed to be active in some but not all estrogen reacting tissues have come in use. Tibolone has estrogenic and androgenic properties, but has been shown to induce apoptosis in breast tissue, and not increase breast density or breast epithelial proliferation (Cline 2002, Lundström 2002, Conner 2004).

One of the first reports of an increased risk of breast cancer with menopausal hormone therapy was published in 1980 (Ross 1980). In 1991 and 1993, two meta-analyses found increased risks among current users of menopausal hormone therapy (Steinberg 1991, Colditz 1993). In 1997, when the Collaborative group on breast cancer published a large meta-analysis clearly showing an association with breast cancer, this risk was taken more seriously (Collaborative Group on Hormonal Factors in Breast Cancer 1997), but still the believed positive effects on cardiovascular disease and other health events made many health professionals to advocate its use (Rozenberg 1998). The Swedish recommendations were more cautious, and menopausal hormone therapy was not recommended for prevention of cardiovascular disease (SBU 1996).

Defined daily doses/1000 persons of estrogen-progestin in Sweden

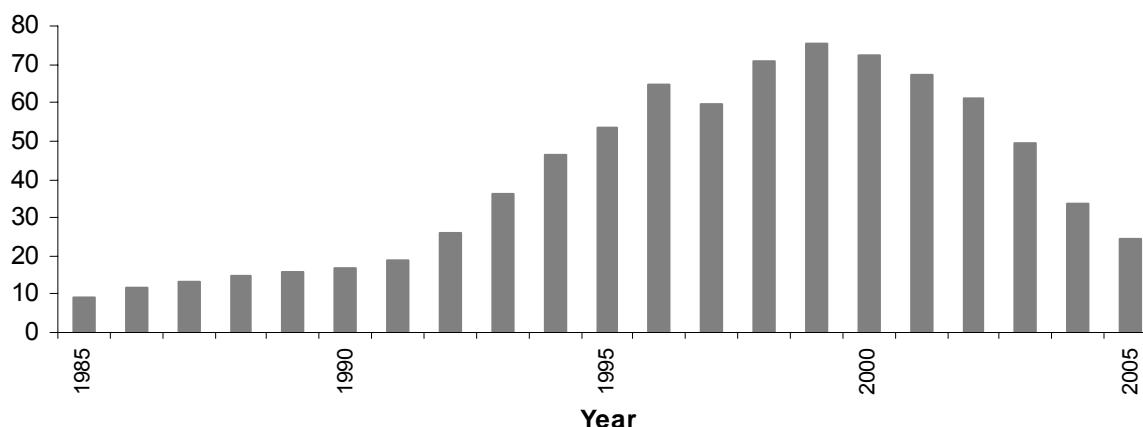


Fig. 5. Use of estrogen-progestin hormone therapy in Sweden 1985-2005. Adapted from statistics from Apoteket AB, 2005.

In 1998, HERS, a randomized study on secondary prevention of cardiovascular events with estrogen (conjugated equine estrogen) combined with progestin (medroxyprogesterone) in the US showed no benefit of menopausal hormone treatment (Hulley 1998). In 2002, results were published from the Women's Health Initiative, a randomized study with estrogen (conjugated equine estrogen) combined with progestin (medroxyprogesterone) compared with placebo given to healthy postmenopausal women (Rossouw 2002). The study was stopped because of too many breast cancer cases and a negative balance on the global risk-benefit index. Treated women had increased risks not only for breast cancer, but also for coronary heart disease, pulmonary embolism and stroke. On the other hand, treated women had reduced risks of endometrial cancer, colon cancer and hip fractures. Later results from WHI showed no effect on cognitive performance, and possibly an increased risk of dementia (Shumaker 2003). These results made the use of hormone therapy drop quickly in the US (Stefanick 2005). In Sweden, the decline started three years earlier (Fig. 5), probably due to the awareness among Swedish gynecologists of the findings of increased breast cancer risk with estrogen alone and estrogen-progestin reported in 1999 from our case-control study (Magnusson 1999a).

A large observational study from the UK, the Million Women Study, was published in 2003 (Beral 2003). This study confirmed the results from many previous observational studies, the first published almost ten years earlier, that estrogen in combination with progestin conferred a higher risk of breast cancer than estrogen alone (Newcomb 1995, Collaborative Group on Hormonal Factors in Breast Cancer 1997, Magnusson 1999a, Ross 2000, Schairer 2000). The Million Women study also showed that the risks were similar regardless of type of estrogen (equine estrogen or estradiol) or progestin (medroxyprogesterone acetate, levonorgestrel, or norethisterone), and for sequential or continuous administration of progestin. Surprisingly, tibolone was associated with an increased risk of breast cancer, despite its lack of proliferative effects on the breast. This might be due to indication bias, because women at high risk of breast cancer could be overrepresented among tibolone users.

Breast cancer risks have generally been found to be higher among current than past users of menopausal hormone therapy (Colditz 2005). More than five years after stopping therapy, the risk increase has leveled off (Beral 2003). The risk seems to increase linearly with duration of

use (Collaborative Group on Hormonal Factors in Breast Cancer 1997). In a meta-analysis on estrogen-progestin use, the increased breast cancer risk was estimated to 7.6% per year of treatment, and the estimate was significantly lower in American studies than in European studies (Lee 2005).

Estrogen alone seems to confer a lower risk of breast cancer compared to estrogen-progestin treatment. Annual increases in breast cancer risk has been estimated to 0-9% per year for estrogen-progestin therapy, and 0-3% per year for estrogen alone therapy (Greiser 2005). In the Women's Health Initiative study, estrogen alone was associated with a non-significantly decreased risk of breast cancer (Anderson 2004). Mean body mass index (BMI, body weight in kg/square height in m (kg/m^2)) levels are usually higher in US studies compared to European studies, but this cannot be the only explanation for the discrepancies in risk between the US and Europe.

There is an interaction between menopausal hormone therapy and obesity. Lean women have higher risks associated with menopausal hormone therapy compared to obese women (Magnusson 1999a, Lahmann 2004, Feigelson 2006). The generally proposed mechanism is that adipose tissue produces estrogen in postmenopausal women, and it has been shown that obese women have increased levels of estradiol and reduced levels of serum hormone binding globulin, leading to increased levels of free estradiol (Bezemer 2005). It has also been shown that the increased risk of breast cancer among obese postmenopausal women can largely be explained by increased levels of free serum estradiol levels (Key 2003, Rinaldi 2006).

As breast cell proliferation has been shown to peak when endogenous progesterone levels are high in the second phase of the menstrual cycle, estrogen-progestin therapy is likely to confer a higher breast cancer risk compared to estrogen alone therapy. Treatment with estrogen-progestin among postmenopausal women significantly increased breast cell proliferation, measured as Ki-67/MIB-1 activity three months after initiation of therapy, and was stable three months thereafter (Conner 2003).

Diet

Large efforts have been made to find associations between dietary fat intake and breast cancer risk. The hypothesis that fat intake would be related to breast cancer came from the ecological finding that in countries with high fat consumption, the breast cancer mortality rates were high (Armstrong 1975). Results from large cohort studies have generally shown no association between overall fat intake and breast cancer risk (Adami 2002, Holmes 2004a). However, in a recent randomized trial in the US, women in the dietary modification group, who had a lower overall fat intake than the control women had a non-significantly reduced risk of breast cancer after 8.1 years of follow-up (Prentice 2006). On the other hand, postmenopausal obesity is an established risk factor for breast cancer (Key 2003). More data is needed to assess the risks of saturated and mono- and poly-unsaturated fats and breast cancer risks (Prentice 2006).

No associations have been found between any kinds of dietary fibers and breast cancer (Adami 2002, Terry 2002a, Holmes 2004b). Phytoestrogens in soy products are consumed mainly in Asia, where breast cancer incidence is low. Besides, phytoestrogens can bind to estrogen receptors, so there is evidently a theoretical basis for an association with breast cancer (Adami 2002). In Asia some studies have found protective effects of different phytoestrogens (Adami 2002, Holmes 2004a), but more studies are needed before conclusions can be drawn.

Alcohol

Moderate alcohol intake has consistently been linked to an increased risk of both pre- and postmenopausal breast cancer (Adami 2002, Hamajima 2002). An interesting finding though, is that in one study on women who were alcoholics, no increased risk was found (Kuper 2000). Alcohol increases bioavailable estrogen in both pre- and postmenopausal women (Reichman 1993, Onland-Moret 2005). Alcohol also reduces folate absorption, thereby possibly increasing the risk of DNA mutations, and one study has found an increased risk for breast cancer with high alcohol intake only among women with a low folate intake (Baglietto 2005).

Smoking

Smoking is not considered an established risk factor for breast cancer. A large meta-analysis failed to show any association between ever or current smoking and breast cancer after restricting to women not drinking alcohol (Hamajima 2002). On the other hand, a concurrent review concluded that smoking might be a risk factor in certain subgroups, namely in women who start at an early age, women who smoke for a long time, and passive smokers (Terry 2002b). More recent studies have found associations for women who started smoking at a young age, before first full-term pregnancy, and for long-term smokers (Band 2002, Reynolds 2004, Gram 2005, Li 2005). The risk increases are however modest, with relative risks generally below 1.5 for high-risk groups.

It has been proposed that smoking is a risk factor, initiating breast cancer in developing breast tissue among teenage smokers, but a protective factor among older women reducing the risk due to “anti-estrogenic” effects (Band 2002). Smoking has not been found to affect circulating estrogen levels (Terry 2002b), but anti-estrogenic mimicking effects are earlier menopause, increased osteoporosis, and reduced risk of endometrial carcinoma (Baron 1990).

Reproductive factors

Age at menarche

A young age at menarche is consistently associated with an increased breast cancer risk. The mechanism is not clear, but an increased exposure to ovarian hormones has been proposed. On the other hand, in a large Danish nested case-cohort study, the association between age at menarche and breast cancer disappeared after adjusting for age at peak growth (Ahlgren 2004).

Age at first birth and parity

Births confer an increased breast cancer risk in the first ten years, and a reduced risk thereafter (Lambe 1994). The pattern is most pronounced for the first birth, but similar for subsequent births. As most breast cancers occur among older women, the overall effect of births is a reduced risk of breast cancer among parous women (Collaborative Group on Hormonal Factors in Breast Cancer 2002). Each additional birth has similar, but smaller effect (Wohlfahrt 2001, Collaborative Group on Hormonal Factors in Breast Cancer 2002). The increased risk in the years after a birth is thought to be an effect of ovarian hormones promoting preclinical cancers (Lambe 1994). The long-term protective effect on the other hand, could be due to the final differentiation of the breast gland during a full-term pregnancy (Russo 2005). Induced or spontaneous abortions do not seem to be associated with breast cancer risk (Melbye 1997, Beral 2003, Erlandsson 2003, Reeves 2006).

Breast-feeding

The effect of breast-feeding on breast cancer risk has not been easy to assess. Breast-feeding is correlated to parity and other reproductive factors related to breast cancer risk. Additionally, in the western world, long-time breast-feeding has not been common during the last decades. A meta-analysis on 50 000 cases and 97 000 controls from the Collaborative group indicated that each year of breast-feeding reduces the risk of breast cancer by 4.3% (95% CI 2.9-5.8) (Collaborative Group on Hormonal Factors in Breast Cancer 2002). This was not modified by age at diagnosis, parity, age at first birth, or ethnicity. Differentiation of the breast gland or reduced number of ovulatory cycles is possible mechanisms.

Menopause and oophorectomy

A higher age at menopause increases breast cancer risk (Kelsey 1993, Magnusson 1999b). Surgically induced menopause, by oophorectomy before the age of 50 reduces the risk of breast cancer (Spicer 1993, Olson 2004), and before the age of 35 halves breast cancer risk (MacMahon 2006).

Perinatal factors

High birth weight as well as high birth length seem to be associated with an increased risk mainly of premenopausal breast cancer (Ahlgren 2004, Forman 2005). Preeclampsia is consistently associated with a reduced risk of breast cancer, both in the mother and the offspring (Forman 2005). Possible mechanisms could be related to levels of estrogens or IGF-1 in the mother or the fetus (Kaijser 2000, Mucci 2003, Forman 2005). Being breast-fed, mother's age, being a twin, or gestational age do not seem to be associated with breast cancer risk (Adami 2002, Hemminki 2002a, Kaijser 2003, Forman 2005).

Anthropometric measures

A Danish study on growth patterns and breast cancer found that early age at peak growth is a risk factor for breast cancer (Ahlgren 2004).

Height

Tall height has consistently been associated with increased breast cancer risk among pre- and postmenopausal women (Tretli 1989, Brinton 1992, Magnusson 1998b, Ahlgren 2004, Lahmann 2004). An increase of five cm in length is associated with 5-15% increased risk of breast cancer. A common genetic cause and increased number of cells at risk are possible mechanisms. High energy intake has been proposed as an explanation, but as childhood obesity is inversely associated with breast cancer risk (Ahlgren 2004), and Dutch women exposed to severe famine in 1944-1945 before the age of nine have increased breast cancer risk (Elias 2004), this seems unlikely.

BMI and weight gain

A high BMI during childhood and adolescence reduces the risk of both pre- and postmenopausal breast cancer (Brinton 1992, Magnusson 1998b, Ahlgren 2004, Baer 2005). It could be that estrogens produced in fat tissue promote early differentiation of breast epithelium (Ahlgren 2004). BMI is weakly inversely related to premenopausal breast cancer (Ursin 1995, Lahmann 2004). A proposed mechanism is that obese premenopausal women have hyperinsulinemia, leading to impaired ovarian function and thereby reduced progesterin levels (Stoll 1997).

For postmenopausal breast cancer on the other hand, high BMI is a risk factor of breast cancer, with a magnitude of around 15% per five kg/m² (Ziegler 1996, Magnusson 1998b, Lahmann 2004, Suzuki 2006). The association can largely be explained by the increase in circulating levels of estrogens and decreased levels of serum hormone binding globulin (Key 2003, Rinaldi 2006). Adult weight gain and waist-hip ratio are other related measures of obesity associated with postmenopausal breast cancer, and some investigators have reported that adult weight gain explains the association between postmenopausal high BMI and breast cancer risk (Magnusson 1998b, Feigelson 2004). Menopausal hormone therapy interacts with obesity on breast cancer risk (see page 20).

Diabetes

Diabetes type 2 seems to moderately increase the risk by around 10-20% after adjustment for obesity (Michels 2003, Wolf 2005). Proposed mechanisms are increased insulin levels, increased insulin like growth factor (IGF-1) levels, or altered regulation of sex hormones (Wolf 2005).

Physical activity

Physical activity seems to be protective for both pre- and postmenopausal breast cancer (Vainio 2002, Lagerros 2004), but a recent large cohort study in Sweden and Norway including more than 1,000 cases, found no association (Margolis 2005). Physical activity has been proposed to affect risk of postmenopausal more than premenopausal breast cancer (Friedenreich 2004). Physical activity, like nutrition, is a life-long exposure, associated with other life-style factors, making studies on this exposure full of methodological problems. Whether physical activity under certain periods of life is more important is not clarified. Proposed mechanisms are reduced levels of ovarian hormones, or reduced insulin resistance leading to lower insulin levels, and reduced IGF-1 levels (Friedenreich 2004). Physical activity in postmenopausal women may reduce BMI, which is an established risk factor for postmenopausal breast cancer (Key 2003).

Ionizing radiation

Ionizing radiation causes somatic mutations and is a risk factor for breast cancer. This has been shown for instance among patients medically treated with radiation for childhood or adult cancer (Boice 1992, Travis 2005), among women exposed to several radiologic examinations (Morin Doody 2000), and among the Japanese atomic bomb survivors (Land 1995). The association seems to be dose-linear without a lower threshold (Ronckers 2005). Women exposed before the age of 20 are at higher risk, while exposure after the age of 50 does not seem to increase the breast cancer risk (Preston 2002, Ronckers 2005).

Benign breast disease

Benign breast diseases are common. Most benign breast tumors are nonproliferative (e.g. cysts, fibroadenomas), and these women do not seem to be at an increased risk of breast cancer (Hartmann 2005). Women operated for proliferative benign tumors, such as hyperplasia, papilloma, and radial scar, seem to be at 1.5-1.9-fold increased risk of breast cancer, and women operated for tumors with atypical hyperplasia have a 4-6-fold increased risk (London 1992, Hartmann 2005). The breast cancer is only slightly more likely to occur in the same breast as the benign tumor (Hartmann 2005). This indicates that benign breast tumors are indicators of increased risk, rather than precursor lesions of breast cancer.

Mammographic breast density

X-rays pass easily through fat tissue, which thus appears black on a mammographic exam. Epithelial and stromal tissue is denser, and appears as white areas on the mammography. In 1976, Wolfe proposed a classification of breast density in four groups, and showed that high density was related to increased breast cancer risk (Wolfe 1976). With the introduction of computers, quantitative measures of the proportion of dense area of the breast, or the absolute areas of dense and non-dense areas have been shown to better predict breast cancer risk (Boyd 2005). Currently, three-dimensional methods of assessing breast density are developed (Jeffreys 2006). The relative risk of breast cancer in women with $\geq 75\%$ compared with 1-10% dense area seem to be three to six-fold higher (Boyd 2005). The proportion of women with breast density $>50\%$ has been estimated to be around 30% (Boyd 1998, Boyd 2005). Many factors are associated with breast density, like age, menopausal status, parity, smoking, heredity, and use of HT (Boyd 2005). For some of these factors, breast density might be an intermediate step to breast cancer, but they do not explain more than a small part of the variation in breast density. Breast density area as well as breast size are proxies for total number of breast cells. Number of cells at risk is a biologically plausible underlying mechanism for these risk factors (Adami 1998).

Etiology of breast cancer defined by tumor characteristics

Compared to lung cancer and esophageal cancer, breast cancers are less heterogeneous tumors, for instance breast cancers are almost exclusively adenocarcinomas. They also differ less in way of growth and other features, so usually breast cancers have been studied as one single entity. However, the etiology of different subgroups of breast cancer currently draws increasing interest. Studying etiology of subgroups of breast cancer might reveal associations hidden when breast cancer is regarded as a single entity. A compelling problem is the rareness of some subgroups, which makes the power in single studies low.

Grade

Young age at first birth (Largent 2005), and a birth within two years before diagnosis (Kroman 1997, Daling 2002b) has been associated with high grade tumors. In the US, African American women have a lower incidence of breast cancer but a poorer prognosis compared to white women and tumors in African American women have been shown to be of higher grade compared to tumors in white women (Chlebowski 2005). Use of menopausal hormone therapy has been linked to low grade tumors in most (Harding 1996, Holli 1998, Esteve 2002, Sacchini 2002, Gertig 2003, Stahlberg 2004), but not all studies (Stallard 2000, Kerlikowske 2003).

Morphology

In 1982, one of the first reports of etiology of histology-defined breast cancer reported that increasing age at first birth was more strongly associated with lobular compared to ductal breast cancer (LiVolsi 1982). This has been refuted by two (Ewertz 1988, Li 2003c) and confirmed in three other studies (Stalsberg 1989, Wohlfahrt 1999, Ursin 2005), though often with non-significant heterogeneity. For other reproductive factors; like number of births, breast feeding, age at menarche, and age at menopause; no or only slight differences between histological groups of breast cancer have been found (LiVolsi 1982, Stalsberg 1989, Wohlfahrt 1999, Li 2003c, Ursin 2005). Height and BMI have been similarly related to ductal and lobular breast cancer in one study (Li 2003c). Alcohol intake has been more strongly linked to lobular than ductal postmenopausal breast cancer (Li 2003d, Li 2006b). Family

history has been more strongly associated with lobular compared to ductal breast cancer in a US register study (Allen-Brady 2005), but not in a Swedish register study (Hemminki 2002b).

In 1999, an association between menopausal hormone therapy and tumors of favorable histology was reported (Gapstur 1999). One year later, Li et al reported that estrogen-progestin therapy was associated with an increased risk of lobular, but not ductal breast cancer (Li 2000). Several reports have confirmed this finding of a heterogeneity (Manjer 2001, Chen C 2002, Newcomb 2002, Daling 2002a, Newcomer 2003, Li 2003e), with few exceptions (Ursin 2002, Lee 2006). Whether menopausal hormone therapy is associated only with lobular cancer or with both lobular and ductal cancer, and whether estrogen alone confers a higher risk for lobular than ductal remains unanswered. One Swedish cohort study with few tubular cases reported a very strong association with tubular cancer (Manjer 2001), but otherwise little is known with regards to the etiology of tubular cancer.

Estrogen and Progesterone receptors

As many risk factors for breast cancer are related to hormones, stronger associations with hormone receptor positive tumors seem likely. Initially, most investigators reported results by ER status. In 1991 the first report on risk factors of both ER and PR status was published (Kreiger 1991). In recent years, investigators have reported ER and PR separately, but joint ER and PR status is now the most common way of defining receptor status in epidemiological etiological research.

Older age at first birth has rather consistently been related to ER+ tumors, with no or little association with ER- tumors (Althuis 2004, Ursin 2005). A birth within two years before diagnosis has been associated with ER- and PR- tumors (Olson 1998, Daling 2002b). When comparing parous with nulliparous women, or increasing number of births, most (Potter 1995, Yoo 1997, Wohlfahrt 1999, Cotterchio 2003, McCredie 2003) but not all (Ursin 2005) studies found similarly reduced risks regardless of receptor status. Postmenopausal obesity (measured as BMI, weight gain or waist/hip ratio) has been linked to receptor positive tumors, and possibly more strongly to the PR+ tumor groups (Enger 2000, Huang 2000, Yoo 2001, Sellers 2002, Colditz 2004, Feigelson 2006, Suzuki 2006).

Recent findings consistently link estrogen-progestin therapy to tumors expressing at least one receptor (Ursin 2002, Daling 2003, Li 2003e, Chen 2004, Lee 2006). The findings for estrogen alone therapy is more inconsistent. Some studies have found a stronger association with ER+PR+ tumors (Ursin 2002, Chen 2006, Lee 2006), while other US studies have reported no association regardless of receptor status (Daling 2003, Li 2003e). In the Women's Health Initiative – a randomized clinical trial, no association between estrogen with or without progestin and receptor status was found (Chlebowski 2003, Stefanick 2006). However, these studies generated rather few cases, and a large proportion of participants were noncompliant to the randomization.

Many risk factors seem similarly related to receptor-defined breast cancer, for instance alcohol intake, family history, smoking, benign breast disease, age at menarche, and age at menopause (Kreiger 1991, Potter 1995, Yoo 1997, Cotterchio 2003, Althuis 2004, Colditz 2004).

HER2/neu

The literature is sparse with regards to risk factors for breast cancer defined by HER2/neu status. In one study, most risk factors (menopause, menarche, menopausal hormone therapy,

lactation, benign breast disease, family history) were similarly related to HER2/neu positive and negative tumors, except that a high BMI was more strongly associated with HER2/neu+ tumors (Tsakountakis 2005). Another study reported no significant differences at all (Huang 2000).

Breast cancer survival and mortality

Usually, the word survival is used to define the rate of deaths (of a specific disease or due to all causes) among persons diagnosed with a disease, counted from the time of the diagnosis. The word mortality is used to define the rate of deaths in a population or a cohort (including diseased and non-diseased). The mortality is combined effect of incidence and survival. Both mortality and survival are interesting, but they are relevant in different settings. When assessing the effect of breast cancer screening, survival is of no value, as screening aims at an earlier diagnosis, thereby prolonging survival. Mortality is the only possibility to evaluate screening efficiency. On the other hand, in the clinical setting, the probability of surviving is the relevant question. If risk factors for breast cancer are not related to survival, the effect on incidence and mortality are similar. However, if the survival is influenced, the effect of a risk factor on the incidence is different from the effect on mortality.

Most observational cohort studies have not found increased breast cancer mortality among menopausal hormone therapy users, despite the increased breast cancer risk (Willis 1996, Grodstein 1997). Before initiating menopausal hormone therapy use, women often perform a mammographic examination, thereby excluding some women from the menopausal hormone therapy group with breast cancer. In line with this and with the increased risk of breast cancer with increasing duration of menopausal hormone therapy, the Nurses' Health study found increased breast cancer mortality among women on current use ≥ 5 years (Colditz 1995). In the Million Women Study, current users were also found to have increased breast cancer mortality (Beral 2003).

By definition, prognostic factors influence survival, but in addition other factors, mainly risk factors for breast cancer, influence breast cancer survival.

Recent high BMI is associated with poorer breast cancer specific survival (Chlebowski 2002). A lower proportion of screened women, and thereby a delayed survival could be an explanation, but the poorer survival remained after adjusting for detection method in one study (Hall 1999).

A low compared to high socioeconomic status has been related to poorer breast cancer specific survival (Karjalainen 1990, Coleman 2004, Lagerlund 2005, Bouchardy 2006). Later detection, different tumor characteristics, comorbidity, and type and quality of treatment are proposed explanations (Vågerö 1987).

A birth less than 2-5 years before diagnosis has been shown to be related to a poorer survival (Kroman 1997, Olson 1998, Reeves 2000, Daling 2002b), and women diagnosed during pregnancy have an even worse prognosis (Clark 1989, Guinee 1994, Bonnier 1997). The poor prognosis seems to be explained by worsened tumor biology rather than delayed diagnosis (Daling 2002b).

In 1989, Bergkvist, Adami, Persson et al. were the first to report menopausal hormone therapy to be associated with a favorable survival (Bergkvist 1989). The study lacked information on mammographic examinations, leaving the possibility of lead time bias. As they assessed

relative survival, they could not exclude a reduced number of cardiovascular deaths to be the explanation. Six other studies have confirmed the improved survival among women using menopausal hormone therapy (Strickland 1992, Fowble 1999, Jernström 1999, Schairer 1999, Fletcher 2005), while two have failed (Ewertz 1991, Bonnier 1998). That women currently using menopausal hormone therapy have a more favorable survival seems likely. In the two screening-based studies, where the possibility of lead-time bias was small, women on menopausal hormone therapy had a significantly favorable survival (Schairer 1999, Fletcher 2005). Three studies assessed the effects of recency of use, and two of them found past users to have similar survival as never users (Bergkvist 1989, Strickland 1992), while one study reported short-term favorable survival, but attenuated compared to current users (Schairer 1999).

Aims

The general aim of this thesis was to better understand how menopausal hormone therapy and other established risk factors for breast cancer influence both the biology of the tumor as well as breast cancer survival. Our main hypothesis was that breast cancers associated with menopausal hormone therapy have a less malignant feature and a better survival. Other risk factors for breast cancer, like reproductive and anthropometric factors may also influence tumor biology and survival. We tested these hypotheses by studying how hormone-related risk factors were associated with breast-cancer histology and receptor status and how they affect survival after breast cancer diagnosis.

The specific questions were:

- Does menopausal hormone therapy and other risk factors affect the risk of histology-defined breast cancer (Paper II)
- Does menopausal hormone therapy and other risk factors affect the risk of receptor-defined breast cancer (Paper III)
- Does age at first birth, number of births, and time between last birth and diagnosis affect survival in breast cancer (Paper I)
- Does menopausal hormone therapy affect tumor characteristics and survival in breast cancer (Paper IV)

Subjects and methods

Sweden gives excellent opportunities for epidemiological research. There are several population-based registers and a health care system with equal access regardless of socioeconomic and geographical factors.

The **National registration number** is an identification number that is assigned to all Swedish citizens since 1947. The first six digits are created by the date of birth and the four additional digits hold information on sex and a control digit. The registration number is used in all Swedish registers, in health care and other institutions. This number makes it possible to safely link information from different sources.

Swedish Registers

The Swedish Cancer Register was established in 1958, and is kept by the National Board of Health and Welfare. Cancers are encoded according to the seventh edition of International Classification of Diseases (ICD-7). Six regional registries collect registration of newly diagnosed cancer cases all over Sweden. Reporting of new cancer cases is mandatory for clinicians, pathologists and cytologists, and in 1978, 96% of all cases were included in the register (Mattsson 1984). Of the four percent that was not included, tumors without histological verification and tumors among patients 75 years or older were overrepresented. Autopsy cases are flagged, but cases verified only at death certificates are not included due to the large uncertainty of the diagnosis in these cases. A validation study on breast cancer cases in the Malmö region compared patient records and register information. They found 99% of invasive breast cancers from the patient records in the register (Garne 1995).

The Swedish Cause of Death Register was established in 1952, and is kept by the National Board of Health and Welfare. It holds information on date and cause of death on all deceased individuals resident in Sweden at the time of death. Cause of death is reported by treating physician, and coded according to the current version of ICD. The completeness was estimated to be 0.4% in 2003 (Socialstyrelsen 2003).

The Register of the Population and Population Changes is held by Statistics Sweden, a governmental agency responsible for population statistics in Sweden. It provides current information on place of residency for all Swedish residents, and information on date of emigration since 1968. Statistics Sweden also holds historical information on place of residency Jan 1st each year.

The Multi-Generation Register was created in 1994 by linking data from several population-based registers. The register provides information on index-persons, resident in Sweden in 1961 or later, and born in 1932 or later and their parents. The parents had to be alive in 1947 to have received a national registration number. Information on birth order is included, so information on siblings can be created. Adoptions are flagged. Children born in 1955 or later are to more than 99% linked to their mothers (Lars Caderius, Statistics Sweden, personal communication).

Paper 1

Study cohort

We used Swedish population-based registries to create our cohort. We identified 32,003 women born in 1932 or later with a first diagnosis of invasive breast cancer in the Swedish Cancer Register in 1958-1997. Follow-up was ascertained by linkages to the Cause of Death Register and the Register of the Population and Population Changes. Date and underlying cause of death was retrieved, and six women who emigrated or had immigrated before diagnoses and 15 women diagnosed at autopsy were excluded. The final study cohort consisted of 27,796 women. Information on date of birth and number of births including multiple births was retrieved from the Multi-Generation Register. No woman was older than 65 years at diagnosis as women had to be born in 1932 or later to be included in the Multi-Generation Register. Women who gave birth within ten months after breast cancer diagnosis were defined as pregnant at diagnosis. Follow-up started at date of diagnosis, and ended at the date of emigration, death or end of follow-up on December 31, 1997, whichever came first.

Statistical analysis

Mortality was calculated as breast cancer specific deaths, so women who died from other causes were censored at the date of death. Annual mortality rates were calculated as the number of women deceased due to breast cancer per 100 person-years. We used 45 years as a cut-off point for showing different effects of parity before and after the fertile period of life. The hazard ratios for breast cancer specific death were investigated with the Cox proportional hazards method, including age at diagnosis (<36, 36-45, >45 years), calendar period of diagnosis (1958-1970, 1971-1980, 1981-1990, and 1991-1997), age at first birth (<20, 20-29, and >29 years) and time since last birth (1st, 2nd-3rd, 4th-10th, and ≥11th years after birth) as variables. All analyses were performed using SAS procedure PROC PHREG.

Papers II-IV

Subjects

These studies are extensions of a case-control study among all Swedish residents born in Sweden and aged 50 to 74 years (Magnusson 1998a, Magnusson 1998b, Magnusson 1999a, Magnusson 1999b). Women diagnosed with incident primary invasive breast cancer between October 1993 and March 1995 were contacted via their doctors and asked for written consent to be approached with a mailed questionnaire. The study identified 3,979 women with breast cancer of whom 3,345 (84%) participated. The primary reasons for non-participation were patient's refusal or doctor's refusal because of the patient's poor health. The mean interval from diagnosis to data collection was 4.3 months.

Controls were frequency-matched by the expected age distribution among the cases and identified through the Swedish National Population Register holding data on national registration number, name, address, and place of birth of all Swedish residents. The response rate among controls was 82% (3,455/4,188).

Classifications

In order to assure accurate classification of age at menopause and avoid confounding we restricted the analyses of age at menopause to women with known age at menopause that had not used menopausal hormone therapy before menopause. The influence of recent BMI (kg/m²) as well as weight gain from age 18 to one year before data collection was evaluated

among never users of menopausal hormone therapy which interacts with the association between body fatness and breast cancer risk (Magnusson 1999a). The interaction between hormone therapy and BMI on risk of histology-defined breast cancer was studied separately. Alcohol has been reported to interact with menopausal hormone therapy to increase the risk of certain groups of receptor-defined breast cancer (Gapstur 1995, Suzuki 2005), so we stratified the analyses of alcohol use according to ever or never use of estrogen +/-progestin. We included alcohol use, hormone use and an interaction term in a model for each receptor-defined group compared with controls to test for interaction. Tumors detected through the national mammography screening program or through other health check-ups (mostly due to menopausal hormone therapy or employment-related) were defined as screening-detected and other tumors as non-screening detected.

We categorized menopausal hormone therapy into four types; estrogen alone (mainly estradiol or conjugated estrogens), estrogen-progestin (mainly estradiol combined with levonorgestrel or norethisterone acetate), orally administered estriol, or local estrogen treatment. Use of only one type of therapy was defined as exclusive use. Estrogen-progestin therapy was also classified according to regimen, that is, as sequential (progestin taken less than 16 days per 28 days) or continuous use (19 or more days per 28 days). We explored the influence of all use as well as exclusive use of each type of therapy. Exclusive use was further classified into current or past use (last use ≤ 6 or >6 months before reference date) and short- or long-term use (<5 or ≥ 5 years). All use of each type was only assessed as ever and current use, as the estimates for past use and duration of use could be severely influenced by the other therapies. Exposure to hormone therapy after a reference date (defined in cases as date of diagnosis minus 3 months; and in controls as the date of questionnaire arrival minus mean time from diagnosis to questionnaire arrival in cases, minus an additional 3 months), was censored.

Pike et al. pointed out that use of menopausal hormone therapy was correlated to young age at menopause (Pike 1998). Young age at menopause confers a lower risk of breast cancer. If studies of hormone therapy and breast cancer risk do not adjust for age at menopause, they will underestimate the breast cancer risk. Therefore, in the analyses on menopausal hormone therapy in paper II, women with an unknown age at menopause were excluded (296 cases and 301 controls). In paper III, the estimates for breast cancer risks associated with hormone therapy did not change after exclusion of women with unknown age at menopause, and therefore these women were kept in the analyses.

Histology was classified after the invasive component as ductal (n=1,888), lobular (n=308), tubular ($\geq 90\%$ tubular component, n=93), mixed lobulo-ductal (n=58), medullary (n=29), mucinous (n=61), papillary (n=10), adenocarcinoma not otherwise specified (n=122), or unspecified tumors (n=38). Information on histology was missing in 36 cases because the medical record was not identified, or the woman had not had a breast cancer operation. We analyzed ductal, lobular, and tubular cases. Adenocarcinoma not otherwise specified was not analyzed as we considered it an undefined subtype, and the other subtypes were too few to be analyzed separately.

ER and PR content of breast tumors were routinely measured in Sweden at the time of the study, but was often not performed on tumors ≤ 1 cm in size due to lack of tumor tissue. Receptor analyses were usually performed by one laboratory within each region. All seven laboratories in Sweden analyzing estrogen receptor (ER) and progesterone receptor (PR) content used an enzyme immuno-assay (Abbott Laboratories) on cytosol samples. The method used for assessing ER content was ER α specific (Brouillet 2001). Three laboratories

reported amount of receptor per μg DNA, three laboratories reported amount of receptor per mg protein, and one laboratory reported both. Quantitative receptor content was available for 67% of the tumors for both estrogen and progesterone receptors. In 4% and 3% of the tumors, for estrogen and progesterone receptors, respectively, we did not obtain any quantitative value, but information on qualitative tumor status (strongly positive, positive, weakly positive, or negative) was given instead. We defined receptor positive tumors as ≥ 0.05 fmol receptor/ μg DNA, or ≥ 10 fmol receptor/mg protein. For the laboratory reporting both analyses, the proportion of receptor positive tumors was most similar to the proportion among the other laboratories when measured as amount of receptor per μg DNA. Hence, we used these values. Tumors with qualitative information were defined as receptor negative if they were classified as negative, and otherwise they were classified as positive.

Grade was classified according to the Nottingham histological grade or Bloom-Richardson scale into three groups (Elston 1991). High S-phase fraction was defined as $\geq 9\%$. Adjuvant endocrine therapy, almost exclusively tamoxifen, affects survival only of ER+ tumors (EBCTCG 2005). Therefore, we classified as follows; (0) untreated tumors and treated ER- tumors, (1) treated ER unknown, and (2) treated ER+ tumors.

Information on mammographic history was defined as missing either if the patient's record was missing, or if we found no examinations in any of the units, but the woman had reported at least one mammography. Recent mammography was defined as mammography within 2 years and 2 months before diagnosis (yes/no), in order to cover the normal 2-year interval of mammographic screening plus 2 months delay.

Exclusions

Women previously diagnosed with invasive cancer (other than non-melanoma skin cancer) were excluded from the study (112 cases and 91 controls). Menopause was defined as the age at last menstrual period or age at bilateral oophorectomy, if one year or more prior to data collection. Premenopausal women (198 cases and 152 controls) as well as women below the age of 55 years with unknown age at menopause (202 cases and 101 controls) were excluded. Following a decision of the ethical review board of the University of Lund, written informed consent to retrieve this information was sought from cases in that region ($n=563$), among whom 58 women did not provide informed consent. Information from the medical records led us to further exclude 58 cases with non-invasive breast cancer, 35 cases with previous cancer, one case with a cancer diagnosis other than breast cancer, and 19 cases diagnosed before or after the study period. Women with missing information on BMI (14 cases and 45 controls) or age at first birth (5 cases and 1 control) were excluded from the study. Thus, the final analytical sample comprised 2,643 cases and 3,065 controls.

In paper II, 38 cases were not included in the analyses due to missing information on histology, and 316 cases were not included due to rare or unspecified histological groups.

In paper III, 789 tumors had unknown receptor status. They were included as a separate group in the analyses.

In paper IV, women with unknown age at first birth or unknown BMI was not excluded, and 2,660 postmenopausal breast cancer cases were analyzed.

Data collection

Data on sociodemographic, anthropometric, reproductive, and menstrual factors, use of oral contraceptives, medical history, lifetime physical activity and smoking habits, as well as recent (one year before data collection) dietary habits and alcohol use were collected by means of a postal questionnaire. Detailed information on use of menopausal hormone therapy, including timing and type for each treatment episode, was also requested. A color chart displaying all preparations ever marketed in Sweden was included with the questionnaire to aid recall. In addition, approximately 50% of both cases and controls were contacted by telephone to complete missing or ambiguous responses, mainly on the use of menopausal hormone therapy. Of all eligible controls, 14% did not return the questionnaire, but agreed to a telephone interview covering the most important items including use of menopausal hormone therapy.

We collected information on primary surgery, adjuvant treatment (endocrine therapy, chemotherapy, and radiotherapy) and tumor characteristics from patient records primarily from surgical and oncological units throughout Sweden.

From Statistics Sweden we obtained information on place of residence for all women between 1988 and 1995. Since, according to Swedish regulation, medical care is provided at the hospital closest to home, we could identify the units where possible mammographic examinations had been performed. We visited 66 of the 68 units performing mammographies in Sweden at that time, and collected information on date and reason for the mammographies (screening or referral) performed from 5 years before diagnosis, excluding 3 months right before diagnosis to avoid registering diagnostic examinations. We compared this information with the questionnaire information on reported mammographies during the last 5 years before diagnosis.

Statistical analysis

Papers II-III

We used unconditional logistic regression to estimate odds ratios (OR) with associated 95% confidence intervals (95% CI) separately for each subgroup of breast cancer compared to controls. To formally test heterogeneity, whether effect estimates were similar between tumor groups, we fitted adjusted case-case-models and estimated the tests for association with the ductal and ER-PR- tumors as the reference groups in paper II and III, respectively. In paper II we used the p-value for each exposure category, while in paper III we used the global p-value for each exposure. In paper III we used chi-square tests to evaluate the statistical significance of associations between missing receptor status information, or receptor status category, and categorical variables such as age group and various tumor characteristics. As missingness was found to be related to tumor size, this was included as a covariate in the models. To test whether the observed heterogeneity could be due to earlier detection of ER+PR+ tumors compared to ER-PR- tumors, we also performed models additionally including screening or non-screening detection and lymph node involvement. P-values from Wald statistics for the heterogeneity were calculated.

We adjusted all estimates for age and assessed the potential confounding effects of a variety of other risk factors, including number of births, age at first birth, age at menopause, bilateral oophorectomy, menopausal symptoms, recent BMI, height, socioeconomic status, recent smoking, recent alcohol intake, and ever use of estrogen-progestin. Factors were defined as confounders, and included in the final models when adjustment introduced a change of age-

adjusted point estimates of more than 10%. We used the SAS System, version 9.1 (SAS Institute, Carey, NC) for all analyses.

Paper IV

Follow-up started on the day of diagnosis, defined as first cytological or histopathological report of cancer. The outcome was breast cancer death (174.9 in ICD-9, and C50.9 in ICD-10). End of follow-up was defined as date of death, emigration, or December 31st 2003, whichever came first. One woman emigrated, 414 died from breast cancer, and 269 died from other causes, during 21,938 person-years of follow-up. Among women alive at December 31st 2003, median follow-up was 9 years and 7 months.

We calculated breast cancer mortality rates by background and tumor characteristics as number of breast cancer deaths per 100 person-years. Chi-square tests were performed to detect statistically different distributions of tumor characteristics between groups of menopausal hormone therapy users and non-users, as well as between groups of current menopausal hormone therapy users. We used polytomous multiple logistic regression to estimate odds ratios with 95% CI for the associations between duration of current menopausal hormone therapy and tumor characteristics. Age at diagnosis and recent mammography were included as covariates. We used the Kaplan-Meier method for assessing cause-specific survival distributions in relation to use of menopausal hormone therapy. We modeled cause-specific mortality using the Cox proportional hazards model (Cox 1972) and estimated hazard ratios (mortality rate ratios) to compare categories of menopausal hormone therapy users to never users. Potential confounders were recent mammography, socioeconomic status, age at first birth, parity, BMI, height, recent alcohol intake, recent smoking, age at menopause, age at menarche, type of menopause (surgical, non-surgical), benign breast disease, and family history. If the factor was associated with breast cancer mortality, crude and stratified Cox models were compared.

Tumor characteristics and treatment are intermediates between menopausal hormone therapy exposure and breast cancer survival, and were thus included in Cox models to assess the extent to which they could explain the observed associations. We tested the proportional hazards assumption of the final model of ≥ 5 years duration of current menopausal hormone therapy versus no menopausal hormone therapy by dividing follow-up time into two periods, before and after 5 years after diagnosis.

Results

Menopausal hormone therapy (Papers II-IV)

Estrogen alone (mainly estradiol) was similarly related to the risk of histological and receptor-defined subtypes of breast cancer. We did multivariate analyses of risk of different tumor characteristics within the cases, and found no significant associations with tumor grade, S-phase fraction, tumor size, or lymph node involvement (results not included in any paper). In short, estrogen alone did not seem to influence tumor biology.

Table 1. Estrogen-progestin therapy and risk of histology-defined breast cancer

	Ductal	Lobular	Tubular
	OR (95% CI)	OR (95% CI)	OR (95% CI)
No use	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)
<5 years use	1.4 (1.1-1.8)	1.8 (1.1-3.1)	1.9 (0.8-4.7)
≥5 years use	2.3 (1.6-3.3)	5.6 (3.2-9.7)	6.5 (2.8-14.9)

Adjusted for age, recent BMI, age at first birth, and age at menopause

Estrogen-progestin (mainly estradiol and testosterone-derived progestin) was associated with increased risk of ductal, lobular, and tubular breast cancer (Table 1). The risks associated with long-term (≥5 years) estrogen-progestin therapy were significantly stronger for lobular and tubular compared to ductal cancer. We found no significant differences in the heterogeneity between lobular and ductal cancer between sequential and continuous menopausal hormone therapy use. When we analyzed breast cancer risk by receptor status, we found increased risks of all groups except the ER-PR- group (Table 2). The risks were borderline significantly stronger for ER+PR+ tumors compared to ER-PR- tumors.

Table 2. Estrogen-progestin therapy and risk of receptor-defined breast cancer

	ER-PR-	ER+PR-	ER-PR+	ER+PR+
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
No use	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)
<5 years use	1.2 (0.8-1.8)	1.6 (1.0-2.6)	2.5 (1.2-5.1)	1.4 (1.1-1.9)
≥5 years use	1.3 (0.7-2.5)	2.3 (1.3-4.1)	2.6 (0.9-7.5)	3.0 (2.1-4.1)

Adjusted for age, recent BMI, and age at first birth

In multivariate analyses within the cases we found current long-term (≥5 years) estrogen-progestin users compared to never users to be at significantly reduced risks for tumors of grade 2 (OR 0.5, 95% CI 0.3-0.7) and grade 3 (OR 0.3, 95% CI 0.2-0.5) compared to grade 1. Current long-term users were also at reduced risk of low compared to high S-phase fraction (OR 0.3, 95% CI 0.1-0.5). We found no significant associations between estrogen-progestin use and tumor size, lymph node involvement, or ploidy.

In paper II, we assessed the interaction between BMI and menopausal hormone therapy by studying risk of ductal or lobular breast cancer with ever or never use of estrogen-progestin

therapy, stratifying BMI in three categories. The increased risk of both ductal and lobular breast cancer seemed confined to women with a BMI ≤ 27 .

Ever use of oral estriol was associated with a doubled risk of lobular breast cancer, but no increased risk of ductal or tubular breast cancer. The risk was confined to short-term and past users, which is contrary to the pattern seen for the association between hormone therapy and breast cancer risk. We found no increased risk with oral estriol when we studied receptor-defined breast cancer. Local estrogen was not found to be associated with any subtype of breast cancer.

Other exogenous breast cancer risk factors (Papers II-III)

Oral contraceptives were not associated with overall risk of postmenopausal breast cancer (Magnusson 1999b), and was reassuringly not found to be linked to any histological or receptor-defined subgroup of breast cancer. Recent alcohol intake was significantly associated with tubular cancer, and seemed non-significantly related to lobular breast cancer but not ductal breast cancer. When we subdivided tumors by receptor status, recent alcohol intake seemed non-significantly associated with ER-PR- tumors but not the other receptor-defined groups. Recent smoking was not found to be associated with any histological or receptor-defined subgroup of breast cancer.

Endogenous breast cancer risk factors (Papers II-III)

Age at first birth seemed more strongly associated with the two ER+ groups, (p for heterogeneity =0.059 between ER+PR+ and ER-PR-). In addition, age at first birth seemed non-significantly more strongly related to lobular compared to ductal histology. BMI and adult weight gain seemed to be more strongly linked to the PR+ tumor groups, and not differ by histology. Having a mother or sister diagnosed with breast cancer and having been operated for benign breast disease were borderline significantly more strongly related to lobular than ductal cancer, but did not differ by receptor status. The other endogenous risk factors seemed similarly associated with the different receptor- and histology-defined tumor groups.

Births and prognosis (Paper I)

Among the 27,727 women, 15% were nulliparous, 19.1% were uniparous and 65.5% were multiparous. During the follow-up period, 6,568 patients died, and 89% of these deaths were due to breast cancer. The median follow-up time was 4.6 years.

Table 3. Hazard ratios of dying from breast cancer in relation to time since last birth

Time since last birth, years	HR* (95% CI)	HR ¹ (95% CI)
1 st	1.66 (1.37-2.00)	1.72 (1.42-2.09)
2 nd	1.40 (1.18-1.67)	1.44 (1.20-1.73)
3 rd	1.35 (1.13-1.61)	1.39 (1.17-1.67)
4 th —5 th	1.19 (1.04-1.36)	1.22 (1.07-1.40)
6 th -10 th	1.13 (1.04-1.23)	1.16 (1.06-1.27)
$\geq 11^{\text{th}}$	1.0 (Reference)	1.0 (Reference)
No births	1.17 (1.08-1.26)	-

*Adjusted for age at diagnosis and calendar period

¹Adjusted for age at diagnosis, calendar period, and age at first birth

Women diagnosed during pregnancy had a doubled risk of dying from breast cancer compared to women diagnosed without being pregnant. For the other analyses, only births before diagnosis were taken into account. Women with a last birth within 10 years of breast cancer diagnosis had a significantly higher risk of dying from breast cancer compared to women with more than 10 years between last birth and subsequent breast cancer (Table 3). We found a slightly but significantly worse prognosis among women with a first birth before the age of 20 compared to 20-24 years (Table 4). Nulliparous women compared to women with a first birth at 20-24 years had a similar survival if diagnosed ≤ 45 years, but a worse prognosis if diagnosed at an older age (Table 4). We stratified the effect of time since last birth on age at first birth, and found increased breast cancer mortality with short time between last birth and diagnosis regardless of age at first birth.

Table 4. Hazard ratios of dying from breast cancer in relation to age at first birth, by age at diagnosis

	HR* (95% CI)	HR ¹ (95% CI)
Age at first birth (years), women diagnosed ≤ 45 years		
<20	1.15 (1.03-1.28)	1.16 (1.05-1.29)
20-24	1.0 (Reference)	1.0 (Reference)
25-29	1.05 (0.96-1.15)	1.00 (0.92-1.09)
30-34	1.06 (0.93-1.54)	0.95 (0.83-1.09)
>34	1.20 (0.93-1.54)	0.94 (0.72-1.23)
No births	1.01 (0.92-1.12)	-
Age at first birth (years), women diagnosed >45 years		
<20	1.13 (0.99-1.28)	1.12 (0.99-1.28)
20-24	1.0 (Reference)	1.0 (Reference)
25-29	0.93 (0.83-1.03)	0.93 (0.83-1.03)
30-34	0.95 (0.81-1.12)	0.94 (0.81-1.11)
>34	1.01 (0.79-1.29)	0.95 (0.74-1.23)
No births	1.25 (1.11-1.41)	-

*Adjusted for age at diagnosis and calendar period

¹Adjusted for age at diagnosis, calendar period, and time since last birth

Menopausal hormone therapy and prognosis (Paper IV)

Oral estriol and local estrogen were not associated with breast cancer survival, and were therefore not defined as menopausal hormone therapy in the following analyses. We found that current, but not past, use compared to no menopausal hormone therapy use was associated with a decreased risk of breast cancer death (HR for current use 0.57, 95% CI 0.41-0.79, adjusted for age at diagnosis and recent mammography). We subdivided current use by duration, and found long-term (≥ 5 years) users to have a more favorable prognosis compared to short-term (<5 years) current users (HR for long-term 0.41, 95% CI 0.25-0.67 and short-term 0.78, 95% CI 0.51-1.19).

When we subdivided current use by regimen, continuous estrogen-progestin therapy seemed to have a slightly more favorable prognosis compared to sequential estrogen-progestin and

estrogen alone therapy, but as the duration of use differed between regimens and duration of use affected survival, we assessed the hazard ratio per year of menopausal hormone therapy use compared to no use, assuming a linear relationship between duration of use and breast cancer survival. The estimates per year of use were similar for sequential (HR 0.89, 95% CI 0.80-1.00) and continuous (HR 0.90, 95% CI 0.84-0.97) estrogen-progestin use, and (HR 0.97, 95% CI 0.90-1.03) for estrogen alone use.

These estimates have been adjusted for age at diagnosis and recent mammography, and may still suffer from lead time bias. Therefore, we adjusted also for tumor size and lymph node involvement, which both reflects early or late detection, but also the underlying tumor biology. As expected, the results were attenuated, but still there was a significantly reduced risk of breast cancer death among menopausal hormone therapy users measured as current, long-term, or continuous estrogen-progestin users compared to non users. The hazard ratio for the first five years after diagnosis was 0.54 (95% CI 0.34-0.87), and 0.80 (95% CI 0.36-1.48) for the period thereafter, when comparing current users to non-users and adjusting for age at diagnosis, recent mammography, tumor size, and lymph node involvement. The proportional hazards assumption was thus not significantly rejected, but the favorable effect of menopausal hormone therapy seemed to be larger in the first five years after diagnosis than later during follow-up.

Due to a large proportion of missing information on grade, S-phase fraction, ploidy, and receptor status we were not able to include all tumor characteristics simultaneously in one model. Including ER status did not affect the survival estimates (data not shown). Adding grade and S-phase fraction to a model with age at diagnosis, recent mammography, tumor size, and lymph node involvement increased the hazard ratio from 0.43 (95% CI 0.15-1.23) to 0.51 (95% CI 0.14-1.84) for current long-term use compared to no menopausal hormone therapy use.

General discussion

Methodological considerations

Internal validity

Internal validity can be defined as the absence of systematic errors within the study. The classification of systematic errors is not clear cut; roughly they are divided into bias and confounding. The magnitude of systematic errors is not affected by sample size, which is different from chance variation, which is reduced with increased sample size.

Selection bias

Bias is a distortion of the association under study, and selection bias refers to problems related to selection and participation.

In a case-control study, one of the central issues is how to select controls. Ideally, the controls should reflect the source population, and more specifically, the exposure prevalence in the source population. In the case-control study (papers II-III), women were selected from the continuously updated Swedish Population Register, resulting in a low risk of selection bias compared to for instance hospital-based case-control studies using controls from the same hospital as the cases.

The cases in the case-control study were recruited from the Regional Cancer Registers, where almost all breast cancer cases among women below the age of 75 years are recorded. The participation proportions in the study were relatively high, 84% among cases, and 82% among controls, but selection bias could still be a problem. One common reason for non-participation among cases was the patient's poor health, and we know that cases had larger tumors and a higher proportion of lymph node involvement, so we have included somewhat less advanced tumors in our study. Apart from information of poor health, and tumor characteristics among cases, we had no information on nonresponders among cases or controls. In a validation study of a case-control study of premenopausal breast cancer, age, relative weight, smoking, family history of breast cancer, number of births, and age at first birth did not differ between responders and nonresponders. However, some differences were found between responders and non-responders, but as the differences were similar among cases and controls, they did not seem introduce a large selection bias (Madigan 2000). To cause a bias in the study of an association between menopausal hormone therapy and breast cancer survival, nonrespondents should have a different association compared to responders. The most likely scenario is that women using menopausal hormone therapy are more likely to participate regardless of tumor severity at diagnosis, while non-users of menopausal hormone therapy with severe tumors would be less likely to respond. If this is true, our estimates of the association between hormone replacement therapy and either tumor characteristics or survival are conservative.

Diagnostic bias

If exposures of interest affect the possibility of being diagnosed, this may lead to diagnostic bias. In our study, especially use of menopausal hormone therapy, but also to a lesser degree high socioeconomic status may increase the use of mammographic examinations, increasing the possibility of being diagnosed with breast cancer. This would overestimate the breast cancer risk related to use of menopausal hormone therapy. In the initial case-control study, adjustments were made for number of mammographies before diagnosis without affecting the

estimates (Magnusson 1999a). We still cannot rule out that over-detection of breast cancer among menopausal hormone therapy users have affected our risk estimates for subgroup-defined breast cancer in papers II-III. However, different risks between subgroups, which were the main aim of paper II and III, would require differential detection depending on tumor characteristics. In paper III, breast cancer was defined by ER and PR status. This information was missing in 30% of the cases. Missing information was explained by tumor size, as small tumors were more likely to lack receptor information. When we stratified on tumor size, we could assume missingness to be at random. The heterogeneity tests were stratified for tumor status, thus trying to reduce the bias due to missingness. Mammographies have lower sensitivity for lobular than ductal tumors, but this would only underestimate the heterogeneity between lobular and ductal cancer.

Lead time bias

In survival studies, follow-up starts at diagnosis. If the diagnosis for instance is a hip fracture, the time of diagnosis is usually evident. Breast cancers, on the other hand, are known to exist for years before being diagnosed (Robbins 1989), and we know that a mammographic examination might put this point of time months or even years before the tumor would be discovered by palpation (Howard 1987). Even if the tumor is lethal, the survival time, counted from the day of diagnosis, has been prolonged, a problem called lead time. Compared to non-users, women using hormone therapy are more likely to visit a doctor and be examined with mammography, thus introducing a possibility of lead time bias. On the other hand, hormone therapy increases the radiological density of the breast thereby lowering the sensitivity of mammography and possibly delaying time of diagnosis (Banks 2001a). Due to this uncertainty regarding start of follow-up (time of the diagnosis) in paper IV, we collected information on mammographies performed in the years before diagnosis. We analyzed the data adjusted for a recent mammography, and we also restricted to women with a recent mammography. We performed models with and without adjustment for tumor size and lymph node involvement, but these variables are both related to lead time and to tumor biology, and including them results in estimates partly adjusted for tumor biology. The reduced sensitivity of mammographies in women with menopausal hormone therapy was not possible to assess, but would only have attenuated our results of improved survival among women with current menopausal hormone therapy.

Length bias

Tumors have different growth rates, a fact difficult to study, as they are excised as soon as they are discovered. Fast-growing tumors have a short preclinical phase, and are thus more likely to give symptoms before they could have been detected at mammographic screening. Consequently, tumors detected at screening are more slow-growing compared to tumors detected in the interval between screening occasions. Thus, in our population-based observational study, way of detection (screening or non-screening) is a prognostic factor, not only because women attending screening are healthier and tumors are detected earlier (lead time), but also because screening is more likely to detect slow-growing tumors.

Follow-up bias

In cohort studies, individuals not followed until the end of the study without having the event studied are censored. In paper I and IV, follow-up was ascertained through the Cause of Death Register, and the Register of Population and Population changes. Very few women emigrated, so the main cause of censoring was death due to another cause than breast cancer. The assumption of noninformative censoring may be violated if there is dependency between the cause of death under study and another cause of death. This can occur if there is a common

risk factor associated with both the diagnosis and other causes of death, like smoking is associated both with lung cancer and cardiovascular death. At least three risk factors for breast cancer are risk factors for other causes of death. Low age at menopause is a risk factor for cardiovascular death, and a high BMI is a risk factor for cardiovascular and diabetes-related death, but none of them is a very strong risk factor for breast cancer. Ionizing radiation is a risk factor for many causes of death, but is unlikely to be a cause of more than a few cases in our studies. In addition, most women in our studies died from breast cancer. In conclusion, competing risks due to common risk factors are not likely to influence our results.

Misclassification

In our studies, we rely on information from registers, questionnaires, and patient records. This information might not be correct, a problem referred to in epidemiological studies as misclassification. Both exposures and outcomes can be misclassified. If the exposure misclassification is unrelated to the outcome, or vice versa, the misclassification is nondifferential. In most cases, nondifferential misclassification will bias the estimates towards the null value. If there are more than two categories of exposure, non-differential misclassification can also bias the estimates for some of the categories away from the null, but in practice, this is not a severe threat to most studies. One should however remember, that nondifferential misclassification of a confounder leads to poorer confounding control, which might bias the estimate away from or towards the null value.

In paper III, the outcome was defined by ER and PR status. One laboratory, Uppsala, reported the amount of receptor content both by amount of DNA and by amount of protein. The Pearson correlation coefficient between these two variables (dichotomized into positive or negative) was 0.36 for ER and 0.42 for PR. This rather poor correlation between two different ways of reporting amount of receptor indicates that the receptor status measurements thus probably suffered from considerable misclassification. As this measurement was not related to our exposures, it was nondifferential and some of our estimates of heterogeneity were probably substantially biased towards the null.

In papers II-IV, exposure information was self-reported, but probably reliable for most exposures. Validation studies have found self-reported menopausal hormone therapy to be accurate (Persson 1987, Banks 2001b)

Recall bias

Differential misclassification, when the exposure misclassification is related to the outcome, or the outcome misclassification is related to the exposure, can bias estimates away from or towards the null.

Recall bias occurs in case-control studies, when cases give information about exposures after being diagnosed. The diagnosis might influence the answers, leading to differential misclassification. We cannot exclude the possibility of recall bias, in papers II-III, but when we assessed the difference of effect between subgroups of breast cancer, all women were diagnosed with breast cancer, and thus recall bias due to different tumor characteristics must be considered unlikely.

Confounding

Associations between exposure and outcome can be affected by a third variable, a confounder. The confounder must be a risk factor of the outcome, associated with the exposure, and not caused by the exposure or the outcome. A factor that is caused by the exposure and causing

the outcome is an intermediate factor and not a confounder. Tumor characteristics are intermediate factors between exposures and breast cancer deaths.

In paper I, we lacked information on possible risk factors for breast cancer and breast cancer prognosis. Socioeconomic status is sometimes found to be a risk factor for poor breast cancer prognosis (Karjalainen 1990, Lagerlund 2005), and is also associated with teenage pregnancies (Olausson 2001). The associations found between teenage pregnancies compared to older mothers, and two births compared to other number of births could be confounded by socioeconomic status that we could not account for.

In papers II-IV, we had information on many risk factors for breast cancer. In the statistical analyses, we were able to assess the influence of these possible confounders, and mostly they did not affect our estimates more than marginally. In the final models, some factors were included as they confounded a few of the estimates.

Precision

Random error, the influence of chance, leads to uncertainty of the estimates found in a study. Assuming no systematic bias, which means that our sample is a random sample of our study population, a 95% confidence interval around the point estimate includes the true population value in 19 out of 20 times. This interval depends on study sample size (larger the smaller sample), frequency of exposure and outcome (larger with exposure and/or outcome proportions close to 0 or 1), and variability in the population (larger the larger variability). The 95% level is standard in non-genetic epidemiologic research, but this level is just an arbitrary cut-off level, easily changed. Theoretically, chance can never be ruled out as an explanation to an observed finding, but with increasing precision, the point estimate will converge towards the true value, with a decreased random error.

In the register-based study on births and breast cancer survival, more than 27,000 cases were followed for a median of 4.6 years, and a substantial number of women had given birth recently before the diagnosis, so precision was not a major problem. In the case-control study on breast cancer, the study was initially planned to assess risks of breast cancer. When we subdivided breast cancer cases into histological and receptor-defined subgroups, we knew we would have little precision to estimate risks for some of the subgroups, but still we thought we would add some knowledge about risk factors and tumor characteristics. Estimating interactions between menopausal hormone therapy and other risk factors, and assessing menopausal hormone therapy by recency or duration by subgroups of breast cancer was severely hampered by low power.

With the 95% confidence interval, 1 of 20 intervals does not include the true population estimate, and with hundreds of estimates, this will most likely affect quite a few intervals. If there were no associations, a study with many comparisons would be likely to find some falsely significant estimates. This is often referred to as the “multiple comparison problem”, where a problem arises especially if the significant estimates are selected and interpreted as true findings. In papers II and III, we assessed the influence of known risk factors on the risk of subgroups of breast cancer, with more than a hundred estimates. We knew beforehand that most assessed factors were associated with breast cancer, so we expected to obtain many significant findings. We also knew that some of the confidence intervals most likely did not include the true value. With this in mind, the results must be cautiously interpreted. Previous or future similar findings in other studies increase the likelihood that our findings are true.

Interaction

Epidemiologists should be aware of the different meanings of the term interaction. Statistical interaction is the departure from the underlying model, which is important to bear in mind when trying to fit the statistical models. The underlying statistical model is usually multiplicative, but can also be additive. Two effects that do not interact under the multiplicative scale interact under the additive scale and vice versa. Biological interaction refers to two or more causes acting together (synergistic or antagonistic) in an individual to cause a disease. According to Rothman, two independent (non-interacting) causes add risks to each other, while biological interaction is departure from additivity of effects (Rothman 1998). Compared to the main effects in a study, analyses of interaction between two variables always have a much lower power (Brookes 2004), often resulting in nonsignificant results with wide confidence intervals. Assessing departure from additivity in the commonly used models logistic regression and Cox models requires some extra work, but is feasible (Andersson 2005). When relative risks are below 2, the difference between the additive and the multiplicative scale is not very large. Assuming a relative risk of 2 for exposures A and B, assuming additivity, exposure to both A and B yields a relative risk of 3, while assuming multiplicativity the relative risk would be 4.

Regardless of the underlying assumptions of interactions, the conclusions for public health are the same. If two single factors are associated with a fourfold increased risk of disease, and those exposed to both factors have a sixteenfold increased risk, the public health implication would be to try to avoid one of the risk factors regardless of assuming biological interaction as departure from additivity or not (Clayton 2001). When relative risks are similar in different age-strata, this means that pooling the results is feasible, but also implies that there probably is a biological interaction between the risk factor and age.

External validity

External validity is the possibility to generalize the finding to other populations than the one under study. The first requirement is internal validity and enough precision. If the study is internally valid, the external validity depends on knowledge on (or assumptions of) biological interactions with other exposures that might vary between populations. In the study of breast cancer survival in relation to menopausal hormone therapy, 16% of all eligible breast cancer cases did not participate. They had somewhat larger tumors and a higher proportion of lymph node involvement, so the study is less reliable for those with worse tumor characteristics. Regarding menopausal hormone therapy, European studies find higher risk estimates than US studies, which thus should be explained by interactions (apart from the fact that different kinds of estrogens and progestins are used), such as a higher mean BMI in the US.

Study design

Generally, cohort studies are expensive and time-consuming for rare outcomes, requiring exposure information on a large number of individuals to obtain an adequate number of persons with the outcome of interest. Register-based studies overcome this drawback of cohort studies, being rather cheap and covering information on many individuals. In paper I, using Swedish population-based registries on births, breast cancer diagnosis, and deaths gave us good power to address the question of prognosis in relation to the uncommon event of breast cancer shortly after a birth. One disadvantage was the lack of information on socioeconomic status. After publishing the paper, we realized that we could have added information on level of employment in the household from the Swedish censuses of 1960, 1970, 1980, and 1990 for confounding control of socioeconomic status.

In papers I and IV, we used breast cancer specific mortality. Deaths due to other causes were censored at the day of death. Cause of death can be misclassified, so that for instance a woman dying from another cause but once diagnosed with breast cancer is misclassified as dead due to breast cancer. However, a validation study on breast cancer deaths in the Swedish Cause of Death Register compared information from patient records with register information, and reported a high concordance (Nystrom 1995). In most discordant cases, women had distant metastasis. We could have used all-cause or relative mortality. All-cause mortality, including all causes of death, can be problematic if the exposure under study is related to mortality due to other causes, like obesity. If a large proportion of deaths are not due to the disease under study, all-cause mortality will generally underestimate differences compared to cause-specific mortality. Relative mortality compares the mortality rate in cases with age- and calendar-specific mortality in the source population. It is comparable with standardized incidence ratios, in that it compares observed deaths with expected deaths. This method is preferable when cause of death classification is unreliable. However, similar to all-cause mortality it is inferior compared to cause-specific death when the cases are expected to have a different overall mortality than the source-population.

In papers II and III, we used a case-control study initially designed for studying risk factors for breast cancer. We subdivided breast cancer by histology and receptor status, respectively. The case-control study is a good design for rare outcomes, and multiple exposures can be assessed, although retrospectively. The case-control study tries to imitate a cohort study, but instead of collecting exposure information on all individuals in the population, controls are selected to reflect the exposure prevalence in the source population. Random selection of controls and high participation proportion are necessary requirements for reducing bias in a case-control study. In our Swedish setting, we were able to randomly select controls, and the participation rate was 82% which is relatively high but far from perfect, leading to the possibility of selection bias between participants and non-participants. The study was not initially planned for studying subgroups of breast cancer, but still we were able to find some heterogeneity even if the precision was low for some rare subgroups of breast cancer.

In paper IV, we used the cases from the case-control study and studied tumor characteristics not studied in paper II or III, and breast cancer survival. Thanks to the Swedish registries, we had only one patient lost to follow-up due to emigration, and we could study breast cancer deaths rather than all deaths. The main problem in the design of the study was how to address the lead time bias, that women using menopausal hormone therapy were more likely to attend mammographic examinations and thereby being diagnosed earlier. Therefore, we decided to collect information on mammographic examinations from all 68 units in Sweden that performed mammographic examinations, which made it possible to restrict to women who had performed a recent mammography, or to include recent mammography as a covariate in the models.

Discussion of the findings

Menopausal hormone therapy, risk and prognosis

Estrogen alone did not seem to influence tumor biology, and had a small, if any, influence on breast cancer survival. Results from other studies are conflicting. Some studies report a subgroup association with ER+ (Ursin 2002, Chen 2006) or with lobular cancer (Li 2000, Newcomer 2003, Li 2003e), but no study has found both associations in the same study. Two studies have reported estrogen alone to be associated with breast cancers of low grade (Harding 1996, Holli 1998), while one large study found similar associations with low- and

high-grade breast cancers (Kerlikowske 2003). Risk factors for tumors may influence a certain carcinogenic pathway, leading to a specific tumor biology. The risk factor should by this mechanism influence only cases where it caused the tumor. For a risk factor that increases the risk only slightly for a tumor, most exposed cases are not caused by the risk factor. Estrogen alone is a weaker breast cancer risk factor compared to estrogen-progestin therapy (Collaborative Group on Hormonal Factors in Breast Cancer 1997, Ross 2000, Schairer 2000, Li 2003e), although our study has reported a stronger association than most other studies (Magnusson 1999a, Greiser 2005). Thus, an association between estrogen alone and certain biological characteristics seems less plausible, or at least more difficult to find.

Our finding that estrogen-progestin therapy is associated with lobular, tubular, or receptor positive breast cancer is supported by most previous studies (Li 2000, Manjer 2001, Chen C 2002, Newcomb 2002, Ursin 2002:Daling, 2003 #263, Daling 2002a, Newcomer 2003, Li 2003e, Chen 2004, Garcia-Closas 2006, Lee 2006, Li 2006b). Most (Harding 1996, Holli 1998, Esteve 2002, Sacchini 2002, Gertig 2003, Stahlberg 2004, Garcia-Closas 2006), but not all (Stallard 2000, Kerlikowske 2003), investigators report an association between menopausal hormone therapy and low grade tumors, but few have been able to separate estrogen alone and estrogen-progestin. Our findings were most pronounced among current or longterm users of estrogen-progestin menopausal hormone therapy, which also are the factors linked to the highest risk of breast cancer (Collaborative Group on Hormonal Factors in Breast Cancer 1997).

In a recently published study, a new polytomous regression model to assess multiple tumor characteristics simultaneously was used (Garcia-Closas 2006). They reported current menopausal hormone therapy to be more strongly related to lobular and tubular breast cancer (compared to ductal), to low grade (compared to high), and to small tumors (<2 cm), but when all characteristics were evaluated simultaneously, the histology was no longer important. Tumor size might be more related to lead time (Webster 2005), but grade should not be affected by lead time according to the theory that grade and receptor status are determined before tumors are usually detected (Lacroix 2004, Webster 2005). This would imply that even if there was a remaining effect of lead time, grade is related to menopausal hormone therapy, and that the association between menopausal hormone therapy and lobular and tubular histology would be explained by grade.

Regarding breast cancer survival in relation to menopausal hormone therapy, we found an improved survival among current, but not past menopausal hormone therapy use, compared to never use. This is supported by most previous studies (Bergkvist 1989, Strickland 1992, Fowble 1999, Jernström 1999, Schairer 1999, Fletcher 2005). Studying the influence of menopausal hormone therapy on breast cancer is accompanied by a number of possible biases to handle. We have tried to account for the lead time and healthy women bias, but as this is not a randomized study, we cannot rule out residual bias. Still, the differences between various regimens, the stronger effect for current compared to past use, as well as the influence of duration of use imply that biological effects on the tumor are more likely explanations for our findings than bias.

Oral estriol was not found to be associated with breast cancer in the initial case-control study (Magnusson 1999a). Despite this we found an association with ever use of oral estriol and lobular breast cancer. As the strongest associations were among short-term or past users, the findings are however difficult to interpret. The result could be due to chance, or a real biologic effect. We found no associations between oral estriol and receptor status, tumor

grade, or breast cancer survival. Local estrogens are absorbed systemically (Heimer 1984), but we found no association with any subgroup of breast cancer, or breast cancer survival.

Other exogenous breast cancer risk factors

We found no associations between use of oral contraceptives and specific subgroups of breast cancer in our study on postmenopausal women. As there was no overall risk increase this is reasonable, but one study has reported an increased risk of lobular breast cancer in women aged at least 65 years (Li 2003c). Recent alcohol consumption is a risk factor for breast cancer, and we found a stronger association with tubular cancer, not earlier reported. Li et al. found alcohol consumption to increase the risk mainly for lobular cancer (Li 2003d), and our data did not contradict this, with no association with ductal, and a nonsignificant association with lobular cancer.

We found no signs of heterogeneity with recent alcohol consumption or smoking on receptor-defined breast cancer, supported by conclusions from a review (Althuis 2004). A possible interaction between menopausal hormone therapy and alcohol on breast cancer risk, supposing that both act via hormonal mechanisms, has been proposed, but refuted in a meta-analysis (Smith-Warner 1998), and in the Nurses' Health Study (Chen W 2002). An interaction between menopausal hormone therapy and alcohol has been reported for ER+PR+ tumors and ER-PR- tumors but not ER+PR- tumors (Gapstur 1995), and recently in a Swedish cohort study, but only for ER+ tumors (Suzuki 2005). Similar to results from the Nurses' Health Study we found no signs of interaction for any receptor-defined subgroup of breast cancer (Colditz 2004).

Endogenous breast cancer risk factors

High age at first birth increased the risk mainly for ER+ tumors, and seemed to be more strongly linked to lobular compared to ductal tumors. These findings are consistent with the majority of previous reports (LiVolsi 1982, Stalsberg 1989, Wohlfahrt 1999, Althuis 2004, Ursin 2005). Other reproductive factors (number of births, age at menarche, age at menopause) were not linked to any specific tumor subgroup, also consistent with most of the previous literature (LiVolsi 1982, Stalsberg 1989, Potter 1995, Yoo 1997, Wohlfahrt 1999, Cotterchio 2003, Li 2003c, Ursin 2005). This could indicate that high age at first birth increases breast cancer risk via other mechanisms than increasing number of children and age at menarche and menopause.

Postmenopausal obesity, measured as BMI or adult weight gain, was found to be associated especially with PR+ tumors, while no association was found with any histological subgroup. This result is consistent with previous literature which has linked postmenopausal obesity to receptor positive and sometimes more specifically PR+ tumors (Enger 2000, Huang 2000, Yoo 2001, Sellers 2002, Li 2003c, Colditz 2004, Feigelson 2006, Suzuki 2006).

Postmenopausal obese compared to slim women have higher levels of bioavailable estrogens (Lukanova 2004), a fact that largely seems to explain the association between postmenopausal obesity and breast cancer (Key 2003, Rinaldi 2006). Estrogen up-regulates the PR expression via the ER in normal breast tissue (Allred 2001). Thus, it could be that PR is more expressed both in normal and cancer tissue in the breast. Receptor positive tumors are associated with a better survival in the first years after diagnosis (EBCTCG 2005, Anderson 2006), so the association between postmenopausal obesity and receptor positive tumors is puzzling, as obesity is related to poor breast cancer survival, even after adjustment for mammographic screening (Chlebowski 2002).

We found a potential association between tall height and receptor positive tumors but not with any histological subgroup, somewhat conflicting with other reports which have found no differences with regard to receptor status or histology (Li 2003c, Li 2006b).

Births and prognosis

Our finding of a doubled risk of breast cancer death among women diagnosed with breast cancer was expected, as this has been consistently reported (Clark 1989, Guinee 1994, Bonnier 1997). Women diagnosed up to ten years after last birth were found to have a poorer survival compared to women with a longer time between last birth and diagnosis, consistent with other reports (Kroman 1997, Olson 1998, Reeves 2000, Daling 2002b, Whiteman 2004). In our study, we had no information on tumor characteristics. Women diagnosed during or within two years after a pregnancy have been found to more often have ER- tumors, high S-phase fraction, higher tumor grade, and larger tumors than other women of the same age (Bonnier 1997, Olson 1998, Daling 2002b). All differences in tumor biology are not captured by current tumor characteristics, as a current or recent pregnancy has independent prognostic value after adjustments for tumor characteristics (Bonnier 1997, Kroman 1997, Daling 2002b, Whiteman 2004). Differences in delays before detection are likely during pregnancy or lactation, but not thereafter (Lambe 1995). Thus, the successively improved survival with increasing time since last birth up to ten years after diagnosis cannot be explained by lead time bias. A biological influence on the tumor is a more likely explanation.

We and others have found that the survival in parous and nulliparous women differ by age at diagnoses. Among older women, parous women have a better prognosis (Papatestas 1980), while among younger women, nulliparous women have a better survival compared to parous women (Black 1983, Mohle-Boetani 1988, Olson 1998). This might be due to the effect of time since last birth.

We found women with a first birth before the age of 20 to have a poorer prognosis compared to other women, supported by others (Schouten 1997, Kroman 1998). An early first birth is a well-known protective factor against breast cancer (Lambe 1994). A high age at first birth seems related to increased risk mainly of receptor positive breast cancer (Althuis 2004), and an early first birth has been associated with high grade tumors (Largent 2005). Hence, it could be that the poorer prognosis among women with an early first birth is due to tumor biology. However, we cannot rule out a confounding effect of low socioeconomic status (perhaps via delayed diagnosis), which has been associated both with teenage births (Olausson 2001) and with poorer breast cancer survival (Lagerlund 2005).

Final remarks and future research

In my thesis I have evaluated the influence of breast cancer risk factors on breast tumor characteristics and survival. There is not a simple association between the influence of an exposure on breast cancer risk and the influence of the same exposure on survival. Many risk factors for breast cancer do not seem to affect the tumor characteristics or the survival. Some risk factors, like postmenopausal obesity and a recent birth, increase the risk and deteriorate the survival. On the other hand, menopausal hormone therapy increases the risk and improves the survival, and an early first birth decreases the risk while deteriorating the survival. These different patterns indicate that there are diverse mechanisms involved.

In science an answer to one question quickly leads to a new one. Regarding the epidemiology of menopausal hormone therapy and breast cancer risk, the initial research question was whether ever use was related to an increased risk of breast cancer. Thereafter more refined

analyses were performed: of menopausal hormone therapy by type and administration of estrogen and progestin, as well as by duration, and recency of use. Interactions between menopausal hormone therapy and other factors have also been studied, and menopausal hormone therapy and BMI were found to interact. In the last years, also the breast cancer outcome has been scrutinized in more detail, where the potential risk associated with a variety of factors have been reported for breast cancer defined by receptor status, histology, and other tumor characteristics. These are the questions evaluated in this thesis. The next question is how these different tumor characteristics are related to each other. The epidemiology will need help from other areas to disentangle this, and parallel with the development in epidemiology is the progress in the genetic field. Hopefully in a couple of years we will know much more about the molecular pathways of breast tumors, and in what ways different tumor characteristics are related. Currently we know that receptor status, grade and tumor size are correlated, but not how. In the future, we might know how risk factors influence the molecular events leading to tumors with certain biological characteristics. This would radically improve the knowledge of how to prevent breast cancer.

Leaving utopia, and looking at the closer future, my thesis work has given rise to the following questions and hopes:

- Due to the bad precision when studying subgroups of breast cancer, meta-analyses of the associations between risk factors and histology-defined and receptor-defined breast cancer would be desirable. This would also allow some precision in the analyses of tumors defined by different tumor characteristics simultaneously.
- Can newer tumor markers or gene expression analyses of tumors explain more of the differences in survival found in relation to an early first birth, a recent birth, obesity, or menopausal hormone therapy?
- Is the breast cancer risk associated with menopausal hormone therapy mediated via increased mammographic breast density?
- Is current alcohol consumption related to tumor grade and breast cancer survival?

Conclusions

Some risk factors are associated with certain subgroups of breast cancer, and may thereby influence the survival.

- Estrogen-progestin menopausal hormone therapy is associated with breast cancers of certain characteristics: lobular or tubular histology, receptor positive, low grade, and low S-phase fraction.
- Estrogen-progestin menopausal hormone therapy is associated with improved breast cancer survival, which seems to be due to an influence of the tumor biology.
- Estrogen alone menopausal hormone therapy does not seem to influence the tumor biology, and has little, if any, favorable effect on breast cancer survival.
- High age at first birth increases the risk mainly for estrogen receptor positive, and possibly lobular, tumors.
- Women with a first birth before the age of 20 had a poorer breast cancer survival compared to women with older age at first birth.
- Women diagnosed with breast cancer within ten years after last birth have a poorer breast cancer survival compared to women with a longer interval between last birth and diagnosis.
- Number of births, age at menarche, and age at menopause were not linked to any subgroup of breast cancer.
- Adult weight gain of at least 30 kg seemed to increase the risk mainly for progesterone receptor positive tumors, but was similarly linked to ductal and lobular breast cancer.
- Recent alcohol intake increased the risk for tubular, and possibly lobular, breast cancer, but not ductal breast cancer.

Svensk sammanfattning

Bröstcancer är den vanligaste cancerformen hos kvinnor i Sverige och övriga västvärlden. De senaste 50 åren har allt fler kvinnor drabbats. Delvis beror det på att vi lever längre, samt att man med mammografi diagnostiserar tumörer som förut förblev oupptäckta, men också på att vi förändrat våra levnadsvanor. Att föda färre barn senare i livet ökar risken för bröstcancer jämfört med många barnafödslar med start i tonåren. Hormonbehandling i klimakteriet ökar också risken för bröstcancer, och det finns indikationer på att riskökningen särskilt gäller bröstcancer med vissa tumörkaraktäristika. Det finns också studier som talar för att kvinnor som behandlats med hormoner i klimakteriet har bättre överlevnad i bröstcancer jämfört med andra kvinnor. Syftet med denna avhandling var att studera hur riskfaktorer påverkar tumörkaraktäristika och prognos.

Vi genomförde en registerstudie där vi utnyttjade data från cancerregistret, dödsorsaksregistret, befolkningsregistret och flergenerationsregistret. Vi studerade prognosen i bröstcancer för drygt 27.000 kvinnor, födda i Sverige 1932 eller senare, som fick bröstcancer 1958-1997. Från flergenerationsregistret fick vi information om när kvinnorna fick barn. Vi fann att kvinnor som fick bröstcancer inom tio år efter att de fött barn hade sämre prognos jämfört med kvinnor som hade längre tid mellan senaste barn och diagnos. Prognosen förbättrades successivt med ökande tid mellan barnafödelse och diagnos. Den successivt förbättrade prognosen med ökande tid sedan barnafödelse under så lång tid som tio år talar för att tumörbiologin påverkas av graviditeten. Vi fann också att kvinnor som fick sitt första barn före 20 års ålder hade sämre prognos än kvinnor som fick sitt första barn senare i livet. Vi saknade uppgifter om socioekonomiska faktorer, och kan inte utesluta att sådana skillnader kan förklara den sämre prognosen hos kvinnor med tonårsgraviditeter, tex via senarelagd diagnos.

Tre artiklar i avhandlingen baserades på en fall-kontrollstudie av bröstcancer som genomfördes i Sverige 1993-1995 hos kvinnor mellan 50 och 74 år. Kvinnor som fick bröstcancer för första gången och lika många kvinnor som inte fått bröstcancer i samma ålder tillfrågades om att delta i en studie genom att svara på ett frågeformulär. Drygt 3.000 fall och 3.000 kontroller deltog. Mitt avhandlingsarbete inleddes med att vi samlade in information från medicinska journaler om tumörkaraktäristika, behandling och återfall. Dessutom besökte vi alla enheter som genomförde mammografier i Sverige under tidigt 1990-tal. Där inhämtade vi information om datum för, och orsak till, genomförda mammografier hos kvinnorna med bröstcancer under 5 år före diagnos.

Vi studerade de tre vanligaste histologiska grupperna av bröstcancer, duktala (1.888 stycken), lobulära (308 stycken) och tubulära (93 stycken) och jämförde med 3065 kontroller. Vi fann att hormonbehandling i klimakteriet med östrogen-progestin ökade risken för alla typerna, men ökningen var starkare för lobulär och tubulär jämfört med duktal bröstcancer. Behandling med enbart östrogen ökade risken lika mycket för både duktal och lobulär cancer. Hög ålder vid första barnet verkade öka risken mer för lobulär än för duktal bröstcancer. Kvinnor med den högsta alkoholkonsumtion ett år före studien hade högre risk att drabbas av tubulär och icke signifikant ökad risk att få lobulär cancer, men vi såg inget samband mellan alkoholkonsumtion och duktal bröstcancer. Övriga riskfaktorer som vi studerade var likartat associerade med de histologiska grupperna av bröstcancer.

Vi delade upp kvinnorna med bröstcancer i fyra grupper, beroende på om tumörerna uttryckte östrogen- eller progesteronreceptorer och jämförde med kontrollerna. Vi fann att hormonbehandling i klimakteriet med östrogen-progestin ökade risken för alla tumörgrupper utom den som var negativ för både östrogen- och progesteronreceptorer. Hormonbehandling med enbart östrogen ökade risken likartat för alla grupper av receptordefinierad bröstcancer. Vi fann också att hög ålder vid första barnet medförde en riskökning enbart för östrogenreceptor-positiv bröstcancer, samt att viktuppgång på minst 30 kg i vuxen ålder ökade risken för progesteronreceptorpositiv bröstcancer. Övriga riskfaktorer var inte associerade med någon särskild grupp av receptordefinierad bröstcancer.

Vi ville studera i detalj hur hormonbehandling i klimakteriet påverkade prognosen i bröstcancer, och om eventuella skillnader kunde förklaras av tumörkaraktäristika. Eftersom tidpunkten när diagnosen ställs påverkas av om man genomgått mammografi eller inte justerade vi våra analyser för genomförd mammografi inom två år och två månader före diagnos. Vi fann att kvinnor som hade slutat med hormonbehandling minst ett halvår före diagnos hade likartade tumörkaraktäristika och överlevnad som icke hormonanvändare. Kvinnor som använde hormonbehandling fram till diagnos hade oftare tumörer med låg grad och låg s-fas, medan tumörstorlek och lymfkörtelmetastasering inte skilde sig mot kvinnor som inte använt hormonbehandling. Tumörer med låg grad och låg s-fas växer långsammare och är mer lika normal bröstvävnad jämfört med tumörer med hög grad och hög s-fas. Mer än fem års användning fram till diagnos medförde ännu gynnsammare tumörkaraktäristika.

Vi fann att prognosen i bröstcancer var kraftigt förbättrad hos kvinnor som använde hormonbehandling fram till diagnos, och att långtids-användning medförde den största förbättringen. När vi studerade typer av behandling fann vi först att kontinuerligt progestin med östrogen verkade medföra bättre prognos än de andra två typerna, men när vi justerade för behandlingstid fick de två kombinationsbehandlingarna ungefär samma prognos och bättre jämfört med enbart östrogen. Skillnader i prognos förklarades delvis av tumörkaraktäristika, men vi tror att hormonbehandlingen medför förändringar i tumörbiologin som inte fångas av de tumörkaraktäristika vi hade tillgång till i vår studie.

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