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Markers of immune competence and the clinical course of breast cancer

PhD dissertation

Lone Winther Lietzen

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Thesis papers

I Lietzen LW, Cronin-Fenton DP, Ejlertsen B, Christiansen P, Sørensen HT, Lash TL. Age–at–Diagnosis and Proportion of Lymph Node Positive Breast Cancer: a Danish nationwide descriptive study. In draft.

II Lietzen LW, Cronin-Fenton DP, Christiansen P, Sørensen HT, Lash TL. Autoimmune Diseases and Breast Cancer Recurrence: A Danish nationwide prospective cohort study. **Breast Cancer Res Treat.** 2015 Jan;149(2):497-504. doi: 10.1007/s10549-014-3258-2. Epub 2015 Jan 3.

III Winther Lietzen L., Cronin-Fenton D, Garne JP, Kroman N, Silliman R, Lash TL. Predictors of re-operation due to post-surgical bleeding in breast cancer patients: a Danish population-based cohort study. **Eur J Surg Oncol.** 2012 May;38(5):407-12. Epub 2012 Mar 17.

IV Lietzen LW, Ahern T, Jensen AB, Christiansen P, Sørensen HT, Lash TL, Cronin-Fenton DP. Glucocorticoid Prescriptions and Breast Cancer Recurrence: A Danish nationwide prospective cohort study. **Ann Oncol.** 2014 Dec;25(12):2419-25. doi: 10.1093/annonc/mdu453. Epub 2014 Sep 15.

Abbreviations

AD	Autoimmune Disease
AUPD	The Aarhus University Prescription Database
ATC	Anatomical Therapeutic Chemical
BC	Breast Cancer
CI	Confidence Interval
CNS	Central Nervous System
CPR	Civil Personal Registration
CRS	Civil Registration System
DBCG	Danish Breast Cancer Cooperative Group
DCR	Danish Cancer Registry
DNPreR	The Danish National Prescription Registry
DNRP	The Danish National Patient Registry
DNPR	The Danish National Pathology Registry
GC	Glucocorticoid
HR	Hazard Ratio
ICD	International Classification of Disease
NSAID	Non-Steroidal Anti-Inflammatory Drug
RR	Relative Risk
TNM	Tumor, Nodes, Metastases
SNOMED	Systematized Nomenclature of Medicine

Table of Contents

1. Introduction

Breast cancer was one of the first cancers to be described in the literature. Documents dating back to ancient Egyptian papyrus from 1600 B.C. and papers from Hippocrates in 460 B.C. refer to breast cancer as “bulging tumors” in the breast or ”excess of black bile” — and there was no cure [1]. The history of the disease up to modern times is cruel and includes disabling surgery without anesthetics and little chance of survival. Since the 1970’s prognosis after breast cancer treatment has improved facilitated by earlier detection via screening mammography, a multidisciplinary approach to cancer care, and more effective surgery and adjuvant treatments [2-4]. The disease is still the most common cancer among women in the western world and in 2009, accounted for 29% of incident cancers and 15% of cancer deaths among women in the Nordic countries [4].

In Denmark the incidence of breast cancer has increased by 1.5-2 % per year since the 1960’s [4] with a peak from 2008-2010 among 50-69 year old women due to the introduction of a national mammography screening program [5]. Today, almost 5000 women in Denmark are diagnosed with breast cancer each year. That means that 1 in 9-10 women will get the diagnosis during their lifetime. The incidence of breast cancer increases with increasing age up to approximately age 65 [4, 6]. Due to the aging population, breast cancer will increasingly become a disease affecting the lives of especially older women in Denmark [7].

Old-age survival has improved substantially during the past 70 years in developed countries [8] and the life expectancy of a newborn Danish girl today is 81.9 years [9]. Overall, the number of people aged 65 or older in Denmark is expected to increase from 16% today to 25% by 2042 [9]. Cancer is a disease of the elderly. Median age at the time of any cancer diagnosis in industrialized countries is around 70 years of age [10], among Danish women with breast cancer it is 63 years [11].

Why do elderly people have a higher risk of getting cancer? This may be due to several reasons — for example susceptibility of aging cells to environmental carcinogens and increased duration of carcinogenesis — but it might also be due to reduced immune function in the elderly — a phenomenon known as immunosenescence or immune-aging [8, 10]. This theory is debated and it is fair to wonder why the overall cancer incidence rates diminish after 80 years whereas immunosenescence continues to progress in the subsequent decades [12].

Though survival after breast cancer diagnosis continues to increase [11, 13] we still do not have the full understanding of the factors that determine who will survive their cancer disease-free and why.

This dissertation therefore aimed to examine different aspects of the potential associations between immune markers and in four epidemiological studies focused on age, immunosuppressive diseases, and immunosuppressive drugs and their potential impact on the clinical course of breast cancer.

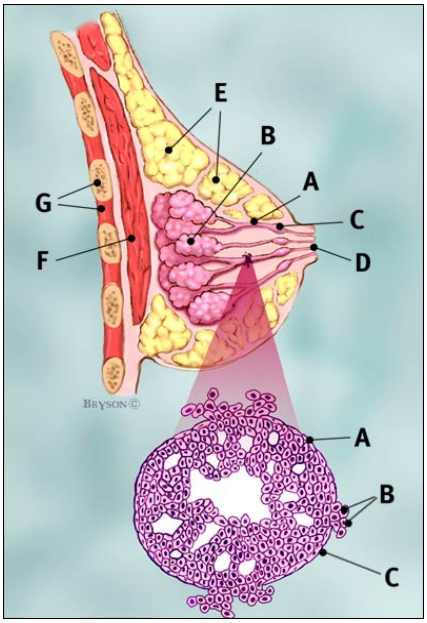
Study I examined the relation between age-at-diagnosis and the stage of breast cancer disease. Study II [14] examined the relation between autoimmune diseases (ADs) and breast cancer recurrence. Study III [15] and IV [16] examined the impact of glucocorticoids (GCs) on the clinical course of breast cancer; namely the risk of re-operation due to post-surgical bleeding in breast cancer patients, and the risk of breast cancer recurrence.

2. Background

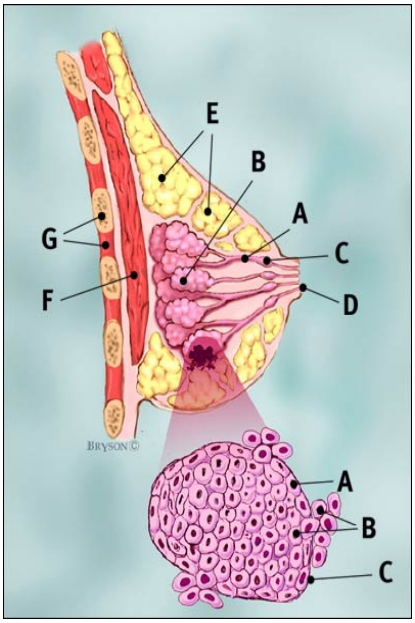
It is beyond the scope of this thesis to give a comprehensive review of breast cancer development and treatment, but, a short introduction is warranted.

2.1 Breast cancer types and treatment

When the term breast cancer is used in this dissertation and the associated articles it refers to a heterogeneous group of malignant tumors in the female breast [17]. The pathological development of the tumor divides into categories depending on the site of origin. The two main categories are ductal carcinomas (Figure 1.1) with origin in the epithelia cells in the milk ducts (app. 85 % of cases in Denmark) and lobular carcinomas (Figure 1.2) with origin in the epithelia in the lobules (app. 10% in Denmark) [18].



1. Ductal carcinoma



2. Lobular carcinoma

Figure 1. Pictures 1 and 2 illustrate where ductal carcinomas and lobular carcinomas develop in the female breast, respectively. Breast profiles: A: ducts, B: lobules, C: dilated section of duct to hold milk, D: nipple, E: fat tissue, F: pectoral major muscle and G: chest wall/rib cage. Enlargement: A: normal cell, B: ductal cancer cells (picture 1) or lobular cancer cells (picture 2) breaking through the basement membrane, C: basement membrane (Illustrations are used with permission from M.A.M.S. Mary Bryson) [17, 19].

Breast cancer tumors are further categorized according to tumor specific characteristics as for example estrogen receptor (ER) status, human epidermal growth factor receptor 2 (HER2) status, and tumor size. Based on tumor specific and individual patient characteristics and patient wishes, treatment is planned in a

multidisciplinary team. Treatment options include surgery, neo-adjuvant and adjuvant chemotherapy, radiation therapy, biological treatment with monoclonal anti-bodies against the HER2-receptor, and anti-hormone therapy [20].

2.2 Risk factors

Numerous risk factors for breast cancer have been identified and evaluated in the literature. Some factors, like sex, age, family history, and genetic predisposition are not modifiable, while other factors, like alcohol consumption, age at first child birth, obesity, and exposure to drugs containing hormones are modifiable and potentially amenable to public health interventions. Table 1 provides an overview of established breast cancer risk factors and the impact each factor has on the relative risk of the disease.

Table 1. Established risk factors for breast cancer in women and the magnitude of increased relative risk [21] (The table is adapted from "Breast Cancer Facts & Figures 2013-2014. Atlanta: American Cancer Society, Inc. 2013" [21]).

Relative Risk	Factor
>4.0	• Age (65+ vs. <65 years, although risk increases across all ages until age 80) • Certain inherited genetic mutations for breast cancer (*BRCA1* and/or *BRCA2*) • Biopsy-confirmed atypical hyperplasia • Lobular carcinoma in situ • Mammographically dense breasts • Family history of early onset (<40 years) breast cancer • Two or more first-degree relatives with breast cancer diagnosed at an early age
2.1-4.0	• Family history of breast cancer (40+ years) • High endogenous estrogen or testosterone levels (postmenopausal) • High-dose radiation to chest • One first-degree relative with breast cancer
1.1-2.0	• Alcohol consumption • Diethylstilbestrol (DES) exposure • Early menarche (<12 years) • Height (tall) • High socioeconomic status • Late age at first full-term pregnancy (>30 years) • Late menopause (>55 years) • Never breastfed a child • No full-term pregnancies • Obesity (postmenopausal)/adult weight gain • Personal history of endometrial, ovary, or colon cancer • Recent and long-term use of menopausal hormone therapy containing estrogen and progestin • Recent oral contraceptive use

2.3 Prognosis

Prognosis is estimating the risk of future outcome in individuals based on their clinical and non-clinical characteristics following the onset of an illness [22, 23]. Natural history refers to the course of un-treated disease, while clinical course refers to the course of a disease that has come under medical care and has been treated in different ways that affect the subsequent course of events [24]. In this dissertation prognosis refers to clinical course.

Cancer prognosis can be described in several ways. Fletcher and Fletcher summarized outcomes of disease as “the 5 D’s”; Death, Disease, Discomfort, Disability, and Dissatisfaction [25]. In breast cancer studies the 5 D’s could refer to for example survival [13], mortality [26], recurrence [16, 27, 28], complications to treatment [15, 29], and quality of life [30].

Breast cancer prognosis is determined by multiple prognostic factors. These include for example disease stage — in particular axillary lymph node involvement, histological type, tumor grade, hormonal receptor status, age-at-diagnosis, treatment, comorbidities, and co-medications [2, 16, 27, 31-34]. Some of these prognostic factors are sensitive to socioeconomic status, for example by differences in stage at diagnosis or determining the level of compliance to treatment [35, 36]. Prognostic factors should not be confused with risk factors, as demonstrated in figure 2, although one should note that some factors such as for example age might be related to both risk and prognosis.

In Denmark, the 5 year overall survival of women <70 years with breast cancer has improved from 65% for women diagnosed in the period 1977-1981 to 81% for those diagnosed in the period 2002-2006 [2]. The 5 year overall survival of Danish women of any age diagnosed with breast cancer improved from 70% in 1998-2000 to 75% (predicted) in 2007-2009 [13]. A similar pattern is seen in many other western countries [37]. However, breast cancer patients >70 years have not enjoyed the same survival benefits that younger breast cancer patients have garnered in the past decades [26, 38].

2.4 Etiology versus prediction

It is important to distinguish between differences in etiology and prediction [22]. Etiological studies are characterized by an *a priori* defined hypothesis about a potential causal association between exposure and outcome. A prediction study provides risk estimates and aims to predict patient outcome based on a number of prediction factors and variables that do not necessarily influence the outcome as a cause [22].

In this dissertation study II and IV are etiological studies as they examine potential association between exposure (AD and GC) and outcome (breast cancer recurrence). Despite the title of Study III, it is not designed as a classical prediction study, rather a cohort study, which aims to investigate the potential causal

association between exposure (GC) and outcome (re-operation due to bleeding) using stratification to control for a selected group of confounding factors, all of which changed the effect estimates by more than 10%. Each study type has strengths and limitations. The model/method is chosen based on the research question asked and the available data.

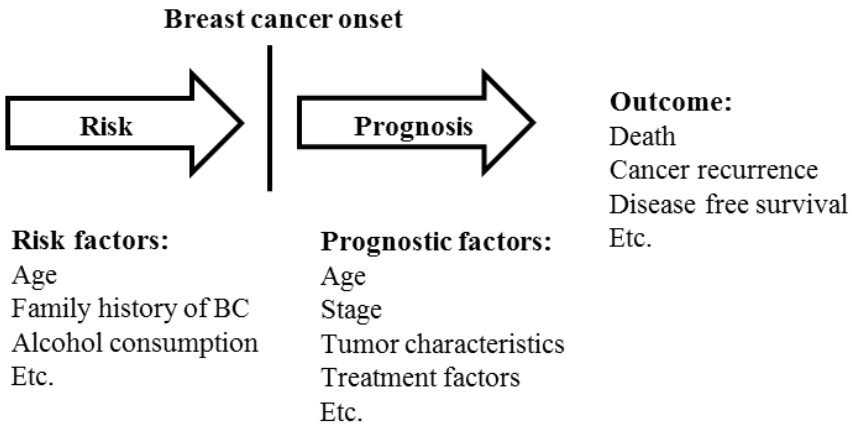


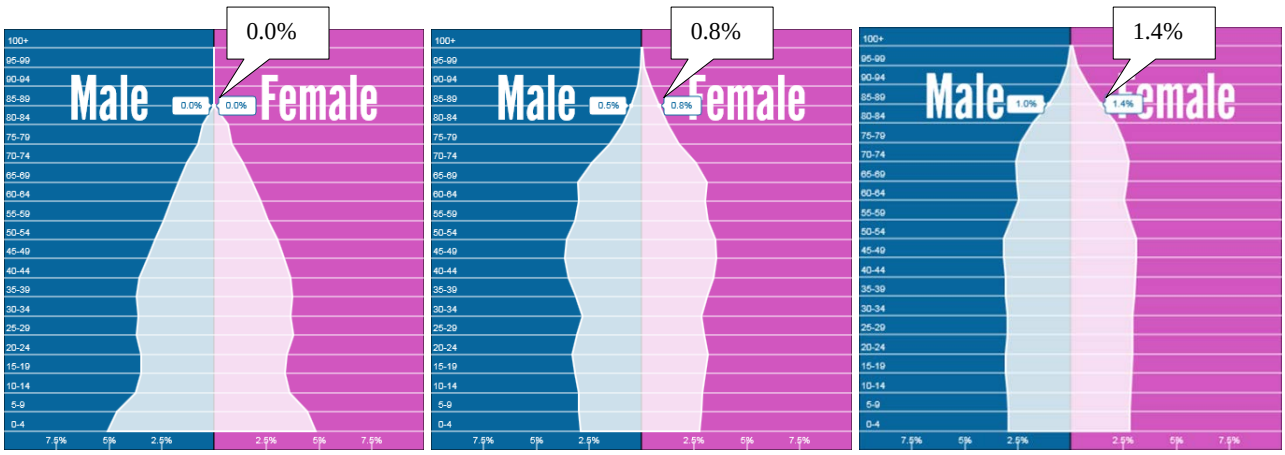
Figure 2. Concept of breast cancer risk, prognosis and outcome (the figure is inspired from [24]).

2.5 Aging population

Breast cancer affects women in a wide age range but incidence peaks among women in the 60s [10]. In Denmark, approximately 25% of breast cancer cases occur in women <50 years, and 20% of cases occur in women >75 years [2]. The incidence of breast cancer has continuously increased over time, largely due to population aging, but also due to changes in reproductive patterns, menopausal hormone use, and rising prevalence of obesity [21]. The incidence of breast cancer among 50-69 year old women peaked expectedly during the screening prevalence round, and thereafter returned to the same level as in the pre-screening phase.

Today, 16% of the Danish population is aged >65 years — age distribution prognosis predicts that around 2040 25% of the population will be >65 years (Figure 3) [9, 39]. In other parts of the western world the predictions of age-development and cancer incidence are similar to those in Denmark [40].

Due to the demographic development in the population of the western world over the past decades, breast cancer rates are likely to increase (Figure 4). More elderly women will likely present with breast cancer and comorbidities [41] and more women will survive breast cancer [13]. This will impose an increased burden on the health services [40, 42].



1. Year 1950 2. Year 2015 3. Year 2045

Figure 3. 1: Population pyramid showing the age distribution of Danes in 1950, *N*= 4.268.000. 2: Population pyramid showing the age distribution of Danes in 2015 *N*=5.661.000. 3: Population pyramid showing the projected age distribution of Danes in 2045 *N*=6.277.000 (Illustrations are used with permission from Martin De Wulf [39]).

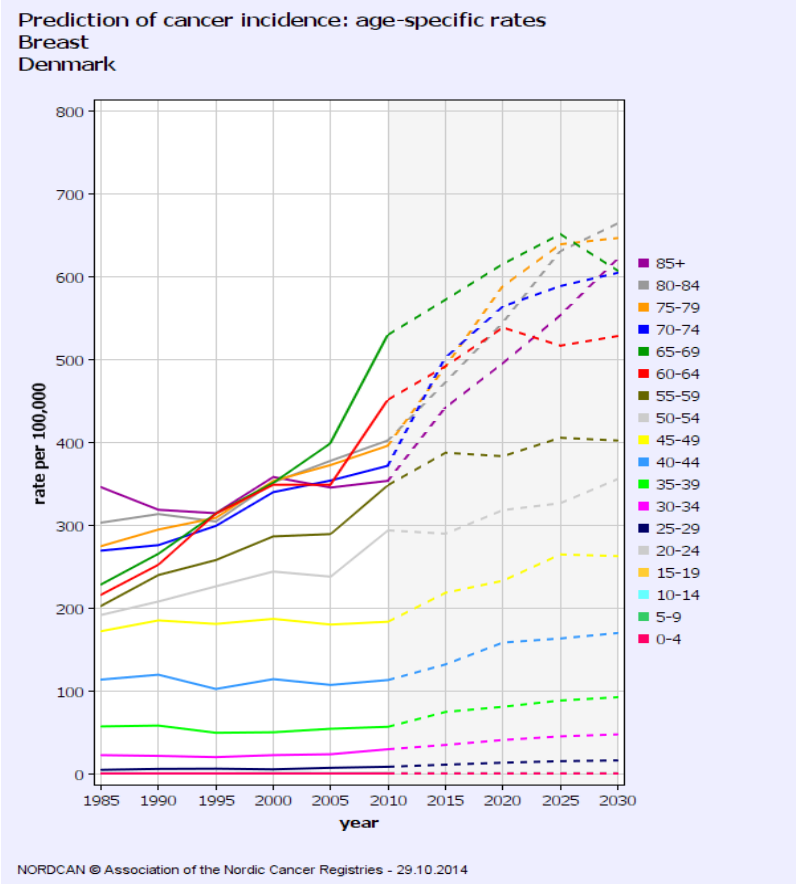


Figure 4. Projected rates of age-specific breast cancer incidence in Denmark through 2030 [3].

2.6 Immune function and cancer

The main function of the immune system is to maintain tissue homeostasis, to protect against pathogens and to eliminate damaged cells [43]. The immune system can broadly be divided into an innate and an adaptive component, with extensive crosstalk between them (Figure 5) [44]. The innate or inborn immune system provides an immediate and none-specific response to stimuli and builds no immunological memory. The adaptive or acquired immune system reacts slower but with a highly pathogen and antigen specific response that leads to immunological memory [44].

The immune system regulating in the initial protection of the body against cancer cells, but also mediates the process where the cancer cells breach the host's immune defense and become immunologically acceptable in the body allowing them to grow without elimination [45]. The interplay between the immune system's dual role in cancer prevention and cancer development has been described by Dunn *et al.* as the "three E's of Cancer Immunoediting" [45]. In this model, they describe three phases of tumorigenesis – Elimination, Equilibrium, and Escape. In short, during the elimination phase, the immune system detects and eliminates tumor cells. In the equilibrium phase, the tumor cell persists without expansion. In the escape phase, tumor cells can progressively grow evading immunosurveillance [45, 46]. It is suggested that transition from the elimination phase into the equilibrium and escape phases can be triggered by an impaired immune function; for example among people receiving immunosuppressive therapy [45]. This theory is supported by studies that suggest that cancer development [43, 47] and cancer progression [48] are associated with dysregulation or imbalance of the immune response. Any imbalance between the innate and adaptive immune function is thought to impact tumor progression [48].

In this dissertation we were interested in examining the potential association between markers of immune competence and the clinical course of breast cancer by using proxies for impaired immune function. When the term "marker" is used it refers to exposures – both iatrogenic (GCs) and intrinsic (age and ADs) — that are known to impair the body's immune competence.

The term "immune competence" is thought of as a description of how well the immune system functions in the body. As described below, age, ADs, and GCs all disturb homeostasis making the body less resistant to disease. We describe this as impaired immune competence.

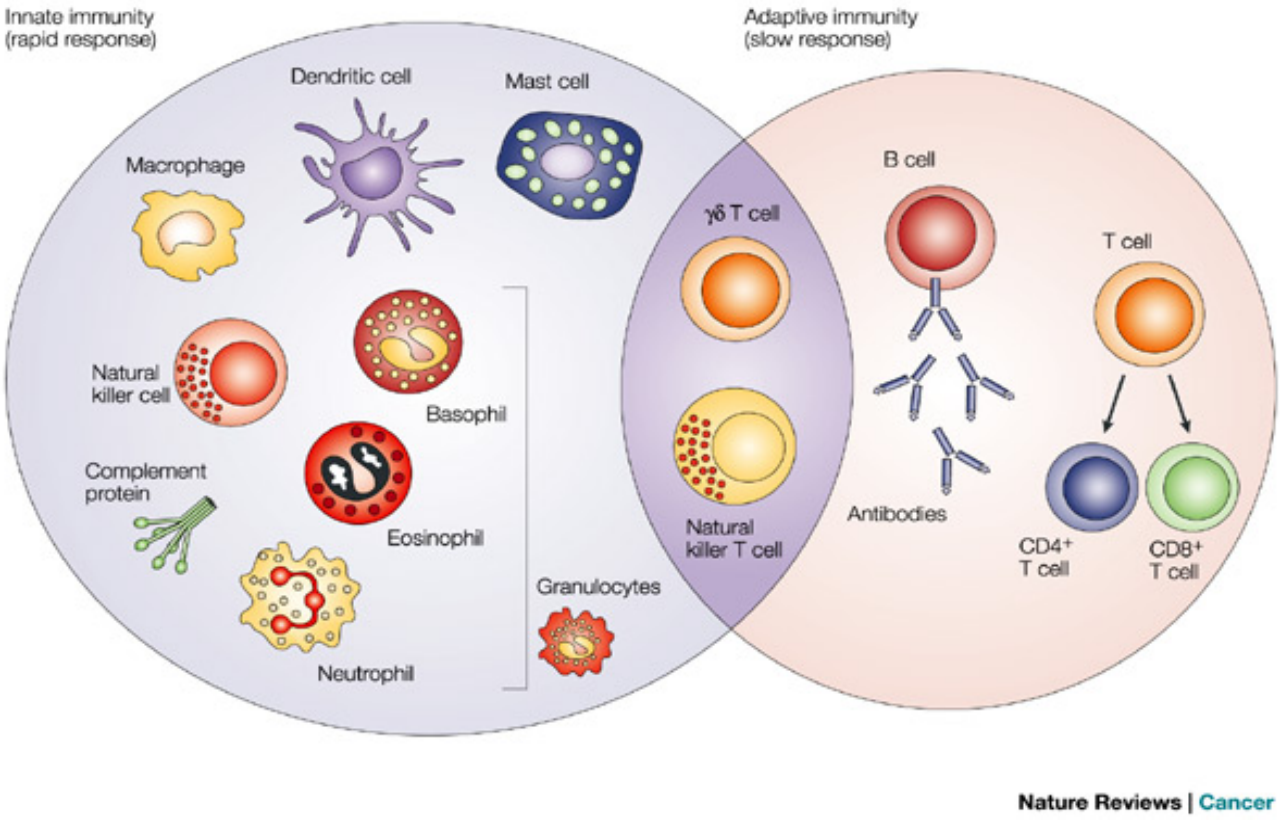


Figure 5. Overview of cell types representing the innate and adaptive immune system. Immunosenescence is mainly driven by alterations in the T-cell line. (Illustration used with permission from Professor Dranoff [44]).

2.7 Aging immune system

From approximately age 65 the balance in the immune system distorts. The changes known as immunosenescence, or immune-aging, represent a continuum of changes that are related to age-related pathology [8, 10]. This dissertation does not aim to review the biological complexity and the controversies behind the term immunosenescence, however, we will provide a brief introduction to the phenomenon.

Age-associated immune alterations have been related to the increase of infections, tumors, and ADs in the elderly population [46, 49]. Also, there is an emerging understanding that the geriatric syndrome of frailty could in part be characterized by inflammatory mechanisms and changes in immune competence associated with aging [8, 46].

Some of the changes in the aging immune system are based on inevitable physiological changes that accompany aging, such as the involution of thymus leading to a decrease in naïve T-cells [10, 49] — cells

that differentiate into various T-helper (Th) cells with distinct biological functions while passing by the thymus [50]. Other changes like the chronic low-grade inflammation or "inflammaging" in the elderly are related to partly modifiable factors such as diet and exercise and partly to the shift in distribution of immune cells [8, 10, 49].

The changes in the distribution of immune cells affect primarily the adaptive immune system and have a smaller effect on the innate immune system [49]. Besides the lower production of naïve T-cells in the adaptive immune system, the T-cell balance shifts from dominating cell-mediated immunity (Th-1 cells attacking mainly intracellular pathogens) toward a humoral immune response (Th-2 cells attacking mainly extracellular pathogens).

The net effect of all the age-related changes in immune competence on cancer occurrence and prognosis is not well understood [43]. Consequences of immunosenescence on breast cancer risk and prognosis are debated and no consensus has been established. Some experimental studies suggest that responses to chemotherapy and radiotherapy may require an intact immune system [10, 51, 52] while other experimental studies show that chemotherapy effectively targets tumor cells, regardless of the condition of the adaptive immune system [53].

As the majority of women with a breast cancer diagnosis are 65 years and older, it is important to investigate whether age-related changes in immune function could impact the clinical stage and thereby the clinical course of breast cancer. Based on the above description and previous studies [54], we have used age ≥70 as a proxy for impaired immune function in study I.

2.8 Autoimmune diseases

ADs comprise a large group of heterogeneous diseases in which immune function is misdirected so it attacks the healthy organs that the immune system is meant to protect [55] – the adaptive specific immune system confuses self with non-self. AD can affect either single or multiple organ systems [55]. The prevalence of AD is 5–10% in industrialized countries, and women are affected approximately 10 times more often than men [56].

We have used diagnosis of AD as a proxy for impaired immune function in study II. Patients with ADs may have compromised immune function due to intrinsic changes in the immune system, immunosuppressive drugs [55], or both [57]. As described above, there are mechanisms by which compromised immune function may increase or decrease risk of breast cancer and breast cancer outcome (see section 2.11.2). Some studies have suggested a protective effect of ADs on breast cancer risk (see section 2.11.2), while the effect of impaired immune function due to AD on breast cancer recurrence is not known.
In this dissertation the term AD includes a group of 30 selected diseases. The diseases are included after

review of the literature within this field [55, 56, 58] and evaluation of the diseases with specialist colleagues in the six chosen categories where the diseases are classified due to the organ system or tissue of origin i.e. 1. Non-malignant hematological diseases, 2. endocrine diseases, 3. central nervous system (CNS) /neuromuscular system diseases, 4. gastrointestinal/hepato-biliary diseases, 5. skin diseases, and 6. connective tissue diseases (Appendix 1).

2.9 Glucocorticoid

Cortisol is a naturally occurring physiologic GC synthesized in the adrenal cortex as a part of the hypothalamic-pituitary-adrenal axis [59, 60]. It acts to maintain homeostasis in the body. Synthetic GCs belong to the same steroid superfamily as estrogens, which are known to play a role in breast cancer development [61]. They are a class of steroid hormones frequently prescribed for their anti-inflammatory properties and can be administered via numerous routes [62, 63].

GCs have strong anti–inflammatory effects in situations of ongoing inflammation. As such, GCs are standard treatment in many acute and chronic inflammatory diseases, ADs, and lymphoid malignancies [60]. Their role in the immune system is less well understood but seems to have dual actions [60]. GCs prepare the innate immune system for rapid activation and enhance a pro–inflammatory action. In contrast, GCs are thought to repress the adaptive immune system and help restore homeostasis by exerting anti–inflammatory effects [60]. Therefore, when the immune response reacts to, for example, bacterial infection, the pro- or anti-inflammatory effect may depend on the phase of the response in which the GC is administered [60] and the dose introduced [64]. Moreover, GCs are used to treat lymphoid cancers where they induce apoptosis. However, some of these cancers develop resistance to GC and the mechanism behind this is largely unknown [65] .

In women with breast cancer, GCs are often used to prevent surgery-induced and chemotherapy-induced nausea and emesis [66-68].

The prevalence of GC use increases with age, due to a higher prevalence of multi morbidity among the elderly population [69]. Unfortunately, GCs can induce many serious side-effects when used in high doses or over long time periods [64]. The side-effects can be grouped into eight categories [62] (Table 2).

We used prescription of GCs as a potential predictor of breast cancer surgery outcome in study III and as a proxy for impaired immune function in study IV.

Table 2. Overview of glucocorticoid side-effect categories (Table based on [62])

	Category	Examples of side effects
1	Impairment of the hypothalamic-pituitary-adrenal axis function	Impairment of the body's own production of cortisol making the patient vulnerable to e.g. stress in connection with surgery
2	Iatrogenic hyper-corticism	Osteoporosis, striae cutis, dys-regulated diabetes, blurring of infection symptoms, activation of latent infections, Cushingoid fat distribution and psychiatric symptoms
3	Pseudotumor cerebri	Headache (mainly in children)
4	Steroid pseudoreumatism	Diffuse muscle and joint pain
5	Impairment of the protein synthesis	Leading to impaired wound healing, fragile vessels, osteoporosis, and skin and muscle atrophy
6	Inhibition of height growth in children	
7	Changes in glucose metabolism and lipid metabolism	Hypercholesterolemia and
8	Changes in the hematopoietic system	Increased destruction of blood cells and impaired production of new blood cells

2.10 Literature search strategy

The literature search strategy aimed to identify English or Scandinavian language literature related to the four included studies. The database PubMed was searched for studies published up to and including November 2014.

Medical Subject Headings (MeSH) and "free text" were used in combinations to build a structured literature search. The following terms were used in combination:
"Breast Neoplasms" [Mesh], "breast cancer" (free text), "Lymph node" [Mesh], "Lymph node" (free text), "Autoimmune Diseases" [Mesh], "Glucocorticoids" [Mesh], "Glucocorticoids" (free text), "Recurrence" [Mesh], "Outcome" (free text), "Reoperation" [Mesh], "Reoperation" (free text), and "Immunosenescence" (free text).

There was an overwhelming amount of literature on several associations and based on review and applied limitations the main literature was narrowed down. On PubMed we filtered the results to include articles with an abstract in English, human research, reviews, and articles published within the past 10 years.

Other sources of literature were found on web pages of official health authorities, health care organizations, patient organizations, and in the reference list of retrieved literature. Further, books and digital literature recommended by supervisors and collaborators were reviewed.

2.11 Existing literature and limitations

2.11.1 Study I (Age and lymph node)

In a 2009 issue of the Journal of Clinical Oncology, Wildiers *et al.* suggested that until age 70 years, increasing age was associated with a decreasing proportion of breast cancer patients diagnosed with positive lymph nodes [54]. However, among women aged over 70 years, increasing age was associated with an increasing proportion of breast cancer diagnoses with positive lymph nodes even in women with small tumors. The trend from the curve on prevalence of lymph node involvement by Wildiers *et al.*[54] shows approximately prevalence 0.58 at 30 years, 0.35 at 65 years, and 0.5 at 90 years. Wildiers *et al.* hypothesized that the increasing proportion of node-positive tumors with increasing age may be explained by declining immunocompetence i.e. immunosenescence, in the older women [54]. In this study, only women with pathologically determined lymph node status were included [70, 71] and the excluded group of women was not described. Critics of the study argued that the increase in lymph node involvement in elderly women might be explained by an unequal age distribution of women who get a full stage evaluation at time of breast cancer diagnosis. Here the older women are missing out. Further differences in choice of surgery in elderly women, less access to screening programs, or different pathology regarding estrogen receptor status and other tumor characteristics are mentioned as possible explanations of the result [70, 71].

Lymph node status at diagnosis is an important prognostic factor of breast cancer outcome [32, 72, 73]. We already know some factors related to the prevalence of positive lymph nodes in registered cohorts, such as use of screening [74], choice of surgical methods [75], and perhaps also calendar time. Advanced age in breast cancer is associated with favorable biological features of the tumor — such as hormone sensitivity — but also with less aggressive treatment [7].

Several studies [33, 76-78] including a Danish study [79] have shown that older women are less likely to get a full lymph node evaluation at time of diagnosis.

Based on the theory by Wildiers *et al.*[54], we aimed to describe the relation between age-at-diagnosis and proportion of lymph node positive breast cancer, by replicating and improving [80] the Belgian group's study by taking the characteristics of the women with no registered evaluation of axillary lymph node status into account. More knowledge about whether older women with suppressed immune function present at a later stage at diagnosis is of importance for understanding the role of immune function in breast cancer survival. Immunosenescence could be one explanation of why older women with breast cancer have not enjoyed the same gains in survival that younger women with breast cancer have garnered in the past decades.

We have therefore conducted a descriptive study with prospectively collected data from a large cohort of Danish women with breast cancer.

2.11.2 Study II (Autoimmune diseases and recurrence)

Many reports indicate that AD diagnoses are associated with an altered risk of cancer development [57, 81-85]. Regarding breast cancer, the overall weight of the evidence suggests a protective effect of ADs on breast cancer risk [55, 82, 86]. The mechanism behind this remains unknown, but it has been speculated that immunosuppressive medications could lead to earlier menopause and thereby lower risk of breast cancer [55]. Further in some — but not all — studies, NSAIDs, which are commonly used to treat ADs, show protective effects on breast cancer risk and recurrence [55, 87-89]. As described in the background section, impaired immune function has also been associated with increased risk of cancer and cancer progression.

To date, only one epidemiological study has examined breast cancer prognosis among patients with ADs as a group [55]. Moreover, the research has been mainly focused on selected AD types [90-93].

Therefore, we aimed to investigate the potential association between AD and the risk of breast cancer recurrence using a large cohort of women with breast cancer registered in Danish national population-based and medical registries.

2.11.3 Study III (Glucocorticoid and re-operation due to bleeding)

Almost all breast cancer patients undergo surgery, either breast conserving surgery, mastectomy, or infrequently, both [41]. Although re-operation due to postsurgical bleeding is a rare complication, it delays hospital discharge, usually requires general anesthesia, and therefore is associated with substantial costs to both the patient and healthcare system.

An earlier study from our group found an increased adjusted risk of re-operation due to post-surgical bleeding among current users of selective serotonin reuptake inhibitors [29].

Some side effects associated with use of GC can impact post-operative complications in patients, for example impaired wound healing, fragile skin and vessels, and blurred infection symptoms that delays relevant antibiotic treatment (see background section 2.9) [62, 94].

Animal models have shown that even a single dose of dexamethasone, a highly potent GC, can delay wound healing [95]. Despite this, few population-based studies, with conflicting results, have investigated the impact of GC use on postoperative complications [96-98].

Age-related alterations in the coagulation and immune system are thought to influence wound healing. Most theories about wound healing and bleeding are based on animal studies, as human studies tend to have a high

degree of confounding problems such as differences in nutrition and vascular insufficiency and also difficulty in identifying identical wounds [99]. A reduced ability to heal wounds could increase the risk of the wound re-opening and bleeding after an operation. Experiments show that the inflammatory phase of wound healing (Figure 6) among elderly people is accompanied by a decline in macrophage function leading to slower wound healing. Next, cell proliferation is affected by aging both because of declined fibroblast proliferation and migration to the wound. The rate of epithelialization of open wounds is therefore slowed in older individuals compared to that in young. Aging is furthermore associated with significantly reduced levels of wound matrix constituents, including collagen, basement membrane components, glucosaminoglycans, and fibronectin leading to decline in anastomotic strength and collagen metabolism [99]. In contrast, cancer is associated with a hyper-coagulant stage. This results in clotting activation, which may play a role in tumor progression. As such, research suggests that treatment with anti-coagulants may reduce cancer spread [100].

Surgery wound →Coagulation →Inflammation →Fibroplasia →Remodeling

Figure 6. Overview of wound healing phases. The in vivo process is not sequential but rather different processes working simultaneously and this division into four stages is only to clarify a complex process [99].

Despite the high incidence rate of breast cancer, the frequent use of GCs, and the risk of post-operative bleeding, no study has investigated GC as potential predictor of the risk of post-surgical bleeding. We therefore aimed to conduct a large population-based cohort study with prospectively collected data to examine the potential association between GC and post-surgical bleeding in a population-based cohort of Danish breast cancer patients.

2.11.4 Study IV (Glucocorticoid and recurrence)

Given their immunosuppressive effects, use of GCs may promote tumorigenesis by facilitating tumor cell evasion of immune surveillance [101, 102]. We previously found no evidence of an effect of GCs on breast cancer risk [103, 104]. However, GCs are often used to manage side effects of breast cancer treatment (see section 2.9). It is therefore important to increase understanding of the potential effects of GCs on breast cancer cell growth after breast cancer diagnosis as this has not been fully elucidated [101, 105]. If GCs increase the risk of breast cancer recurrence, this has important clinical implications when deciding the most appropriate treatments for patients with breast cancer and a need for GC treatment due to other diseases or

conditions. A laboratory-based study of human breast cancer cells found that treatment with GCs induced a better prognostic profile in ER-negative tumor cells (cells became more differentiated and less invasive), but not in ER-positive cells, compared with untreated ER-negative and ER-positive cells, respectively [106]. In contrast, GCs have also been shown to inhibit the cytotoxic effects of chemotherapy in human breast cancer cell culture models and these studies have raised concern about the safety of GC use in breast cancer treatment [107-109]. Previous phamaco-epidemiological studies by our group have shown a protective effect of simvastatin on breast cancer recurrence [27] but no effect of beta-blockers or other antihypertensives on breast cancer recurrence [28].

However, to our knowledge, the impact of GCs on breast cancer prognosis has never been investigated. We therefore planned to investigate the potential association between GC use and breast cancer recurrence in a large population-based cohort of breast cancer patients, using high-quality clinical data with complete follow-up.

2.12 Hypotheses and objectives

Based on the described background knowledge we decided to evaluate markers of immune competence and the clinical course of breast cancer. Increasing age, AD diagnoses, and prescriptions of GCs are used as proxies for altered immune function. We had the following hypotheses and objectives:

2.12.1 Study I (Age and lymph node)

Hypothesis: Increasing age relates to declining immune function and is associated with increasing proportion of lymph-positive disease at breast cancer diagnosis.

Specific aim: To replicate and improve the Wildiers *et al.* study [54] by evaluating whether the prevalence of node-positive disease among all women with breast cancer decreases to age 70 years, and then increases after age 70 years.

2.12.2 Study II (Autoimmune diseases and recurrence)

Hypothesis: AD diagnoses impair immune function and are associated with an increased risk of breast cancer recurrence.

Specific aim: To evaluate whether women with breast cancer and an AD have an increased risk of breast cancer recurrence compared with breast cancer patients who do not have an AD.

2.12.3 Study III (Glucocorticoid and reoperation due to bleeding)

Hypothesis: Use of GC is associated with the risk of reoperation due to post-surgical bleeding.

Specific aim: To evaluate if prescription of GCs is associated with re-operation due to post-surgical bleeding in women undergoing surgery for breast cancer.

2.12.4 Study IV (Glucocorticoid and recurrence)

Hypothesis: Use of GC impairs the immune function and is associated with a higher risk of breast cancer recurrence.

Specific aim: To evaluate whether prescription of GCs among women with a breast cancer diagnosis increases the risk of breast cancer recurrence compared with breast cancer patients without prescription of GCs.

The four studies in this thesis are based on data linked on an individual-level from medical and population-based registries in Denmark.

3. Materials and methods

3.1 Setting

The studies were nested in partly overlapping cohorts of women with an invasive breast cancer diagnosis in Denmark. The country counts ~ 5.6 million inhabitants including ~2.8 million females. The National Health Service provides tax-supported high-quality health care to the Danish population, including access to hospital care and partial reimbursement for prescribed medications. Denmark has a long tradition of collecting administrative and medical data in national registries. Today, there are more than 60 medical registries collecting disease-specific and personal information from Danish citizens [110]. Data in registries together with the comprehensive administrative system make Danes a well described population that is very suitable for epidemiological studies [111, 112].

3.2 Data sources

Data used in this dissertation were retrieved from population-based administrative and medical registries, and linked by a unique registration number (Figure 7). Relevant diagnostic codes and drug codes used are listed in Appendices 1–7.

The Civil Registration System (Study I, II, III, and IV)

The Civil Registration System (CRS) is the key to epidemiological research in Denmark. Since 1968, a 10-digit unique civil personal registration (CPR) number has been assigned to all Danish residents at birth or immigration, permitting unambiguous individual-level data linkage across Danish medical registries [111]. This database is updated daily and contains information about vital status and migration.

The Danish Breast Cancer Cooperative Group (Study I, II, and IV)

Since 1977, the Danish Breast Cancer Cooperative Group database (DBCG) has prospectively registered nearly all invasive breast cancers diagnosed in Denmark [74, 113-115]. The registry includes clinical data from pathological departments, breast surgical departments, and oncology departments all over Denmark. Completeness of breast cancer registration by the DBCG has improved over time, from 87% in 1986 [115] to approximately 94% in 2013 [116] when compared to the Danish National Pathology Registry (see below).

The Danish National Pathology Registry (Study I)

The Danish National Pathology Registry (DNPR) was founded in 1997 when several local registries were merged to a national registry and reporting from all pathological departments in Denmark became mandatory. The registration of pathology specimen characteristics is complete since 1997 [117].

The Danish National Patient Registry (Study II, III, and IV)

The Danish National Patient Registry (DNRP) has registered diagnoses on all non-psychiatric hospitalizations since 1977 and outpatient contacts since 1995. Since 2003 data from Danish private hospitals were added to the registry. Today, the private hospitals in Denmark account for approximately 2% of the total hospital activity counted in admissions and cost [118]. With the CPR number as the basis, the DNRP records the date of each hospital visit, duration of hospital stay, site of stay, procedures, and discharge diagnoses entered by physicians [119]. The diagnoses are recorded according to World Health Organization's (WHO's) *International Classification of Diseases* (ICD) version 8 until 1994 and version 10 since then.

The Danish National Prescription Registry (Study IV)

The Danish National Prescription Registry (DNPreR) is maintained by Statistics Denmark and has automatically recorded detailed information on all prescriptions redeemed at Danish pharmacies since 1995 [120]. The registry holds detailed information about dispensed prescriptions, including full Anatomical Therapeutic Chemical (ATC) codes, date, and quantity dispensed.

The Aarhus University Prescription Database (Study III)

The Aarhus University Prescription Database (AUPD) tracks prescriptions for reimbursed drugs redeemed at pharmacies located in the North and Central Denmark Region by residents in these regions. These regions cover a population of ~1.8 million inhabitants — approximately 33% of the Danish population [110].

The Danish Cancer Registry (Study III)

The Danish Cancer Registry (DCR) has recorded malignant neoplasms in Denmark since 1943. It is based on notifications from departments, specialists, and autopsy reports [121, 122]. In 1987 reporting became mandatory for all Danish doctors. Available data includes date of cancer diagnosis, cancer type and site, primary histology, and tumor spread at diagnosis. Since 2004 the registry has included information about TNM staging [110].

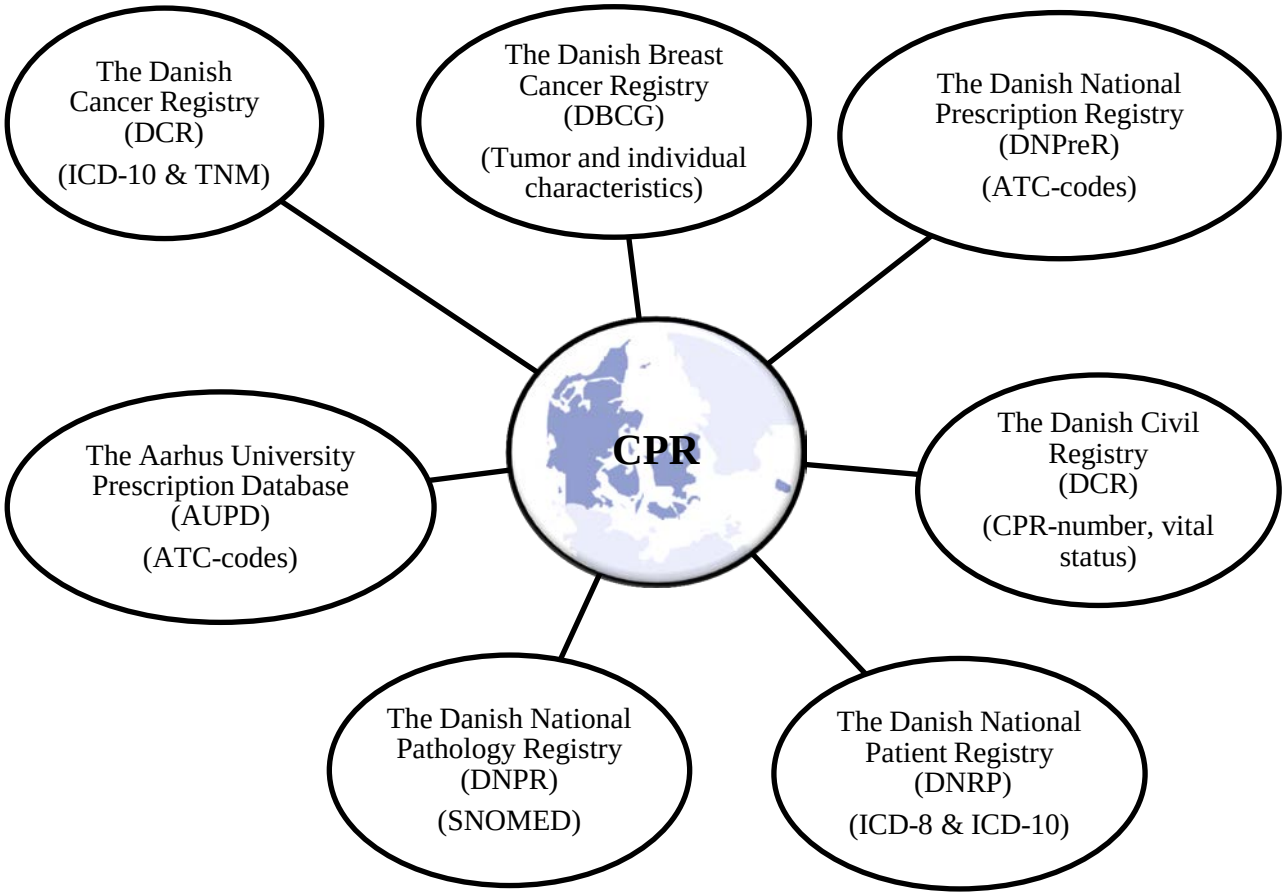


Figure 7. Overview of the registries used in this dissertation, registry abbreviations, and the main variables used in the studies.

Abbreviations: CPR, civil registration number; ICD, International Classification of Diseases; ATC, Anatomical Therapeutic Chemical Classification System; SNOMED, Systematized Nomenclature of Medicine.

3.3 Study design

Study I is a descriptive study. Study II is a population-based etiological cohort study. Studies III and IV are population-based pharmacoepidemiological cohort studies (See section 2.4).

3.4 Study populations

In study I (Age and lymph nodes) we enrolled all women ≥18 years old with an invasive breast cancer diagnosis registered in the DBCG or DNPR in the period 2000–2013 (Appendix 2). We defined the index date as the date of breast cancer diagnosis in the DBCG registry or the date of diagnosis in the DNPR among the 6.5% of women only registered in this registry. The DBCG registry validates their pathology data using the DNPR [116].

In study II (Autoimmune diseases and recurrence) we enrolled all women ≥18 years old with a stage I–III breast cancer diagnosis in the DBCG registry between 1 January 1980 and 31 December 2007. Data were merged with information about this cohort from the DNRP. Index date was 60 days after date of primary breast cancer surgery.

In study III (Glucocorticoid and reoperation due to bleeding) we enrolled all women ≥18 years old with a stage I–III breast cancer diagnosis who received mastectomy or a breast conserving surgery recorded in the DNRP between 1 January 1996 and 31 December 2009. Data were merged with information from AUPD. Index date was date of primary breast cancer surgery. A sub-cohort diagnosed between 1 January 2004 and 31 December 2008 was merged with data from the DCR to evaluate the impact of tumor size on the risk of post-surgical bleeding.

In study IV (Glucocorticoid and recurrence) we enrolled all women ≥18 years old with a stage I–III breast cancer diagnosis in the DBCG registry in the period 1 January 1996 to 31 December 2003. Index date was date of surgery. Data were merged with information about the cohort from the DNDRP and the DNRP.

3.5 Main exposures

The exposure in study I (Age and lymph node) was age-at-diagnosis.

The exposure in study II (Autoimmune diseases and recurrence) was presence or absence of one or more of 30 selected ADs. To achieve accurate exposure time, follow-up time was split on date of AD diagnosis so unexposed and exposed time for each woman could be calculated. If AD was diagnosed before date of breast cancer surgery or within the first 60 days after surgery, follow-up time would begin on day 60 after first surgery. If a woman had AD diagnosis more than 60 days after surgery, her person-time between day 60 after surgery and AD diagnosis would be categorized as unexposed person-time, and her person-time from date of AD diagnosis forward would be categorized as exposed person-time. If a woman had more than one AD diagnosis, the date of the first registered AD would be used. If a woman had more than one diagnosis registered as the first AD diagnosis, the diagnosis with the highest frequency among the first diagnoses was chosen. A woman was regarded as exposed from the start of the exposure until end of follow-up (see section 3.7).

The exposure in study III (Glucocorticoid and reoperation due to bleeding) was redeemed prescription of GCs (for specific ATC codes see Appendix 3). The drugs were categorized in two ways. First, we classified the drugs according to never or ever use. To make a sensitivity analysis, we further categorized the drugs according to the temporality of use: current users (any prescription for systemic GCs within 90, 180, or 360 days before initial breast cancer surgery) and former users (prescription for systemic GCs only more than 90, 180, or 360 days before initial breast cancer surgery).

The exposure in study IV (Glucocorticoid and recurrence) was redeemed prescription of GCs. The drugs were categorized in several ways. First, we classified GC use as a time-varying dichotomous variable updated yearly after breast cancer surgery. The 1-year lagged exposure time model has been used in previous studies by our group [27, 28]. It was added to allow the effect of the drug to accrue, to allow for a reasonable induction period for an effect of the drugs and co-prescriptions on recurrence, and guarded against potential weakening of the effect and the possibility that imminent recurrence affected prescription patterns [123, 124]. In each yearly interval, women were classified as exposed to GCs if they had at least one prescription registered in the DNPreR with an ATC code corresponding to a systemic, inhaled, or intestinal-acting GC (for specific ATC codes see Appendix 5). Women who were prescribed a GC were assumed to be exposed, and women who did not redeem a GC prescription were classified as non-users. GCs were further categorized according to three sub-groups based on route of administration: systemic (pills and injections), inhaled (inhalants), and intestinal-acting (foam and suppositories).

Prednisolone-equivalent cumulative doses were used to perform dose-response calculations for systemic GCs, based on the methods of Sørensen *et al*. [104]. The cumulative dose was calculated as the product of the number of pills (or injections) dispensed, the dose per pill (or injection), and the prednisolone-equivalent conversion factor associated with each prescription's ATC code [104]. These values were aggregated and updated in each follow-up cycle according to the following categories of use: non-use, 1-999 mg, 1000-4999 mg, or ≥5000 mg. Duration of GC use was estimated by the cumulative number of years exposed to GC, ranging from 0-10 years.

3.6 Main outcomes

In study I, the main outcome was the presence of at least one positive axillary lymph node at diagnosis. Data about lymph node status were categorized in three groups: (1) known lymph node positive, according to pathologic record (2) known lymph node negative, according to pathologic record, and (3) no registered pathologic lymph node status, but confirmed diagnosis of invasive breast cancer. It is likely that this last group of women with "unknown lymph node status" had node status evaluated by surgery, palpation, or ultrasound of the axilla. In other words, though not registered, they likely received a clinical lymph node evaluation, but this lymph node status was not pathologically evaluated or registered.

In study II and IV, the main outcome was breast cancer recurrence from index date and up to a maximum of ten years after diagnosis. Recurrence was defined according to the DBCG criteria as any local, regional, or distant recurrence, or cancer of the contralateral breast [114].

In study III, the main outcome was reoperation due to post-surgical bleeding within 14 days of primary breast cancer surgery (see Appendix 4 for ICD codes). Almost 80% of operations due to post-surgical bleeding were performed within the first 14 days after primary surgery;

3.7 Follow-up

In study II, the cohort was followed from 60 days after index date (date of primary breast cancer surgery) until breast cancer recurrence, death, emigration, 10 years of follow-up or 31 December 2009, whichever came first.

In study III, the cohort was followed from date of primary breast cancer surgery until reoperation due to post-surgical bleeding or 14 days of follow-up, whichever came first.

In study IV, the cohort was followed from the date of primary breast cancer surgery until breast cancer recurrence, death, emigration, 10 years of follow-up or 31 December 2009, whichever came first.

3.8 Statistical methods

In all four studies, we used frequency tables to show the baseline characteristics of the women in the cohorts. In all studies we presented patient and treatment characteristics according to the exposure — lymph node status in study I, AD in study II and GC prescriptions in study III and IV. In study II and IV we also presented tumor characteristics.

3.8.1 Graphics (Study I)

In study I, we computed a stacked bar chart showing the distribution of age-at-diagnosis and axillary lymph node status. We further computed an area chart with age-at-diagnosis on the x-axis and proportion of lymph node status at time of diagnosis on the y-axis. The proportions in this chart are smoothed across five years, with weights of 1, 2, 3, 2, 1 assigned respectively to the first through fifth years and the mid-year used as the plotting point. Finally, we made a trend line to describe the development of lymph node status distribution from 2000-2013 and stratified the trend line figures by age <70 years and ≥ 70 years at diagnosis.

3.8.2 Cox regression (Studies II and IV)

In study II, we computed 10-year recurrence hazard ratios (HR) and 95% confidence intervals (95% CI) for exposure to any AD and exposure by AD categorized by organ or tissue of origin in unadjusted and multivariable Cox regression models. In all models, competing risk of death was taken into account (See section 6.3.8) [125].

In study IV, we computed 10-year recurrence HRs and 95% CI for the three GC groups (systemic, inhaled, and intestinal-acting) in unadjusted and multivariable Cox regression models, with medication exposures characterized as time-varying covariates lagged by 1 year. Exposure of GC and recurrence was handled as a dichotomous variable in each exposure year. We lagged GC exposure by 1 year to allow the effect of the drug to accrue. Accordingly, GC exposure in the year before surgery was modeled for its association with recurrence in the first year after surgery; GC exposure in the first year after surgery was modeled for its

association with recurrence in the second year after surgery. This procedure was followed for the whole follow-up period. The lagged exposure time allowed for a reasonable induction period for an effect of GC and co-prescriptions on recurrence, and guarded against the possibility that imminent recurrence affected prescription patterns.

We repeated the unadjusted and multivariable lagged Cox regression models to estimate the 10-year HR of recurrence and 95% CI for equivalent cumulative dose categories, using nonusers as the reference group, and to measure the cumulative number of years exposed to GC, and the rate of breast cancer recurrence. All multivariable Cox regressions were restricted to women with no missing information about any potential confounders.

In both study II and IV we tested the proportionality of hazards by evaluating the significance of the interaction between exposure and the logarithm of person-time, and saw no evidence of a departure from proportionality.

3.8.3 Logistic regression (Study III)

In study III, we investigated the potential association between GC and the risk of post-surgical bleeding. We collected information about a large number of potential confounders of this association. We tabulated contingency tables for the main variables from which we calculated the risk of reoperation due to post-surgical bleeding according to use of GCs. We computed the crude risk difference and risk ratio and their 95% CI when estimating the association between systemic acting GC prescription and post-operative bleeding. We then stratified the contingency tables according to each of the possible confounding variables to examine the strength of association and fitted multiple logistic regression models to the data to compute the odds ratio and associated 95% CI controlling for confounders. Given that re-operation for post-surgical bleeding was rare in all combinations of the independent variables, these adjusted odds ratios provided an estimate of the adjusted risk ratios (aRR). Only age and surgery type changed the estimate by more than 10%, and they were of *a priori* interest, so we stratified by these two variables to clarify the impact the individual variable had on the estimate, and adjusted for no other variables in the final model.

3.8.4 Stratified analysis

In study III, we found that only surgery type (mastectomy or breast conserving surgery) and age (more or less than 80 years of age) changed the estimates by more than 10% (effect measure modification, see section 6.3.9). We decided that a 10 % change was a sufficient impact on the result and, guided by the results, we stratified by these two variables to control confounding [126].

In study IV, we included analyses stratified by treatment with chemotherapy (yes or no) and ER status (positive or negative) to evaluate whether the association between the exposure and outcome varied in

subgroups. Women who receive adjuvant chemotherapy are at higher risk of recurrence and also receive substantial doses of unmeasured GCs while hospitalized. Also, a previous experimental study suggested that ER status was important in relation to the potential effect of GCs on breast cancer recurrence [106].

Analyses were conducted with SAS version 9.3 (SAS institute Inc., Cary, NC, USA), and Stata 11 (StataCorp, College Station, TX, USA).

3.9 Confounders

We considered potential confounders as variables that are associated with the outcome, not in the causal pathway between the exposure and outcome, and are equally distributed between exposure and reference groups [127] (See section 6.3.8).

In studies II and IV, we used the DNRP to obtain data to create the Charlson Comorbidity Index score (design variable, modified in study II) [128] (Appendix 7). In study IV we further obtained data from the DNRP on relevant non-Charlson comorbidities (Appendix 6).

In studies II and IV, we used the DBCG registry to retrieve information about stage at diagnosis, menopausal status at diagnosis, Union for International Cancer Control (UICC) stage (design variable), receipt of adjuvant chemotherapy, and type of primary surgery received. In study IV we further retrieved the following information from the DBCG registry; pre-diagnosis combination hormone replacement therapy, histological grade (design variable), ER status and receipt of adjuvant endocrine therapy (conjugated, design variable).

For study IV we retrieved information from the DNPreR about potential confounding co-prescriptions (any beta-blockers, angiotensin-converting-enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), acetylsalicylic acids (ASAs), and simvastatin) (see Appendix 5 for specific ATC codes).

For study III we retrieved information from the AUPD of any platelet inhibitors, vitamin K antagonists, oral anti-coagulants, non-steroidal anti-inflammatory drugs (NSAIDs) (non-aspirin NSAIDs, excluding selective Cox-2 inhibitors, as these have pro-thrombotic side effects [129], SSRI anti-depressants, and non-SSRI anti-depressants (tri-cyclic anti-depressants (TCA), tetracyclic anti-depressants) (See section 10.3for specific ATC codes).

Table 3. Overview of study designs.

	Study I	Study II	Study III	Study IV
Topic	Age-at-diagnosis and proportion of lymph node positive breast cancer	Autoimmune diseases and risk of breast cancer recurrence	Glucocorticoid and risk of reoperation due to post-surgical bleeding	Glucocorticoid and risk of breast cancer recurrence
Design	Descriptive cohort study	Cohort study	Cohort study	Cohort study
Inclusion criteria	Invasive BC diagnosis in DNPR or DBCG	Stage I-III BC in DBCG	Registration of breast cancer surgery code in DNRP	Stage I-III BC in DBCG
Study period	2000–2013	1980–2009	1996–2009	1996–2008
Sample size	62,393	78,095	19,919	18,251
Data source	DBCG and DNPR	DBCG and DNRP	DNRP, AUPD, and DCR	DBCG, DNPreR, and DNRP
Independent variables (exposure)	Age-at-diagnosis	Autoimmune disease diagnosis	Drugs containing glucocorticoid	Drugs containing glucocorticoid
Dependent variables (outcome)	Axillary lymph node status	BC recurrence	Reoperation due to post-surgical bleeding	BC recurrence

For explanation of abbreviations, see page VII.

4. Ethics

The studies included in this dissertation did not involve any contact with patients or any intervention, it was therefore not necessary to obtain permission from the Danish Scientific Ethics Committee.

The following approvals of the studies were obtained:

Study :I Approved by the Danish Data Protection Agency record number: 2013-41-1760 and by the DBCG Steering Committee.

Study II: Approved by the Danish Data Protection Agency record number: 2013-41-1759 and by the DBCG Steering Committee.

Study III: Approved by the Danish Data Protection Agency record number: 2004-41-4693.

Study IV: Approved by the Danish Data Protection Agency record number: 2006-41-6387 and by the DBCG Steering Committee.

5. Results

5.1 Study I (Age and lymph nodes)

We included 62,393 women 18 years or older with a diagnosis of invasive breast cancer in Denmark between 2000 and 2013. The age of all women in the cohort ranged from 18 to 105 and median age was 62. 18,286 (29%) of the enrolled women were ≥70 years old. Women in the unknown lymph node status group were older than the rest of the cohort, with a median age of 76 years.

In Table 4 and Figures 8 and 9 we present the cohort age distribution and lymph node evaluation characteristics. Overall, 8,981 (14%) had no registered lymph node status — 61% of this group were among women aged ≥70. Women with tumors >20 mm were more likely to have received a mastectomy and to have positive lymph node status.

The proportion of patients with positive lymph node status decreased from 41% to 33% from 2000-2013, while the proportion with unknown lymph node status was stable at 16% over the time period 2000-2013 – but with a temporary dip to 12% around 2009. When these results are stratified by age <70 and ≥70 years, the trend among the younger group is similar to the trend in the entire cohort with a decrease in the proportion of patients with positive lymph node status from 49% to 34% from 2004 to 2013 (Figure 10.A). Among women ≥70 years the proportion of patients with positive lymph node status increased to 40% through 2009 and thereafter decreased to 31% by 2013. In this elderly group the proportion of patients with unknown status decreased from 36% in 2002 to 27% in 2009 and then increased to 32% by 2013 (Figure 10.B).

In the group of women <70 years old, more than half of the tumors were <20mm of size. In the group of women ≥70 years old, the registered tumor size distribution moves toward larger tumors and an increasing proportion with unknown tumor size. This result follows the trend in Table 4, where out of 4,082 women only registered in the DNPR, 2,241 (55%) were women ≥70 years and so had no recorded tumor size.

Table 4. Cohort characteristics and distribution of axillary lymph node status evaluation at diagnosis in a cohort of Danish women with an invasive breast cancer diagnosis 2000–2013. N=62,393. *BCS, Breast conserving surgery. **Surgery type is only registered in the DBCG and not in DNPR. ***Technique not fully implemented in Denmark until 2006.

	N (column %) (*N*=62,393)	**Positive lymph node status N (column %) (row %) (*N*=25,689)**	**Negative lymph node status N (column %) (row %) (*N*=27,723)**	**Unknown lymph node status N (column %) (row %) (*N*=8,981)**	**DNPR only N (column %) (row %) (*N*=4,082)**
Age category					
18–29	202 (0.3)	99 (0.4) (49)	80 (0.3) (40)	23 (0.3) (11)	16 (0.4) (7.9)
30–39	2,258 (3.6)	1,164 (4.5) (52)	912 (3.3) (40)	182 (2.0) (8.0)	108 (2.7) (4.8)
40–49	8,189 (13)	4,063 (16) (50)	3,551 (13) (43)	575 (6.4) (7.0)	300 (7.4) (3.7)
50–59	15,089 (24)	6,691 (26) (44)	7,279 (26) (48)	1,119 (12) (13)	584 (14) (3.9)
60–69	18,369 (29)	7,195 (28) (39)	9,594 (35) (52)	1,580 (18) (8.6)	833 (20) (4.5)
70–79	10,644 (17)	4,307 (17) (41)	4,527 (16) (43)	1,810 (20) (17)	938 (23) (8.8)
80–89	6,496 (10)	1,989 (7.7) (31)	1,671 (6.0) (26)	2,836 (32) (44)	1,018 (25) (16)
≥90	1,146 (1.8)	181 (0.7) (16)	109 (0.4) (9.5)	856 (10) (75)	285 (7.0) (25)
Surgery type					
Mastectomy	23,904 (38)	13,890 (54) (58)	9,190 (33) (38)	824 (9.2) (3.4)	0
BCS*	29,112 (47)	9,966 (39) (34)	18,138 (65) (78)	1,008 (11) (3.4)	0
BCS* & Mastectomy	836 (1.3)	468 (1.8) (56)	356 (1.3) (43)	12 (0.1) (1.4)	0
Missing information**	8,541 (14)	1,365 (5.3) (16)	39 (0.1) (0.4)	7,137 (80) (84)	4,082 (100) (48)
Estrogen receptor					
Positive	45,017 (72)	20,127 (78) (45)	22,880 (83) (51)	2,010 (22) (4.4)	0
Negative	9,229 (15)	4,368 (17) (47)	4,563 (17) (49)	298 (3.3) (3.2)	0
Missing information	8,147 (13)	1,194 (4.7) (15)	280 (1.0) (3.4)	6,673 (74) (82)	4,082 (100) (50)

Tumor size					
≤20 mm	31,681 (51)	10,722 (42) (34)	20,118 (73) (64)	841 (9.4) (2.7)	0
21–50 mm	18,477 (30)	11,090 (43) (60)	6,728 (24) (36)	659 (7.3) (3.6)	0
>50 mm	1,790 (2.9)	1,417 (5.5) (79)	297 (1.1) (17)	76 (0.9) (4.2)	0
Unknown	10,445 (17)	2,460 (10) (24)	580 (2.1) (5.6)	7,405 (83) (71)	4,082 (100) (39)
Sentinel node technique* **					
Used	32,595 (52)	12,008 (47) (37)	20,492 (74) (63)	95 (1.1) (0.2)	0
Unknown	29,798 (48)	13,681 (53) (46)	7,231 (26) (24)	8,886 (99) (30)	4,082 (100) (14)
Year of diagnosis					
2000	3,799 (6.1)	1,562 (6.1) (41)	1,628 (5.9) (43)	609 (6.8) (16)	349 (8.5) (9.1)
2001	3,763 (6.2)	1,552 (6.0) (41)	1,727 (6.2) (46)	569 (6.3) (15)	321 (7.9) (8.5)
2002	4,289 (6.6)	1,808 (7.0) (43)	1,665 (6.0) (39)	646 (7.2) (15)	387 (9.5) (9.0)
2003	4,141 (6.4)	1,761 (6.9) (43)	1,609 (5.8) (39)	610 (6.8) (15)	378 (9.3) (9.3)
2004	4,058 (6.4)	1,799 (7.0) (44)	1,594 (5.8) (39)	566 (6.3) (14)	162 (4.0) (4.0)
2005	4,066 (6.3)	1,778 (6.9) (44)	1,578 (5.7) (39)	585 (6.5) (14)	274 (6.7) (6.7)
2006	4,220 (6.6)	1,835 (7.1) (43)	1,713 (6.2) (41)	597 (6.7) (14)	277 (6.8) (6.6)
2007	4,227 (6.7)	1,853 (7.2) (44)	1,734 (6.3) (41)	589 (6.6) (14)	313 (7.7) (7.4)
2008	4,857 (7.7)	2,100 (8.2) (43)	2,072 (7.5) (43)	635 (7.1) (13)	332 (8.1) (6.8)
2009	5,877 (9.5)	2,413 (9.4) (41)	2,821 (10) (48)	699 (7.8) (12)	311 (7.6) (5.3)
2010	5,227 (8.5)	2,149 (8.4) (41)	2,465 (8.9) (47)	689 (7.7) (13)	230 (5.6) (4.4)
2011	4,733 (7.6)	1,779 (6.9) (38)	2,285 (8.2) (48)	693 (7.7) (15)	275 (6.7) (5.8)
2012	4,692 (7.5)	1,663 (6.5) (35)	2,345 (8.5) (50)	684 (7.6) (15)	217 (5.3) (4.6)
2013	4,934 (7.9)	1,637 (6.4) (33)	2,487 (9.0) (50)	810 (9.0) (16)	256 (6.3) (5.1)

Figure 8. Frequencies of age-at-diagnosis and lymph node status at diagnosis in a cohort of 62,393 women with invasive breast cancer diagnosed 2000–2013 in Denmark.

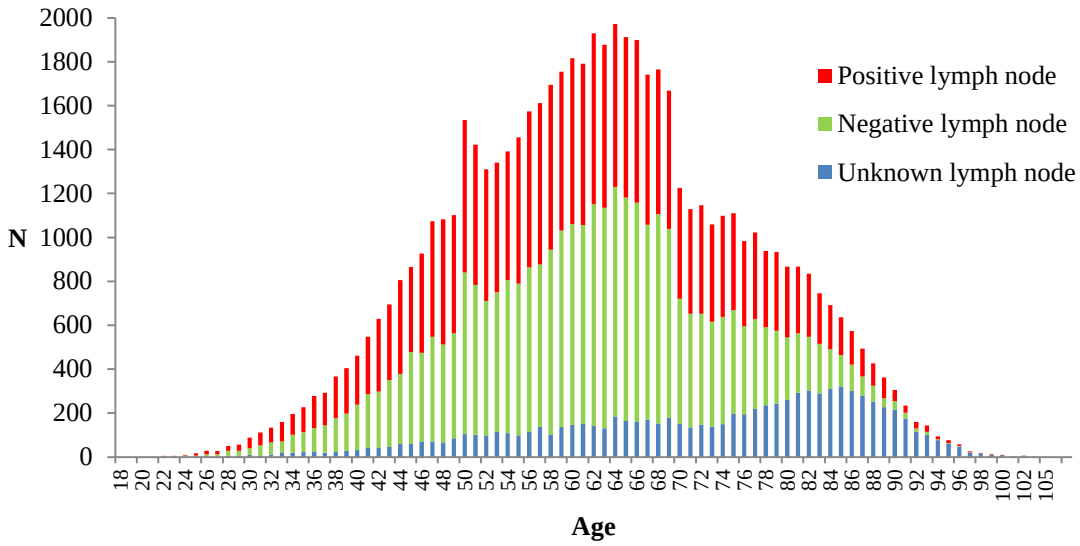


Figure 9. Area–chart showing distribution of lymph node status at time of diagnosis according to age at diagnosis. Cohort of 62,393 women with an invasive breast cancer diagnosis in Denmark 2000-2013.

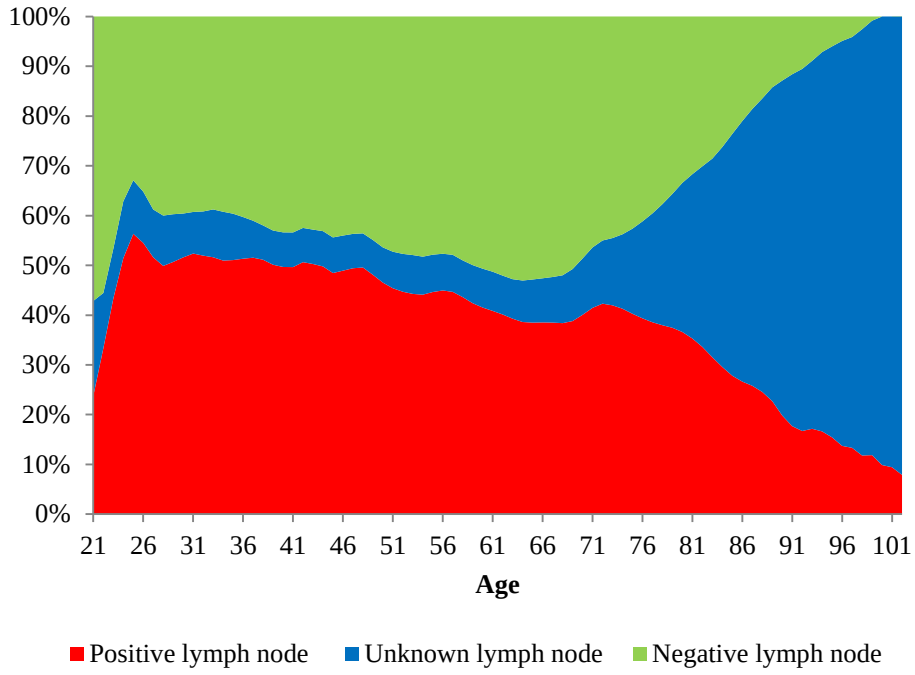
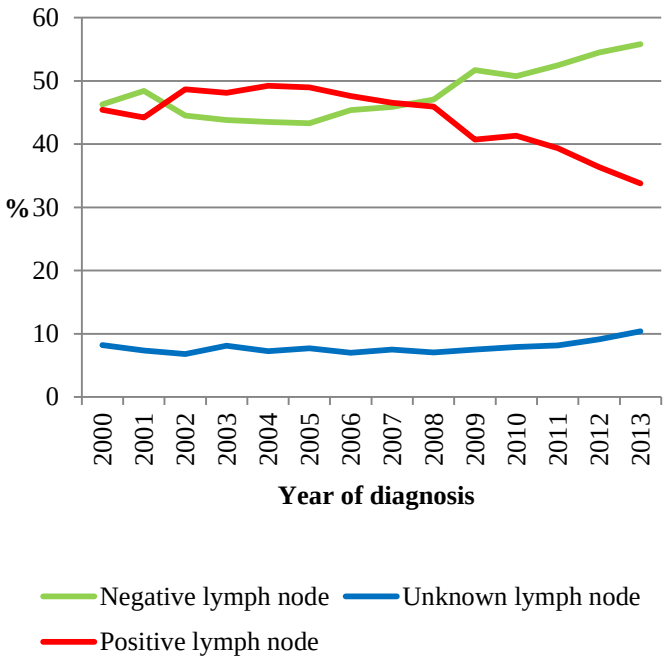
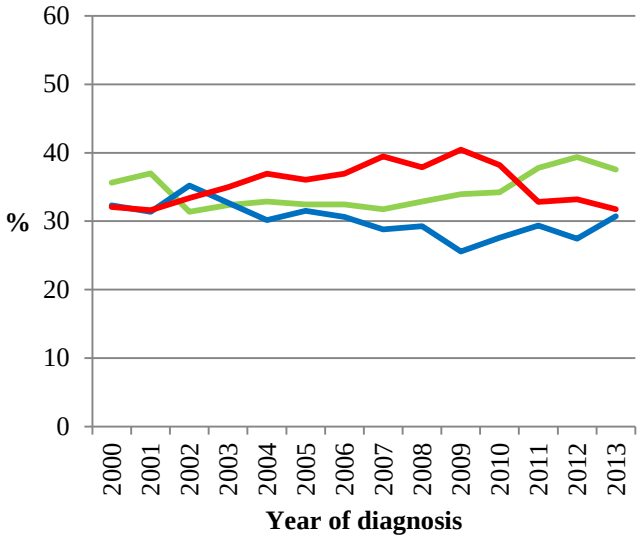


Figure 10. Time trend of lymph node evaluation from 2000–2013 stratified by age <70 or ≥70. (*N*=62,393).

A: Women <70 years (*N*=44,107):



B: Women ≥70 years (*N*=18,286):



5.2 Study II (Autoimmune diseases and recurrence)

This study included 78,095 women ≥18 years old with stage I-III incident breast cancer. Characteristics of the cohort are presented in table 5. The median age at diagnosis was 61 years (range 19-102) and the median follow-up time was 7.7 years. 8.6% of the study population had at least one AD (range 0-8) at some point during follow-up. 17.3% of the cohort developed recurrent breast cancer disease within the first 10 years after diagnosis.

In appendix 1, the frequencies of the 30 selected autoimmune exposure diseases are presented in six categories based on the organ system or tissue of disease origin. The most frequent ADs in the cohort are diabetes I diagnosed in 1,390 women (1.8% of cohort), rheumatoid arthritis diagnosed in 1,300 women (1.7% of the cohort) and Grave's disease diagnosed in 1,247 women (1.6% of the cohort). When considering the frequency of first AD, diabetes I is still most frequent with 1,338 diagnosed women, but Grave's disease is slightly more frequent than rheumatoid arthritis as a first AD diagnosis with 1,218 and 1,144 diagnosed women respectively.

The crude Cox regression model is an evaluation of the presence of at least one AD and the risk of breast cancer recurrence (Table 6). The overall $HR_{unadjusted}$= 0.83 (95% CI 0.77, 0.89). We adjusted the results for potential confounders (age, stage, chemotherapy, surgery type, menopausal status, modified Charlson Comorbidity Index (excluding exposure diseases) and found $HR_{adjusted}$= 0.97 (95% CI 0.90, 1.04). We also adjusted the result for competing risk of death among the women with an AD and found the results robust to this with a $HR_{adjusted}$= 0.96 (95% CI 0.89, 1.04) including adjustment for competing risk of death. This adjusted near-null result was robust across sub-categories of ADs according to organ of origin, except for in the CNS / neuromuscular system diseases category the result showed a protective effect against breast cancer recurrence with $HR_{adjusted}$= 0.56 (95% CI 0.40, 0.78) including adjustment for competing risk of death.

Table 5. Baseline characteristics of operable stage I, II, or III breast cancer patients diagnosed in Denmark from 1980 to 2007, by presence of autoimmune disease (AD) (*N*=78,095).

Characteristics	Women, N (%)		Recurrence, N (%)		Total person-years, N (%)	
	+AD (*N*=6,716)	No AD (*N*=71,379)	+AD (*N*=759)	No AD (*N*=13,545)	+AD (*N*=108,300,000)	No AD (*N*=1,081,000,000)
Age at diagnosis, y						
≤29	8 (0.1)	311 (0.4)	4 (0.5)	112 (0.8)	134,286 (0.1)	4,592,062 (0.4)
30-39	168 (2.5)	3,612 (5.1)	37 (4.9)	1,171 (8.7)	2,597,414 (2.4)	53,103,333 (4.9)
40-49	786 (12)	12,770 (18)	122 (16)	3,054 (23)	12,691,266 (12)	192,300,000 (18)
50-59	1,407 (21)	17,659 (25)	217 (29)	3,965 (29)	23,206,460 (21)	273,400,000 (25)
60-69	1,853 (28)	17,406 (24)	263 (35)	3,798 (28)	30,203,853 (28)	265,500,000 (25)
70-79	1,642 (25)	12,974 (18)	96 (13)	1,271 (9.4)	26,085,294 (24)	192,800,000 (18)
≥80	852 (13)	6,647 (9.3)	20 (2.6)	174 (1.3)	13,406,176 (12)	99,745,394 (9.2)

Characteristics	Women, N (%)		Recurrence, N (%)		Total person-years, N (%)	
	+AD (*N*=6,716)	No AD (*N*=71,379)	+AD (*N*=759)	No AD (*N*=13,545)	+AD (*N*=108,300,000)	No AD (*N*=1,081,000,000)
Menopausal status at diagnosis						
Premenopausal	1,180 (18)	20,550 (29)	181 (24)	5,146 (38)	19,006,674 (18)	307,500,000 (28)
Postmenopausal	5,530 (82)	50,712 (71)	577 (76)	8,391 (62)	89,224,059 (82)	772,000,000 (71)
Missing	6	117	1	8	25,305 (0.0)	400,376 (0.04)
UICC stage						
I	2,139 (32)	21,749 (31)	191 (25)	3,164 (23)	35,011,432 (32)	338,500,000 (31)
II	2,736 (41)	29,631 (42)	306 (40)	5,765 (43)	44,347,007 (41)	449,600,000 (42)
III	1,046 (16)	12,511 (18)	234 (31)	4,106 (30)	16,601,880 (15)	183,600,000 (17)
Missing	795 (12)	7,488 (11)	28 (3.7)	509 (3.8)	12,364,430 (11)	109,711,163 (10)
ER/adjuvant ET status						
ER-/ET-	981 (15)	10,811 (15)	131 (17)	2,313 (17)	16,092,439 (15)	170,200,000 (16)
ER+/ET-	2,636 (39)	26,112 (37)	222 (29)	4,120 (30)	42,114,282 (39)	395,000,000 (37)
ER+/ET+	1,902 (28)	17,586 (25)	281 (37)	3,487 (26)	33,662,995 (31)	303,400,000 (28)
ER-/ET+	28 (0.4)	397 (0.6)	8 (1.0)	181 (1.3)	437,295 (0.4)	5,321,303 (0.5)
Missing*	1,902 (17)	16,473 (23)	117 (15)	3,444 (25)	15,992,989 (15)	207,078,697 (19)
Type of primary therapy						
Mastectomy	3,115 (46)	30,959 (43)	281 (37)	5,380 (40)	48,603,211 (45)	451,700,000 (42)
Mastectomy + RT	1,595 (24)	20,672 (29)	314 (41)	5,849 (43)	25,034,628 (23)	297,400,000 (28)
BCS	36 (0.5)	837 (1.2)	1 (0.1)	168 (1.2)	453,997 (0.4)	10,382,758 (1.0)
BCS + RT	1,661 (25)	15,887 (22)	157 (21)	2,070 (15)	29,348,175 (27)	276,500,000 (26)
No operation or RT	299 (4.5)	2,875 (4.0)	5 (0.7)	68 (0.5)	4,750,448 (4.4)	43,731,399 (4.1)
Only RT	10 (0.2)	149 (0.2)	1 (0.1)	10 (0.1)	134,290 (0.1)	1,792,705 (0.2)
Adjuvant chemotherapy						
Yes	1,037 (15)	14,374 (20)	170 (22)	3,920 (29)	17,721,164 (16)	230,800,000 (21)
No	5,679 (85)	57,005 (80)	589 (78)	9,625 (71)	90,603,584 (84)	850,600,000 (79)
Modified Charlson Comorbidity Index**						
0	3,842 (57)	59,859 (84)	487 (64)	12,384 (91)	61,108,675 (56)	900,400,000 (83)
1	1,439 (21)	5,886 (8,3)	168 (22)	711 (5,3)	23,715,893 (22)	92,566,752 (8.6)
2	769 (12)	3,668 (5.1)	62 (8.2)	309 (2,3)	12,513,044 (12)	57,713,661 (5.3)
3+	666 (10)	1,966 (2.8)	42 (5.5)	141 (1.0)	10,987,137 (10)	30,788,132 (2.8)
Calendar year of diagnosis						
1980-1989	1,108 (17)	20,047 (28)	146 (19)	5,283 (39)	13,002,340 (12)	227,000,000 (21)
1990-1999	2,371 (35)	25,631 (36)	346 (46)	5,557 (41)	36,572,791 (34)	386,900,000 (36)
2000-2007	3,237 (48)	25,701 (36)	269 (35)	2,705 (20)	58,749,617 (54)	467,600,000 (43)

Abbreviations: UICC, Union for International Cancer Control; ER, estrogen receptor status; ET, adjuvant endocrine therapy; BCS, breast-conserving surgery; RT, radiation therapy

*missing is primarily due to missing information about receptor status.

** The Charlson Comorbidity Index has been modified so it does not include any of the ICD-8 and ICD-10 codes included in the autoimmune exposure variables (see appendix 7 for ICD-8 and ICD-10 codes).

Table 6. 10-year risk of breast cancer recurrence in a cohort of 78,095 women with breast cancer diagnosed in Denmark 1980–2007. Listed by the organ or tissue of origin of first autoimmune disease. Risk calculated from the date of the first autoimmune disease diagnosis.

Autoimmune disease	***N***	**Unadjusted HR (95% CI)**	**Adjusted HR* (95% CI)**	**Unadjusted HR (+competing risk of death analysis) (95% CI)**	**Adjusted HR* (+competing risk of death analysis) (95% CI)**
No autoimmune disease	71,379	1(reference)	1(reference)	1 (reference)	1 (reference)
Any autoimmune disease	6,716	0.83 (0.77, 0.89)	0.97 (0.90, 1.04)	0.76 (0.71, 0.82)	0.96 (0.89, 1.04)
Non-malignant hematological diseases	69	0.75 (0.34, 1.67)	0.76 (0.34, 1.69)	0.69 (0.31, 1.54)	0.80 (0.36, 1.81)
Endocrine diseases	2,704	0.82 (0.73, 0.93)	0.97 (0.86, 1.09)	0.76 (0.67, 0.85)	0.98 (0.87, 1.10)
Central nervous system /neuromuscular system diseases	297	0.75 (0.53, 1.05)	0.65 (0.47, 0.92)	0.69 (0.49, 0.96)	0.56 (0.40, 0.78)
Gastrointestinal/hepato-billiary system diseases	872	0.76 (0.62, 0.92)	0.86 (0.70, 1.06)	0.75 (0.61, 0.92)	0.87 (0.70, 1.07)
Skin diseases	399	0.91 (0.69, 1.20)	0.94 (0.71, 1.24)	0.88 (0.67, 1.16)	0.92 (0.71, 1.24)
Connective tissue diseases	2,369	0.87 (0.77, 0.98)	1.10 (0.97,1.24)	0.80 (0.71, 0.90)	1.11 (0.98, 1.25)

*Adjusted for age, stage, chemotherapy, surgery type, menopausal status, modified Charlson Comorbidity Index, and competing risk of death.

5.3 Study III (Glucocorticoids and re-operation due to bleeding)

Characteristics

The study included 19,919 women ≥18 years old with stage I-III incident breast cancer. Median age at diagnosis was 57 (18-100 years). Baseline characteristics of the cohort are presented in table 7. 1,412 women were 80 years or older and two-thirds of them had mastectomy as their initial surgery. Among women less than 80 years, one-third had mastectomy as their initial surgery. 356 women aged 80 years or older and 3,217 women aged less than 80 years had a history of GC use. 149 women had a GC prescription in the 90 days before their initial breast cancer operation.

508 of 19,919 women (2.6%) were re-operated due to post-surgical bleeding within 14 days of their initial operation. Of these women 247 were re-operated on day 0, 165 were operated on day 1, 35 were operated on day 3, 15 were operated day 4-7, and 30 were operated on day 8-14.

The crude risk of re-operation was 2.5% among never users of GCs, 2.6% among ever users of GCs, and 4.0% among current users of GCs. The mean number of GC prescriptions per women in ever users was between 2 and 3, regardless of age or surgery type.

Stratified analysis and logistic regression

When stratifying contingency tables according to each possible confounding variable only age category, surgery type, and GC use affected the risk of re-operation. No other measured covariate was an important confounder or predictor of risk of re-operation. Older women had a higher risk of re-operation than younger women, regardless of the type of surgery or use of GCs (aRR adjusted for surgery type and GC use= 1.6 (95% CI 1.2, 2.0)). Mastectomy approximately doubled the risk of post-surgical bleeding compared with BCS in both age categories among ever users and never users of GCs (aRR adjusted for age and GC use= 2.3 (95% CI 1.9, 2.7)). Overall, GC use did not affect the risk of re-operation (aRR adjusted for age and surgery type= 0.98 (95% CI 0.78, 1.2)). However, in the women 80 years old or older who received mastectomy, the risk of re-operation increased by 3.3% (95% CI —0.6%, 7.2%) among ever users of GCs compared with never users of GCs (Table 8). Women 80 years or older who were ever users of GCs and who had a mastectomy had an 8.1% risk of re-operation due to post-surgical bleeding, whereas women less than 80 years old who never used GCs and were operated by BCS had a 1.7% risk of re-operation (risk difference= 6.4% (95% CI 3.0%, 7.8%)) (Table 8).

Table 7. Baseline characteristics of 19,919 Danish breast cancer patients treated with surgery in 1996-2009 according to glucocorticoid (GC) prescription.

Characteristics	**No GC Prescription**	**Ever GC Prescription**
	***N*=16,346**	***N*=3,573**
	N (%)	**N (%)**
Age Group		
<40	2375 (15)	296 (8.3)
40-49	3181 (19)	592 (17)
50-59	4111 (25)	840 (24)
60-69	3693 (23)	860 (24)
70-79	1930 (12)	629 (18)
≥80	1056 (6.5)	356 (10)
Temporality of Glucocorticoid Prescription*		
Never	16, 346	0
Current (≤3months)	0	149 (4.2)
Former (>3months)	0	3424 (96)
Current (≤6 months)	0	264 (7.4)
Former (>6 months)	0	3309 (93)
Current (≤12 months)	0	512 (14)
Former (>12 months)	0	3061 (86)
Primary Operation Type		
Breast conserving surgery	10, 577 (65)	2162 (61)
Mastectomy	5769 (35)	1411 (39)
Anti-depressant Prescription		
No	13, 396 (82)	2568 (72)
Yes	2950 (18)	1005 (28)
Oral Anti-coagulants Prescription		
No	16, 312 (99.8)	3564 (99.7)
Yes	34 (0.2)	9 (0.3)
NSAIDs Prescription		
No	7277 (45)	855 (24)
Yes	9069 (55)	2718 (76)

Characteristics	No GC Prescription	Ever GC Prescription
	***N*=16,346**	***N*=3,573**
	N (%)	**N (%)**
Vitamin K Antagonist Prescription		
No	15, 934 (97)	3437 (96)
Yes	412 (3)	136 (3.8)
Platelet Inhibitors Prescription		
No	14, 836 (91)	3064 (86)
Yes	1510 (9.2)	509 (14)
Statin Prescription		
No	14, 933 (91)	3216 (90)
Yes	1413 (8.6)	357 (10)
Comorbid Diseases		
None	14, 753 (90)	2966 (83)
Yes	1593 (9.7)	607 (17)
Diseases		
Liver disease	127 (0.8)	34 (1.0)
Renal disease	112 (0.7)	60 (1.7)
Cancer	954 (5.8)	248 (6.9)
Thrombocytopoenia	11 (0.1)	7 (0.2)
Auto-immune disease	482 (2.9)	294 (8.2)
Vascular disease	15 (0.1)	11 (0.3)

*Sensitivity analyses

Table 8. Risk of re-operation due to post-surgical bleeding in the fourteen days after breast cancer surgery, according to age group, surgery type, and glucocorticoid (GC) prescription. (*N*=19,919).

Age Category	**Women <80 years**				**Women ≥80 years**			
Operation Type	**Mastectomy**		**BCS**		**Mastectomy**		**BCS**	
GC exposure*	**+GC**	**—GC**	**+GC**	**—GC**	**+GC**	**—GC**	**+GC**	**—GC**
Re-operated within 14 days (N)	42	199	29	170	18	34	5	11
Breast cancer Surgery (N)	1188	5059	2029	10,231	223	710	133	346
Risk of re-operation (%)	3.5	3.9	1.4	1.7	8.1	4.8	3.8	3.2

*ever exposed to glucocorticoid.

Abbreviations: BCS: Breast conserving surgery. GC: glucocorticoid

Risk difference for mastectomy versus BCS, adjusted for age group and glucocorticoid use, is 2.3% (95% CI 1.7%, 2.8%).

Risk difference for age <80 years versus age ≥80 years, adjusted for surgery type and glucocorticoid use, is 1.7% (95% CI 0.6%, 2.9%).

Risk difference for glucocorticoid use versus no glucocorticoid use, adjusted for age group and surgery type, is —0.1% (95% CI —0.6%, 0.5%).

5.4 Study IV (Glucocorticoid and recurrence)

The study included 18,773 women ≥18 years old with stage I-III incident breast cancer. After deleting 486 women with only 0 or 1 day of follow up and 36 women with ER-negative tumors who received endocrine therapy (contrary to indication), 18,251 women remained in the cohort. The median age was 57 (21-95 years). There were 3,408 recurrences of breast cancer during 94,345 person-years of follow-up (median = 6.9 years), equaling an incidence rate of 36 recurrences per 1000 person-years. During follow-up, 4,602 women redeemed at least one GC prescription. Users of any GC were more likely to be older, to be postmenopausal at breast cancer diagnosis, and to have more comorbid conditions compared with non-users (Table 9).

Cox regression

The unadjusted Cox regression model indicated no notable association between use of systemic, inhaled, or intestinal-acting GCs and risk of 10-year breast cancer recurrence, compared with non-use (unadjusted $HR_{systemic\ GC}$ = 1.1 (95% CI 0.9, 1.3); unadjusted $HR_{inhaled\ GC}$ = 0.9 (95% CI 0.7, 1.0); unadjusted $HR_{intestinal\ GC}$ =1.0 (95% CI 0.9, 1.2)) (Table 10).

In adjusted models, the association remained near null for GC use and 10-year risk of breast cancer recurrence (adjusted $HR_{systemic\ GC}$ = 1.1 (95% CI 0.9, 1.2); adjusted $HR_{inhaled\ GC}$ = 0.8 (95% CI 0.7, 1.0); and adjusted $HR_{intestinal\ GC}$ = 1.0 (95% CI 0.8, 1.2)) (Table 10). In categories of prednisolone-equivalent dose and duration of GC use the results changed only little and stayed near-null (Table 10).

Stratified analysis

We stratified the analysis for use of chemotherapy and estrogen receptor status. The results suggested the same pattern of associations as in the collapsed data (Table 10).

Table 9. Baseline characteristics and relevant drug exposures among stage I-III breast cancer patients diagnosed in Denmark from 1996 to 2003, by glucocorticoid (GC) use. (*N*=18,251).

Characteristics	Women, N (%)		Recurrence, N (%)		Total person-years, N (%)	
	GC users (*N*=4,602)	Nonusers (*N*=13,649)	GC users (*N*=621)	Nonusers (*N*=2,787)	GC users (*N*=23,004)	Nonusers (*N*=71,341)
Age at diagnosis, y						
<=29	19 (0.4)	51 (0.4)	8 (1.3)	22 (0.8)	100 (0.4)	189 (0.3)
30-39	242 (5.3)	667 (4.9)	48 (7.7)	210 (7.5)	1,234 (5.4)	3,384 (4.7)
40-49	861 (19)	2,593 (19)	102 (16)	528 (19)	4,697 (20)	14,741 (21)
50-59	1,498 (33)	4,576 (34)	187 (30)	960 (35)	7,796 (34)	25,173 (35)
60-69	1,439 (31)	3,969 (29)	200 (32)	780 (28)	6,846 (30)	20,172 (28)
70-79	531 (12)	1,681 (12)	75 (12)	278 (10)	2,297 (10)	7,397 (10)
>=80	12 (0.3)	112 (0.8)	1 (0.2)	9 (0.3)	34 (0.2)	285 (0.4)
Menopausal status at diagnosis						
Premenopausal	1,417 (31)	4,103 (30)	177 (28)	875 (31)	7,827 (34)	23,157 (32)
Postmenopausal	3,184 (69)	9,544 (70)	444 (72)	1,911 (69)	15,174 (66)	48,181 (68)
Missing	1	2	NA	NA	NA	NA
Medical history at diagnosis*						
Myocardial infarction	45 (1.0)	164 (1.2)	6 (1.0)	22 (0.8)	211 (0.9)	678 (1.0)
Congestive heart failure	58 (1.3)	108 (0.8)	6 (1.0)	9 (0.3)	220 (1.0)	379 (0.5)
Peripheral vascular disease	73 (1.6)	186 (1.4)	8 (1.3)	28 (1.0)	319 (1.4)	746 (0.9)
Cerebrovascular disease	124 (2.7)	333 (2.4)	21 (3.4)	58 (2.1)	520 (2.3)	1,427 (1.0)
Chronic pulmonary disease	448 (9.7)	235 (1.7)	66 (11)	44 (1.6)	2,122 (9.2)	1,034 (1.4)
Diabetes without Complications	86 (1.9)	291 (2.1)	15 (2.4)	56 (2.0)	327 (1.4)	1,314 (1.8)
Diabetes w/organ damage	28 (0.6)	108 (0.8)	2 (0.3)	20 (0.7)	99 (0.4)	490 (0.7)
Renal disease	32 (0.7)	59 (0.4)	4 (0.6)	6 (0.2)	152 (0.7)	298 (0.4)
Liver disease(mod./severe)	3 (0.1)	20 (0.2)	0 (0)	0 (0)	4 (0)	91 (0.1)
RA	39 (0.9)	137 (1.0)	8 (1.3)	28 (1.0)	205 (0.1)	620 (0.9)
COPD	285 (6.2)	169 (1.2)	40 (6.4)	28 (1.0)	1,330 (5.8)	743 (1.0)
Asthma	242 (5.3)	71 (0.5)	35 (5.6)	17 (0.6)	1,191 (5.2)	339 (0.5)
IBD	55 (1.2)	52 (0.4)	4 (0.6)	8 (0.3)	272 (1.2)	263 (0.4)
UICC stage						
I	1,889 (41)	4,999 (37)	171 (28)	643 (23)	10,006 (44)	26,688 (37)
II	1,957 (43)	5,991 (44)	247 (40)	1,098 (39)	10,105 (44)	32,668 (46)
III	732 (16)	2,593 (19)	199 (32)	1,033 (37)	2,773 (12)	9,788 (14)
Missing	2	5	NA	NA	NA	NA
Histological grade						
Low	1,290 (28)	3,622 (27)	114 (18)	515 (19)	6,850 (30)	20,864 (29)
Moderate	1,617 (35)	4,854 (36)	230 (37)	1,042 (37)	7,943 (35)	24,756 (35)
High	850 (19)	2,705 (20)	160 (26)	779 (28)	3,959 (17)	12,440 (17)
Missing	845 (18)	2,468 (18)	NA	NA	NA	NA
ER/adjuvant ET status						
ER-/ET-	900 (20)	2,784 (20)	157 (25)	718 (26)	4,384 (19)	13,260 (19)
ER+/ET-	1,415 (31)	4,143 (30)	160 (26)	713 (26)	7,282 (32)	22,569 (32)
ER+/ET+	2,097 (46)	6,197 (45)	271 (44)	1,222 (44)	10,392 (45)	32,798 (46)
Missing	190 (4.1)	525 (3.9)	33 (5.3)	134 (4.8)	NA	NA
Type of primary therapy						
Mastectomy	2,000 (43)	5,857 (43)	289 (47)	1209 (43)	9,859 (43)	29,437 (41)
Mastectomy + RT	998 (22)	3,341 (24)	170 (27)	922 (33)	4,764 (21)	16,252 (23)
BCS + RT	1,603 (35)	4,451 (33)	161 (26)	656 (24)	8,375 (36)	25,652 (36)
Missing	1	0	NA	NA	NA	NA
Adjuvant chemotherapy						
Yes	1,369 (30)	4,071 (30)	211 (34)	976 (35)	7,208 (31)	21,675 (30)
No	3,233 (70)	9,578 (70)	410 (66)	1,811 (65)	15,795 (69)	49,666 (70)
Drug exposure*						
Statins, pre and post**	946 (21)	2,290 (17)	62 (10)	194 (7)	5,360 (23)	15,472 (22)
Simvastatin, pre and post**	857 (19)	2,111 (16)	42 (6.8)	156 (5.6)	4,932 (21)	14,539 (20)
HRT, pre	1,236 (27)	2,855 (21)	142 (33)	477 (17)	6,337 (28)	15,517 (22)
NSAIDs, pre and post	3,414 (53)	8,635 (63)	406 (65)	1,612 (58)	17,920 (78)	48,812 (68)
ASAs, pre and post	1,030 (22)	2,510 (18)	92 (15)	342 (12)	5,410 (24)	14,559 (20)
Alphablockers, pre and post	73 (1.6)	171 (1.3)	4 (0.6)	26 (0.9)	394 (1.7)	995 (1.2)
Anticoagulants, pre and post	1,103 (24)	2,788 (20)	98 (16)	390 (14)	5,734 (25)	15,980 (22)
Antidiabetics, pre and post	86 (1.9)	297 (2.3)	13 (2.1)	40 (1.4)	412 (1.8)	1,590 (2.2)
ACE inhibitors, pre and post	845 (18)	2,203 (16)	84 (14)	245 (8.8)	4,552 (20)	13,565 (19)
Angiotensin Receptor Blocker, pre and post	621 (14)	1,357 (9.9)	58 (9.3)	160 (5.7)	3,407 (15)	8,200 (12)

Characteristics	Women, N (%)		Recurrence, N (%)		Total person-years, N (%)	
	GC users (*N*=4,602)	Nonusers (*N*=13,649)	GC users (*N*=621)	Nonusers (*N*=2,787)	GC users (*N*=23,004)	Nonusers (*N*=71,341)
Beta-blockers, pre and post	995 (22)	2,613 (19)	85 (14)	380 (14)	5,262 (23)	15,119 (21)
COPD drugs, pre and post	1,685 (37)	1,107 (8.1)	244 (39)	155 (5.6)	8,352 (36)	6,035 (8.5)
Immune drugs***	48 (1)	48 (0.04)	3 (0.4)	6 (0.2)	298 (1.3)	258 (0.4)

Abbreviations: RA, Rheumatoid Arthritis; COPD, Chronic Obstructive Pulmonary Disease; IBD, Inflammatory Bowel Disease; ER, estrogen receptor status; ET, adjuvant endocrine therapy; BCS, breast-conserving surgery; RT, radiation therapy; ACE, angiotensin-converting enzyme; HRT, combination hormone replacement therapy; NSAIDs, non-steroidal anti-inflammatory drugs; ASAs, acetyl salicylic acids (high and low dose) .

*Proportions of patients, recurrences, and person-years calculated with denominators equal to sums within GC exposure groups because categories are not mutually exclusive

**One year before diagnosis and up to ten years after diagnosis.

*** Methotrexate and azathioprine

Table 10. HR and 95% CI for GC exposures (according to route of administration), stratified by presence/absence of chemotherapy and positive/negative Estrogen Receptor (ER) status, and for categories of prednisolone-equivalent doses (only systemic GC) and cumulative increase in GC exposure over 10 years. Reference group is non-users. Stage I-III breast cancer patients diagnosed in Denmark, 1996 to 2003 (*N*= 18,251).

	Unadjusted[§] HR (95% CI)		Adjusted[§*] HR (95% CI)	
Systemic GC	1.1 (0.9,1.3)		1.1 (0.9,1.3)	
Inhaled GC	0.9 (0.7,1.0)		0.9 (0.7,1.0)	
Intestinal GC	1.0 (0.9,1.2)		1.0 (0.8,1.2)	
	Chemotherapy	**No chemotherapy**	**Chemotherapy**	**No chemotherapy**
Systemic GC	1.1 (0.9, 1.4)	1.0 (0.9, 1.2)	1.1 (0.9, 1.4)	1.0 (0.8, 1.2)
Inhaled GC	0.9 (0.6, 1.2)	0.9 (0.7, 1.1)	0.9 (0.6, 1.3)	0.8 (0.7, 1.0)
Intestinal GC	0.9 (0.7, 1.2)	1.1 (0.9, 1.3)	0.9 (0.6, 1.2)	1.0 (0.8, 1.3)
	ER positive	**ER negative**	**ER positive**	**ER negative**
Systemic GC	1.1 (0.9,1.3)	1.1(0.8,1.4)	1.1(0.9,1.3)	1.0(0.8,1.4)
Inhaled GC	0.9 (0.7,1.1)	0.8(0.6,1.2)	0.8(0.7,1.0)	1.0(0.7,1.4)
Intestinal GC	1.0 (0.8,1.2)	1.0(0.7,1.4)	1.0(0.8,1.2)	1.0(0.7,1.4)
Prednisolone-equivalent dose**				
1-999 mg	0.9 (0.8, 1.0)		0.9 (0.8, 1.1)	
1000-4999 mg	0.9 (0.8, 1.1)		0.8 (0.7, 1.0)	
≥5000 mg	1.0 (0.7, 1.5)		0.9 (0.6, 1.4)	
Cumulative increase in duration of GC exposure over a 10-year period ^	1.0 (0.9, 1.0)		1.1 (0.9, 1.3)	

§Models incorporating yearly updated drug exposure, lagged by 1 year.

*Models adjusted for age at diagnosis (continuous), menopausal status at diagnosis, UICC stage (design variables), histological grade (design variables), ER status and receipt of adjuvant endocrine therapy (conjugated, design variables), receipt of adjuvant chemotherapy, type of primary surgery received, Charlson Comorbidity Index score (design variables), pre-diagnosis combination HRT, and co-prescriptions (time-varying, updated yearly, and lagged by 1 year) of any β-blockers, ACE inhibitors, ARBs, ASAs, and simvastatin.

**Applies only to systemic GCs.

^The cumulative increase in the duration of GC exposure over a 10-year period was updated yearly. GC exposure was lagged by one year.

6. Discussion

6.1 Main conclusions

We have evaluated age, ADs, and GCs as proxies for impaired immune function and its potential relation to the clinical course of breast cancer.

Due to a lack of data about axillary lymph node status among women >70 years we cannot make any conclusions about the potential relationship between age and immune competence based on the age-related pattern of node status by pathologic evaluation. Compared to the study by Wildiers *et al.* [54] that we aimed to replicate and improve, we described the full cohort including the cohort with no information about lymph node status and found that age >70 was associated with increasing risk of un–staged breast cancer.

However, our findings provide near–null evidence to support an association between ADs or GC prescriptions and breast cancer recurrence. Our findings suggest that GC prescriptions are associated with an increased risk of reoperation due to postsurgical bleeding among women >80 years who received a mastectomy.

6.2 Comparison with existing literature

6.2.1 Study I (Age and lymph node)

Our results support the critique of the study that we aimed to replicate and improve [80], i.e. that we note an increased prevalence of pathologically un–staged breast cancer with increasing age. The Wildiers *et al.* study [54] evoked some controversy [70, 71] and an earlier attempt to replicate the results [130]. The key limitation of the Wildiers *et al.* study [54] is that it gives no information on women who were excluded from the study due to missing information on lymph node status. It is known from the literature that in general, among breast cancer patients, the majority of unknown lymph node status is found in the elderly population [71, 79]. Seen in the light of our results, factors other than the immunosenescence theory may explain the Wildiers *et al.* study result [54] – for example the possible increase in lymph node involvement in elderly women might be due to differences in choice of surgery in elderly women, less access to screening programs, or different pathological markers such as estrogen receptor status and other tumor characteristics than among younger women with breast cancer [70]. Elderly patients with good prognostic markers such as small tumors, ER-positive tumors, and clinically node negative breast cancer might be under-registered as they are not offered standard treatment. Also, one could speculate that the increasing rate of un-staged breast cancer with increasing age could be due to higher levels of comorbidity among the elderly population, and following possible unwillingness to treat or receive treatment among this group. Finally, lack of reporting of women >70 years to the DBCG registry could be a problem based on delayed implementation of guidelines dictating that all women with invasive breast cancer should be reported regardless of age [114].

Our findings concur with those from a study that also failed to replicate the Wildiers *et al.* findings [130]. Another study of the relation between age and lymph node status was restricted to development of extent of disease among especially women <40 years of age [131]. The distorted distribution of unknown lymph node status found in our study makes it plausible that the included cohort in the Wildiers *et al*.'s study [54] was a selected group of elderly women with complete pathologic information [71]. In our study, the distortion of the available lymph node information makes it impossible for us to make any conclusions about the immune competence of the older women in regard to their lymph node status at breast cancer diagnosis. Our findings also call into question this interpretation of the Wildiers *et al.* study results [54]. Our results show the same stage distribution pattern as a previous Danish study where mainly elderly women were not registered in the DBCG database [79].

Full access to information about tumor size would have been of value to this study, but we did not have complete and precise information on tumor size for the whole cohort. The majority of the cohort, though, comprised women registered in the DBCG, which provided information about tumor size.

Over the study period from 2000 through 2013, diagnostic procedures have evolved. Most importantly, the sentinel node technique was introduced in Denmark in 2002 [75]. Since 2004, this technique has been standard procedure in the entire country [75, 132]. Surgical treatment preferences and guidelines have also transitioned from predominantly mastectomies to breast conserving surgery — also in the elderly [133]. We note a small stage migration toward increasing proportions of node positive breast cancers since the sentinel node evaluation was introduced as standard care [134] but no change in the number of unknown lymph node evaluation pattern over time. The breast cancer treatment recommendations in Denmark since 2006 [75] state that all women with a diagnosis of invasive breast cancer should be offered a full diagnostic evaluation regardless of age at diagnosis, similar to the US guidelines [135-137]. However, the guidelines in Denmark and the USA also acknowledge that exceptions to guideline treatment can be made upon assessment of important comorbidity or very high age. Our study was unable to account for physician or patient preference, which may have impacted on our finding.

6.2.2 Study II (Autoimmune diseases and recurrence)

Our results support findings by Hemminki *et al*., who analyzed the risk of death due to female cancer in a large Swedish cohort of women with ADs [55]. They used Cox regression to estimate HRs interpreted as mortality rate ratios (MRR) for deaths in different female cancers while deaths from other causes were censored. In their study, the risk of breast cancer specific death among women with an AD compared to that expected based on the general population was near-null, $HR_{adjusted}$= 0.95 (95% CI 0.89, 1.02) [55].

To our knowledge, no previous studies have evaluated the risk of breast cancer recurrence associated with AD. Few studies have evaluated breast cancer survival associated with selected AD diseases among breast cancer patients. A study among autoimmune hypo-thyroidism patients (Hashimoto's thyroiditis) with a specific drug use profile showed little evidence of an effect on breast cancer specific mortality HR= 0.92 (95%CI 0.71, 1.18) [91]. A study among breast cancer patients with and without inflammatory bowel disease showed no substantial difference in all-cause mortality rates $MRR_{adjusted,crohn's\ disease}$= 1.22 (95% CI 0.85,1.75) and $MRR_{adjusted,ulcerative\ colitis}$= 1.09 (95% CI 0.86, 1.38) [90]. However, one study found poorer survival among breast cancer patients with rheumatoid arthritis than without rheumatoid arthritis $HR_{breast\ cancer\ specific}$= 1.55 (95% CI 1.40, 1.71) [93]. Further, a recent Swedish study has evaluated the risk of breast cancer recurrence in breast cancer patients with RA who received tumor necrosis factor inhibitor and found $HR_{adjusted}$= 1.1 (95% CI 0.4, 2.8) [92], comparable to our results for the connective tissue AD category. However, the lack of precision of the Swedish study, highlighted by the width of the Swedish study's 95% CI, allows for a wide range of consistent estimates.

There is a general concern about the immunosuppressive approaches to AD management. It is difficult to differentiate between the effect of the AD and its impact on the immune system from the iatrogenic effect of the treatment for the AD, which suppresses immune function [55, 81]. Our study IV shows, in an overlapping cohort of patients, no evidence of an association between prescriptions for the immunosuppressive drugs most commonly used to treat AD — GCs — and risk of breast cancer recurrence [16]. Other studies have investigated the relation between use of other immunosuppressive drugs and risk of cancer and concluded that this potential association should be considered for each individual drug and AD [82, 138].

6.2.3 Study III (Glucocorticoid and post-surgical bleeding)

We found an overall higher risk of re-operation due to bleeding among all women who had a mastectomy regardless of age. Many patient-specific factors such as tumor size, tumor location, and comorbidity, must be considered by the patient, surgeon, and oncologist when deciding on the appropriate surgical procedure for an individual patient [113]. Although mastectomy is a more extensive and invasive operation than BCS, studies suggest that older breast cancer patients are more likely to receive a mastectomy than younger women [133, 139], which is consistent with the distribution of surgery type in our study. Older women often prefer mastectomy to avoid radiation therapy [41, 139], according to Danish guidelines, the presence of comorbidity and older age should weigh in favor of choosing mastectomy without radiation therapy [20, 140].

Considering the age-related changes in wound healing described in the background section, it is likely that the physiologic delay of the healing process in older persons becomes evident only in the presence of conditions that exert their own, negative influence on wound repair. Such an influence could be from GC use

as this drug group inhibits the inflammatory phase of wound healing and large doses of GCs reduce collagen synthesis and wound strength [99, 141]. In addition, surgeons often find that older women have more fragile and atherosclerotic vessels than younger women, and it can be more difficult to achieve hemostasis using electric coagulation or ligation. These theoretical and clinical experiences support our finding that older women are at higher risk of re-operation than younger women, regardless of the type of surgery.

Women 80 years old or older represent a highly heterogeneous population in terms of frailty and comorbid disease. Comprehensive geriatric assessment tools [142] can be helpful to assess these conditions and to evaluate an individual patient's risk before deciding on a treatment regime [143]. The important point, though, is that history of GC use may be a predictor of higher risk of re-operation in women 80 years of age or older who receive a mastectomy.

6.2.4 Study IV (Glucocorticoid and recurrence)

To the best of our knowledge, this is the first study to evaluate the association between GC use and breast cancer recurrence. Our study is the first to examine directly the use of GCs and breast cancer recurrence, rather than diseases potentially treated with GC prescription [55]. The results from our study II [14] about ADs and risk of recurrence and a Swedish study by Hemminki *et al.*[55] are population-based studies that indirectly support our results finding no effect of ADs that are commonly treated with GC and the risk of breast cancer recurrence.

Our results do not support the concerns raised by a German research group in a laboratory-based setting, who suggested that GC impairs the action of chemotherapy and may therefore impact cancer prognosis in different solid cancers including breast cancer [101, 107-109, 144-146].

6.3 Methodological considerations

The estimates presented in our studies are products of the study design, the study conduct, and the data analysis [147]. The aim of our studies was to produce precise and valid estimates of the associations between the chosen exposures and outcomes. Also, that the studies would be reproducible in relevant target populations. To achieve these goals, we have some methodological considerations that will be discussed below.

6.3.1 Validity

Validity of epidemiological estimations can be divided into internal validity that pertain to the members of the source population, and external validity or generalizability that pertains to people outside the source population [147]. To evaluate the internal validity of each of the four studies, the risk of systematic and random errors must be addressed.

Systematic error refers to selection bias, information bias, and uncontrolled confounding. Selection bias and information bias occur due to systematic errors in the study design. Such bias cannot be controlled by conventional statistical methods. Confounding by measured confounders, on the other hand, can be controlled by both study design and statistical analysis as outlined below [147].

Random error occurs due to variability in data that is present simply by chance. This kind of error can be reduced if the study population is sufficiently large.

6.3.2 Selection bias and generalizability

By selection bias, we refer to the systematic error associated with the selection of the study participants [127]. As described in the methods section, we based all four studies on population-based design and the coverage of breast cancer diagnoses in Denmark is virtually complete. The validity of the DBCG registry is very high for patients diagnosed up to age 75. Overall, the positive predictive value for classification of breast cancer recurrence was found to be 99.4% using medical records as a gold standard [148]. However, in study I we showed that the completeness of elderly women with breast cancer diagnosis is not complete in the DBCG registry. Although this is not a selection bias, it may affect the generalizability, so one must bear this in mind when interpreting the results from study II and IV.

We ensured accurate and complete follow up of the cohorts and incorporated comprehensive information on potential confounders, including comorbid diseases in studies II, III, and IV.

6.3.3 Information bias

Information bias occurs when exposure or outcome data are measured incorrectly, e.g. a woman is regarded as unexposed although she was truly exposed. Information bias of this type can be referred to as misclassification [127].

Misclassification can be either differential or non-differential referring to the mechanism for the misclassification. Differential misclassification occurs when the misclassification is different in the exposed group than the unexposed group. An example of differential misclassification is recall-bias. In our studies, recall-bias was eliminated by use of prospectively collected mandatory registered prescription data. All data were collected from population-based registries that have a completeness approaching 100% [119]. Non-differential misclassification is difficult to avoid in epidemiologic studies. Below we discuss how non-differential misclassification may have influenced the exposures and outcomes in our studies and thereby influenced our results.

6.3.4 Misclassification of exposure

Autoimmune diseases

In study II our exposure, AD, could be misclassified due to several reasons. We only have access to discharge information from hospitals and out-patient clinics. Our data shows an increasing prevalence of AD disease, from 5.2% to 11.2%, over calendar time from 1980–1989 to 2000–2007. This change is most likely due to access to data from out-patient clinics from 1995 onwards. Some ADs are managed solely by primary care physicians, and we did not have access to these data. Further, symptoms of ADs can be common and possibly be misinterpreted, which might lead to misclassification or under-diagnosis. The time of diagnosis might also be delayed due to nonspecific or vague symptoms. Accordingly, a Danish study calculated the total number of persons alive in Denmark on 31 December 2001 with one or more AD registered in the DNPR and found that lifetime prevalence of AD was 5.2% [56]. Cooper *et al.* find this prevalence underestimated and based on a literature review they calculated a corrected prevalence of 7.6–9.4% of ADs in Denmark among both men and women [58]. In our cohort we found an AD prevalence of 8.6%. Due to the above mentioned risk of misclassification, our AD-exposed person time may be underestimated. However, women with a breast cancer diagnosis will be seen in a hospital and any known comorbidity, including an AD, would most likely be registered in the DNPR in the beginning of the follow up period. Working with AD as a dichotomous variable, we do not include any information about severity of the disease or potential presence of several diseases. A measure of severity would have allowed us to control for this potential confounder by stratification.

ADs – and sub-groups of ADs based on organ or tissue of origin – have different etiology. In this study they are also represented in very different numbers in the cohort. These circumstances can potentially lead to information bias and should be considered when interpreting study II.

Glucocorticoid

In studies III and IV there were several ways we could have misclassified the GC exposure and the potential confounder drugs.

Locally administered GCs acting on the ear, nose, eye, or skin were not included among the exposures in study III or IV, as they are not thought to act systemically [62]. Low-dose, locally administered GCs are available in limited supply over-the-counter in Denmark, while systemically acting GCs are only available by prescription. Any use of over-the-counter GCs in our patient cohort was likely to have a minimal effect on our recurrence estimates.

Another concern is our reliance on redeemed prescriptions as a measure of drug use. We had no information on treatment compliance. However, because patients have to pay a portion of the cost of their prescription medication, it is likely that redeemed prescriptions reflect actual use [149]. GC dosing varies a lot depending on the administrative route and indication for treatment. A set dose can be taken on a regular basis, or the dose may fluctuate according to variation in the severity of symptoms. Among women who took inhaled or intestinal-acting drugs, the exact bio-availability is not known. We therefore restricted our dose-response analysis to systemically administered GCs.

Except for NSAIDs and aspirin, all the potentially confounding drugs are only available by prescription in Denmark. Residual confounding due to over-the-counter aspirin use is a potential concern. However, patients are reimbursed a proportion of the cost of prescribed medicine, so long-term, continuous use of aspirin is likely to be via prescription. In study III adjustment for prescribed NSAIDs had little effect on the estimated associations.

Finally, we were unable to assess in-hospital drug use, which may have impacted our estimates. As mentioned in the background section, GCs are used in-hospital peri-operatively to reduce postoperative nausea and vomiting (PONV) [66] and to reduce chemotherapy-induced nausea and vomiting [68].

A previous medical record review of 150 breast cancer patients diagnosed between 2004 and 2008 conducted by members of our group gave us an insight on the extent of GC use in-hospital. Not all 150 randomly selected breast cancer patients had full information available, but only 3 of 136 women who underwent surgery received perioperative dexamethasone (a very potent GC), and only one of these patients was aged over 80 years. None of the exposed women had a re-operation due to post-surgical bleeding. “Stress doses” of GC will only be administered to current long term users (2 of 136 in our medical record review). This

category of women are already a part of the exposed cohort, and receipt of the stress dose is potentially, therefore, part of the underlying causal mechanism.

The medical record review further showed that all women who received chemotherapy were treated with systemic GC to alleviate treatment-related cytotoxic reactions [15]. As described in the methods section, we stratified our estimates in study IV by receipt of chemotherapy to address this potential exposure misclassification; we found no change in the effect estimate.

Finally, due to the lack of information about in-hospital drug use, we have no information about the use of low molecular weight heparins (LMWH) as deep vein thrombosis prophylaxis, although we know from clinical experience and the literature that LMWH is given to all cancer patients undergoing surgery as a daily injection throughout their in-hospital stay [150]. We furthermore have the experience that it is mostly elderly people who are receiving long term oral anticoagulation therapy, so a higher dose of LMWH among this group of older women may explain part of the higher risk of operation due to re-bleeding in this age category.

6.3.5 Misclassification of outcome

In the CRS information about emigration, birth and death is recorded with negligible error [151]. Below is a discussion of the potential misclassification of outcomes in all four studies.

Lymph node status

In study I, we learned that there was a lack of registration of the elderly in the key database. It is possible that it is not all of the women with unknown lymph node status who actually did not get a pathological evaluation of the axillary lymph nodes — we do not know the validity of this variable. We can only speculate about the reasons for the high proportion of elderly women registered with unknown lymph node status (See section 6.4).

Recurrence

A major strength of studies II and IV is the high quality information about breast cancer recurrence in the DBCG. During the first five years following diagnosis, women in the DBCG registry undergo physical examination every 3–6 months to detect recurrences and an annual exam in years 6–10 following diagnosis, also to detect recurrences. A mammography is carried out every second year [114]. Recurrences diagnosed between examinations are reported to the DBCG.

Re-operation due to post-surgical bleeding

As we use data from the DNRP we do not have information about the extent of bleeding or postoperative complications leading to the re-operation. All breast cancer patients who received a re-operation for post-surgical bleeding have had a surgical code. In Denmark, it is general practice to code the diagnosis hematoma when performing an invasive procedure such as a puncture. If the surgeon has to put the woman in general anesthesia, then the procedure would get a surgical code (Personal communication with breast surgeon Jens Peter Garne, 2011). All of our cases of surgery for re-bleeding had a surgical code. Hence, the vast majority of the patients registered as cases of surgery for re-bleeding in our study were likely treated under general anesthesia.

6.3.6 Detection bias

Detection bias is a sub-type of information bias/misclassification and is traditionally described in situations where the probability of identifying the diseased people is conditional on the clinical information collected, which is different in the categories of the risk factor [152, 153]. The term can also be used about the opposite situation where it describes a diagnostic neglect [154]. Detection bias can arise when an exposed group is either followed closer with a following higher risk of detection of outcome or the opposite situation where the group is followed less intensely with a lower detection of outcome. In study II, our results remained null when analyzed by AD organ/tissue category, except for the CNS / neuromuscular system category. The decreased rate of recurrence observed in patients with CNS diseases may be due to detection bias. Most of these women had multiple sclerosis (MS) (N=272); 25 had myasthenia gravis. MS is a chronic disease that often presents in early adulthood and progresses into a severely debilitating and disabling disease [84]. Any evidence of breast cancer recurrence in a patient with severe MS may have been overlooked by patients and physicians [154] at least in the short term.

6.3.7 Handling of missing information

In study I we considered using multiple imputation to compensate for the lack of specific outcome information [155]. However, we did not impute the missing data because we lacked the outcome variable in a very large proportion of a selected group of our population, i.e. the women >70 years. We did not have enough basic information about the reasons why some elderly women were not lymph node evaluated to make a precise imputation of the lymph node status.

In study IV in the final model, we only included women with complete information.

6.3.8 Confounding

In observational studies, like ours, confounding is inevitable as the exposure is not randomly assigned in the study design. The group exposed to the variable of interest may therefore differ from the un-exposed group in ways that are related to the outcome. The potential association between exposure and outcome (causal) can become confused or distorted by the effect of other factors, i.e. confounding. To be a confounding factor a number of properties apply; 1. A confounder must be an independent cause or proxy/marker of the disease, 2. It must be imbalanced across exposure categories, and 3. It must not occur on the causal pathway between exposure and outcome [127].

In the present studies, several factors were considered potential confounding factors. It is possible to control for these factors by both study design (randomization, restriction, and matching) and statistical analysis (standardization, stratification and adjustment). We have tried to reduce potential confounding in both the design and analysis phases of our studies (See section 3.9).

In the design of study II, III, and IV we restricted the cohorts to include only women with breast cancer stage I–III. In study IV we restricted the Cox regression model to women with no missing information about any potential confounders. Also in study IV, we designed the model to estimate GC and other covariates as time-varying covariates lagged by 1.

In the analyses part we have worked with both adjustment and stratification in various combinations as described below.

In study II and IV, we adjusted the Cox proportional hazards models for potential confounders (including co-medications in study IV) (See section 3.9). In study II we lacked information about drugs used to treat ADs. NSAIDs and aspirin are commonly used as treatment for diseases such as rheumatoid arthritis and have previously been investigated and suggested to act protectively against breast cancer recurrence [156]. However, in an overlapping population, we observed near null evidence to support a protective association between NSAID prescriptions and breast cancer recurrence, even among new users (Cronin-Fenton *et al*. 2014, in draft). We therefore do not expect use of these drugs to confound our results. Use of GCs and other immunosuppressive drugs could have been interesting components of the analysis. However, our study IV about use of GCs and risk of breast cancer recurrence showed little effect of GCs on breast cancer risk.

In study II, the women who were exposed to one or more AD could potentially have been at a risk of dying of the AD before they had a recurrence of breast cancer. To take account of this potential risk of dying before breast cancer recurrence we used the Fine and Grey model for competing risk of death as it was provided by the STATA software [157].

In study IV, confounding by indication may have impacted our findings. By this we mean that the women could be treated with GCs due to another factor potentially associated with the risk of breast cancer recurrence [158]. This kind of confounding can be difficult to adjust for [158]. In this case GCs could be associated with e.g. treatment of ADs — and if these diseases were associated with breast cancer recurrence it would impact our results. We did not have access to information about indications for the GC prescriptions. However, the results were robust to stratification by subgroups of GC administrative route, and the drugs containing GC are prescribed for a very diverse group of diseases and conditions. Therefore, we do not think that confounding by indication affected our estimates.

Likewise in study IV, we had a concern about reverse causation, e.g. that GC was prescribed because of imminent breast cancer recurrence. However, from clinical experience we know that GCs would rarely be prescribed to a breast cancer survivor due to nonspecific symptoms — that could be related to recurrence — without a clinical evaluation of recurrence. Our lagged exposure in study IV also reduced the likelihood of reverse causation.

In study III, we computed crude risk differences and risk ratios and 95% CI of all potential confounders of the association between GC and re-operation due to bleeding and included only confounders that changed the estimate by more than 10% (see methods section). The included variables were 1. Age used as a dichotomous variable ≥80 years of age or <80 years of age as this age cut-off has previously been used to distinguish between older study participants [41], 2. Surgery type used as a dichotomous variable (breast conserving surgery versus mastectomy), and 3. Ever or never use of GC prescriptions. We used a stratified analysis to control for these potential confounders. Although our results suggested that GC use did not affect the risk of re-operation due to post-surgical bleeding, when stratifying by age and surgery type we saw more clearly how each variable contributed to the risk of re-operation due to bleeding.

In study IV, we stratified the results by use of chemotherapy and the tumor specific estrogen-receptor status to control for confounding (See section 3.9). The stratification did not show any effect of these variables on recurrence rate.

6.3.9 Effect measure modification

Effect measure modification is a term used to describe the situation where a measure of effect changes over values of some other variable [159]. For example, in study I, age is our exposure and lymph node status our outcome. Here age changes the chance of getting a lymph node evaluation – but from our data we do not know if age changes the risk of actually having a positive lymph node status at diagnosis.

In study III, we evaluated several potential confounders and found effect measure modification when evaluating age categories (over and under 80 years) and surgery type (mastectomy or breast conserving

surgery). Consequently, we stratified by these two variables in the final model to compute measures of risk difference.

6.3.10 Immortal person-time

When designing a cohort study, sometimes the design requires that all participants survive a certain time period in order to be eligible for inclusion in the study [160, 161]. That is the case in our study II where the DBCG defined recurrent disease as any locoregional or distant recurrence or contralateral cancer that occurred more than 60 days after diagnosis [162]. This time period ensures that final stage is assessed and the recurrence is true recurrence and not a late change in initial stage. This requirement of survival for 60 days until a person can get at risk of a recurrence presupposes a special study design to avoid immortal person time. In this case we had to postpone the start of follow up for everybody (exposed and unexposed) until 60 days after diagnosis — so the time before the eligibility criterion was met was excluded from the calculation of exposed time. If we did not take this into consideration and started the follow up at time of diagnosis, the exposed time would weight too much in favor of not getting the outcome.

6.3.11 Precision

We assess the precision of the associations in the studies by providing a 95% CI. The narrower the 95% CI, the better the precision of the result is. All four studies in this dissertation included large sample size and complete cohorts retrieved from high quality and valid national population-based registries reducing the impact of random error. In studies II, III, and IV the primary findings have narrow 95% CI and this is also the case for most of the analyses conducted in subgroups. However, for some subgroups of ADs in study II, such as the non-hematological diseases, there were few patients and therefore the precision of the estimates was low.

6.4 Clinical implication and future research

Cancer is becoming an increasing worldwide problem associated with increasing health care costs, low quality of life, and premature death. Understanding of the potential association between the immune system and clinical course of cancer is an important factor on the pathway to improve the treatment and outcome of cancer. The purpose of this dissertation was to evaluate if impaired immune function was associated with the clinical course of breast cancer using proxies of immune function in epidemiological studies. Based on these four population-based studies it is not possible to give a clear answer to this question. However, the investigations presented in this dissertation add to the existing literature with respect to prognostic factors and predictors of the clinical course of breast cancer. We have gained valuable knowledge that can help women with breast cancer and their physicians better assess the individual risk profile and make the best possible choices about treatment options.

Nonetheless, several questions remain:

- Why are elderly women less likely to have a full stage evaluation? Is it possible to increase the proportion of older women who get full stage evaluation and will it impact on breast cancer outcome in older women?
- Do immunosuppressive treatment regimens other than GCs alter the risk of breast cancer recurrence?
- Does the different biological etiology of ADs and different patient characteristics within each disease category matter to breast cancer outcome measures?
- Are other immune diseases and immunosuppressive conditions such as HIV, solid organ transplantation, and immunodeficiency disorders associated with breast cancer recurrence?
- What is the prevalence of ADs if cases in primary health care sector are measured and would this change our results in study II?
- Would it improve survival of older women with breast cancer and multimorbidity or polyphamacy if they receive geriatric care in connection with cancer treatment?
- Is re-operation of breast cancer patients associated with breast cancer outcome?

In order to address these questions, it will be necessary to supplement existing data with more detailed clinical data and maybe clinical intervention studies. The Danish nationwide administrative and medical registries contain comprehensive information about health care and cover the entire population, together with tax-supported and equal access to high quality health care, provide a unique opportunity for answering some of these questions.

7. Summary in English

Breast cancer is the most common cancer among women in the Western world. Though survival after breast cancer diagnosis continues to increase, we still do not have the full overview of the factors that determine who will survive their cancer disease–free and why. The aim of this dissertation was to explore the potential associations between immune function and clinical course of breast cancer in four epidemiological studies using age–at–diagnosis, immunosuppressive diseases, and immunosuppressive drugs as markers of declining immune competence.

We conducted one descriptive cohort study (study I) and three cohort studies (studies II, III, and IV). We used Danish administrative and medical registries to secure information on breast cancer diagnosis and retrieve data on redeemed prescriptions, comorbidities, and breast cancer outcomes. The statistical methods used included graphics, Cox proportional hazards regression, and logistic regression.

In study I (2000–2013), we included 62,393 women with an invasive breast cancer diagnosis. Previous research has suggested that women >70 years have a higher risk of lymph node positive disease at diagnosis than do women <70years even in small tumors. Also, this result could be explained by impaired immune response among women >70 years. To test this hypothesis, we aimed to describe the potential relationship between age–at–diagnosis and the proportion of breast cancer patients with lymph node positive disease. Our results showed that the proportion of women with unknown pathologic lymph node status increases markedly after age 70. Therefore, our data could not provide enough information about the lymph node status among elderly women with breast cancer to evaluate the potential association between age–at–diagnosis and node–status mediated by declining function of the immune system.

In study II (1980–2009), we included 78,095 women with breast cancer to investigate if autoimmune diseases are associated with breast cancer recurrence. We found no association between exposure to autoimmune diseases and risk of breast cancer recurrence. In subcategories based on organ or tissue of origin, the risk estimates remained near null, with the possible exception of autoimmune diseases affecting the CNS and neuromuscular system.

In study III (1996–2009), we included 19,919 women who had surgery for a first diagnosis of breast cancer and investigated if use of glucocorticoid was associated with the risk of re–operation due to post–surgical bleeding. Our results showed that women 80 years or older who had a mastectomy and ever used glucocorticoids had a higher risk of re–operation due to post–surgical bleeding than those women aged <80 years who received breast conserving surgery and never used glucocorticoids.

In study IV (1996–2008), we included 18,251 women with stage I–III breast cancer to investigate if use of glucocorticoid prescriptions is associated with breast cancer recurrence. Neither overall glucocorticoid use,

nor sub–categories of glucocorticoids, was associated with breast cancer recurrence. The results were robust to stratification by use of chemotherapy or by estrogen receptor status.

All studies were observational using medical and administrative registries and were therefore subject to bias and confounding. In particular misclassification related to information about actual use of drugs, lymph node status, and information about autoimmune diseases may explain our results.

8. Dansk resume

Brystkræft er den hyppigste kræftform blandt kvinder i den vestlige verden. Selvom overlevelsen efter en brystkræftdiagnose fortsat stiger, har vi stadig ikke det fulde overblik over, hvilke faktorer, der bestemmer hvem, der overlever deres kræftsygdom uden tilbagefald og hvorfor de overlever. Formålet med denne afhandling var at undersøge mulige sammenhænge mellem immunfunktion og det kliniske udfald af brystkræft i fire epidemiologiske studier, ved brug af alder–ved–diagnose, immunsupprimerende sygdomme og immunsupprimerende lægemidler som markører for vigende immunforsvar.

Studierne i denne afhandling består af et deskriptivt kohortestudie (studie I) og tre kohortestudier (studie II, III og IV). Vi brugte danske administrative og medicinske registre til at identificere kvinder med en invasiv brystkræftdiagnose og til at indhente information omkring indløste recepter, komorbiditet og det kliniske forløb af brystkræft. De anvendte statistiske metoder omfattede grafisk fremstilling, Cox regression og logistisk regression.

I studie I (2000–2013) indkluderede vi 62.393 kvinder med en invasiv brystkræftdiagnose. Tidligere forskning har foreslået, at kvinder >70 år har højere risiko for at have lymfeknudepositiv sygdom på diagnosetidspunktet end kvinder <70 år – selv ved små tumorer. Desuden, at dette resultat kunne bero på, at kvinder >70 år har et vigende immunforsvar. For at teste denne hypotese ønskede vi at beskrive den potentielle sammenhæng mellem alder–ved–diagnosetidspunktet og andelen af lymfeknudepositiv brystkræft. Vores resultater viste, at andelen af kvinder med ukendt patologisk lymfeknudestatus steg markant fra 70 års alderen. Derfor kunne vores data ikke give nok information omkring lymfeknudestatus hos ældre kvinder til at kunne sige noget om en mulig association mellem alder–ved–diagnose og lymfeknudestatus medieret af faldende immunfunktion.

I studie II (1980–2009) inkluderede vi 78.095 kvinder med brystkræft for at undersøge om autoimmune sygdomme er associerede med brystkræfttilbagefald. Vi fandt ingen sammenhæng mellem det at have en autoimmun sygdom og risikoen for brystkræfttilbagefald. Risikoen forblev nul når vi inddelte i underkategorier af autoimmune sygdomme baseret på det organ eller væv, hvor sygdommen er opstået, med en mulig undtagelse af sydomme i centralnervesystemet og det neuromuskulære system.

I studie III (1996–2009) inkluderede vi 19.919 kvinder, som havde fået kirurgisk behandling for primær brystkræft og undersøgte, om brugen af glucocorticoider var associeret med risiko for reoperation pga. post–operativ blødning. Vores resultater viste, at kvinder over 80 år, som fik udført en mastektomi og på et tidspunkt havde brugt glucocorticoider, havde en højere risiko for re–operation pga. post–operativ blødning end kvinder under 80 år, som fik udført en brystbevarende operation og som aldrig havde brugt glucocorticoider.

I studie IV (1996–2008) inkluderede vi 18.251 kvinder med stadie I–III brystkræft for at undersøge om brug af glucocorticoider var associeret med brystkræfttilbagefald. Vi fandt ikke holdepunkter for en association mellem hverken brug af nogen form for glucocorticoider eller sub–kategorier af glucocorticoider. Resultatet var robust for stratificering i forhold til brug af kemoterapi og østrogenreceptorstatus.

Alle undersøgelserne var observationelle og der blev anvendt medicinske og administrative registre. Studierne er derfor udsat for bias og confounding. Særligt misklassifikation relateret til information omkring faktisk forbrug af medicin, lymfeknudestatus og information omkring autoimmune sygdomme kan muligvis forklare vores resultater.

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10. Appendices

10.1 Appendix 1

Study II (Autoimmune and recurrence). Distribution of autoimmune diseases in a cohort of 78,095 women with stage I–III breast cancer diagnosed in Denmark 1980–2007, by presence of recurrence.

	Regardless of diagnosis order*		**First AD diagnosis****	
Auto immune disease	**Recurrence*** ***N*(row%)**	**No recurrence*** ***N*(row%)**	**Recurrence** ***N*(row%)**	**No recurrence** ***N*(row%)**
No autoimmune disease	13,545(19)	57,834(81)		
Non-malignant hematological diseases				
Autoimmune hemolytic anemia	3(9)	31(91)	3(10)	27(90)
Idiopathic Thrombocytopenic Purpura (ITP)	5(10)	45(90)	3(8)	36(92)
Endocrine diseases				
Grave's disease	160(13)	1,087(87)	154(13)	1,064(87)
Autoimmune thyroiditis	14(11)	115(89)	13(11)	104(89)
Addison's disease	7(19)	29(81)	6(18)	27(82)
Diabetes I	130(9)	1,260(91)	120(9)	1,218(91)
Central nervous /neuromuscular diseases				
Multiple sclerosis	34(11)	268(89)	33(12)	239(88)
Myasthenia gravis	2(5)	35(95)	1(4)	24(96)
Gastrointestinal / hepato-billiary diseases				
Pernicious anemia	20(11)	170(89)	16(11)	126(89)
Coeliac disease	4(7)	53(93)	4(10)	37(90)
Ulcerative colitis	63(11)	508(89)	53(12)	408(88)
Crohn's disease	22(9)	233(91)	16(9)	158(91)
Primary biliary cirrhosis	5(12)	36(88)	3(10)	27(90)
Autoimmune hepatitis	1(3)	36(97)	1(4)	23(96)
Skin diseases				
Pemphigus/pemphigoid	1(3)	39(97)	0(0)	29(100)
Dermatitis herpetiformis	2(11)	16(89)	2(13)	13(87)
Psoriasis	56(13)	387(87)	45(13)	298(87)
Vitiligo	4(17)	19(83)	3(25)	9(75)
Connective tissue diseases				
Scleroderma	10(16)	54(84)	9(20)	36(80)
Juvenile Rheumatoid Arthritis	2(12)	15(88)	1(8)	11(92)
Rheumatoid arthritis	173(13)	1,127(87)	152(13)	992(87)
Ankylosing spondylitis	7(17)	34(83)	6(17)	29(83)
Polymyositis/dermatomyositis	6(14)	36(86)	4(12)	29(88)
Systemic lupus erythematosus	20(19)	85(81)	13(19)	57(81)
Sjögren's syndrome	6(11)	48(89)	3(14)	19(86)
Sarcoidosis	28(16)	150(84)	25(16)	129(84)
Polyarthritis nodosa	2(8)	23(92)	0(0)	17(100)
Wegener's granulomatosis	4(22)	14(78)	3(21)	11(79)
Temporal arteritis / polymyalgia rheumatic	69(8)	796(92)	58(8)	685(92)
Mixed connective tissue disorder	13(11)	102(89)	9(11)	75(89)

*Women in the study had 0-8 autoimmune diseases and in this table all diseases are presented why the number of diseases will add up to more than the number of women with an autoimmune disease.

** In cases where a woman had more than one AD diagnosed on the same first day we made the rule that the disease with the highest incidence as first AD diagnosis among women with only one AD was counted as the first AD.

10.2 Appendix 2

Study I (Age and lymph node). Variables collected from The Danish Breast Cancer Cooperative Group (DBCG) and The Danish National Pathology Registry (DNPR) in a cohort of 62,393 women with invasive breast cancer diagnosed 2000–2013 in Denmark.

	DBCG	**DNPR**
Variables in registry	Tumor characteristics	SNOMED
Age and time	CPR and date of first registration (i.e. date of surgery if surgery was performed)	CPR and date of sample arrival
Tumor specific	Surgery type, sentinel node technic, estrogen receptor status, and tumor size	None
Breast cancer and lymph node	This registry only includes persons with an invasive breast cancer diagnosis. We requested only women. Lymph node evaluation variable: ”Nodepos”	We requested only women. Site codes (breast): T04* Cancer codes: M80xxY, M81xxY, M82xxY, M83xxY, M84xxY, M85xxY

10.3 Appendix 3

Study III (Glucocorticoid and re-operation due to bleeding). Anatomical Therapeutic Chemical (ATC) codes used to identify exposure drugs (containing glucocorticoids) and potential confounder drugs from the Aarhus University Prescription Database.

Exposure drugs	**ATC codes**
Systemic glucocorticoids	H02AB, H02BX01
Inhaled glucocorticoids	R01AD02, R01AD03, R01AD06, R01AD52, R01AD53, R01AD60, R03BA01, R03BA02, R03BA05, R03BA07
Other glucocorticoids	A07EA01, A07EA04, C05AA01, C05AA04
Potential confounder drugs	**ATC codes**
Aspirin low dose	B01AC06, N02BA01
Vitamin K antagonists	B01AB01, B01AA03, B01AA04, B01AC07, B01AC04
Oral anti-coagulants	B01AB02, B01AB04, B01AB05, B01AB08, B01AB10, B01AC06, B01AC09, B01AC13, B01AC14, B01AC16, B01AC17, B01AC30, B01AD01, B01AD02, B01AD04, B01AD07, B01AD10, B01AD11, B01AE04, B01AE05, B01AX03, B01AX05
NSAIDs	M01A, excluding selective Cox-2 inhibitors
Anti-depressants	N06AA01, N06AA02, N06AA03, N06AA04, N06AA07, N06AA09, N06AA10, N06AA11, N06AA12, N06AA16, N06AA17, N06AA21, N06AX03, N06AX21, N06AX16, N06AX11, N06AX18, N06AF01, N06AG02, N06AB03, N06AB04, N06AB05, N06AB06, N06AB08, N06AB10

10.4 Appendix 4

Study III (Glucocorticoid and re-operation due to bleeding). International Classification of Diseases (ICD) codes used to identify relevant diagnoses and surgical procedures from the National Registry of Patients.

Diagnosis/procedure	**ICD-10 codes**
First diagnosis of breast cancer	C50.0–50.6, C50.8 and C50.9
Primary surgery type	Mastectomy: KHAC Breast conserving surgery: KHAB
Re-operation due to bleeding	KHWD00 and KHWE00

10.5 Appendix 5

Study IV (Glucocorticoid and recurrence). ATC codes from the Danish National Prescription Registry.

Exposure drugs	**ATC codes**
Systemic glucocorticoids	H02AB
Inhaled glucocorticoids	R03BA
Intestinal-acting glucocorticoids	A07EA01, A07EA02, A07EA06, C05AA01, C05AA05, C05AA08

Potential confounder drugs	**ATC codes**
Postmenopausal hormone replacement therapy	G03C, G03D, G03F
NSAIDs	M01A
Aspirin	B01AC06, N02BA01, N02BA51
Statins	C10AA (simvastatin: C10AA01)
ARBs	C09C, C09D
ACE inhibitors	C09A, C09B, C02E, C02L
Anticoagulants	B01A
Betablockers	C07
Methotrexate	L01BA01
Azathioprine	L04AX, L04AX01
Valproic acid	N03AG01
Anti-arrhythmic drugs	C01EB10, C01BD01, C01BD07, C01BC04, C01BB01, N01BB02, C01BC03, C01BG11, C01AA05
Angina drugs	C01DA, C01DX16
Anti-diabetics	A10A
Migraine drugs	N02CC, N02CA, N02CX01, N02CX02, N07CA03, N03AX11
COPD drugs	R03AB, R03AC

10.6 Appendix 6

Study IV (Glucocorticoid and recurrence). International Classification of Diseases (ICD) codes on relevant non-Charlson comorbidities from the Danish National Registry of Patients used to adjust for or stratify past medical history in studies IV.

Non-Charlson comorbidities	**ICD-8**	**ICD-10**
Chronic Obstructive Pulmonary Disease	490,491, 492	J40, J41, J42, J43, J44, J47
Parkinson's disease	342	G20
Asthma	493	J45, J46
Inflammatory Bowel Disease	563.01, 563.02, 563.09, 563.19	K50, M07.4, M07.5, K51
Asthma	493	J45, J46
Arrhythmia	427.90, 427.97	I47, I48, I49.
Rheumatoid Arthritis	712.09, 712.19, 712.29, 712.39, 712.59	M05-M08, G73.7D, I32.8A, I39.8E, I41.8A, I52.8A

10.7 Appendix 7

Studies II and IV (Autoimmune and recurrence & Glucocorticoid and recurrence). The Charlson Comorbidity Index (CCI) was used to adjust for or stratify past medical history in studies II and IV. International Classification of Diseases (ICD) codes were retrieved from the Danish National Registry of Patients. In study II the CCI was modified to not include any autoimmune disease diagnoses used as exposure in the study.

Codes excluded as comorbidity in study II: ICD-8: 135.99, 249, 446.09, 712.09, 712.19, 712.29, 712.39, 712.59, 734.19, 716.09, 716.19, 734.00, and 734.90. ICD-10: DE10, DM05-06, DM08, DM30, DM32-3, DD86, and DC50.

Charlson Comorbidity Index and comorbidity groups:

Charlson comorbidity category	**ICD-8**	**ICD-10**	**Charlson comorbidity index score**	**Comorbidity groups**
Myocardial infarction	410	I21; I22; I23	1	Myocardial infarction
Congestive heart failure	427.09; 427.10; 427.11; 427.19; 428.99; 782.49	I50; I11.0; I13.0; I13.2	1	Congestive heart failure
Peripheral vascular disease	440; 441; 442; 443; 444; 445	I70; I71; I72; I73; I74; I77	1	Peripheral vascular disease
Cerebrovascular disease	430–438	I60–I69; G45; G46	1	Cerebrovascular disease
Dementia	290.09–290.19; 293.09	F00–F03; F05.1; G30	1	–
Chronic pulmonary disease	490–493; 515–518	J40–J47; J60–J67; J68.4; J70.1; J70.3; J84.1; J92.0; J96.1; J98.2; J98.3	1	Chronic pulmonary disease
Connective tissue disease	712; 716; 734; 446; 135.99	M05; M06; M08; M09; M30; M31; M32; M33; M34; M35; M36; D86	1	–
Ulcer disease	530.91; 530.98; 531–534	K22.1; K25–K28	1	Peptic ulcer disease
Mild liver disease	571; 573.01; 573.04	B18; K70.0–K70.3; K70.9; K71; K73; K74; K76.0	1	Liver disease
Diabetes type 1	249.00; 249.06; 249.07;	E10.0, E10.1; E10.9	1	Diabetes

Charlson comorbidity category	ICD-8	ICD-10	Charlson comorbidity index score	Comorbidity groups
	249.09			
Diabetes type 2	250.00; 250.06; 250.07; 250.09	E11.0; E11.1; E11.9		
Hemiplegia	344	G81; G82	2	–
Moderate to severe renal disease	403; 404; 580–583; 584; 590.09; 593.19; 753.10–753.19; 792	I12; I13; N00–N05; N07; N11; N14; N17–N19; Q61	2	Renal disease
Diabetes with end organ damage type 1	249.01–249.05; 249.08	E10.2–E10.8	2	Diabetes
type 2	250.01–250.05; 250.08	E11.2–E11.8		
Any tumor	140–194	C00–C75	2	Cancer
Leukemia	204–207	C91–C95	2	Cancer
Lymphoma	200–203; 275.59	C81–C85; C88; C90; C96	2	Cancer
Moderate to severe liver disease	070.00; 070.02; 070.04; 070.06; 070.08; 573.00; 456.00–456.09	B15.0; B16.0; B16.2; B19.0; K70.4; K72; K76.6; I85	3	Liver disease
Metastatic solid tumor	195–198; 199	C76–C80	6	Cancer
AIDS	079.83	B21–B24	6	–

11. Thesis papers

Paper I

Paper II

Paper III

Paper IV

Paper I

Age at Diagnosis and Proportion of Node–Positive Breast Cancer Cases: A Danish Population–based Study

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Abstract

Background

Lymph node status (LNS) at diagnosis is an important prognostic factor for survival among women with breast cancer. An earlier study reported that age >70 years at diagnosis was associated with positive axillary LNS and hypothesized that this could be explained by declining immune competence in older adults—also known as immunosenescence. The earlier study was restricted to women with known LNS. We aimed to replicate and improve the earlier study by examining the association between age at diagnosis and LNS, including unknown status, in a large population–based cohort of women diagnosed with breast cancer.

Methods

We used nationwide medical registries in Denmark to assemble a cohort of women diagnosed with breast cancer during 2000–2013. We examined time trends for age at diagnosis and distribution of lymph node evaluation, using a stacked bar chart and an area chart. Women with no registered pathologic lymph node evaluation were classified as having unknown pathologic LNS.

Results

The study included 62,393 women with invasive breast cancer. Of these, 29% were ≥70 years old. 14% had unknown pathologic LNS and of these 61% were aged ≥70. We observed that the overall proportion of patients with positive LNS decreased from 41% - 33% from 2000-2013, while the proportion with unknown pathologic LNS was stable on 16% with a dip to 12% in 2009.

Conclusion

Women over age 70 with breast cancer are less likely than younger women to undergo pathologic evaluation of their axillary lymph nodes. Without complete pathologic data, it is not possible to evaluate accurately the immunosenescence hypothesis.

Article

Background

Lymph node status at diagnosis is an important prognostic factor for breast cancer outcome [1]. A Belgian study [2] reported that up to age 70, increasing age is associated with a decreasing proportion of breast cancer patients diagnosed with positive lymph nodes. In contrast, among women aged over 70 years, increasing age was associated with a rising proportion of lymph–node positive breast cancer diagnoses. This earlier study included only women with pathologically determined lymph node status [3, 4]. It was hypothesized that the increasing proportion of lymph-node positive disease among the elderly could be explained by a declining immune function, a phenomenon known as immunosenescence. Immunosenescence, or immune-aging, represents a continuum of changes that are related to age-related pathology [5, 6]. The changes in the distribution of immune cells affect primarily the adaptive immune system and have a smaller effect on the innate immune system [7]. The net effect of all the age-related changes in immune competence on cancer occurrence and prognosis is incompletely understood [8]. Consequences of immunosenescence on breast cancer risk and prognosis have been debated and no consensus has been established [8].

Several other studies [9-12], including a Danish study [13], have found that older women are less likely to receive a full lymph node evaluation at diagnosis. In the Wildiers study [2] the age distribution of the excluded women with no lymph node evaluation was not presented, but according to another study from the same cohort, 13% of women aged 70-79 and 41% of women 80 years or older did not receive an axillary lymph node evaluation [4, 14]. If a large proportion of the women >70 years of age are excluded from a cohort due to missing information about lymph node status, the age-related trends in positive node status may be distorted by the missing information. The age-related decline in the proportion of women with pathologic node evaluation may have biased the findings of the Belgian study [2].

The present study aimed to examine the relation between age at diagnosis and proportion of patients with lymph–node positive breast cancer, replicating the earlier Belgian study [2], but also taking into account the characteristics of the women without pathological evaluation of axillary lymph node status. We conducted a prevalence study with prospectively collected data from a large cohort of Danish women with breast cancer.

Materials and Methods

Setting and study population

We conducted our study in Denmark, which has a population of approximately 5.6 million persons. At birth, all Danes are assigned a unique personal civil registration number (CPR number) by the Danish Civil Registration System (CRS), which was founded in 1968. It covers all Danish citizens and legal residents [15, 16]. The CPR number allows unambiguous linkage among all Danish registries. The Danish National Health Service provides high–quality tax–supported health care for all residents, guaranteeing free access to hospitals and general practitioners.

Registries and cohort enrollment

We identified all Danish women aged ≥18 years diagnosed with primary invasive breast cancer from 1 January 2000 to 31 December 2013 by accessing information from the Danish Breast Cancer Cooperative Group Registry (DBCG) and the Danish National Pathology Registry (DNPR).

Danish Breast Cancer Cooperative Group

Since it was established in 1977, the DBCG has collected clinical data on most invasive breast cancers diagnosed in Denmark. Data on tumor and patient characteristics are collected routinely using DBCG standardized registration forms completed by treating physicians in pathology, surgery, and oncology departments throughout Denmark. Registration of breast cancers in the DBCG was 94% complete compared to the DNPR in 2011 [17]. Despite the accuracy of DBCG data, the registry lacks complete information on women in the >70 age group [18]. Clinicians have been encouraged since 2000 to register all women with an invasive breast cancer diagnosis in the DBCG regardless of age and treatment protocol [18], but older patients are still sometimes never registered. Before 2000, research protocols run by the DBCG had an upper age limit; 69 years before 1988 and 74 years from 1989–2000. These upper age limits were due to age-limits in randomized clinical trials implemented using the DBCG registry.

We retrieved information on date of diagnosis, treatment and tumor characteristics, and pathologic lymph node status from the DBCG (see Appendix for details).

Danish National Pathology Registry

The DNPR was founded in 1997 when several local registries were merged into a national registry and reporting from all pathology departments in Denmark became mandatory. The DNPR provides information using the Danish version of the Systemized Nomenclature of Medicine (SNOMED) codes [19] describing pathological and anatomical diagnostic tests. The DNRP contains complete data since 1997 [19, 20]. This

registry provided information on CPR, date of diagnostic test for invasive breast cancer, and SNOMED code for invasive breast cancer (see Appendix for details).

Variable definitions

We examined age at breast cancer diagnosis by year (continuous) and by decade of age (18–29, 30–39, 40–49, 50–59, 60–69, 70–79, 80–89, and ≥90 years). Time was measured by calendar year from 2000 to 2013. Data on lymph node status were categorized into three groups: (1) known lymph–node positive, according to pathologic record (2) known lymph–node negative, according to pathologic record, and (3) no registered pathologic lymph node status, but confirmed diagnosis of invasive breast cancer. It is likely that women in the third category received a clinical lymph node evaluation by means of surgery, palpation, or ultrasound of the axilla, but their lymph node status was not pathologically evaluated or recorded.

Before introduction of the sentinel lymph node biopsy technique into routine clinical practice in Denmark in the early 2000s [21, 22], decisions about axillary lymph node dissection were based on clinical evaluation of the patient by palpation and ultrasound [23].

For women in our cohort, information about tumor size was available only in the DBCG registry. We categorized tumor size as recorded in the registry to fit the definitions used for TNM staging [24], *i.e*., T1: tumors ≤20 mm; T2: tumors 21 mm–50 mm; T3: tumors >50 mm; and tumor size unknown.

Data analysis

The DBCG and DNPR datasets were merged using the patient's unique CPR number. All data on lymph node status in the DBCG registry were compared to DNPR data and augmented accordingly so that any potential extra information registered in the DNPR about lymph node evaluation or other pathological information was added to the information already in the DBCG registry. For women with invasive breast cancer but without registration of lymph node status in either the DBCG or the DNPR, the pathologic lymph node status was categorized as unknown.

Frequencies and proportions of characteristics of women in the cohort were tabulated according to lymph node status and database (Table 1). We used a stacked bar chart to show the distribution of age at diagnosis and axillary lymph node status (Figure 1). We also created an area chart with age at diagnosis on the x–axis and proportion of women with lymph node status at time of diagnosis on the y–axis (Figure 2). The proportions in this chart were smoothed across five years, with weights of 1, 2, 3, 2, and 1, assigned respectively to the first through fifth years, with the mid–year used as the plotting point on the x-axis. Finally, we generated a trend line (Figure 3) to describe lymph node status distribution from 2000 to 2013, stratified by age <70 years and ≥70 years at diagnosis.

Ethics

The study was approved by the DBCG Registry Board and the Danish Data Protection Agency (record number: 2013–41–1760).

Results

The study included 62,393 women aged 18 years or older diagnosed with invasive breast cancer in Denmark between 2000 and 2013 and registered in DBCG and/or DNPR. The age range was 18 to 105 years and the median age was 62 years. Among enrolled women, 18,286 (29%) were ≥70 years old. Women with unknown pathologic lymph node status were older than the rest of the cohort, with a median age of 76 years.

Table 1 and Figures 1and 2, present the cohort's age distribution and the characteristics of lymph node evaluation. Overall, 8,981 (14%) had no registered pathologic lymph node status. Of this group, 61% were women aged ≥70. Women with tumors >20 mm were more likely to have received a mastectomy and to have positive lymph node status.

The proportion of patients with positive lymph node status decreased from 41% to 33% from 2000-2013, while the proportion with unknown pathologic lymph node status was stable at 16% over the time period 2000-2013 – but with a temporary dip to 12% around 2009. When these results are stratified by age <70 years and ≥70 years, the trend among the younger group is similar with a decrease in the proportion of patients with positive lymph node status from 49% to 34% from 2004 to 2013 (Figure 3a). Among women ≥70 years, the proportion with positive lymph node status increased to 40% though 2009 and thereafter decreased to 31% by 2013. In this elderly group, the proportion with unknown pathologic lymph node status decreased from 36% in 2002 to 27% in 2009 and then increased to 32% in 2013 (Figure 3b).

In the group of women <70 years old, more than half of tumors were <20 mm in size. In the group of women ≥70 years old, the registered tumor size distribution moves towards larger tumors and an increasing proportion of women with unknown tumor size. This result follows the trend in Table 1, where out of 4,082 women only registered in the DNPR, 2,241 (55%) were women ≥70 years and had no recorded tumor size.

Discussion

In this Danish population–based study, we found that women diagnosed with invasive breast cancer after age 70 were less likely to have a recorded pathologic axillary lymph node evaluation than women diagnosed before age 70. Unknown lymph node status stems largely from lack of pathologic node evaluation, so older women received a full prognostic evaluation less often than younger women. This finding is consistent with previous American and Danish studies [9, 10, 13, 25, 26].

Wildier's hypothesis that increasing proportion of women with node–positive tumors at older ages may be explained by declining immunocompetence in the elderly or immunosenescence [2] was criticized in letters [3] [4]. The letters argued that the apparent higher proportion of patients with positive lymph node status among elderly women may be explained by differences in choice of surgery, less access to screening programs, or different pathology regarding estrogen receptor status and other tumor characteristics. Other researchers attempting to replicate the Wildiers study [2] could not confirm its results [27] or restricted their study to younger age groups [28]. In our study, the age disparity in available lymph node information makes it impossible to make an inference about the immune competence of older women and its effect on their lymph node status at diagnosis. This calls into question as well the immune competence hypothesis put forth in the Wildiers study [2], which did not account for women with unknown pathologic node status.

The major strengths of our study are its large sample size and complete cohort identification using high quality and valid national population–based registries. This study also has some important limitations. We did not have complete and precise information on tumor size available for the whole cohort. However, the majority of women in our cohort were registered in the DBCG, which provided information about tumor size, as well as information on use of the sentinel node technique since 2002. Our data showed the same pattern as that in a previous Danish study, in which predominantly older women were not registered in the DBCG database [13].

Over the 2000–2013 study period, diagnostic procedures evolved [29]. Most importantly, the sentinel node technique was introduced and became standard procedure in the entire country since 2004 [21, 22]. Surgical treatment preferences and guidelines also changed, from a predominance of mastectomies to breast–conserving surgery as the preferred treatment. This trend also occurred among older women [30].

Some women with clinically negative lymph node status evaluated by palpation may be misclassified. Had they received a definite pathological evaluation, they would have been classified in the lymph–node positive category [9, 31, 32]. In earlier studies, between 17% and 27% of clinically evaluated node–negative women were classified as node positive when their tumors were pathologically staged [9, 31, 32]. The misclassification rate has not been adequately characterized as a function of age at diagnosis.

During the 2000–2013 period, the proportion of patients aged <70 years with positive lymph nodes decreased. This decrease is most likely due to opportunistic screening and the introduction of a national breast cancer screening program. Beginning in 2007, women aged 50–69 were invited biennially to undergo screening mammography. This program likely underlies the sudden increase in frequency of breast cancer diagnoses at age 50, as seen in Figure 1. Introduction of screening was followed by a larger proportion of women with lower stage than before screening was introduced [33]. The increasing proportion of patients with unknown pathologic lymph node status during the two last years of the study period] could stem from

delayed reporting of pathologic staging results to the registry. Among women ≥70 years, the trend of an increased proportion of lymph–node negative breast cancer is less marked, possibly because women in this age group are not invited to the screening program. It is clear that the proportion of patients with unknown pathologic lymph node status is around 30% among women ≥70 years, in contrast to about 8% among women <70 years old.

Sentinel node evaluation became the standard of care during the study period. The breast cancer treatment recommendations issued in Denmark in 2006 state that all women diagnosed with invasive breast cancer should be offered a full diagnostic evaluation regardless of age [21]. Further knowledge is needed about how and why patients and their physicians decide on diagnostic procedures and breast cancer treatment. It also would be important to examine whether women with less than definitive staging have a worse outcome than fully staged women in the same age category, since previous research results are inconsistent [13, 22, 26, 34, 35].

Access to data about comorbidity would potentially have helped us to better understand the characteristics the group of women with unknown pathologic lymph node status. From the literature we know that especially elderly women with breast cancer also have a higher level of comorbidity [36]. A high level of comorbidity may impact the choice of treatment among both the patient and the treating physician and would potentially be the reason why a woman does not receive any surgery or limited surgery. Increasing age and comorbidity influence the competing risks of death among women with breast cancer and might cause a woman to die of non-cancer related reasons before a potential recurrence would be experienced [37]. However, knowledge about comorbidity would not have solved the main problem in this study, namely the lack of comprehensive information about lymph node status in the elderly.

Conclusion

Our results, based on two comprehensive population–based registries in Denmark, do not provide enough information about lymph node status among elderly breast cancer patients to evaluate the potential association between age at diagnosis and node status, potentially mediated by declining function of the immune system. The Belgian study [2] also cannot provide convincing evidence about this association, since it excluded women with unknown pathologic node status. More complete data are needed to evaluate any potential impact of immunosenescence on stage in older women diagnosed with breast cancer. Further information also is needed to understand the relation between age at diagnosis and incomplete pathology information on node status.

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Table 1. Cohort characteristics and distribution of axillary lymph node status evaluation at diagnosis in a cohort of Danish women with an invasive breast cancer diagnosis 2000–2013. N=62,393.

	N (column %) (*N*=62,393)	**Positive lymph node status** **N (column %) (row %) (*N*=25,689)**	**Negative lymph node status** **N (column %) (row %) (*N*=27,723)**	**Unknown pathologic lymph node status** **N (column %) (row %) (*N*=8,981)**	**DNPR only** **N (column %) (row %) (*N*=4,082)**
Age category					
18–29	202 (0.3)	99 (0.4) (49)	80 (0.3) (40)	23 (0.3) (11)	16 (0.4) (7.9)
30–39	2,258 (3.6)	1,164 (4.5) (52)	912 (3.3) (40)	182 (2.0) (8.0)	108 (2.7) (4.8)
40–49	8,189 (13)	4,063 (16) (50)	3,551 (13) (43)	575 (6.4) (7.0)	300 (7.4) (3.7)
50–59	15,089 (24)	6,691 (26) (44)	7,279 (26) (48)	1,119 (12) (13)	584 (14) (3.9)
60–69	18,369 (29)	7,195 (28) (39)	9,594 (35) (52)	1,580 (18) (8.6)	833 (20) (4.5)
70–79	10,644 (17)	4,307 (17) (41)	4,527 (16) (43)	1,810 (20) (17)	938 (23) (8.8)
80–89	6,496 (10)	1,989 (7.7) (31)	1,671 (6.0) (26)	2,836 (32) (44)	1,018 (25) (16)
≥90	1,146 (1.8)	181 (0.7) (16)	109 (0.4) (9.5)	856 (10) (75)	285 (7.0) (25)
Surgery type					
Mastectomy	23,904 (38)	13,890 (54) (58)	9,190 (33) (38)	824 (9.2) (3.4)	0
BCS*	29,112 (47)	9,966 (39) (34)	18,138 (65) (78)	1,008 (11) (3.4)	0
BCS* & Mastectomy	836 (1.3)	468 (1.8) (56)	356 (1.3) (43)	12 (0.1) (1.4)	0
Missing information**	8,541 (14)	1,365 (5.3) (16)	39 (0.1) (0.4)	7,137 (80) (84)	4,082 (100) (48)
Estrogen receptor					
Positive	45,017 (72)	20,127 (78) (45)	22,880 (83) (51)	2,010 (22) (4.4)	0
Negative	9,229 (15)	4,368 (17) (47)	4,563 (17) (49)	298 (3.3) (3.2)	0

	N (column %) (*N*=62,393)	Positive lymph node status N (column %) (row %) (*N*=25,689)	Negative lymph node status N (column %) (row %) (*N*=27,723)	Unknown pathologic lymph node status N (column %) (row %) (*N*=8,981)	DNPR only N (column %) (row %) (*N*=4,082)
Missing information	8,147 (13)	1,194 (4.7) (15)	280 (1.0) (3.4)	6,673 (74) (82)	4,082 (100) (50)
Tumor size					
≤20 mm	31,681 (51)	10,722 (42) (34)	20,118 (73) (64)	841 (9.4) (2.7)	0
21–50 mm	18,477 (30)	11,090 (43) (60)	6,728 (24) (36)	659 (7.3) (3.6)	0
>50 mm	1,790 (2.9)	1,417 (5.5) (79)	297 (1.1) (17)	76 (0.9) (4.2)	0
Unknown	10,445 (17)	2,460 (10) (24)	580 (2.1) (5.6)	7,405 (83) (71)	4,082 (100) (39)
Sentinel node technique* **					
Used	32,595 (52)	12,008 (47) (37)	20,492 (74) (63)	95 (1.1) (0.2)	0
Unknown	29,798 (48)	13,681 (53) (46)	7,231 (26) (24)	8,886 (99) (30)	4,082 (100) (14)
Year of diagnosis					
2000	3,799 (6.1)	1,562 (6.1) (41)	1,628 (5.9) (43)	609 (6.8) (16)	349 (8.5) (9.1)
2001	3,763 (6.2)	1,552 (6.0) (41)	1,727 (6.2) (46)	569 (6.3) (15)	321 (7.9) (8.5)
2002	4,289 (6.6)	1,808 (7.0) (43)	1,665 (6.0) (39)	646 (7.2) (15)	387 (9.5) (9.0)
2003	4,141 (6.4)	1,761 (6.9) (43)	1,609 (5.8) (39)	610 (6.8) (15)	378 (9.3) (9.3)
2004	4,058 (6.4)	1,799 (7.0) (44)	1,594 (5.8) (39)	566 (6.3) (14)	162 (4.0) (4.0)
2005	4,066 (6.3)	1,778 (6.9) (44)	1,578 (5.7) (39)	585 (6.5) (14)	274 (6.7) (6.7)
2006	4,220 (6.6)	1,835 (7.1) (43)	1,713 (6.2) (41)	597 (6.7) (14)	277 (6.8) (6.6)
2007	4,227 (6.7)	1,853 (7.2) (44)	1,734 (6.3) (41)	589 (6.6) (14)	313 (7.7) (7.4)
2008	4,857 (7.7)	2,100 (8.2)	2,072 (7.5)	635 (7.1)	332 (8.1)

	N (column %) (*N*=62,393)	Positive lymph node status N (column %) (row %) (*N*=25,689)	Negative lymph node status N (column %) (row %) (*N*=27,723)	Unknown pathologic lymph node status N (column %) (row %) (*N*=8,981)	DNPR only N (column %) (row %) (*N*=4,082)
		(43)	(43)	(13)	(6.8)
2009	5,877 (9.5)	2,413 (9.4) (41)	2,821 (10) (48)	699 (7.8) (12)	311 (7.6) (5.3)
2010	5,227 (8.5)	2,149 (8.4) (41)	2,465 (8.9) (47)	689 (7.7) (13)	230 (5.6) (4.4)
2011	4,733 (7.6)	1,779 (6.9) (38)	2,285 (8.2) (48)	693 (7.7) (15)	275 (6.7) (5.8)
2012	4,692 (7.5)	1,663 (6.5) (35)	2,345 (8.5) (50)	684 (7.6) (15)	217 (5.3) (4.6)
2013	4,934 (7.9)	1,637 (6.4) (33)	2,487 (9.0) (50)	810 (9.0) (16)	256 (6.3) (5.1)

*BCS, Breast conserving surgery.
**Only registered in the DBCG and not in DNPR.
***Technique not fully implemented in Denmark until 2006.

Figure 1. Frequencies of age at diagnosis and lymph node status at diagnosis in a cohort of 62,393 women with invasive breast cancer diagnosed during 2000–2013 in Denmark.

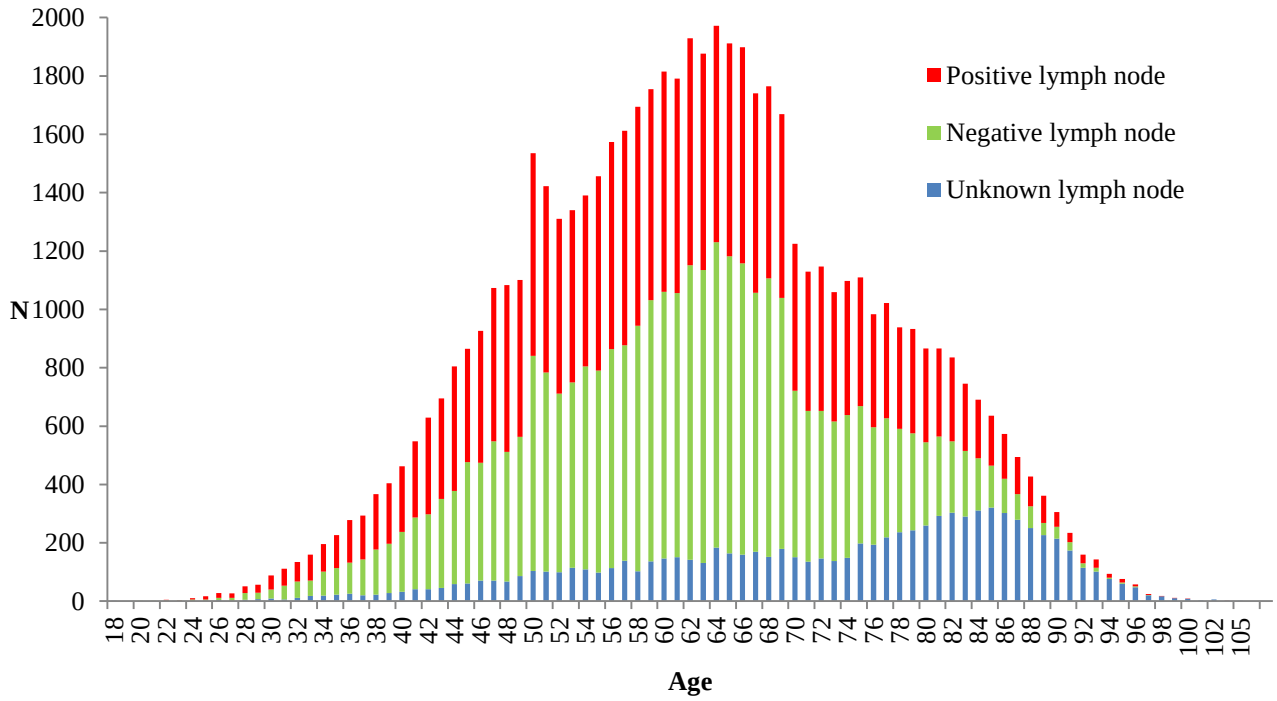


Figure 2. Area–chart showing distribution of lymph node status at diagnosis according to age at diagnosis. Cohort of 62,393 women with an invasive breast cancer diagnosis in Denmark, 2000–2013.

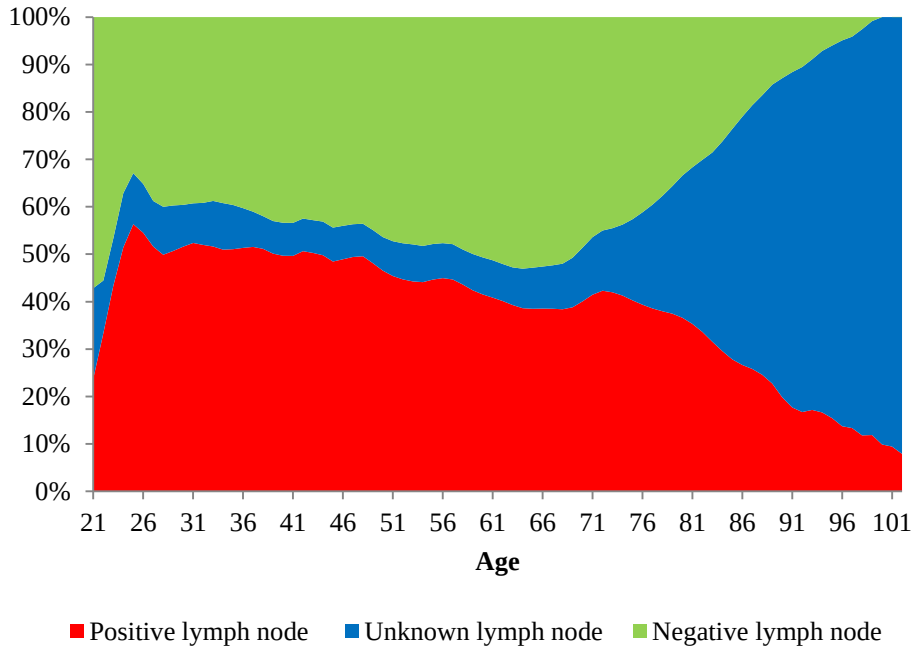
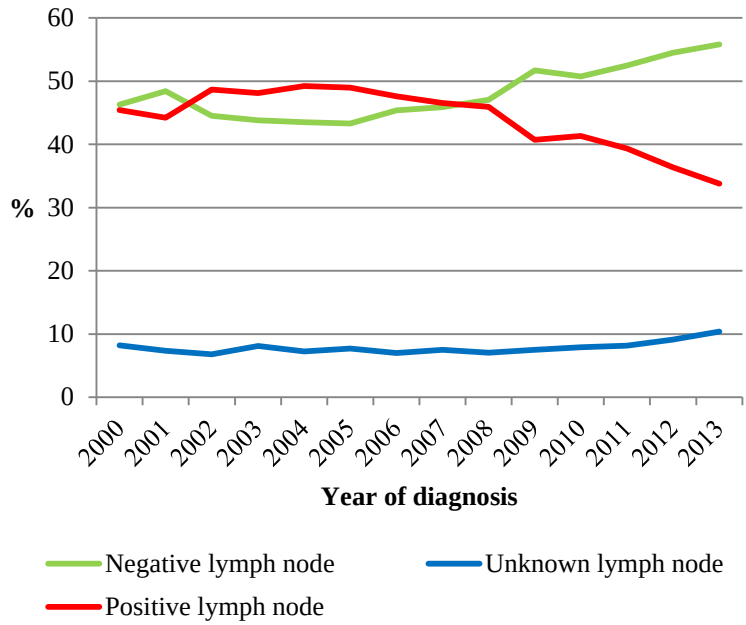
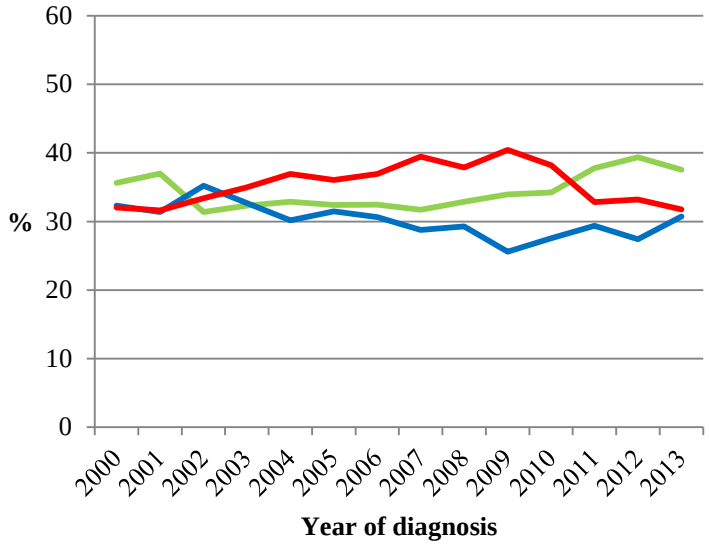


Figure 3. Time trend of lymph node evaluation from 2000 to 2013 stratified by age <70 or ≥70 years. (*N*=62,393).

Women <70 years (*N*=44,107):



Women ≥70 years (*N*=18,286):



Appendix:

Variables collected from the Danish Breast Cancer Cooperative Group (DBCG) and the Danish National Pathology Registry (DNPR) for a cohort of 62,393 women with invasive breast cancer diagnosed during 2000–2013 in Denmark.

	DBCG	**DNPR**
Variables in registry	Tumor characteristics	SNOMED
Age and time	CPR and date of first registration (*i.e.*, date of surgery if surgery was performed)	CPR and date of sample arrival
Tumor–specific	Surgery type, sentinel node technique, estrogen receptor status, and tumor size	None
Breast cancer and lymph node	This registry only includes persons with an invasive breast cancer diagnosis. We requested data only for women. Lymph node evaluation variable: ”Nodepos”	We requested data only for women. Site codes (breast): T04* Cancer codes: M80xxY, M81xxY, M82xxY, M83xxY, M84xxY, M85xxY

Paper II

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EPIDEMIOLOGY

Autoimmune diseases and breast cancer recurrence: a Danish nationwide cohort study

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Abstract Autoimmune diseases (ADs) comprise a large group of heterogeneous diseases in which the immune system attacks healthy organs. Both intrinsic changes in the body and AD treatment can compromise immune function. Impaired immune function could increase the risk of recurrent cancer. We aimed to investigate this hypothesis in a population-based epidemiological study. We examined the risk of breast cancer (BC) recurrence associated with an AD diagnosis among patients with incident stages I–III BC diagnosed during 1980–2007. Data were obtained from Danish population-based medical registries. ADs were categorized dichotomously and according to organ system of origin. Follow-up was up to 10 years or until 31 December 2009. Multivariate Cox proportional hazard regression was used to compute hazard ratios (HRs) and associated 95 % confidence intervals (95 % CIs) to evaluate the association between AD diagnosis and BC recurrence. 78,095 women with stages I–III BC were identified. Median age-at-diagnosis was 61 years (19–102 years), median follow-up was 5.7 years, and 13,545 women had a recurrence during follow-up. 6,716 women had at least one AD. In adjusted models, the association between ADs and BC recurrence was near null: $HR_{adjusted}$ 0.96 (95 % CI 0.89, 1.04). These results held in all AD subcategories, except for central nervous/neuromuscular system ADs, with $HR_{adjusted}$ 0.56 (95 % CI 0.40, 0.78). Among women with BC, a history of at least one AD diagnosis was not associated with BC recurrence, with the possible exception of ADs of the central nervous/neuromuscular system.

Keywords Autoimmune diseases · Breast neoplasm · Clinical epidemiology · Outcome/recurrence · Denmark

Background

Autoimmune diseases (AD) comprise a large group of heterogeneous diseases in which the immune system attacks healthy cells that it is meant to protect [1]. The prevalence of ADs is 5–10 % in industrialized countries, and women are affected approximately ten times more often than men [2]. AD can affect either single or multiple organ systems [1]. Patients with ADs may have compromised immune function due to disease-induced intrinsic changes in the immune system, immunosuppressive drugs [1], or both [3].

Many reports indicate that AD diagnoses are associated with altered risk of cancer development [3–8]. For breast cancer (BC), the overall weight of the evidence suggests a protective effect of ADs or their treatment on BC risk [1, 5, 9]. In contrast, the effect of impaired immune function due to AD on BC recurrence is unknown. Recurrence is determined by multiple factors, such as disease stage, age, treatment factors, co-morbiditites and co-medications [10–15], and compromised immune function also may play a role.

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Research to date has focused mainly on selected AD types [15–18]. Only one epidemiological study has examined BC prognosis among patients with ADs as a group and by AD disease type [1]. We therefore undertook this study to provide more evidence concerning the potential association between AD and risk of BC recurrence, using a large cohort of women with BC registered in Danish national population-based medical registries.

Methods

Setting

We conducted this nationwide cohort study using population-based administrative and medical registries in Denmark, which has approximately 2.8 million female inhabitants. Denmark's National Health Service provides a tax-funded health care system that ensures free and equal care to all Danish citizens [19]. At birth or upon legal immigration, each person is assigned a unique personal registration number that allows unambiguous individual-level linkage among all national registries. We retrieved data from the Danish Breast Cancer Cooperative Group registry (DBCG) [20, 21], the Danish National Patient Registry (DNPR) covering all Danish hospitals [20], and the Danish Civil Registration System (CRS) [19] to conduct the present study.

Study population

We identified all women aged ≥18 years registered in the DBCG with a first incident diagnosis of operable stages I–III BC between 1 January 1980 and 31 December 2007. Since its establishment in 1977, the DBCG has registered almost all women with invasive BC in Denmark. Prespecified data on tumor and patient characteristics are prospectively collected by clinicians in surgery, pathology, and oncology departments. Completeness of registration in the DBCG has increased from 87 % in 1986 to approximately 95 % in 2010 [22]. To detect recurrences, patients undergo a physical examination every 3–6 months during the first 5 years after diagnosis and an annual exam in years 6–10. A mammography is performed every second year [23]. Interval recurrences also are reported to the DBCG.

Autoimmune diseases

ADs are a heterogeneous group of diseases. Those included in the present study represent selected ADs sharing some common disease mechanisms. To identify women with AD, the DBCG study cohort was linked to the DNPR. The DNPR contains information on all non-psychiatric inpatient hospital discharge diagnoses since 1977 and on hospital outpatient clinic diagnoses since 1995. The diagnoses are registered according to the WHO's *International Classification of Diseases* (ICD). Based on the literature [24], we focused on 30 AD diagnoses common enough to be prevalent in our study cohort. The diseases were categorized into six subgroups based on the organ system or tissue of origin: benign hematological diseases, endocrine diseases, central nervous/neuromuscular system diseases, gastrointestinal/hepato-biliary diseases, skin diseases, and connective tissue diseases (see Table 2). We retrieved ICD-8 and ICD-10 codes for all ADs included in the present study. (See Appendix for specific codes.)

Data on comorbid diseases

We also collected information from the DNPR on diagnoses included in the Charlson comorbidity index (CCI) for all women in the study cohort. (See Appendix for ICD-8 and ICD-10 codes.) We modified the CCI to exclude AD diagnoses and BC diagnoses.

Outcome variables

We retrieved data from the DBCG on recurrent disease diagnosed up to ten years after initial BC surgery. We followed patients to recurrence, death, emigration, or 31 December 2009, whichever occurred first. BC recurrence was defined according to DBCG criteria as any local, regional, or distant recurrence, or cancer of the contralateral breast [21]. We retrieved information on death and migration from the CRS. The CRS, established in April 1968, contains information on the vital status of all Danish citizens and is updated daily. Women who emigrated from Denmark or who died without a BC recurrence during the follow-up period were censored on their date of emigration or death [19].

Statistical analyses

We estimated the frequency and proportion of BC patients with and without AD by patient, tumor, and treatment characteristics. We tabulated the prevalence of specific AD diagnoses in the cohort both according to presence of any AD and time to first AD.

To ensure that all patients had a definitive diagnostic stage and thereby avoid misclassification of women with disseminated disease, person-time was calculated from day 60 after BC surgery until end of follow-up. We calculated AD-unexposed and AD-exposed time for each woman based on the date of AD diagnosis. If AD was diagnosed before date of BC surgery or within the first 60 days after surgery, AD-exposed follow-up time began on day 60 after

surgery. If a woman received an AD diagnosis more than 60 days after surgery, her person-time between day 60 after surgery and the AD diagnosis date was categorized as AD-unexposed person-time, and her person-time from date of AD diagnosis forward was categorized as AD-exposed person-time. If a woman had more than one AD diagnosis, the date of the first recorded diagnosis was used. A woman was regarded as AD-exposed from the start of the exposure until end of follow-up. Person-time of women never diagnosed with an AD was categorized as AD-unexposed person-time from day 60 after BC surgery until end of follow-up.

We used unadjusted and multivariable Cox regression models to compute 10-year recurrence hazard ratios (HRs) and 95 % confidence intervals (95 % CIs) for exposure to any AD as well as exposure to ADs categorized by organ or tissue of origin. Competing risk of death was taken into account in all models [25]. The adjusted model included the following potential confounders: age group, presence/absence of chemotherapy, UICC stage, surgery type, modified CCI score, and menopausal status. Age at diagnosis was categorized into decades. Histologic grade was defined as low, moderate, or high. Stage was defined as I, II, or III according to the UICC classification. Estrogen receptor status (ER) and adjuvant endocrine therapy (ET) were summarized using a design variable: ER+/ET+, ER−/ER−, ER+/ET−, and ER−/ET+. Surgery type was defined as mastectomy or breast-conserving surgery in model adjustment and presented as a summarized variable combined with information on radiation therapy in Table 1. Menopausal status was defined as premenopausal or postmenopausal according to the definitions used in the DBCG registry. Treatments with adjuvant chemotherapy and radiation therapy were categorized dichotomously. We tested the proportionality of hazards by evaluating the significance of the interaction between ADs and the logarithm of person-time, and saw no evidence of a departure from proportionality.

Analyses were performed using SAS version 9.3 and STATA version 11.

Results

We identified 78,095 women aged ≥18 years with stages I–III incident BC. Baseline characteristics of the cohort are presented in Table 1. Median age was 61 years (age range 19–102 years). Median follow-up was 5.7 years. Among women in the study cohort, 13,545 developed a BC recurrence during follow-up and 6,716 (8.6 %) had at least one AD (range 0–8 ADs). In general, ADs were more prevalent among women aged 50–79 years and 87 % of the ADs are present among women 50 years and older. The AD-exposed group had a higher prevalence of comorbidities, and more women with an AD diagnosis were postmenopausal. The prevalence of AD increased from 5.2 % to 11.2 % from the earliest to the latest calendar period. The distribution of chemotherapy, stage, ER status, and surgery type was similar across exposure groups.

The frequency of each type of AD is shown in Table 2. The most frequent ADs in the cohort are diabetes I diagnosed in 1,390 women (1.8 % of cohort), rheumatoid arthritis (RA) diagnosed in 1,300 women (1.7 % of the cohort) and Grave's disease diagnosed in 1,247 women (1.6 % of the cohort). When considering the frequency of first AD, diabetes I is still most frequent with 1,338 diagnosed women, but Grave's disease is slightly more frequent than RA as a first AD diagnosis with 1,218 and 1,144 diagnosed women respectively.

The crude Cox regression model suggested an effect of AD on BC recurrence: HR_{crude} 0.83 (95 % CI 0.77, 0.89). The estimate was near null when adjusted for potential confounders: $HR_{adjusted}$ 0.96 (95 % CI 0.89, 1.04) (Table 3). This adjusted near-null result remained robust across subcategories of ADs according to organ or tissue of origin. An exception was the AD category of central nervous/neuromuscular system diseases. For this type of AD, we found a protective effect against BC recurrence, with $HR_{adjusted}$ 0.56 (95 % CI 0.40, 0.78).

Discussion

In this large cohort of Danish BC patients, we found a near-null association between AD diagnosis and risk of BC recurrence. The result remained near null when analyzed by AD organ/tissue category, except for the central nervous system/neuromuscular system category. The decreased rate of recurrence observed in patients with CNS diseases may be due to detection bias. Most of these women had multiple sclerosis (MS) ($N = 272$); 25 had myasthenia gravis. MS is a chronic disease that often presents in early adulthood and progresses into a severely debilitating and disabling disease [7]. Any evidence of BC recurrence in a patient with severe MS may have been overlooked by patients and physicians [26].

Our results support Hemminiki et al.*'s* study, which analyzed risk of death due to female cancers in a large Swedish cohort of women with ADs [1]. The study used Cox regression to compute HRs interpreted as mortality rate ratios (MRRs) for deaths from different female cancers, while censoring deaths from other causes. The study found that the risk of breast cancer-specific death among women with an AD compared to that expected based on the general population was near null ($HR_{adjusted}$ 0.95 (95 % CI 0.89, 1.02)) [1].

Table 1 Baseline characteristics of women with operable stages I, II, or III breast cancer diagnosed in Denmark from 1980 to 2007, by presence of autoimmune disease (AD). $N = 78{,}095$

Characteristics	Women no. (%)		Recurrence no. (%)		Total person-years no. (%)	
	+AD ($N = 6{,}716$)	No AD ($N = 71{,}379$)	+AD ($N = 759$)	No AD ($N = 13{,}545$)	+AD ($N = 108{,}300{,}000$)	No AD ($N = 1{,}081{,}000{,}000$)
Age at diagnosis, years						
≤29	8 (0.1)	311 (0.4)	4 (0.5)	112 (0.8)	134,286 (0.1)	4,592,062 (0.4)
30–39	168 (2.5)	3,612 (5.1)	37 (4.9)	1,171 (8.7)	2,597,414 (2.4)	53,103,333 (4.9)
40–49	786 (12)	12,770 (18)	122 (16)	3,054 (23)	12,691,266 (12)	192,300,000 (18)
50–59	1,407 (21)	17,659 (25)	217 (29)	3,965 (29)	23,206,460 (21)	273,400,000 (25)
60–69	1,853 (28)	17,406 (24)	263 (35)	3,798 (28)	30,203,853 (28)	265,500,000 (25)
70–79	1,642 (25)	12,974 (18)	96 (13)	1,271 (9.4)	26,085,294 (24)	192,800,000 (18)
≥80	852 (13)	6,647 (9.3)	20 (2.6)	174 (1.3)	13,406,176 (12)	99,745,394 (9.2)
Menopausal status at diagnosis						
Premenopausal	1,180 (18)	20,550 (29)	181 (24)	5,146 (38)	19,006,674 (18)	307,500,000 (28)
Postmenopausal	5,530 (82)	50,712 (71)	577 (76)	8,391 (62)	89,224,059 (82)	772,000,000 (71)
Missing	6	117	1	8	25,305 (0.0)	400,376 (0.04)
UICC stage						
I	2,139 (32)	21,749 (31)	191 (25)	3,164 (23)	35,011,432 (32)	338,500,000 (31)
II	2,736 (41)	29,631 (42)	306 (40)	5,765 (43)	44,347,007 (41)	449,600,000 (42)
III	1,046 (16)	12,511 (18)	234 (31)	4,106 (30)	16,601,880 (15)	183,600,000 (17)
Missing	795 (12)	7,488 (11)	28 (3.7)	509 (3.8)	12,364,430 (11)	109,711,163 (10)
ER/adjuvant ET status						
ER−/ET−	981 (15)	10,811 (15)	131 (17)	2,313 (17)	16,092,439 (15)	170,200,000 (16)
ER+/ET−	2,636 (39)	26,112 (37)	222 (29)	4,120 (30)	42,114,282 (39)	395,000,000 (37)
ER+/ET+	1,902 (28)	17,586 (25)	281 (37)	3,487 (26)	33,662,995 (31)	303,400,000 (28)
ER−/ET+	28 (0.4)	397 (0.6)	8 (1.0)	181 (1.3)	437,295 (0.4)	5,321,303 (0.5)
Missing*	1,902 (17)	16,473 (23)	117 (15)	3,444 (25)	15,992,989 (15)	207,078,697 (19)
Type of primary therapy						
Mastectomy	3,115 (46)	30,959 (43)	281 (37)	5,380 (40)	48,603,211 (45)	451,700,000 (42)
Mastectomy + RT	1,595 (24)	20,672 (29)	314 (41)	5,849 (43)	25,034,628 (23)	297,400,000 (28)
BCS	36 (0.5)	837 (1.2)	1 (0.1)	168 (1.2)	453,997 (0.4)	10,382,758 (1.0)
BCS + RT	1,661 (25)	15,887 (22)	157 (21)	2,070 (15)	29,348,175 (27)	276,500,000 (26)
No operation or RT	299 (4.5)	2,875 (4.0)	5 (0.7)	68 (0.5)	4,750,448 (4.4)	43,731,399 (4.1)
Only RT	10 (0.2)	149 (0.2)	1 (0.1)	10 (0.1)	134,290 (0.1)	1,792,705 (0.2)
Adjuvant chemotherapy						
Yes	1,037 (15)	14,374 (20)	170 (22)	3,920 (29)	17,721,164 (16)	230,800,000 (21)
No	5,679 (85)	57,005 (80)	589 (78)	9,625 (71)	90,603,584 (84)	850,600,000 (79)
Modified charlson comorbidity index score**						
0	3,842 (57)	59,859 (84)	487 (64)	12,384 (91)	61,108,675 (56)	900,400,000 (83)
1	1,439 (21)	5,886 (8,3)	168 (22)	711 (5,3)	23,715,893 (22)	92,566,752 (8.6)
2	769 (12)	3,668 (5.1)	62 (8.2)	309 (2,3)	12,513,044 (12)	57,713,661 (5.3)
3+	666 (10)	1,966 (2.8)	42 (5.5)	141 (1.0)	10,987,137 (10)	30,788,132 (2.8)
Calendar year of diagnosis						
1980–1989	1,108 (17)	20,047 (28)	146 (19)	5,283 (39)	13,002,340 (12)	227,000,000 (21)
1990–1999	2,371 (35)	25,631 (36)	346 (46)	5,557 (41)	36,572,791 (34)	386,900,000 (36)
2000–2007	3,237 (48)	25,701 (36)	269 (35)	2,705 (20)	58,749,617 (54)	467,600,000 (43)

UICC Union for International Cancer Control, *ER* estrogen receptor status, *ET* adjuvant endocrine therapy, *BCS* breast-conserving surgery, *RT*, radiation therapy

* Missing data are primarily due to missing information on receptor status

** The Charlson comorbidity index was modified to exclude the ICD-8 and ICD-10 codes for autoimmune exposure variables (see Appendix for ICD-8 and ICD-10 codes)

Table 2 Distribution of autoimmune diseases in a cohort of 78,095 women diagnosed with stages I–III breast cancer in Denmark during 1980–2007, by presence/absence of recurrence

Autoimmune disease	Autoimmune diseases regardless of diagnosis order*		First AD diagnosis**	
	Recurrence* *N* (row %)	No recurrence* *N* (row %)	Recurrence *N* (row %)	No recurrence *N* (row %)
No autoimmune disease	13,545(19)	57,834(81)		
Non-malignant hematological diseases				
Autoimmune hemolytic anemia	3(9)	31(91)	3(10)	27(90)
Idiopathic thrombocytopenic purpura (ITP)	5(10)	45(90)	3(8)	36(92)
Endocrine diseases				
Grave's disease	160(13)	1,087(87)	154(13)	1,064(87)
Autoimmune thyroiditis	14(11)	115(89)	13(11)	104(89)
Addison's disease	7(19)	29(81)	6(18)	27(82)
Diabetes type I	130(9)	1,260(91)	120(9)	1,218(91)
Central nervous/neuromuscular diseases				
Multiple sclerosis	34(11)	268(89)	33(12)	239(88)
Myasthenia gravis	2(5)	35(95)	1(4)	24(96)
Gastrointestinal/hepato-billiary diseases				
Pernicious anemia	20(11)	170(89)	16(11)	126(89)
Coeliac disease	4(7)	53(93)	4(10)	37(90)
Ulcerative colitis	63(11)	508(89)	53(12)	408(88)
Crohn's disease	22(9)	233(91)	16(9)	158(91)
Primary biliary cirrhosis	5(12)	36(88)	3(10)	27(90)
Autoimmune hepatitis	1(3)	36(97)	1(4)	23(96)
Skin diseases				
Pemphigus/pemphigoid	1(3)	39(97)	0(0)	29(100)
Dermatitis herpetiformis	2(11)	16(89)	2(13)	13(87)
Psoriasis	56(13)	387(87)	45(13)	298(87)
Vitiligo	4(17)	19(83)	3(25)	9(75)
Connective tissue diseases				
Scleroderma	10(16)	54(84)	9(20)	36(80)
Juvenile rheumatoid arthritis	2(12)	15(88)	1(8)	11(92)
Rheumatoid arthritis	173(13)	1,127(87)	152(13)	992(87)
Ankylosing spondylitis	7(17)	34(83)	6(17)	29(83)
Polymyositis/dermatomyositis	6(14)	36(86)	4(12)	29(88)
Systemic lupus erythematosus	20(19)	85(81)	13(19)	57(81)
Sjögren's syndrome	6(11)	48(89)	3(14)	19(86)
Sarcoidosis	28(16)	150(84)	25(16)	129(84)
Polyarthritis nodosa	2(8)	23(92)	0(0)	17(100)
Wegener's granulomatosis	4(22)	14(78)	3(21)	11(79)
Temporal arteritis/rheumatic polymyalgia	69(8)	796(92)	58(8)	685(92)
Mixed connective tissue disorder	13(11)	102(89)	9(11)	75(89)

* Women in the study had 0–8 autoimmune diseases and diagnoses of all these diseases are presented in this Table. For this reason, the number of diseases adds up to more than the number of affected women

** In cases in which a woman had more than one AD diagnosed on the same day, the disease with the highest incidence as first AD diagnosis among women with only one AD was counted as the first AD

No previous studies have evaluated the risk of BC recurrence associated with AD in general or among categories of ADs. A few studies have evaluated BC survival associated with specific ADs among BC patients. Thus a study of patients with autoimmune hypothyroidism and a specific drug use profile reported a BC-specific mortality HR of 0.92 (95 % CI 0.71, 1.18) [16]. A study conducted among BC patients with and without inflammatory bowel

Table 3 Ten-year risk of breast cancer recurrence in a cohort of 78,095 women diagnosed in Denmark during 1980–2007, by organ or tissue of origin of first autoimmune disease. Risk was calculated from the date of the first registered autoimmune disease diagnosis

Autoimmune disease	*N*	Crude HR (95 % CI)	Adjusted HR* including competing risk of death (95 % CI)
No autoimmune disease	71,379	1 (reference)	1 (reference)
Any autoimmune disease	6,716	0.83 (0.77, 0.89)	0.96 (0.89, 1.04)
Non-malignant hematological diseases	69	0.75 (0.34, 1.67)	0.80 (0.36, 1.81)
Endocrine diseases	2,704	0.82 (0.73, 0.93)	0.98 (0.87, 1.10)
Central nervous/neuromuscular system diseases	297	0.75 (0.53, 1.05)	0.56 (0.40, 0.78)
Gastrointestinal/hepato-billiary system diseases	872	0.76 (0.62, 0.92)	0.87 (0.70, 1.07)
Skin diseases	399	0.91 (0.69, 1.20)	0.92 (0.71, 1.24)
Connective tissue diseases	2,369	0.87 (0.77, 0.98)	1.11 (0.98, 1.25)

* Adjusted for age, stage, chemotherapy, surgery type, menopausal status, modified Charlson comorbidity index score, and competing risk of death

disease found a possible difference in all-cause mortality rates among women with Crohn's disease ($MRR_{adjusted,\ Crohn's\ disease} = 1.22$ (95 % CI 0.85, 1.75)) and no association among women with ulcerative colitis ($MRR_{adjusted,\ ulcerative\ colitis} = 1.09$ (95 % CI 0.86, 1.38)) [15]. Another study reported poorer survival among BC patients with RA than among those without this condition ($HR_{breast\text{-}cancer\ specific}$ 1.55 (95 % CI 1.40, 1.71)) [18]. Furthermore, a recent Swedish study that evaluated risk of BC recurrence in RA patients treated with tumor necrosis factor inhibitors reported an $HR_{adjusted}$ of 1.1 (95 % CI 0.4, 2.8) [17], comparable to our results for the connective tissue AD category, with the caveat that the width of the Swedish study's confidence interval allows for a wide range of consistent estimates.

Immunosuppressive approaches to AD management have raised concerns [1, 4, 9]. It is difficult to differentiate between the effect of the AD and its impact on the immune system on one hand, and the iatrogenic effect of the treatment for the AD, which suppresses immune function, on the other hand [1, 4]. Previous research by our group in an overlapping cohort of patients likewise showed no evidence of an association between prescriptions for the immunosuppressive drugs most commonly used to treat AD—glucocorticoids—and risk of BC recurrence [14]. Non-steroidal anti-inflammatory drugs (NSAIDs) are used especially to treat connective tissue diseases and have been associated with decreased risk of BC recurrence and breast cancer-specific death [27]. However, in an overlapping population, we found little evidence to support a protective association between NSAID prescriptions and BC recurrence (Cronin-Fenton et al. 2014, in draft). We therefore do not expect use of these drugs to confound our results. Other studies that investigated the relation between use of other immunosuppressive drugs and risk of cancer concluded that this potential association should be considered for each individual drug and disease [5, 28].

The validity of our findings depends on several factors. Our registry-based study population reduced the risk of selection bias due to differential loss-to-follow-up. The validity of data from the DBCG registry is very high—the positive predictive value for classification of BC recurrence was found to be 99.4 % using medical records as a gold standard [23]. We also incorporated comprehensive information on potential confounders, including comorbid diseases. A concern is that our AD exposure could be misclassified, for several reasons. We only had access to discharge information from hospitals and outpatient specialist clinics. Our data showed an increasing AD prevalence, from 5.2 to 11.2 %, over calendar time from 1980–1989 to 2000–2007. This is likely due to the addition of data from outpatient clinics starting in 1995 and the lack of access to data for patients whose ADs were managed solely by general practitioners. As well, in Denmark most patients are referred to hospital-based care (inpatient or outpatient) by a general practitioner. If the referral process delays the entry date of an AD diagnosis into the DNPR, left-truncation of DNPR data would occur, resulting in shortened measurement of exposure time. Symptoms of ADs also can be non-specific and misinterpreted, leading to misclassification or under-diagnosis. Another consideration is that time of diagnosis could be delayed due to nonspecific or vague symptoms. A Danish study that calculated the total number of persons alive in Denmark on 31 December 2001 with one or more AD diagnoses registered in the DNPR found a lifetime AD prevalence of 5.2 % [2]. Cooper et al. considered this prevalence to be an underestimation. Based on a literature review, they calculated a corrected AD prevalence of 7.6–9.4 % among both men and women in Denmark [24]. In our cohort the AD prevalence was 8.6 %.

Due to the risk of misclassification discussed above, our AD-exposed person-time may have been slightly underestimated. However, women with a BC diagnosis receive care in a hospital and any known comorbidity, including an AD, likely would be registered in the DNPR in the beginning of the follow- up period.

Another concern is that we lacked information about severity of the disease or potential presence of several diseases with accompanying treatments. In our cohort we found women with up to eight different AD diagnoses. We did not have any available measure of severity and had to handle AD as a dichotomous variable.

Conclusion

In this large prospective population-based cohort study we found no association between exposure to AD and risk of BC recurrence among women. In subcategories based on organ or tissue of origin, the risk estimates remained near null, with the possible exception of ADs affecting the central nervous and neuromuscular system. For women with a BC diagnosis and their clinicians, this finding is reassuring when assessing the risk of recurrence in the clinical setting.

Conflict of interest The authors have declared no conflicts of interest.

Ethics The study was approved by the Board of the DBCG Registry and the Danish Data Protection Agency [journal number: 2013-41-1759].

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Paper III

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Predictors of re-operation due to post-surgical bleeding in breast cancer patients: A Danish population-based cohort study

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Abstract

Aim: To assess the risk of re-operation due to post-surgical bleeding after initial breast cancer surgery and to identify predictors of re-operation.
Methods: We conducted a population-based study in Denmark. Patients were categorized according to age group, surgery type, and glucocorticoid use before surgery: never, current (0–90 days), and former (>90 days). We calculated the risk of re-operation due to post-surgical bleeding within 14 days after surgery, risk differences, and risk ratios of re-operation associated with age group, surgery type, and glucocorticoid use.
Results: 19,919 women were studied; 508 were re-operated. 3573 of the 19,919 women ever used glucocorticoids. Older age and mastectomy increased the risk of post-surgical bleeding compared with breast conserving surgery and younger age among both ever and never users of glucocorticoids. The crude risk of re-operation was 2.5% among never users of glucocorticoids, 2.6% among ever users and 4.0% among current users. Women aged ≥80 who were ever users of glucocorticoids and who had a mastectomy had 8.1% risk of re-operation due to post-surgical bleeding, whereas women <80 years old who never used glucocorticoids and who had breast conserving surgery had a 1.7% risk of re-operation.
Conclusions: Older age, mastectomy, and – in some women – glucocorticoid use add an extra risk of re-operation due to bleeding. Clinicians and their patients can use this information to evaluate the patient-specific risk of this complication.

Keywords: Epidemiology; Breast neoplasms; Glucocorticoid; Post-surgical bleeding; Aging; Denmark

Introduction

Breast cancer is the most common cancer among women in industrialized countries.[1] Almost all breast cancer patients undergo surgery, either breast conserving surgery or mastectomy. Although re-operation due to post-surgical bleeding is a rare complication, it delays hospital discharge, usually requires general anesthesia, and therefore is associated with substantial costs to both the patient and healthcare system. Predictors of re-operation due to bleeding after breast cancer surgery have not previously been identified.

Synthetic glucocorticoids are among the most frequently used drugs to lower the general immune response to inflammation.[2,3] Some of the side effects associated with use of synthetic glucocorticoids include delayed wound healing, upper gastrointestinal bleeding, skin atrophy, striae cutis and masked and increased activation of microbial infections. These can result in substantial post-operative complications in patients.[2–4] Animal models have shown that even a single dose of dexamethasone, a highly potent glucocorticoid, can delay wound healing.[5] Despite this, few population-based studies, with conflicting results, have investigated the impact of glucocorticoid use on post-operative complications.[6–9]

Despite the high incidence rate of breast cancer and the risk of post-operative bleeding, no study has investigated

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predictors of the risk of post-surgical bleeding. We therefore conducted a large population-based cohort study with prospectively collected data to examine predictors of post-surgical bleeding – with special focus on age, surgery type, and glucocorticoid use – in a population-based cohort of Danish breast cancer patients.

Methods

Study population

Following a design we used earlier to study the association between selective serotonin reuptake inhibitor (SSRI) anti-depressant prescriptions and re-operation due to bleeding,[10] we conducted this population-based cohort study among residents of the North and Central Denmark Regions, which have a total population of 1.8 million inhabitants. A unique civil personal registration (CPR) number has been assigned to all Danish citizens and immigrants since 1968 by the Danish Civil Registration System.[11] This number encodes sex and date of birth, the latter allowing calculation of age at the date of breast cancer surgery. The CPR number also facilitates precise linkage between population-based registries. All non-psychiatric hospitalizations are registered to individual patients in the Danish National Patient Registry (DNPR).[12,13] The DNPR has registered all inpatient procedures in Danish public hospitals since 1977 and all outpatient procedures since 1995. Information is recorded in the DNPR immediately after discharge or outpatient visit and includes CPR number, dates of admission and discharge, and up to 20 diagnostic codes categorized according to the International Classification of Disease (ICD). Using the DNPR, we identified 19,919 women who had surgery for a first diagnosis of breast cancer (ICD-10 codes C50.0–50.6, C50.8 & C50.9) from 1 January 1996 through 31 December 2009, the time period during which we could link to complete prescription history by the methods described below. From the DNPR we also collected data on the type of primary breast cancer-directed surgery in accordance with the Danish Classification of Surgical Procedures and Therapy[14] – mastectomy (code KHAC) or breast conserving surgery (BCS) (code KHAB). All breast cancer patients were treated initially with either mastectomy or BCS.

Prescription data

All pharmacies in the North and Central Denmark Regions use computerized accounting systems connected with the National Health Service to record for each prescription the patient's CPR number, type and quantity of medication dispensed (tablet and package size), and prescription data according to the drug's Anatomical Therapeutic Classification (ATC).[15] Information on prescriptions for refundable drugs is forwarded electronically to the National Health Service and to a research database at Aarhus University, with complete coverage in the region since 1998.[12] The National Health Service refunds a proportion of the cost of prescribed drugs. Our target drug class was systemically absorbed glucocorticoids. We therefore limited the definition of exposure to the following classes of glucocorticoids: systemic hormones (ATC code H02AB and H02BX), glucocorticoids for rectal application (ATC codes A07EA and C05AA), and inhalation glucocorticoids (ATC code R03AD and R03BA). A complete list of individual drug types can be found in Appendix Table 1.

Data on potential predictors

To account for factors that may be associated with a prescription for systemic glucocorticoids and with post-surgical bleeding, we acquired data on age at breast cancer surgery and on any (ever) preoperative use of platelet inhibitors, vitamin K antagonists, oral anti-coagulants, non-steroidal anti-inflammatory drugs (NSAIDS) (non-aspirin NSAID, excluding selective Cox-2 inhibitors, as these have prothrombotic side effects[16]), SSRI anti-depressants, and non-SSRI anti-depressants (tri-cyclic anti-depressants (TCA), tetracyclical anti-depressants) (see Appendix for specific ATC codes).

We obtained information on specific precedent comorbidities from the DNPR including liver disease, uremia, other cancers, renal disease, autoimmune diseases, thrombocytopenia, and vasculitis; all of which can cause bleeding.

To obtain information on stage of breast cancer we used data from the Danish Cancer Registry (DCR) and linked to DNPR data. The DCR has existed since 1943 and from 1987 reporting became mandatory for all Danish doctors. The reporting delay in DCR is approximately 2 years and at the time of our data retrieval we could collect information up to 31 December 2008. TNM staging was registered beginning 1 January 2004.[12] With the DNPR data as the underlying basis to identify breast cancer diagnoses, we searched for matching CPR numbers on women who had stage information in the DCR and only one breast cancer diagnosis since 2004. By this strategy, we identified 5037 women. This subpopulation was used to perform the statistical analysis examining the effect of confounding by disease stage.

Post-operative bleeding outcomes

Information on re-operation due to post-surgical bleeding within 14 days of primary breast cancer-directed surgery was retrieved from the DNPR (codes: KHWD00, KHWE00).

Variable definition

Consistent with earlier definitions of older women with breast cancer we stratified the study population by age into two categories: women <80 years and women 80 years or older.[16] Surgery type was dichotomized as mastectomy or breast conserving surgery. Patients were categorized according to their use of glucocorticoids (never/ever use)

Table 1
Baseline characteristics of 19,919 Danish breast cancer patients treated in 1996–2009 according to glucocorticoid (GC) prescription.

Characteristics	No GC prescription *N*	Ever GC prescription *N*
Age group		
<40	2375	296
40–49	3181	592
50–59	4111	840
60–69	3693	860
70–79	1930	629
≥80	1056	356
Temporality of glucocorticoid prescription		
Never	16,346	0
Current (≤3months)	0	149
Former (>3months)	0	3424
Current (≤6 months)	0	264
Former (>6 months)	0	3309
Current (≤12 months)	0	512
Former (>12 months)	0	3061
Primary operation type		
BCS[a]	10,577	2162
Mastectomy	5769	1411
Anti-depressant prescription		
No	13,396	2568
Yes	2950	1005
Oral anti-coagulants prescription		
No	16,312	3564
Yes	34	9
NSAIDs prescription		
No	7277	855
Yes	9069	2718
Vitamin K antagonist prescription		
No	15,934	3437
Yes	412	136
Platelet inhibitors prescription		
No	14,836	3064
Yes	1510	509
Statin prescription		
No	14,933	3216
Yes	1413	357
Comorbid diseases		
None	14,753	2966
Yes	1593	607
Comorbid diseases		
Liver disease	127	34
Renal disease	112	60
Cancer	954	248
Thrombocytopoenia	11	7
Auto-immune disease	482	294
Vascular disease	15	11

[a] Breast conserving surgery.

and according to the temporality of use: current users (any prescription for systemic glucocorticoids within 90 days before initial breast cancer surgery) and former users (prescription for systemic glucocorticoids only more than 90 days before initial breast cancer surgery).

Statistical analyses

Follow-up began on the date of primary breast cancer surgery and continued until 14 days after the operation. Almost 80% of the re-operations due to post-surgical bleeding were performed within this period. We analyzed the data first by tabulating contingency tables for the main study variables, from which we calculated the risk of re-operation due to post-surgical bleeding according to use of glucocorticoids. We computed the crude risk difference and risk ratio and their 95% confidence intervals (95% CI) associating systemic glucocorticoid prescription with post-operative bleeding. We then stratified the contingency tables according to each of the possible confounding variables to examine the strength of confounding and fitted multiple logistic regression models to the data to compute the odds ratio and associated 95% CI controlling for confounders. Given that re-operation for post-surgical bleeding was rare in all combinations of the independent variables, these adjusted odds ratios provided an estimate of the adjusted risk ratios (aRR). Only age and surgery type were confounders, and they were of *a priori* interest, so we stratified by these two variables to adjust all results for confounding by them, and adjusted for no other variables.

All data analyses were performed using STATA 11.0 (Stata.Corp LP, Texas, USA).

Ethics

Studies based on registry data do not require formal ethical approval under Danish law. However, the project was approved by the Danish Data Protection Agency.

Results

Characteristics of the cohort of 19,919 breast cancer patients according to use of systemically acting and respiratory synthetic glucocorticoid prescription are presented in Table 1. 1412 of the women were 80 years or older and two-thirds of them had mastectomy as their initial surgery. Among women less than 80 years, one-third had mastectomy as their initial surgery. 356 of women 80 years or older and 3217 of women aged less than 80 years had a history of glucocorticoid use. 149 women had a glucocorticoid prescription in the 90 days before their initial breast cancer operation.

508 of 19,919 women (2.6%) were re-operated due to post-surgical bleeding within 14 days of their initial operation. The crude risk of re-operation was 2.5% among never users of glucocorticoids, 2.6% among ever users of glucocorticoids, and 4.0% among current users of glucocorticoids. The mean number of glucocorticoid prescriptions per women in ever users was between 2 and 3, regardless of age or surgery type.

Age category, surgery type, and glucocorticoid use affected the risk of re-operation. No other measured covariate was an important confounder or predictor of risk of re-operation. Older women had a higher risk of re-operation than younger women, regardless of the type of surgery or use of glucocorticoids (aRR adjusted for surgery type and glucocorticoid use = 1.6, 95% CI 1.2, 2.0). Mastectomy

approximately doubled the risk of post-surgical bleeding compared with BCS in both age categories among ever users and never users of glucocorticoids (aRR adjusted for age and glucocorticoid use = 2.3, 95% CI 1.9, 2.7). Overall, glucocorticoid use did not affect the risk of re-operation (aRR adjusted for age and surgery type = 0.98, 95% CI 0.78, 1.2). However, in the women 80 years old or older who received mastectomy, the risk of re-operation increased by 3.3% (95% CI −0.6%, 7.2%) among ever users of glucocorticoids compared with never users of glucocorticoids (Table 2). Women 80 years or older who were ever users of glucocorticoids and who had a mastectomy had an 8.1% risk of re-operation due to post-surgical bleeding, whereas women less than 80 years old who never used glucocorticoids and were operated by BCS had a 1.7% risk of re-operation (risk difference = 6.4%, 95% CI 3.0%, 7.8%) (Table 2).

Discussion

In this study, we found that women 80 years or older who had a mastectomy and ever used glucocorticoids had a higher risk of re-operation due to post-surgical bleeding than women less than 80 years who had BCS and never used glucocorticoids. The higher risk of re-operation associated with older age, surgery type, and glucocorticoid use should be considered from several perspectives.

First, we found an overall higher risk of re-operation due to bleeding among all women who had a mastectomy regardless of age. Many patient-specific factors such as tumor size, precise location of the tumor, and comorbidity, must be considered by the patient, surgeon, and oncologist when deciding on the appropriate surgical procedure for an individual patient. Although mastectomy is a more extensive and invasive operation than BCS, studies suggest that older breast cancer patients are more likely to receive a mastectomy than younger women,[17,18] which is consistent with the distribution of surgery type in our study. Older women often prefer mastectomy to avoid radiation therapy[17,18] and, according to Danish guidelines, the presence of comorbidity and older age should weigh in favor of choosing mastectomy without radiation therapy.[19,20]

Second, age-related alterations in the coagulation and immune system influence wound healing. A declined ability to heal wounds could increase the risk of the wound re-opening and bleed after an operation. In the inflammatory phase of wound healing older persons experience a decline in macrophage function leading to a slower wound healing. Next, cell proliferation is affected by aging both because of declined fibroblast proliferation and migration to the wound. The rate of epithelialization of open wounds is therefore slowed in older persons compared to that in young individuals. Aging is furthermore associated with significantly reduced levels of wound matrix constituents, including collagen, basement membrane components, glucosaminoglycans, and fibronectin leading to decline in anastomotic strength and collagen metabolism. However, it is likely that the physiologic delay of the healing process in older persons becomes evident only in the presence of conditions that exert their own, negative influence on wound repair. Such an influence could be from glucocorticoid use as this drug group inhibits the inflammatory phase of wound healing and large doses of glucocorticoids reduce collagen synthesis and wound strength.[4,21] In addition, surgeons often find that older women have more fragile and atherosclerotic vessels than younger women, and it can be more difficult to achieve hemostasis using electric coagulation or ligation. These theoretical and clinical experiences support our finding that older women are at higher risk of re-operation than younger women, regardless of the type of surgery.

Women 80 years old or older represent a highly heterogeneous population in terms of frailty and comorbid diseases. Comprehensive geriatric assessment tools[22] can be helpful to assess these conditions and to evaluate an individual patient's risk before deciding on a treatment regime.[23] The important point, though, is that history of glucocorticoid use may be a predictor of higher risk of re-operation in women 80 years of age or older who receive a mastectomy and the present result adds further knowledge to the modern approach considering the oldest old women for BCS if possible.

The main strengths of our study include its large size and population-based setting with complete prescription and follow-up data. Recall-bias was eliminated by use of prospectively collected prescription data before the initial breast cancer surgery date and all data were collected from population-based registries that have a completeness approaching 100%.[13] The CPR number facilitated individual-level linkage across the population-based registries.[24]

Our study has some limitations. We were unable to account for glucocorticoids and NSAIDs bought "over the counter," as we have no data on these purchases. However, glucocorticoids are available only in low-dose topical preparations over the counter in Denmark, and adjustment for

Table 2
Risk of re-operation due to post-surgical bleeding in the fourteen days after breast cancer surgery, according to age group, surgery type, and glucocorticoid (GC) prescription.

Age category	Women <80 years				Women ≥80 years			
Operation type	Mastectomy		BCS		Mastectomy		BCS	
GC exposure[a]	+GC	−GC	+GC	−GC	+GC	−GC	+GC	−GC
No re-operated within 14 days	42	199	29	170	18	34	5	11
No operated	1188	5059	2029	10,231	223	710	133	346
Risk of re-operation (%)	3.5	3.9	1.4	1.7	8.1	4.8	3.8	3.2

Risk difference for mastectomy versus BCS, adjusted for age group and glucocorticoid use, is 2.3% (CI 95%: 1.7%; 2.8%).
Risk difference for age <80 years versus age ≥80 years, adjusted for surgery type and glucocorticoid use, is 1.7% (95% CI 0.6%, 2.9%).
Risk difference for glucocorticoid use versus no glucocorticoid use, adjusted for age group and surgery type, is −0.1% (CI 95%: −0.6%; 0.5%).
[a] Ever exposed to glucocorticoid.

prescribed NSAIDs had little effect on our estimated associations. In addition, the Danish healthcare system covers a proportion of the costs of prescribed medicine, which provides an incentive to use prescribed medications rather than over the counter medications. Furthermore, patients must pay part of the cost for a dispensed prescription; therefore our estimates are likely to reflect actual use. As we use data from the DNRP we do not have information about the extent of bleeding or post-operative complications leading to the re-operation. All breast cancer patients who received a re-operation for post-surgical bleeding had a surgical code. In Denmark, it is general practice to code the diagnosis hematoma when performing an invasive procedure such as a puncture. If the surgeon has to put the woman in general anesthesia, then the procedure would get a surgical code. All of our cases of surgery for re-bleeding had a surgical code. Hence, the vast majority of the patients registered as cases of surgery for re-bleeding in our study were likely treated under general anesthesia.

Finally, we were unable to assess in-hospital use of medications, which may have impacted on our estimates. Consequently we have no information about the use of low molecular weight heparins (LMWH) as deep vein thrombosis prophylaxis, although we know from clinical experience that LMWH is given to all cancer patients undergoing surgery as a daily injection throughout their in-hospital stay. Patients in current oral anticoagulation therapy would have been given double dose LMWH during the intermission of the oral therapy. We furthermore have the experience that it is mostly elderly people who are receiving long term oral anticoagulation therapy, so a higher dose of LMWH among this group of older women may explain part of the higher risk of operation due to re-bleeding in this age category. Dexamethasone used in-hospital perioperatively to reduce post-operative nausea and vomiting (PONV) – including among breast cancer surgical patients[25] – and "stress doses" of glucocorticoid given perioperatively to long term glucocorticoid users with impaired adrenal gland function, are additional examples of medications administered in-hospital and therefore not registered in the prescription databases. Unpublished results from a medical record review we conducted on 136 breast cancer patients operated between 2004 and 2008 showed that only 3 of these patients received perioperative dexamethasone. Only one of these patients was aged over 80 years, and none of the three patients had a re-operation due to post-surgical bleeding. "Stress doses" of glucocorticoid will only be administered to current long term users (2 of 136 in our medical record review). This category of women are already a part of the exposed cohort, and receipt of the stress dose is potentially, therefore, part of the underlying causal mechanism.

Conclusion

Treatment of breast cancer patients often takes place in a very complex setting, which requires a focus on both details of the individual patient, the general perspective, and clinical experience. Our results add important new clinical information regarding the risk of post-surgical complications. Women 80 years of age or older who undergo mastectomy and are users of glucocorticoids have an excess risk of re-operation due to bleeding. Clinicians and patients can use this information to evaluate the individual risk estimate for re-operation.

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Conflict of interest

None of the authors received any fees, honoraria, grants or consultancies that would constitute a conflict of interest with the current study. The Department of Clinical Epidemiology, Aarhus University Hospital, receives funding for other studies from pharmaceutical companies in the form of research grants to (and administered by) Aarhus University. None of these studies have any relation to the present study.

Appendix

ATC codes for glucocorticoids

MA07EA01 prednisolon, MA07EA04 betamethason, MC05AA01 hydrocortison, MC05AA04 prednisolon, MH02AB glucocorticoides, MH02AB01 betamethason, MH02AB02 dexamethason, MH02AB04 methylprednisolon, MH02AB06 prednisolon, MH02AB07 prednison, MH02AB08 triamcinolon, MH02AB09 hydrocortison, MH02BX01 methylprednisolon – combinations, MR01AD02 prednisolon, MR01AD03 dexamethason, MR01AD06 betamethason, MR01AD52 prednisolon – combinations, MR01AD53 dexamethason – combinations, MR01AD60 hydrocortison – combinations, MR03BA01 beclomethason, MR03BA02 budesonid, MR03BA05 Fluticason, MR03BA07 Mometason.

ATC codes for potential confounding drugs

Platelet inhibitors: low-dose aspirin B01AC06 and N02BA01 in tablet sizes of 75, 100 and 150 mg;

Vitamin K antagonists:

(heparin B01AB01; warfarinB01AA03; phenprocoumon B01AA04), dipyridamol B01AC07, clopidogrel B01AC04;

Oral anti-coagulants:

(B01AB02, B01AB04, B01AB05, B01AB08, B01AB10, B01AC06, B01AC09, B01AC13, B01AC14, B01AC16, B01AC17, B01AC30, B01AD01, B01AD02, B01AD04, B01AD07, B01AD10, B01AD11, B01AE04, B01AE05, B01AX03, B01AX05).

NSAIDs: (non-aspirin NSAIDs M01A, excluding selective Cox-2 inhibitors);

Anti-depressants: Tri-cyclic anti-depressants (TCAs): Desipramine N06AA01; imipramin N06AA02; imipramine oxide N06AA03; clomipramin N06AA04; opipramol N06AA07; amitriptylin N06AA09; nortriptylin N06AA10; protriptyline N06AA11; doxepin N06AA12; dosulepin N06AA16; amoxapine N06AA17; Tetracyclical anti-depressants (TeCA): Maprotilin N06AA21, Mianserin N06AX03; Other anti-depressants: Duloxetin N06AX21, venlafaxin N06AX16, Mirtazapin N06AX11, Reboxetin N06AX18, Isocarboxazid N06AF01, Moclobemid N06AG02; Selective serotonin reuptake inhibitors (SSRI): Fluoxetine N06AB03, citalopram N06AB04, paroxetine N06AB05, sertraline N06AB06, fluvoxamine N06AB08, and escitalopram N06AB10.

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Paper IV

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Glucocorticoid prescriptions and breast cancer recurrence: a Danish nationwide prospective cohort study

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Background: Treatment with synthetic glucocorticoids (GCs) depresses the immune response and may therefore modify cancer outcomes. We investigated the association between GC use and breast cancer recurrence.

Materials and methods: We conducted a population-based cohort study to examine the risk of breast cancer recurrence associated with GC use among incident stage I–III female breast cancer patients aged >18 years diagnosed 1996–2003 in Denmark. Data on patients, clinical and treatment factors, recurrence, and comorbidities as well as data on GC prescriptions and potential confounders were obtained from Danish population-based medical registries. GCs were categorized according to administrative route: systemic, inhaled, or intestinal. Women were followed for up to 10 years or until 31 December 2008. We used Cox proportional hazards regression models to compute hazard ratios (HRs) and associated 95% confidence intervals (95% CIs) to evaluate the association between GC use and recurrence. Time-varying drug exposures were lagged by 1 year.

Results: We included 18 251 breast cancer patients. Median recurrence follow-up was 6.9 years; 3408 women developed recurrence during follow-up. Four thousand six hundred two women filled at least one GC prescription after diagnosis. In unadjusted models, no association was observed among users of systemic, inhaled, and intestinal GCs ($HR_{systemic}$ = 1.1, 95% CI 0.9–1.3; $HR_{inhaled}$ = 0.9, 95% CI 0.7–1.0; and $HR_{intestinal}$ = 1.0, 95% CI 0.9–1.2) versus nonusers. In adjusted models, the results were also near null ($HR_{systemic}$ = 1.1, 95% CI 0.9–1.2; $HR_{inhaled}$ = 0.8, 95% CI 0.7–1.0; and $HR_{intestinal}$ = 1.0, 95% CI 0.8–1.2).

Conclusion: We found no evidence of an effect of GC use on breast cancer recurrence.

Key words: breast neoplasm, glucocorticoids, outcome, epidemiology

introduction

Synthetic glucocorticoids (GCs) are frequently prescribed anti-inflammatory drugs [1]. They have a general immunosuppressive effect on a large and diverse set of diseases, but are also associated with many serious side-effects including diabetes, obesity, osteoporosis, fractures, psychosis, and catabolism [1]. In women with breast cancer, GCs are often used to prevent surgery-induced and chemotherapy-induced nausea and emesis [2–4]. Given their immunosuppressive effects, use of GCs may promote tumorigenesis, by facilitating tumor cell evasion of immune surveillance [5, 6].

GCs belong to the same steroid superfamily as estrogens, which are known to play a role in breast cancer development [7], but the potential effect of GCs on breast cancer cell growth has not been fully elucidated [5, 8]. A laboratory-based study of human breast cancer cells found that treatment with GCs induced a better prognostic profile in ER-negative tumor cells (cells became more differentiated and less invasive), but not in ER-positive cells, compared with untreated ER-negative and ER-positive cells, respectively [9]. In contrast, GCs have also been shown to inhibit the cytotoxic effects of chemotherapy in human breast cancer cell culture models [10]. We previously found no evidence of an effect of GCs on breast cancer risk [11, 12]. However, to our knowledge, the impact of GCs on breast cancer prognosis has never been investigated.

We therefore investigated the potential association between GC use and breast cancer recurrence in a large population-

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based cohort of breast cancer patients, using high-quality clinical data with complete follow-up. We hypothesized that GC use would increase the risk of breast cancer recurrence in humans due to impaired immune response.

materials and methods

setting

We conducted a nationwide cohort study using Danish population-based medical registries, covering a population of ~5.6 million persons. Denmark's National Health Service provides tax-supported health care to the Danish population, including access to hospital care and partial reimbursement for prescribed medications. The unique civil personal registration (CPR) number, assigned to all Danish residents at birth or emigration [13], permitted individual-level data linkage across the following Danish registries: the Danish Breast Cancer Cooperative Group (DBCG) registry [14, 15], the Danish National Registry of Patients (DNRP) [16], the Danish National Prescription Registry (DNPR) maintained by Statistics Denmark [17], and the Danish Civil Registration System (DCRS) [18].

study population and data collection

Since 1977, the DBCG has registered nearly all invasive breast cancers diagnosed in Denmark [14, 15, 19]. Completeness of breast cancer registration by the DBCG has improved over time, from 87% in 1986 [19] to ~95% in 2010 [20]. During the first 5 years following diagnosis, women in the DBCG registry undergo physical examination every 3–6 months to detect recurrences and an annual exam in years 6 to 10 following diagnosis, also to detect recurrences. A mammography is carried out every second year [21]. Recurrences diagnosed between examinations are reported to the Registry. Our study included all cases of incident primary female breast cancer stages I, II, or III diagnosed between 1 January 1996 and 31 December 2003 in Denmark and registered in the DBCG. Information on age and menopausal status at diagnosis, date of diagnosis, type of surgery, stage, histological grade, estrogen receptor (ER) status, receipt of adjuvant chemotherapy, endocrine therapy (ET) and/or radiation therapy, and eventual date of recurrence were obtained from the DBCG registry. From the DCRS, we retrieved information on date of birth, death, and emigration.

data on prescriptions

All members of the study cohort were linked to the DBCG and the DNPR. The DNPR has automatically recorded detailed information on all prescriptions redeemed at Danish pharmacies since 1995. We retrieved prescription information on full Anatomical Therapeutic Chemical (ATC) codes, and the date and quantity dispensed for all systemic GCs, inhaled GCs, and intestinal-acting GCs. We also retrieved data on potential confounder drugs, including postmenopausal hormone replacement therapy, NSAIDs, aspirin, statins, anticoagulants, β-blockers, ACE inhibitors, COPD medications (without GC), angiotensin receptor blockers, α-blockers, acetyl salicylic acids, antidiabetic medications, and immune-modulating drugs (methotrexate and azathioprine) (see Appendix I for ATC codes).

data on comorbid diseases

Members of the study cohort were also linked to the DNPR, which has collected information on all diagnoses from nonpsychiatric inpatient hospital admissions since 1977 and from outpatient contacts since 1995. The diagnoses are recorded according to WHO's 'International Classification of Diseases' (ICD). To ascertain information about potential confounding comorbidities, we obtained data on selected ICD diagnoses, including both diseases included in the Charlson Comorbidity Index and additional diseases for which GCs are indicated, and summarized the data for each woman between 1977 and the date of her breast cancer surgery (see Appendix II for ICD-8 and ICD-10 codes).

definition of analytic variables

Age at diagnosis was categorized into decades for stratified analyses, but was used as a continuous variable in regression models. Histologic grade was defined as low, moderate, or high. Receipt of adjuvant chemotherapy and administration of radiation therapy were categorized dichotomously. ER status and ET were summarized using a design variable: ER+/ET+, ER−/ET−, ER+/ET−, and ER−/ET+.

We categorized GC exposure in several ways. First, we classified GC use as a time-varying dichotomous variable updated yearly after breast cancer surgery. In each yearly interval, women were classified as exposed to GCs if they had at least one prescription registered in the DNPR with an ATC code corresponding to a systemic, inhaled, or intestinal-acting GC. Women who were prescribed a GC were assumed to be exposed, and women who did not redeem GC prescription were classified as nonusers. GCs were further categorized according to route of administration: systemic (pills and injections), inhaled (inhalants), and intestinal-acting (foam and suppositories).

Prednisolone-equivalent cumulative doses were used to perform dose–response calculations for systemic GCs, based on the methods of Sørensen et al. [11]. The cumulative dose was calculated as the product of the number of pills (or injections) dispensed, the dose per pill (or injection), and the prednisolone-equivalent conversion factor associated with each prescription's ATC code [11]. These values were aggregated and updated in each follow-up cycle according to the following categories of use: nonuse, 1–999, 1000–4999, or ≥5000 mg. Duration of GC use was estimated by the cumulative number of years exposed to GC, ranging from 0 to 10 years.

outcome data

Breast cancer recurrence was defined according to the DBCG convention as any local, regional, or distant recurrence, or cancer of the contralateral breast [15]. Follow-up of each woman began on the date of primary breast cancer surgery and continued until breast cancer recurrence, death, emigration, accrual of 10 years of follow-up, the last date of follow-up registered in the DBCG, or 31 December 2008 (end of the study period), whichever came first. Patients who died without a breast cancer recurrence or who emigrated from Denmark were censored on their date of death or emigration.

statistical analysis

Frequencies and proportions of patients, recurrences, person-time according to patient, tumor, and treatment characteristics, and exposure to GCs and other medications are presented in Tables 1 and 2. We computed 10-year recurrence hazard ratios (HRs) and 95% confidence intervals (95% CI) for the three GC groups (systemic, inhaled, and intestinal-acting) in unadjusted and multivariable Cox regression models, with medication exposures characterized as time-varying covariates lagged by 1 year. Exposure of GC and recurrence was handled as a dichotomous variable in each exposure year. We lagged GC exposure by 1 year to allow the effect of the drug to accrue. Accordingly, GC exposure in the year before surgery was modeled for its association with recurrence in the first year after surgery; GC exposure in the first year after surgery was modeled for its association with recurrence in the second year after surgery. This procedure was followed for the whole follow-up period. The lagged exposure time allowed for a reasonable induction period for an effect of GC and co-prescriptions on recurrence, and guarded against the possibility that imminent recurrence affected prescription patterns. Since women who receive chemotherapy are at higher risk of recurrence and receive substantial unmeasured doses of GC as inpatients, we stratified analyses by receipt of adjuvant chemotherapy to evaluate

Table 1. Baseline characteristics and relevant drug exposures among stage I–III breast cancer patients diagnosed in Denmark from 1996 to 2003, by glucocorticoid (GC) use ($N = 18\,251$)

Characteristics	Women, No. (%)		Recurrence, No. (%)		Total person-years, No. (%)	
	GC users ($N = 4602$)	Nonusers ($N = 13\,649$)	GC users ($N = 621$)	Nonusers ($N = 2787$)	GC users ($N = 23\,004$)	Nonusers ($N = 71\,341$)
Age at diagnosis (years)						
≤29	19 (0.4)	51 (0.4)	8 (1.3)	22 (0.8)	100 (0.4)	189 (0.3)
30–39	242 (5.3)	667 (4.9)	48 (7.7)	210 (7.5)	1234 (5.4)	3384 (4.7)
40–49	861 (19)	2593 (19)	102 (16)	528 (19)	4697 (20)	14 741 (21)
50–59	1498 (33)	4576 (34)	187 (30)	960 (35)	7796 (34)	25 173 (35)
60–69	1439 (31)	3969 (29)	200 (32)	780 (28)	6846 (30)	20 172 (28)
70–79	531 (12)	1681 (12)	75 (12)	278 (10)	2297 (10)	7397 (10)
≥80	12 (0.3)	112 (0.8)	1 (0.2)	9 (0.3)	34 (0.2)	285 (0.4)
Menopausal status at diagnosis						
Premenopausal	1417 (31)	4103 (30)	177 (28)	875 (31)	7827 (34)	23 157 (32)
Postmenopausal	3184 (69)	9544 (70)	444 (72)	1911 (69)	15 174 (66)	48 181 (68)
Missing	1	2	NA	NA	NA	NA
Medical history at diagnosis[a]						
Myocardial infarction	45 (1.0)	164 (1.2)	6 (1.0)	22 (0.8)	211 (0.9)	678 (1.0)
Congestive heart failure	58 (1.3)	108 (0.8)	6 (1.0)	9 (0.3)	220 (1.0)	379 (0.5)
Peripheral vascular disease	73 (1.6)	186 (1.4)	8 (1.3)	28 (1.0)	319 (1.4)	746 (0.9)
Cerebrovascular disease	124 (2.7)	333 (2.4)	21 (3.4)	58 (2.1)	520 (2.3)	1427 (1.0)
Chronic pulmonary disease	448 (9.7)	235 (1.7)	66 (11)	44 (1.6)	2122 (9.2)	1034 (1.4)
Diabetes without complications	86 (1.9)	291 (2.1)	15 (2.4)	56 (2.0)	327 (1.4)	1314 (1.8)
Diabetes w/organ damage	28 (0.6)	108 (0.8)	2 (0.3)	20 (0.7)	99 (0.4)	490 (0.7)
Renal disease	32 (0.7)	59 (0.4)	4 (0.6)	6 (0.2)	152 (0.7)	298 (0.4)
Liver disease (mod./severe)	3 (0.1)	20 (0.2)	0 (0)	0 (0)	4 (0)	91 (0.1)
RA	39 (0.9)	137 (1.0)	8 (1.3)	28 (1.0)	205 (0.1)	620 (0.9)
COPD	285 (6.2)	169 (1.2)	40 (6.4)	28 (1.0)	1330 (5.8)	743 (1.0)
Asthma	242 (5.3)	71 (0.5)	35 (5.6)	17 (0.6)	1191 (5.2)	339 (0.5)
IBD	55 (1.2)	52 (0.4)	4 (0.6)	8 (0.3)	272 (1.2)	263 (0.4)
UICC stage						
I	1889 (41)	4999 (37)	171 (28)	643 (23)	10 006 (44)	26 688 (37)
II	1957 (43)	5991 (44)	247 (40)	1098 (39)	10 105 (44)	32 668 (46)
III	732 (16)	2593 (19)	199 (32)	1033 (37)	2773 (12)	9788 (14)
Missing	2	5	NA	NA	NA	NA
Histological grade						
Low	1290 (28)	3622 (27)	114 (18)	515 (19)	6850 (30)	20 864 (29)
Moderate	1617 (35)	4854 (36)	230 (37)	1042 (37)	7943 (35)	24 756 (35)
High	850 (19)	2705 (20)	160 (26)	779 (28)	3959 (17)	12 440 (17)
Missing	845 (18)	2468 (18)	NA	NA	NA	NA
ER/adjuvant ET status						
ER−/ET−	900 (20)	2784 (20)	157 (25)	718 (26)	4384 (19)	13 260 (19)
ER+/ET−	1415 (31)	4143 (30)	160 (26)	713 (26)	7282 (32)	22 569 (32)
ER+/ET+	2097 (46)	6197 (45)	271 (44)	1222 (44)	10 392 (45)	32 798 (46)
Missing	190 (4.1)	525 (3.9)	33 (5.3)	134 (4.8)	NA	NA
Type of primary therapy						
Mastectomy	2000 (43)	5857 (43)	289 (47)	1209 (43)	9859 (43)	29 437 (41)
Mastectomy + RT	998 (22)	3341 (24)	170 (27)	922 (33)	4764 (21)	16 252 (23)
BCS + RT	1603 (35)	4451 (33)	161 (26)	656 (24)	8375 (36)	25 652 (36)
Missing	1	0	NA	NA	NA	NA
Adjuvant chemotherapy						
Yes	1369 (30)	4071 (30)	211 (34)	976 (35)	7208 (31)	21 675 (30)
No	3233 (70)	9578 (70)	410 (66)	1811 (65)	15 795 (69)	49 666 (70)
Drug exposure[a]						
Statins, pre and post[b]	946 (21)	2290 (17)	62 (10)	194 (7)	5360 (23)	15 472 (22)
Simvastatin, pre and post[b]	857 (19)	2111 (16)	42 (6.8)	156 (5.6)	4932 (21)	14 539 (20)

Continued

Table 1. *Continued*

Characteristics	Women, No. (%)		Recurrence, No. (%)		Total person-years, No. (%)	
	GC users ($N = 4602$)	Nonusers ($N = 13\,649$)	GC users ($N = 621$)	Nonusers ($N = 2787$)	GC users ($N = 23\,004$)	Nonusers ($N = 71\,341$)
HRT, pre	1236 (27)	2855 (21)	142 (33)	477 (17)	6337 (28)	15 517 (22)
NSAIDs, pre and post	3414 (53)	8635 (63)	406 (65)	1612 (58)	17 920 (78)	48 812 (68)
ASAs, pre and post	1030 (22)	2510 (18)	92 (15)	342 (12)	5410 (24)	14 559 (20)
α-Blockers, pre and post	73 (1.6)	171 (1.3)	4 (0.6)	26 (0.9)	394 (1.7)	995 (1.2)
Anticoagulants, pre and post	1103 (24)	2788 (20)	98 (16)	390 (14)	5734 (25)	15 980 (22)
Antidiabetics, pre and post	86 (1.9)	297 (2.3)	13 (2.1)	40 (1.4)	412 (1.8)	1590 (2.2)
ACE inhibitors, pre and post	845 (18)	2203 (16)	84 (14)	245 (8.8)	4552 (20)	13 565 (19)
Angiotensin receptor blocker, pre and post	621 (14)	1357 (9.9)	58 (9.3)	160 (5.7)	3407 (15)	8200 (12)
β-Blockers, pre and post	995 (22)	2613 (19)	85 (14)	380 (14)	5262 (23)	15119 (21)
COPD drugs, pre and post	1685 (37)	1107 (8.1)	244 (39)	155 (5.6)	8352 (36)	6035 (8.5)
Immune drugs[c]	48 (1)	48 (0.04)	3 (0.4)	6 (0.2)	298 (1.3)	258 (0.4)

[a]Proportions of patients, recurrences, and person-years calculated with denominators equal to sums within GC exposure groups because categories are not mutually exclusive.
[b]One year before diagnosis and up to 10 years after diagnosis.
[c]Methotrexate and azathioprine.
RA, rheumatoid arthritis; COPD, chronic obstructive pulmonary disease; IBD, inflammatory bowel disease; ER, estrogen receptor status; ET, adjuvant endocrine therapy; BCS, breast-conserving surgery; RT, radiation therapy; ACE, angiotensin-converting enzyme; HRT, combination hormone replacement therapy; NSAIDs, nonsteroidal anti-inflammatory drugs; ASAs, acetyl salicylic acids (high and low dose).

modification of the association by this variable. We also stratified our analyses by ER status to investigate the potential relation between ER-negative breast cancer and prognostic profile [9].

We used unadjusted and multivariable Cox regression models to estimate the 10-year HR of recurrence and 95% CI for equivalent cumulative dose categories, using nonusers as the reference group. We also used Cox models to estimate the association between duration of GC use, as a time-varying exposure lagged by 1 year and measuring the cumulative number of years exposed to GC, and the rate of breast cancer recurrence. All multivariable Cox regressions were restricted to women with no missing information about any potential confounders. All statistical analyses were carried out with SAS 9.3.

ethics

The study was approved by the Board of the DBCG Registry and the Danish Data Protection Agency [journal number: 2006-41-6387].

results

The study included 18 773 women with a first incident breast cancer diagnosis. After excluding 486 women with only 0 or 1 day of follow-up and 36 women with ER-negative tumors who received ET (contrary to indication), 18 251 women remained in the cohort. The median age was 57 years (range: 21–95 years). There were 3408 recurrences of breast cancer during 94 345 person-years of follow-up (median = 6.9 years), equaling an incidence rate of 36 recurrences per 1000 person-years. Table 1 presents characteristics of the cohort and the distribution of subjects according to GC exposure and key demographic, tumor, and treatment variables. During follow-up, 4602 women redeemed at least one GC prescription. Users of any GC were more likely to be older, to be postmenopausal at breast cancer diagnosis, and to have more comorbid conditions compared with nonusers (Table 1).

The unadjusted Cox regression model indicated no notable association between use of systemic, inhaled, or intestinal-acting GCs and risk of 10-year breast cancer recurrence, compared with nonuse (unadjusted $HR_{systemic\ GC} = 1.1$, 95% CI 0.9–1.3; unadjusted $HR_{inhaled\ GC} = 0.9$, 95% CI 0.7–1.0; unadjusted $HR_{intestinal\ GC} = 1.0$, 95% CI 0.9–1.2) (Table 2). In adjusted models, the association remained near null for GC use and 10-year risk of breast cancer recurrence (adjusted $HR_{systemic\ GC} = 1.1$, 95% CI 0.9–1.2; adjusted $HR_{inhaled\ GC} = 0.8$, 95% CI 0.7–1.0; and adjusted $HR_{intestinal\ GC} = 1.0$, 95% CI 0.8–1.2) (Table 2).

When we repeated analyses within strata of adjuvant chemotherapy use, we observed the same pattern of associations as in the unstratified models (Table 2). We also repeated our analyses stratifying by ER status, with little change in the effect estimates (Table 2). Furthermore, associations remained near null across categories of cumulative prednisolone-equivalent dose of GC and for the duration of GC exposure (Table 2). We tested the proportionality of hazards by evaluating the significance of the interaction between GC use and the logarithm of person-time, and saw no evidence of a departure from proportionality.

discussion

In this large cohort of breast cancer patients, we observed no evidence of an association between prescriptions for systemic, inhaled, or intestinal-acting GC and risk of breast cancer recurrence. There was also no evidence of a dose–response

Table 2. HR and 95% CI for GC exposures (according to route of administration), stratified by presence/absence of chemotherapy and positive/negative estrogen receptor (ER) status, and for categories of prednisolone-equivalent doses (only systemic GC) and cumulative increase in GC exposure over 10 years

	Unadjusted[a] HR (95% CI)		Adjusted[ab] HR (95% CI)	
Systemic GC	1.1 (0.9–1.3)		1.1 (0.9–1.3)	
Inhaled GC	0.9 (0.7–1.0)		0.9 (0.7–1.0)	
Intestinal GC	1.0 (0.9–1.2)		1.0 (0.8–1.2)	
	Chemotherapy	No chemotherapy	Chemotherapy	No chemotherapy
Systemic GC	1.1 (0.9–1.4)	1.0 (0.9–1.2)	1.1 (0.9–1.4)	1.0 (0.8–1.2)
Inhaled GC	0.9 (0.6–1.2)	0.9 (0.7–1.1)	0.9 (0.6–1.3)	0.8 (0.7–1.0)
Intestinal GC	0.9 (0.7–1.2)	1.1 (0.9–1.3)	0.9 (0.6–1.2)	1.0 (0.8–1.3)
	ER positive	ER negative	ER positive	ER negative
Systemic GC	1.1 (0.9–1.3)	1.1 (0.8–1.4)	1.1 (0.9–1.3)	1.0 (0.8–1.4)
Inhaled GC	0.9 (0.7–1.1)	0.8 (0.6–1.2)	0.8 (0.7–1.0)	1.0 (0.7–1.4)
Intestinal GC	1.0 (0.8–1.2)	1.0 (0.7–1.4)	1.0 (0.8–1.2)	1.0 (0.7–1.4)
Prednisolone-equivalent dose (mg)[c]				
1–999	0.9 (0.8–1.0)		0.9 (0.8–1.1)	
1000–4999	0.9 (0.8–1.1)		0.8 (0.7–1.0)	
≥5000	1.0 (0.7–1.5)		0.9 (0.6–1.4)	
Cumulative increase in duration of GC exposure over a 10-year period[c]	1.0 (0.9–1.0)		1.1 (0.9–1.3)	

Reference group is nonusers. Stage I–III breast cancer patients diagnosed in Denmark, 1996–2003 ($N = 18\,251$).

[a]Models incorporating yearly updated drug exposure, lagged by 1 year.

[a]Models adjusted for age at diagnosis (continuous), menopausal status at diagnosis, UICC stage (design variables), histological grade (design variables), ER status and receipt of adjuvant endocrine therapy (conjugated, design variables), receipt of adjuvant chemotherapy, type of primary surgery received, Charlson Comorbidity Index score (design variables), pre-diagnosis combination HRT, and co-prescriptions (time-varying, updated yearly, and lagged by 1 year) of any β-blockers, ACE inhibitors, ARBs, ASAs, and simvastatin.

[b]Applies only to systemic GCs.

[c]The cumulative increase in the duration of GC exposure over a 10-year period was updated yearly. GC exposure was lagged by 1 year.

relationship. These results remained unchanged after stratification by chemotherapy. To the best of our knowledge, this is the first study to evaluate the association between GC use and breast cancer recurrence.

The validity of our estimates depends on several factors. The large size of the study population, in a country with free and equal access to high-quality health care, reduced the potential for selection bias. CPR numbers facilitated individual-level data linkage across registries, ensuring accurate and complete follow-up of the entire cohort. Use of registry-based prescription records eliminated the potential for differential exposure misclassification due to recall bias. The validity of the DBCG registry data is exceptionally high—the positive predictive value for classification of breast cancer recurrence by the DBCG registry was found to be 99.4%, using medical records as a gold standard [22]. Together with the prospective mandatory registration of prescription data, our study is unlikely to be prone to information bias. The study also benefitted from comprehensive information on potential confounders, including comorbid diseases and prescribed drugs. Except for aspirin, all the potentially confounding drugs are only available by prescription in Denmark. Residual confounding due to over-the-counter aspirin use is a potential concern. However, patients are reimbursed a proportion of the cost of prescribed medicine, so long-term, continuous use of aspirin is likely to be via prescription.

Our use of lagged exposures reduced the likelihood of reverse causation [23, 24]. The 1-year lag time allowed for a reasonable interval for the drug to affect the process of recurrence, but was not too long to weaken any potential association between the exposure drug and the outcome measure.

Locally administered GCs acting on the ear, nose, eye, or skin were not included in the exposure, as they are not thought to act systemically [25]. Low-dose locally administered GCs are available in limited supply over the counter in Denmark, while systemically acting GCs are only available by prescription. Any use of over-the-counter GCs in our patient cohort was likely to have a minimal effect on our recurrence estimates. We also lacked information on in-hospital GC use, which may have biased our estimates. Our previous medical record review of 200 breast cancer patients showed that all women who received chemotherapy were treated with systemic GC to alleviate treatment-related cytotoxic reactions [26]. When we stratified our estimates by receipt of chemotherapy to address this potential exposure misclassification, we found no change in the effect estimate.

Another concern is our reliance on redeemed prescriptions as a measure of drug use. We thus lacked information on compliance with treatment. However, because patients have to pay a portion of the cost of their prescription medication, it is likely that redeemed prescriptions reflect actual use [27]. GC dosing varies depending on the administrative route and indication for treatment. A set dose can be taken on a regular basis, or the dose may fluctuate according to variation in the severity of symptoms. Among women who took inhaled or intestinal-acting drugs, the

exact bioavailability is thus not known. We therefore restricted our dose–response analysis to systemically administered GCs.

We lacked information on HER-2 status and therapies with anti-HER-2 antibodies such as trastuzumab, since this treatment was not introduced into routine care in Denmark until 2006, 3 years after the last woman in our cohort had been diagnosed. The potential interaction between GCs and trastuzumab would be interesting to evaluate in future studies.

Our study is the first to examine directly the use of GCs and breast cancer recurrence, rather than diseases potentially treated with GCs [28]. GCs are widely used co-medications in breast cancer treatment so our findings are reassuring to clinicians and women with breast cancer when assessing the risks of these drugs.

In summary, we found no evidence of any impact of systemic, inhaled, or intestinal GCs on breast cancer recurrence in a nationwide prospective cohort of Danish breast cancer survivors.

funding

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disclosure

The authors have declared no conflicts of interest.

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Paper I

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Declaration of co-authorship

Full name of the PhD student: Lone Winther Lietzen

This declaration concerns the following article/manuscript:

Title:	Age at Diagnosis and Proportion of Node–Positive Breast Cancer Cases: A Danish population–based study
Authors:	Lone Winther Lietzen, Deirdre Cronin–Fenton, Peer Christiansen, Henrik Toft Sørensen, Bent Ejlertsen, Rebecca Silliman, Timothy Lee Lash

The article/manuscript is: Published ☐ Accepted ☐ Submitted ☐ In preparation ☒

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3. Involvement in the experimental work/clinical studies	D
4. Interpretation of the results	C
5. Writing of the first draft of the manuscript	D
6. Finalization of the manuscript and submission	D

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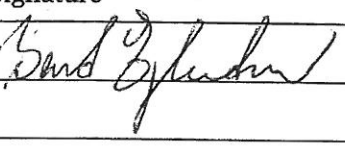
03.02.2015	Bent Ejlertsen	

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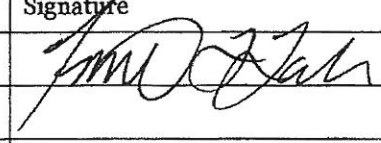
2/2/15	Timothy Lee Lash	

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Paper II

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Declaration of co-authorship

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Authors:	Lone Winther Lietzen, Deirdre Cronin-Fenton, Peer Christiansen, Henrik Toft Sørensen

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16/2 2015	Peer Christiansen	

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03.02.15	Henrik Toft Sørensen	

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Date	Name	Signature
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Paper III

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Declaration of co-authorship

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4. Interpretation of the results	C
5. Writing of the first draft of the manuscript	C
6. Finalization of the manuscript and submission	D

Signatures of the co-authors

Date	Name	Signature
9/2 -2015	Jens Peter Garne	

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Signature of the PhD student

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Declaration of co-authorship

Full name of the PhD student: Lone Winther Lietzen

This declaration concerns the following article/manuscript:

Title:	Predictors of re-operation due to post-surgical bleeding in breast cancer patients: A Danish population-based cohort study
Authors:	Lone Winther Lietzen, Deirdre Cronin-Fenton, Jens Peter Garne, Niels Kroman, Rebecca Silliman, Timothy Lee Lash

The article/manuscript is: Published ☒ Accepted ☐ Submitted ☐ In preparation ☐

If published, state full reference:

Eur J Surg Oncol. 2012 May;38(5):407-12. doi: 10.1016/j.ejso.2012.02.184. Epub 2012 Mar 17.

If accepted or submitted, state journal:

Has the article/manuscript previously been used in other PhD or doctoral dissertations?

No ☒ Yes ☐ If yes, give details:

The PhD student has contributed to the elements of this article/manuscript as follows:

A. No or little contribution
B. Has contributed (10-30 %)
C. Has contributed considerably (40-60 %)
D. Has done most of the work (70-90 %)
E. Has essentially done all the work

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03.02.2015	Niels Kroman	N. Kroman

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2/4/2105	Rebecca Silliman	Rebecca A Silliman

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Paper IV

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Declaration of co-authorship

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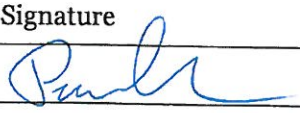
Date	Name	Signature
16/2 2015	Peer Christiansen	

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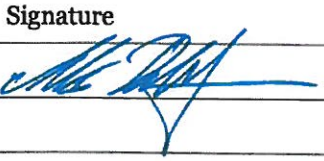
Date	Name	Signature
4.02.2015	Anders Bonde Jensen	

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Date	Name	Signature
03.02.15	Henrik Toft Sørensen	

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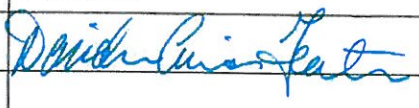
Date	Name	Signature
3/2/2015	Deirdre Cronin-Fenton	

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