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Statistical models of breast cancer tumour growth and spread

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Statistical models of breast cancer tumour growth and spread

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Abstract

In this thesis, we develop statistical methods for studying tumour growth and metastatic lymph node spread in breast cancer. The methods can be used for analysing breast cancer disease progression before diagnosis. They may be used to answer questions such as: For how long does a tumour grow inside of the body before it is detected? How much will the tumour metastasise in the lymph nodes before detection? Or, which women have a high risk of missing breast cancer at mammography screening? These questions are important for studying the effects of mammography screening at an individual level.

We work in a framework called continuous growth models. This is an alternative to Markov models, which is the most commonly used approach for modelling breast cancer disease progression. Standard Markov models assume that all women in each disease state are identical, making the model easy to implement and practically useful. Unfortunately, women with breast cancer are not identical, and relaxing this assumption quickly increases the Markov model's complexity. Continuous growth models are instead more complex at the outset. However, as the number of clinical factors increase, continuous growth models become more flexible and less complex than Markov models.

In **Study I**, we focus on a continuous growth process used for modelling tumour volume at diagnosis. We provide a detailed description of the so called Stable Disease Assumptions that are used for continuous growth modelling. We use them to derive new theoretical results for the model. These are then integrated into our growth model, which helps to simplify and reduce computational complexity of the model. With these results, we were able to greatly reduce the computational time needed for estimating growth parameters. The theoretical results derived in Study I are further used in Studies II and III.

In **Study II**, we extend our continuous growth model to also include a sub-model for metastatic lymph node spread. The result is a joint model of tumour volume and number of lymph node metastases at diagnosis, conditional on mammography screening history and mammographic density. When applied on empirical data on 1860 incident invasive breast cancer cases, our model provides a dramatically better fit than other models in current use. Furthermore, we show that our sub-model of lymph node spread can be estimated independently of the tumour growth process. This property forms the first part of the theoretical basis for study IV. In Study II, we use the property to validate our lymph node spread model on an independent data set, consisting of 3961 women diagnosed with invasive breast cancer.

In **Study III**, we show how to study the effect of other clinical factors on metastatic lymph node spread. Our approach is to regress clinical factors on the proportionality constant in our model for lymph node spread. We illustrate our method by studying the association between hormone replacement therapy (HRT) and tumour growth rate and rate of lymph node spread. Using data from 1631 women diagnosed with breast cancer, we estimate that women using HRT have a 36% lower rate of lymph node spread than non-users (95% confidence interval: 58% to 8%). This can be contrasted with the effect of HRT on tumour growth rate. We estimate growth rates to be 15% slower in HRT users ($p = 0.16$). We also derive theoretical distributions for metastatic lymph node spread at future points in time. We use them to illustrate the potential consequences of false negative screens, in terms of lymph node spread.

In **Study IV**, we use the method developed in Study III to study the association between clinical factors and rate of breast cancer lymph node spread. We use data on 10950 women to study the associations with grade, estrogen receptor (ER) status, progesteron receptor (PR) status, molecular subtype, and a polygenic risk score. We found that grade 2 and 3 tumours, respectively, were associated with 1.63 and 2.17 times faster rates of lymph node spread than grade 1 tumours ($p < 10^{-16}$). ER negative breast cancer was associated with a 1.25 times faster spread than ER positive and PR negative breast cancer was associated with a 1.19 times faster spread than PR positive cancer ($p = 0.0011$, $p = 0.0012$, respectively). Her2-enriched breast cancer was associated with a 1.53 times faster spread than luminal A cancer ($p = 0.00072$).

List of scientific studies

I. Gabriel Isheden and Keith Humphreys
Modelling breast cancer tumour growth for a stable disease population
Statistical methods in medical research 2019;28(3):681–702

II. Gabriel Isheden, Linda Abrahamsson, Therese Andersson, Kamila Czene, and Keith Humphreys
Joint models of tumour size and lymph node spread for incident breast cancer cases in the presence of screening
Statistical methods in medical research 2019;28(12):3822–3842

III. Gabriel Isheden, Kamila Czene, and Keith Humphreys
Random effects models of lymph node metastases in breast cancer: quantifying the roles of covariates and screening using a continuous growth model
Submitted

IV. Gabriel Isheden, Felix Grassman, Kamila Czene, and Keith Humphreys
Lymph node metastases in breast cancer: investigating associations with tumour characteristics, molecular subtypes, and polygenic risk score using a continuous growth model
Manuscript

The articles will be referred to in the text by their Roman numerals, and are reproduced in full at the end of the thesis.

Contents

Chapter 1

From general to personalised screening

Breast cancer is the most common form of cancer among women in the world. It has been estimated that approximately 600 000 women die from breast cancer each year globally (1). In Sweden, around 7500 women are diagnosed every year, out of which 1 400 die due to breast cancer (2). In most cases, breast cancer death is caused by metastases that have spread to other parts of the body, effectively shutting down vital body functions. If breast cancer is found and treated before it has spread, it is more likely to be curable. Therefore, in order to reduce mortality from breast cancer, most western countries have implemented nationwide breast cancer screening programs. Today, approximately half of all diagnosed breast cancers are detected by routine mammography screening.

The first breast cancer screening program in Europe was introduced in a selection of regions of Sweden in 1986 (3). It was based on age, which is the most important breast cancer risk factor in women. The program invited women age 50 to 69 to mammography screening every two years. In the same year, the Stockholm County Council decided to extend the program to include women aged 40 to 74 (4). Today, the Swedish national guidelines (5) are to invite all women age 40 to 74 to mammography screening every 18 to 24 months. Exact practices vary based on age and county. Still today, the general screening recommendations are mainly age based.

The Swedish guidelines on mammography screening (6) were primarily based on the results from the Swedish Two-County Screening Trial (7), that was conducted between 1977 and 1984. Since the 1980s, there has been great progress in our general understanding of cancer (8; 9), and a large number of discoveries concerning breast cancer biology and risk factors have been made. Researchers have discovered rare genetic mutations that lead to a highly increased risk of breast cancer (10; 11), as well as more than 300 common genetic variants that modestly increase risk (12). Hormone replacement therapy, oral contraceptives, and alcohol intake have been found to be associated with increased risk of breast cancer (13; 14; 15), whilst having children, breast feeding them, and staying physically active have been found to be associated with decreasing risk (16; 17; 18). Furthermore, each woman has different risk depending on her breast tissue composition, BMI, age of first menarche, and age of menopause (19; 20; 21).

Thanks to our improved understanding of breast cancer biology, there have been major

breakthroughs in breast cancer treatment. Today breast cancer is subclassified based on biomarkers, such as tumour grade, Ki-67 proliferation marker, estrogen receptor status, progesteron receptor status, and human epidermal growth factor receptor 2 (HER2) status. Breast cancer prognosis differs across subtype, and each subtype responds differently to different treatments. This discovery has lead to the development of less generalised and more personalised treatment, in the form of targeted therapies. One of the more successful examples is trastuzumab, an antibody used for treating HER2-enriched breast cancer. This type of cancer is prone to early metastasis, but since the discovery of trastuzumab, its prognosis has been much improved.

With treatment becoming personalised, and with so many breast cancer risk factors having been discovered, this begs the question: shouldn't breast cancer screening also be personalised?

Before personalised screening can reach practice, it first needs clinical evidence. The gold standard for testing medical hypotheses is randomised clinical trials. However, these are highly resource intensive and expensive to perform. Further, of all possible personalised screening programs, it is unclear which one would be considered optimal. For practical and ethical reasons, a large number of potential screening programs cannot be tested in clinical trials; there is not enough women, and many would receive sub-optimal screening patterns. To use resources effectively, and to reduce harm and suffering, a personalised screening program should be well designed before it is tested. An alternative approach could be to design and scrutinise potential personalised screening programs with the help of computer simulations. A large number of alternative screening programs could be assessed, and the one considered optimal could be tested in a randomised clinical trial employing real human subjects. To create such a simulation program, the natural development of breast cancer could be estimated from a number of different sources, such as clinical trials, expert opinion, and observational screening data. Unfortunately, creating a realistic simulation program is conceptually very difficult, and requires advanced statistical methods (22).

Statistical modelling of breast cancer progression is most commonly done using multi-state Markov models (23). These models assume that breast cancer progresses through a number of pre-specified and homogeneous disease states, and that these same states are shared among all breast cancer patients. For example, the basic model for breast cancer progression assumes three states: no detectable cancer, preclinical cancer detectable by screening, and clinical symptomatic breast cancer (24). This model has been extended to include, for example, states for metastatic lymph node spread (25; 26), and more complex models have been suggested (27). Homogeneous disease states are practical, but they are ultimately a simplification of reality. As I will illustrate in the following sections, the success of screening depends on a large number of biological and technical factors. Many of these are likely interdependent. In order to design personalised screening programs, flexible models are needed that allow for many clinical factors being accounted for. Increasing the number of disease states and clinical factors quickly increases the Markov model's complexity, which makes the model unsuitable for designing individualised screening programs. A recent alternative is continuous growth models. Unlike multi-state models, they aim to capture the underlying continuous process of tumour progression. Continuous growth models are more complex than Markov models at the outset, but as

the number of clinical factors increase, continuous growth models become more flexible and less complex than Markov models. Additionally, continuous growth models can more easily incorporate interdependencies between biological processes. This makes them more suitable for designing individualised screening programs, motivating further research on the topic.

Chapter 2

Aims and structure

My overarching aim is to develop continuous growth models that can be used for studying breast cancer disease progression, based on observational breast cancer screening data. I also want illustrate how the models can be applied to patient data to draw clinically relevant conclusions. The specific aims of each study are:

I. To simplify existing continuous growth models by clarifying their underlying assumptions and by developing new theory.

II. To use the theory developed in Study I to develop joint models of breast cancer tumour growth and metastatic lymph node spread.

III. To show how to model associations between clinical factors and rate of metastatic lymph node spread, and to derive new probability distributions for the number of lymph node metastases at earlier and later points in time.

IV. To use the models from Studies II and III to investigate associations between clinically relevant factors and rate of metastatic lymph node spread.

Studies I - III are highly technical, and require in-depth knowledge of statistics to read. Therefore, prior to and up to the point where the articles are presented, my aim with this thesis is to give the reader a *technical context*, rather than a detailed technical description that very few readers would benefit from. I will mainly focus on discussing *technical factors* that need to be taken into consideration for designing individualised screening programs. Before presenting articles I - IV, I give the following context:

- First, I describe what it means to assess a screening program, and show examples of how it has been done for breast cancer.
- Second, I discuss and try to intuitively explain the major technical and statistical factors contributing to screening effectiveness.
- Third, I introduce natural history models from the literature, i.e. Markov models, continuous growth models, and CISNET models. From this chapter onwards, more detailed statistical knowledge is required.

- Finally, I give a short summary of my papers, and end with a discussion of possible future directions for the research of my thesis.

Chapter 3

How can a breast cancer screening program be assessed?

To assess the effectiveness of breast cancer screening, one has to study its potential costs, harms, and benefits, and weigh them against one another. When considering all the available evidence for and against screening, if the benefits outweighs the costs and harms, then one can say that breast cancer screening is effective. This has been done by several independent research groups. The conclusions vary. The scientific community generally agrees that there are benefits to mammography screening, in the form of prolonged survival for women attending screening. The main point of contention is how big the benefits are in relation to the harms and costs (28). In this section, I will give an overview of how to estimate the survival benefits of screening, based on clinical trials and simulation studies.

In medicine, mortality and survival is studied by collecting data from human subjects over time, recording their times of study entry and death, together with other important medical information. After adjusting for age at entry, and relevant medical factors, one can use this type of data to estimate how long patients live. If this is done for two groups of patients, one being a control group and one receiving mammography screening, then one can study whether screening improves survival.

Survival data has mainly been collected from randomised clinical trials and observational studies. In the first case, data is collected prospectively as part of a randomised controlled experiment. In the latter, medical data is collected retrospectively from women taking part in the normal healthcare system. Both types of data have been used to assess screening. Unfortunately, four severe biases occur when assessing screening based on observational data (29): lead time bias, length bias, overdiagnosis, and self selection bias. These biases occur when survival is measured from time of diagnosis, when comparing breast cancer survival between screen detected cases and symptomatically detected cases (cases found in-between screening rounds or outside of the screening program). For this reason, survival studies based on observational data are considered much less reliable than those based on randomised clinical trials.

Clinical trials

An independent UK panel on breast cancer screening (30) identified 11 completed randomised clinical trials, involving 650 000 women, carried out in Europe and North America between 1963 and 1991. The trials measured overall survival from time of study entry; they compared women who were invited to screening for breast cancer to women who were not invited. Based on these trials, the panel estimated a 20% reduction in breast cancer mortality after 13 years of follow-up in women invited for screening. They noted that the relative reduction in mortality will be higher for women actually attending screening. The systematic review by the Cochrane collaboration (28; 31) critisised a number of the trials for employing sub-optimal randomisation procedures. Based on the three studies that were appropriately randomised, they estimated a 10% reduction in mortality.

Screening trials trials can also give rise to biased mortality ratios when women in the non-screening group receive mammography screens at other hospitals. This bias will not, however, make screening seem more effective, but rather less effective.

Simulation studies

Recognising that there are limitations and flaws in the randomised trials used to evaluate the effects of screening on cancer mortality, there have been various efforts to estimate screening effects based on simulation studies. These can be designed with the help of expert opinion, and can incorporate data from observational screening studies and clinical trials. One of the larger initiatives to assess the effects of screening is the CISNET (Cancer Intervention and Surveillance Modelling Network) consortium.

The CISNET consortium was established in 2000 by the U.S. National Cancer Institute and currently consists of six research groups from Georgetown University, Dana-Farber Cancer Institute, Stanford University, University of Wisconsin, Erasmus MC, and University of Texas MDACC (22). These groups develop simulation-based modelling approaches for investigating the impact of breast cancer interventions, with a focus on prevention, screening, and treatment. Each group has developed models for breast cancer progression, including submodels for incidence, natural history, and survival/treatment. The models have been calibrated against incidence and mortality data from the Surveillance, Epidemiology and End Results (SEER) Program for U.S. women aged at least 25 in 1975.

Comparing breast cancer mortality in 1990 to that in 2000, the CISNET group estimated that breast cancer mortality had decreased by 24%. Each group has concluded that mammography screening has contributed to this decrease. Their assessment of the relative contribution from screening have however differed, ranging from 28% to 65% of the total decrease. In 2018 , CISNET evaluated the contributions of screening and treatment for different molecular subtypes of breast cancer (32). The subtypes were defined based on the tumours estrogen receptor (ER) status and human epidermal growth factor receptor 2 (HER2) status. The group estimated that in 2012, compared to baseline mortality rates, total reduction in mortality rate from interven-

tions was 49%, ranging from 37% for ER-/HER2- breast cancer to 58% for ER+/HER2+ breast cancer. The contributions of screening and treatment were estimated to differ substantially between molecular subtypes. Screening was estimated to have contributed to 31% of the total mortality reduction for ER-/HER2- and to 48% of the reduction for ER+/HER2+ breast cancer.

Chapter 4

Factors contributing to screening effectiveness

Individualised screening programs will need to be well designed before they can be tried out in a clinical setting. A program is more likely to have the intended effectiveness if it is designed based on realistic models of breast cancer disease progression. By realistic, I mean in the sense of capturing mechanisms and factors that are likely to contribute to screening effectiveness. In this section, I will discuss and try to give an intuitive description of the technical and statistical factors that contribute to screening effectiveness. The factors are not presented in any strict logical ordering, so the first factor should not be interpreted as the most important one, and the last factor should not be interpreted as the least important.

Screening effectiveness

The effectiveness of a screening program is very dependent on how *effectiveness* is defined. In other words, screening effectiveness depends on how one weighs the costs and harms of screening with the benefits of screening. Weighing the risks and benefits usually involves a trade-off between competing objectives. For example: maximizing quality of life versus maximizing life expectancy versus minimizing the resources required for screening implementation (33). Screening effectiveness can alternatively be be defined in terms of cost-effectiveness.

New medical interventions, such as drugs, medical equipment, and new procedures, are often evaluated by health authorities/policy-makers in so called cost-effectiveness studies. The aim of such studies is to assess the intervention by estimating all of its future expected costs, health benefits, and risks, and then to quantify them, calculate their present value, and finally to estimate the cost-effectiveness. A positive cost-effectiveness can be interpreted as the monetary amount (dollars) that must be spent in order to gain an additional (quality adjusted) life-year. A small positive value means that small monetary resources need to be spent per life-year saved, and a large value means that large monetary resources are required for a small life gain. The cost-effectiveness ratio is defined as

$$\mathrm{CER} = \frac{C_p}{U_p}, \tag{4.1}$$

where U_p is the present value of future health utilities gained (quantities of health risks and benefits) and C_p is the present value of future intervention costs. Costs and health utilities may be taken from literature reviews or analyses of register data. Cost-effectiveness can be estimated from a health sector perspective, or from a societal perspective. In many cases, interventions are compared to an already existing treatment alternative. In those cases, the incremental cost-effectiveness ratio is used instead. An intervention, or an intervention compared to an alternative, can be said to be cost effective if the cost-effectiveness ratio, or incremental cost-effectiveness ratio, meets a certain threshold. In practice, many health authorities use a 'soft' or informal threshold. In the UK, this threshold is 20 to 30 thousand pound sterling per life-year saved (34), and in Sweden it is 700 000 to 1 220 000 kronor SEK per life-year saved (35). If the cost for each life-year saved is much higher than these numbers, the intervention is generally not accepted.

Cost-effectiveness studies are carried out by health authorities in many countries, but are still considered controversial. One of the ethical concerns is fairness. If cost-effectiveness determines treatment options, people who are ill may be left without treatment. Another concern is connected to the choice of which costs to count, and how to count them. One of the benefits of cost-effectiveness analysis is that it can expose costs, make them explicit, and in doing so improve transparancy in policy making decisions (36).

For the remainder of this chapter I will use the word screening effectiveness to mean the cost-effectiveness of a screening program.

Costs, harms, and benefits

Screening effectiveness depends how the costs, harms, and benefits of screening are counted and measured. The direct costs of screening are the medical costs for equipment, personnel, and maintaining the hospital. The indirect costs may include costs from job time lost, and lost tax revenue. Generally, the cost can be measured economically, e.g. as a monetary (dollar) amount C. Apart from economic costs, the harms of screening may be: overtreatment of slow-growing or in-situ tumours, increased anxiety, and use of ionising radiation (30). If these harms are measured in medical or health economic studies, they may be quantified as life-years lost from screening, or as quality-adjusted life-years lost from screening. In the same way, the benefits of screening can be quantified as life-years gained from screening, or quality-adjusted life-years gained from screening. If health utility is chosen as the quantity for harm, then one also has to define what a quality-adjusted life-year means, and measure it using a health economic instrument, such as EQ-5D (37).

Costs, harms, and benefits can be weighed differently over time, so that those that occur close to the present time have higher value, and those that occur far in the future are given a lower value. Mathematically, this is usually done by discounting future quantities into present valued quantities with a time-increasing discount factor. To clarify, if the expected cost t years

into the future is C_t, then the present value of that cost C_{tp} can be defined as

$$C_{tp} = \frac{C_t}{\lambda^t}, \tag{4.2}$$

where $\lambda \geq 1$ is a discount factor (e.g. $\lambda = 1.03$). Similarly, the total present cost C_p can be defined as the discounted sum of all future costs, from 0 to year T

$$C_p = \sum_{t=0}^{T} C_{tp} = \sum_{t=0}^{T} \frac{C_t}{\lambda^t}. \tag{4.3}$$

Breast cancer risk

Breast cancer risk is, perhaps, the most apparent factor that can contribute to screening effectiveness. I present here a simplified argument of why that is so. Assume that there are two women, A and B, and that the women are identical in every possible way, except that woman A has, at every point in time, an $x > 1$ times higher risk of breast cancer. Assume further that the additional costs for screening the two women is C_S and that the expected health (or health utility) gained from treating them earlier when they have cancer is U. If both women are screened once, the cost-effectiveness ratio of each screening visit is

$$\text{CER} = \frac{C_S}{pqU}, \tag{4.4}$$

where $p > 0$ is each woman's probability of having breast cancer, and q is the average probability of finding the cancer when it is present. At this visit, woman A has a higher probability of having breast cancer than woman B, because of her higher risk. If the costs C_S are positive, then, as long as the net expected health benefit U is positive, equation (4.4) is a decreasing function in p. A small cost-effectiveness ratio means an effective intervention, so it follows explicitly that it is more cost-effective to screen woman A than woman B.

This argument does not hold if the woman has a higher risk from a factor that also impacts costs or health benefits. For example, a predictive risk factor that also predicts life expectancy, such as alcohol use (15; 38), affect the health benefit from the shorter life-span of heavy alcohol drinkers. Similarly, older women who are at a higher risk of breast cancer because of their age might not live long enough to see the benefits of screening and treatment. Even though it does not hold strictly for arbitrary risk factors, the argument is clear for factors that are believed to primarily affect breast cancer risk.

Life expectancy in the absence of breast cancer

Life expectancy plays an important role in screening effectiveness. In terms of life-years saved, a woman who is expected to die naturally, from other causes, shortly after a breast cancer diagnosis, will benefit less from breast cancer treatment. As mentioned above, this fact becomes

important when studying breast cancer risk factors that also shorten women's remaining life spans, such as alcohol use and and age. If such breast cancer risk factors are incorporated into simulation programs used in evaluating screening effectiveness, it is crucial that the shortened life-span is also accounted for.

The importance of life expectancy can be argued for using cost-effectiveness directly. Assume now that two almost identical women A and B have the same breast cancer risk r, and the same costs of screening C_S, but that woman A has a longer life-span. The expected gain in utility for treating woman A, U_A, is higher than the expected gain in utility for treating woman B, U_B. If the probability for each woman having breast cancer is p and the probability of detection is q, then the cost-effectiveness ratio for screening woman A is

$$\text{CER}_A = \frac{C_S}{pqU_A}, \tag{4.5}$$

and the the cost-effectiveness ratio for screening woman A is

$$\text{CER}_B = \frac{C_S}{pqU_B}. \tag{4.6}$$

As long as the utilities for treating women A and B are positive, it holds that

$$\text{CER}_A = \frac{C_S}{pqU_A} < \frac{C_S}{pqU_B} = \text{CER}_B, \tag{4.7}$$

which means that it is more cost-effective to screen woman A than woman B.

Breast cancer treatment

For screening to be effective, treatment must either be less resource intensive or/and more effective at earlier stages of breast cancer progression. If neither is true, then screening cannot be effective.

At one extreme, if there is no effective treatment, or if treatment is too expensive or otherwise unavailable, then finding the tumours through screening will not help reduce mortality. At another extreme, if treatment can fully cure breast cancer at late stages of disease progression, then screening for the cancer will be unnecessary. It should be noted, however, that better treatment does not necessarily decrease screening effectiveness. As long as the treatment works better at earlier stages of breast cancer progression, then screening will have a positive impact on survival, and can still be effective.

Today, treatment of invasive breast cancer depends on the characteristics of the tumour, including the tumour's grade, Ki-67 proliferation marker, estrogen receptor status, progesteron receptor status, and human epidermal growth factor receptor 2 status. New treatment options have been discovered that are well suited for treating certain subtypes of breast cancer, such as trastuzumab for treating HER2-enriched breast cancer. The breast cancer screening trials (30) provided evidence that early treatment improves breast cancer survival. For the newer

treatment options that came after the screening trials, the effect of early treatment on survival has not been established.

Screening method

The choice of screening method influences screening effectiveness through the test's costs, specificity, and sensitivity. Increased costs make the screening method less cost-effective, low specificity adds costs for additional medical examinations and dis-utilities for overtreatment, and a high sensitivity of course increases screening effectiveness by identifying more breast cancer patients.

The standard screening method used today is mammography, a test in which low doses of X-rays are used to examine the breasts. The sensitivity of a mammography screening test depends heavily on the woman's breast tissue composition. The female breast has two main tissue types: fatty tissue, that appears dark on a mammogram; and fibroglandular tissue, that has a bright appearance. Tumours also appear bright on mammograms, and can easily be concealed in fibroglandular tissue. Mammographic density, measured as a function of the pixel intensities in the image, reflects the different tissues in the breast, and is also an important risk factor for breast cancer (19).

Mammography screening sensitivity is one of the major topics in the articles of my thesis. In article I, we sought to understand how mammographic density and tumour volume interact in their effect on screening sensitivity.

Disease progression

The last important factor that I am going to mention is the rate of breast cancer disease progression, which is the main focus of the articles of my thesis.

Virchow (39) hypothesised in 1862 that tumours develop from a single cell. Today, this hypothesis has been shown to be true for the majority of cancers, including breast cancer (40). Breast cancer normally develops in the lobules or ducts of the breast from cells that have mutated. As tumour cells divide, they mutate and get a number of distinct features (9): they resist cell death, get unlimited replicative potential, maintain proliferation, and start evading growth suppressors. As tumours grow, they may start forming their own blood vessels and invade neighbouring tissue. Breast cancer that is not yet invasive is called breast cancer in situ. This type of breast cancer respects tissue boundaries and is localised to the lobules (lobular carcinoma in situ) or ducts (ductal carcinoma in situ). Generally, these do not spread quickly; the prognosis is good for breast cancer in situ. Invasive breast cancer, on the other hand, will start spreading if left untreated, and may metastasise in the lymph nodes close to the breast, or in other parts of the body.

The purpose of screening is to find breast cancer at early stages of disease progression, when curability is high. A woman who attends screening can get a breast cancer diagnosis either as a result of a screening visit or as a result of breast cancer symptoms occurring in-between

two screening round. If a tumour progresses at a very aggressive rate, then the chance for symptomatic tumour detection increases. If a tumour is symptomatically detected in-between screening rounds, then, by definition, the woman who had the tumour was not helped by screening. Consequently, screening effectiveness for an individual woman must depend on how quickly the tumour progresses through its different stages.

Breast cancer progresses on a number of different scales simultaneously. For example, the cancer progresses in terms of the tumour becoming more mutated, larger, more vascularised, and invading more neighboring tissue. Two simplistic yet important scales for understanding screening effectiveness are symptomatic progression and metastatic progression. In terms of symptomatic progression, breast cancer is non-symptomatic at onset, but starts displaying symptoms as the tumour grows. The early breast cancer Markov models modelled symptomatic progression: the cancer progressed from being non-symptomatic and not detectable by screening, into non-symptomatic but detectable by screening, and finally became symptomatic breast cancer (24). In terms of metastatic progression, most breast cancers progress from a localised breast cancer stage, into a regional stage with metastatic lymph node spread, and finally into a distant metastatic stage, with breast cancer metastases in the brain, lungs, liver, or bone marrow. Prognosis is worse for later stages of breast cancer progression.

Some of the most important factors that shed light on the rate of disease progression are: tumour size, lymph node metastases, distant metastases, and molecular subtype. Tumour size is informative of the tumours growth rate. Additionally, tumours are more likely to metastasise at larger sizes, and also become more easily detectable at mammography screening. Lymph node metastases commonly occur before distant metastatic spread, and influence long term prognostis when present at diagnosis (41; 42). Distant metastases cause breast cancer death, and lymph node metastases can be informative of micro metastases in distant regions of the body. Finally, different breast cancer molecular subtypes have different breast cancer prognoses, and likely progress at different rates.

In the next chapter, I will discuss different ways in which breast cancer progression have been modelled in the statistical literature. The discussion will focus on models of *invasive* breast cancer. For a description of models of in situ breast cancer, used by CISNET, see van Ravesteyn et al. (43).

Chapter 5

Natural history models

The aim of my thesis is to develop statistical models of breast cancer disease progression. As I described in the previous section, two of the key components for understanding the natural history of breast cancer are tumour growth and metastatic lymph node spread. In this chapter, I will discuss existing modelling approaches from the literature, focusing on Markov models and continuous growth models. I will also briefly describe the CISNET models.

This chapter is a bit more technical than the previous chapters. Readers without knowledge of statistics may get less benefit from reading it.

Markov models

The Multi-state Markov model has a long history in the analysis of screening data. The basic model for breast cancer assumes three states: no detectable cancer, preclinical cancer detectable by screening, and clinical symptomatic breast cancer (24). Several extensions of the model have been described in the literature; for example Chen et al. (25; 26) propose a five-state model that enriches the preclinical and clinical states by splitting both states into two sub-states, one with positive nodal involvement and one with negative nodal involvement. Parameter estimates of these models have been obtained with several different methods: Duffy fit the 3-state model based on the likelihood of prevalent breast cancer screening cases and interval cases (split into two groups by arrival times), Chen et al (44) fit the 3- and 5-state models based on the likelihood of prevalent and subsequent screens from screening trials, Taghipour et al. (45) fit the 3-state model (with an additional state added for deaths due to other causes) to screening cohort data, and Weedon-Fekjaer et al. (46) fit the 3-state model based on the likelihood of prevalent screening cases and interval cases after the prevalent screen.

Many applications of these models have been published in recent years, for example, for evaluating overdiagnosis (47; 48) and lead time bias when comparing screen detected cancers and cancers found outside of screening programs (27). For a relatively recent review of multi-state Markov models see Uhry et al.(23).

Markov models assume homogeneous disease states. While this is practical, it is a simplification of reality. For example, screening sensitivity is usually assumed to be constant during the

preclinical state. Homogeneous disease states also imply that we can not add risk factors to the model without increasing the complexity of the model significantly. Since we want to model breast cancer progression and take risk factors into consideration at the same time, alternative models are needed to improve our understanding of disease progression with respect to the screening procedure.

Continuous models applied to non-screened populations

An alternative approach to Markov models is to model tumour progression with a continuous growth function, and add other processes on top of the growth function. Bartoszynski et al. (49) proposed several growth functions, such as Gompertz growth, logistic growth, and exponential growth. In the exponential case, they proposed that tumour volume at time t could follow the function

$$V(t,r) = V_0 e^{t/r},\ t \geq 0, \tag{5.1}$$

where r is an individually assigned growth rate and $V_0 = 1000\ \mu m^3$ is the starting volume of the tumour. Furthermore, they allowed the inverse growth rate r to follow a gamma distribution with shape parameter τ_1 and inverse scale parameter τ_2,

$$f_R(r) = \frac{\tau_2^{\tau_1}}{\Gamma(\tau_1)} r^{\tau_1 - 1} e^{-\tau_2 r},\ r \geq 0. \tag{5.2}$$

On top of the growth function, they assumed that eventually, the tumour would be detected spontaneously from its symptoms. In their model, the probability for instantaneous symptomatic detection at time $T_{det} = t$ after tumour initiation depended linearly on the tumour volume

$$P(T_{det} \in [t, t+dt) | T_{det} \geq t, R = r) = \eta V(t,r) dt + O(dt),\ V(t,r) \geq V_0. \tag{5.3}$$

Plevritis et al. (50) described a similar model with exponential growth, gamma distributed inverse growth rate, and a hazard for symptomatic detection proportional to tumour volume. The model differed in the choice of starting volume for the tumour. Instead of $V_0 = 1000\ \mu m^3$, they assumed that the tumour started from an observable volume corresponding to a sphere of diameter $d_0 = 2mm$. Early analyses using models (5.1) - (5.3) were based on data from women diagnosed with breast cancer in non-screened populations (50; 51) and therefore based inference on the density for tumour volume at symptomatic detection, which was shown by Plevritis et al. (50) to be

$$f_{V_{det}}(v) = \eta \tau_1 \frac{\tau_2^{\tau_1}}{(\tau_2 + \eta(v - V_0))^{\tau_1 + 1}}, v > V_0. \tag{5.4}$$

This density was derived from the density for tumour volume at symptomatic detection conditioned on the inverse growth rate

$$f_{V_{det}|R=r}(v) = \eta r \exp(-\eta r (v - V_0)), v > V_0 \tag{5.5}$$

and the joint density for tumour volume at symptomatic detection and the inverse growth rate

$$f_{V_{det},R}(v,r) = \eta \frac{\tau_2^{\tau_1}}{\Gamma(\tau_1)} r^{(\tau_1+1)-1} \exp(-r(\tau_2 + \eta(v - V_0))), v > V_0, r \geq 0. \tag{5.6}$$

Continuous models applied to populations under screening

Weedon-Fekjaer et al.(52; 53) described a continuous growth model that obtained estimates of growth and screening parameters based on screening data. They based tumour growth on a logistic function proposed by Spratt et al. (54), which assumes that the tumour starts at size V_{cell}, corresponding to a cell, and grows to size V_{max} corresponding to a spherical tumour of diameter $d_{max} = 128\ mm$ (40 tumour doublings). The volume $V(t)$ at time t was described by

$$V(t) = \frac{V_{max}}{\left[1 + \left(\left(\frac{V_{max}}{V_{cell}}\right)^{1/c} - 1\right) e^{-\kappa t/c}\right]^c}, \tag{5.7}$$

where κ is a log-normally distributed random effect with log-mean α_1 and log-variance α_2

$$f_\kappa(x) = \frac{1}{x\sqrt{2\pi\alpha_2}} e^{-\frac{(\log x - \alpha 1)^2}{2\alpha_2}}, x > 0. \tag{5.8}$$

Spratt et al. found the constant $c = 4$ to give the best model fit. The same constant was used by Weedon-Fekjaer et al.

In order to model screening data, Weedon-Fekjaer et al. (52; 53) modelled screening sensitivity as function of tumour diameter d,

$$S(d) = \frac{e^{a_1 + a_2 d}}{1 + e^{a_1 + a_2 d}}, \tag{5.9}$$

where a_1 and a_2 are screening parameters.

In the first of these studies, Weedon-Fekjaer et al. (52) estimated α_1, α_2, a_1 and a_2 by combining two likelihoods: one based on the distribution of tumour sizes at the first screening, and one based on the incidence of interval cases. In the second of these studies, Weedon-Fekjaer et al. (53) extended the model by incorporating previous negative screening tests into the likelihood. This was achieved by calculating the distribution of tumour size conditioned on time since the last negative screen.

Abrahamsson and Humphreys (55) described an approach that extends the model of Bartoszynski et al. to screening data, and further build on the ideas presented by Weedon-Fekjaer et al. They modelled tumour progression using (5.1) - (5.3), assuming an initial diameter $d_0 = 0.5\ mm$, and modelled screening sensitivity as a function of both tumour size (d) and

mammographic density (m)

$$S(d,m) = \frac{e^{\alpha_1+\alpha_2 d+\alpha_3 m+\alpha_4 d\cdot m}}{1+e^{\alpha_1+\alpha_2 d+\alpha_3 m+\alpha_4 d\cdot m}}. \tag{5.10}$$

They estimated $\tau_1, \tau_2, \eta, \alpha_1, \alpha_2, \alpha_3$ and α_4 from a sample of incident cases of breast cancer by maximising the product of two likelihoods: one based on the distribution of tumour sizes at screen detection, and one based on the distribution of tumour sizes at symptomatic detection, both conditional on the screening history of the woman (the time and number of previous screens). Because pathologists tend to round tumour diameters to the nearest mm, they express the likelihood function in terms of the probabilities for tumours to be in one of the 24 millimetre size intervals $[0.5, 1.5)$, $[1.5, 2.5)$, $[2.5, 7.5)$, $[7.5, 12.5)$, ..., $[67.5, 72.5)$, $[72.5, 85)$, $[85, 95)$, ..., $[145, 155)$. They expressed the likelihood as

$$L(0|\theta) = \frac{n!}{\Pi_i o_i!}\prod_i p_i^{o_i}, \tag{5.11}$$

where n is the number of cases, o_i is the number of cases in size interval i, p_i is the probability for a tumour to be in size interval i at deteection, and θ is a vector of the unknown parameters. In order to describe how p_i is calculated in the two cases, the authors introduced a very convenient notation:

A_i - The tumour is in size interval i at symptomatic detection.

B_j - The tumour is detected at screening number j (at time point t_j).

$C_{i,t}$ - The tumour is in size interval i at time point t.

$D_{t,f}$ - In the absence of a screening programme, a tumour will be symptomatically detected at time point $t+f$, $f \geq 0$.

B_j^c - The tumour is not detected at screenings 1 to j, $j \geq 1$.

For a woman with screening time $t_1, t_2, ..., t_K$, they showed that in the screen detected case, p_i could be calculated as

$$p_i \propto P(B_K|C_{i,t_k})P(C_{i,t_k})\sum_{s_{-1}\leq i} P(B_{K-1}^c|C_{i,t_k}\cap C_{s_{-1},t_{k-1}})P(C_{s_{-1},t_{k-1}}|C_{i,t_k}), K \geq 1, \tag{5.12}$$

$$p_i \propto P(B_K|C_{i,t_k})P(C_{i,t_k}), K = 1. \tag{5.13}$$

To calculate the probability for the previous missed screens, they followed the trajectory of a tumour passing through the midpoints of size intervals i and s at time points t_k and t_{k-1}, and used the formula

$$P(B_{K-1}^c|C_{i,t_k}\cap C_{s,t_{k-1}}) = (1-P(d_{s_{-1}},m)\cdot(1-P(d_{s_{-2}},m)\cdot ...\cdot(1-P(d_{s_{-K}},m),$$

where $d_{s_{-1}}, ..., d_{s_{-K}}$ are the projected tumour diameters at the previous screens.

In their model, $P(C_{s_{-1},t_{k-1}}|C_{i,t_k})$ was calculated by finding appropriate inverse growrth rates for tumour passing through intervals i and s_{-1} (at both boundary values of the size interval s_{-1}, using the midpoint of interval i) and then using the distribution function for the growth rate conditioned on the tumour being at least in interval i at symptomatic detection. We note, however that this potentially leads to a small bias—the tumour being in size interval i at time point t_k is not equivalent to the tumour being in size interval i, or an interval representing a larger size, at symptomatic detection.

Finally, to calculate $P(C_{i,t_k})$, (the probability density function for tumour size of an undetected breast cancer case) the authors modified a procedure suggested by Weedon-Fekjaer et al. (53). They calculated the quantity via back-calculation, tracking tumours backwards in time from their hypothetical symptomatic detection, i.e. from the tumours expected outcome, had the woman not attended screening. In practice, they performed the numerical calculation

$$P(C_{i,t_k}) \propto \sum_{0\leq f}\sum_{i\leq g} P(C_{i,t_k}|D_{t_k,f} \cap A_g) \cdot P(A_g). \tag{5.14}$$

In order to do this they first derived the density for growth rate conditioned on volume at symptomatic detection, shown under (5.1) - (5.3) to be

$$f_{R|V_{det}=v}(r) = \frac{(\tau_2+\eta(v-V_0))}{\Gamma(\tau_1+1)}\big(r(\tau_2+\eta(v-V_0))\big)^{(\tau_1+1)-1}\exp(-r(\tau_2+\eta(v-V_0))), r \geq 0. \tag{5.15}$$

From this large model, the authors were able to retrieve parameter estimates for the average growth rate of tumours, the time to symptomatic detection, and the effect of tumour diameter and mammographic density on screening sensitivity.

In a later study, Abrahamsson et al. (56) extended their framework to estimate the effects of BMI on tumour growth rate, and the effect of breast size on time to symptomatic detection. These effect were estimated by regressing the log expected value of the inverse growth rate on BMI (b)

$$log\ E[R] = \alpha + \beta b, \tag{5.16}$$

and by regressing on breast size (s) using the log of the effect parameter (η) in (5.3)

$$log\ \eta = \gamma + \delta s. \tag{5.17}$$

We used a related approach to incorporate covariates into models of lymph node spread in Studies III and IV.

Models of metastatic lymph node spread

In the context of continuous growth models, models of metastatic lymph node spread have been proposed by Plevritis et al. (50), Hanin and Yakovlev (51), Shwartz (57), and the CISNET university of Wisconsin group. To model breast tumour spread, the Wisconsin group used the

model proposed by Shwartz (57), which assumes that tumour volume V grows exponentially with an individually assigned growth rate, and that the rate of additional lymph node spread is equal to $\lambda = b_1 + b_2V + b_3V'$. The group has modified the growth component of the model slightly. Instead of exponential tumour growth, they assumed an exponential Gompertz function with decelerating doubling time. In fitting the model to observed breast cancer incidence data, the group found the overall model fit to be inadequate, but subsequently introduced additional, somewhat ad-hoc, assumptions to improve model fit. Firstly, when simulating lymph node progression, they found that the Shwartz model produced too much lymph node spread for large tumours. Consequently, they simulated lymph node spread using an adjusted diameter (reducing the tumour diameter by 25%) in the spread model. At the other end of the spectrum, they assumed that 1% of all invasive tumours had four affected lymph nodes at tumour onset and that 2% had 5 or more affected lymph nodes (in their model, 5 or more affected lymph nodes involved was considered to be synonymous with distant metastatic spread).

Hanin and Yakovlev's model (51) was also based on the model of Shwartz, and assumed that the tumours grow exponentially and that the rate of lymph node spread is proportional only to tumour volume. They introduced a number of additional assumptions and provided a detailed mathematical description of the model. Plevritis et al. described a simpler model in which the hazard of a localised tumour spreading to the lymph nodes is proportional to the volume of the tumour. They also relied on exponential tumour growth.

From the fact that: a) the Wisconsin group had to introduce additional assumptions to fit the Shwartz model to data and b) that the Shwartz model represents a more general case of Hanin and Yakovlev as well as Plevritis et al., it is likely that the mentioned models can be improved. This claim can be further backed up by noting two problems in the models of Shwartz, and Hanin and Yakovlev (see Figure 1).

The first problem is that under the Shwartz model, a slow growing tumour will have, on average, more node spread compared to a fast growing tumour, given that the two tumours are of the same size. This property is not supported by empirical evidence. Slow growing tumour having comparatively higher degree of lymph node spread implies that screen detected cancers would have more lymph node involvement compared to interval cancers, due to length biased sampling. Empirical data shows that this is not true (58). The model of Hanin and Yakovlev is also affected by this problem.

The model of Hanin and Yakovlev exhibits a second problem: namely, that the rate of additional lymph node spread grows excessively with increasing tumour volumes. In their model, the expected number of lymph nodes for tumours of diameters 20 *mm* is approximately 1000 times larger compared to the expected number of lymph nodes in tumours of diameter 2 *mm*. Such an extreme difference is not supported by clinical data. It is not uncommon to find tumour of less than 2 *mm* with one or several lymph nodes affected. Moreover, even, large tumours do not exhibit spread with lymph nodes affected in the thousands. Shwartz (57) found that, when simulating cohorts of symptomatic cancers based on his model, too few affected lymph nodes in tumours with diameters smaller than 1 *mm* were produced. The CISNET Wisconsin group found similar patterns of behaviour in their modified approach. They

also found that their model generated too many lymph nodes in large tumours. Even though this named second problem, strictly speaking, does not have to apply to the Shwartz model, the findings of Shwartz and the CISNET Wisconsin group do indeed indicate that the problem will apply to to the Shwartz model. Together, these problems indicate that new models of lymph node spread are needed.

For a detailed mathematical description of the problems I mentioned above, see article II in this thesis.

CISNET models

To investigate the impact of breast cancer interventions, including prevention, screening, and treatment, the CISNET consortium have developed six different breast cancer progression models. Each model can be divided into components, such as incidence, natural history, and survival/treatment. The CISNET groups use the following types of natural history models:

- The Georgetown University group uses a Markov model with cancer types: ductal carcinoma in situ, local invasive breast cancer, locally spread breast cancer, and breast cancer with distant metastases. They also include pre-clinical states with age-dependent exponentially distributed sojourn times.

- The Dana-Farber Cancer Institute group uses a three-state Markov model with an asymptomatic and clinically undetectable breast cancer state, an asymptomatic but clinically detectable state, and a diagnosed breast cancer state. At diagnosis, the stage of the breast cancer is drawn from a stage distribution based on observed incident breast cancer cases.

- The Stanford University group uses a continuous growth model with exponential tumour growth, with inverse growth rate drawn from a gamma distribution, a hazard of symptomatic clinical detection proportional to current tumour size, and specifies hazard functions for the tumour to spread (similar to those described in Plevritis et al. (50)).

- The University of Wisconsin group uses a continuous growth model with Gompertz growth, growth rate drawn from a lognormal distribution, symptomatic detection following a failure-time model, and lymph node spread following an inhomogenous Poisson process (57).

- The The Erasmus MC group uses the MISCAN breast cancer model (59), in which tumours are assumed to have a fatal diameter, drawn from a weibull distribution.

- The University of Texas MDACC group uses a population based approach where they simulated incident cohorts of breast cancer cases with appropriate tumour characteristics.

These models are calibrated against incidence and mortality data from the Surveillance, Epidemiology and End Results (SEER) Program for U.S. women aged at least 25 in 1975.

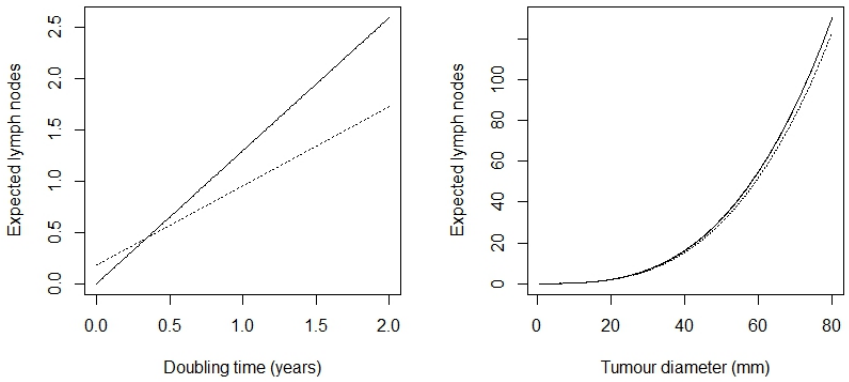

Figure 5.1: To illustrate problems 1 and 2, described previously, I have plotted the lymph node models of Shwartz (solid) and Hanin and Yakovlev (dotted) based on hypothetical parameter values. To the left: doubling time vs. expected number of affected lymph nodes at tumour diameter 12 *mm*. To the right: tumour diameter vs. expected number of affected lymph nodes for a tumour growing with a doubling time of 178 days.

Chapter 6

Summary of studies

Data sources

The analyses presented in this thesis use data from three studies, here abbreviated as CAHRES, ST01-08, and Libro-1. The inclusion-exclusion criteria that were used varied across the different analyses. I therefore only provide a brief summary of the studies/data used. For complete details on the different studies, see articles I-IV.

CAHRES (Cancer and hormone replacement study) is a case control study, consisting of all Swedish born women between the ages of 50 and 74, that were diagnosed with invasive breast cancer in Sweden from October 1993 to March 1995. The study had a participation rate of 84% (n=3345), and patients were matched to randomly selected controls from the general population based on the expected age frequency distribution of the cases. For the purpose of our analyses we use only the cases. In an extension of the CAHRES study, analog mammographic images were retrieved from mammography screening units and radiology departments managing mammography screening in Sweden. Information on tumour size, degree of lymph node spread, screening history, mode of detection, grade, ER status, and PR status was collected from the Swedish Cancer Registry and the Stockholm-Gotland Breast Cancer Registry. The collection of this data has been described previously by Rosenberg et al. (60; 61) and Eriksson et al. (62). In another extension of the CAHRES, DNA samples were collected from a subgroup of the full study population (63). 1500 women were randomly selected, together with all women who had taken hormone replacement therapy (191 cases) and all women with self-reported diabetes (110 cases). These women were contacted by mail and those who consented were given blood sampling kits to be used at their primary health care facility. From all deceased breast cancer cases, attempts were made to retrieve archived tissue samples. Blood samples were collected from 1322 cases and archived tissue was collected for 247 cases (85% of all selected). DNA was isolated from 3 ml of whole blood and from non-malignant cells in the paraffin-embedded tissue samples. DNA samples were genotyped on a custom Illumina iSelect Array (iCOGS) (64).

ST01-08 consists of all women diagnosed with invasive breast cancer in Stockholm from 2001 to 2008. Women were identified through the Stockholm-Gotland Regional Breast cancer

quality register, and information was collected on tumour size, lymph node involvement, grade, ER status, and PR status (65).

Libro-1 has the same study base as ST01-08. All women in the Stockholm-Gotland Regional Breast Cancer quality register, still alive in 2009, diagnosed with invasive breast cancer between between 2001 and 2008, and younger than age 80 at diagnosis were invited to participate in Libro-1. Invitations were mailed out in 2009, and 62% ($n = 5715$) of the invited women consented to take part in the study. These women gave blood specimens for genetic analysis, and were asked to provide web questionnaire information. Of these, 5125 were successfully genotyped in a large-scale genotyping study on breast cancer risk (66). 5122 patients had enough remaining DNA for mutation testing using targeted sequencing. For the women in the Libro-1 study, data on molecular markers were retrieved from medical and pathology records at treating hospitals. From these, molecular subtypes were assigned based on each woman's age at diagnosis and ER, PR, HER2, and Ki67 status, using a random forest algorithm (67).

Study I

Title:
Modelling breast cancer tumour growth for a stable disease population

Background & aims:
Continuous growth models offer a promising framework in which to study the role of individual risk factors on breast cancer progression. However, the underlying assumptions of the model have not been formalised to the same degree as other frameworks have. New formalism could open the door to many new development in the field. We therefore set out to explicitly formulate the underlying assumptions of the model, and to use these to develop new theory. A specific aim was to develop analytical formulas that could be used to simplify the likelihood described in Abrahamsson and Humphreys. In addition, we wanted to correct the small error in the calculation of $P(C_{s_{-1},t_{k-1}}|C_{i,t_k})$, and improve the computational complexity when back-calculating $P(C_{i,t})$; see Chapter 5.

Theoretical results:
We firstly specified three assumptions, which we call stable disease population assumptions. These are that:

A1. the rate of births in the population is constant across calendar time.

A2. the distribution of age at tumour onset is constant across calendar time.

A3. the distribution of time to symptomatic detection is constant across calendar time.

As long as A1 and A2 hold, there is a constant incidence rate, which was stated as an assumption in both Weedon-Fekjaer et al. (53) and Abrahamsson and Humphreys (55).

We next showed that if we think of each individual as belonging to any one of three states at any particular point in time,

$\mathscr{P}_{Before}$ - disease free state (prior to breast cancer tumour onset),

$\mathscr{P}_{Tumour}$ - breast cancer state (as of yet undetected),

$\mathscr{P}_{After}$ - post symptomatic detection state.

and if each individual progress though these three states only once, and if assumptions A1 - A3 hold, then, in the absence of screening and competing events, the following two results follow:

1. The probability for an individual to have an undetected tumour at a particular/current time point is proportional to the time it will spend in tumour growth (i.e. in state $\mathscr{P}_{Tumour}$).

2. For an individual with a currently undetected tumour at time point s, the probability density that tumour onset occurred exactly t' years earlier is $1/t$, given that t is the eventual time it

will spend in $\mathscr{P}_{Tumour}$

With the help of these assumptions, assuming tumour progression following models (5.1) - (5.3), we derived analytical expressions for:

- the probability density function for sizes of undetected tumours,
- the probability density function for growth rate conditioned on tumour size, and
- the probability density function for size at symptomatic detection conditioned on current (undetected) tumour size.

These results were used for simplifying the calculation of the likelihood for screen detected tumour size conditioned on previous screening history; see equations (5.12) and (5.13). We achieved this by exchanging equation (5.14) with the analytical density for size of undetected tumours. This simplification made it possible to vectorise the likelihood calculation. Using the density for growth rate conditioned on tumour size, we were also able to correct the small error in the calculation of $P(C_{s_{-1},t_{k-1}}|C_{i,t_k})$.

Empirical results:
We used the new likelihood to estimate inverse growth rates, individual variation in inverse growth rates, time to symptomatic detection, and screening sensitivity parameters based on a sample of 1901 cases from the CAHRES study. These cases had data on tumour diameter, mode of detection, numbers and times of previous screening visits, and a measure of mammographic density (percent density, PD). After coding the new estimation procedure in *R*, we reduced the computational time by 88% (for obtaining point estimates, using the same starting values).

In our study, screening test sensitivity was modelled in two ways. We modelled screening test sensitivity using a logistic function, which depended on tumour diameter d (in mm), PD m (scaled to [0,1]) and an interaction term expressed as the ratio of PD to tumour diameter squared m/d^2:

$$\frac{\exp(\beta_1 + \beta_2 d + \beta_3 m + \beta_4 m/d^2)}{1 + \exp(\beta_1 + \beta_2 d + \beta_3 m + \beta_4 m/d^2)}, \tag{6.1}$$

where β_1, β_2, β_3, and β_4 are model parameters. The interaction term was included to capture an interplay between tumour size and area mammographic percent density. We compared this to a model where β_4 was set to zero. The addition of the interaction term to the sensitivity model significantly improved model fit (p=0.013, using a likelihood ratio test). This was the first time a screening sensitivity model of this complexity has been fitted to this type of data.

Study II

Title:
Joint models of tumour size and lymph node spread for incident breast cancer cases in the presence of screening

Background & aims:
Breast cancer tumour growth and metastatic lymph node spread are dependent processes. As a tumour grows in size, the probability for lymph node spread increases. In Chapter 5, we described two problems present in models of metastatic lymph node spread from the literature. These are that: (a) the existing models imply that slow growing tumours have a higher degree of lymph node spread, compared to fast growing tumours, and (b) the models imply either an unrealistically high degree of lymph node spread for large tumours or an unrealistically low degree of spread for small tumours. We wanted to create a joint process of tumour growth and metastatic lymph node spread where these two problems were not present, and use these processes to jointly model tumour size and number of lymph node metastases at diagnosis for incident breast cancer cases in the presence of screening.

Theoretical results:
Assuming that tumour progression following models (5.1) - (5.3), we derived three new processes for metastatic lymph node spread. All three were based on inhomogeneous Poisson processes.

In the first model, the Poisson process had an intensity that was proportional to the first derivative, with respect to time, of tumour volume. In the second model, the Poisson process had an intensity that was proportional to the number of times the tumour cells have divided, to the power k, and the rate of cell division in the tumour. In the third model, the Poisson process had an intensity that was proportional to the same quantities as the second process. However, this process differed in that it was possible for different individuals to have different proportionality constants. The proportionality constant was assumed to follow a gamma distribution.

With the help of the theory derived in Study I and assuming a logistic model for screening test sensitivity, we derived the joint likelihood of tumour size and number of lymph node metastases, given a patients screening history, mode of detection, and mammographic density, based on the three different processes. The likelihood was shown to be separable into a lymph node component and a tumour size component. This was a key result for studies III and IV.

Empirical results:
Using data on tumour diameter and number lymph node metastases from a sample of 1860 women from CAHRES, complete with screening history and mammographic density measurements, we found that the best model fit was achieved for the third model, when $k = 4$. We validated this model of lymph node spread on a sample of 3961 cases from ST01-08. The final model did not exhibit problems (a) or (b).

Study III

Title:
Random effects models of lymph node metastases in breast cancer: quantifying the roles of covariates and screening using a continuous growth model

Background & aims:
The number of lymph node metastases present at diagnosis depends on the rate of lymph node spread and the tumour volume, which in turn depends on the rate of tumour growth. We wanted to show how the effect of clinical factors on both tumour growth rate and rate of lymph node spread could be estimated. For this purpose, we chose to estimate the effect of postmenopausal hormone replacement therapy (HRT), which has been shown to increase breast cancer incidence. As a secondary objective, we wanted to estimate the effect of a false negative mammography screening test on number of lymph node metastases.

Theoretical results:
This study had three theoretical results. We first showed how to estimate the effect of clinical factors on the rate of lymph node spread by regressing the clinical factor on the log of the proportionality constant in our Poisson process for breast cancer lymph node spread. Secondly, we derived an analytical expression for the probability of having N_f lymph nodes affected at a future time point t_f, conditional on tumour volume at diagnosis V, number of lymph nodes affected at diagnosis N, and screening history H. Thirdly, we derived the probability of having N_e lymph nodes affected at a previous time point t_e, conditional on tumour volume at diagnosis V, number of lymph nodes affected at diagnosis N, and screening history H. In both of our approaches, screening history H denotes the number and times of previous screening visits and whether the tumour was symptomatically or screening detected.

Empirical results:
Using data on number of lymph node metastases, tumour diameter, and HRT use from 1631 women diagnosed with invasive breast cancer from CAHRES, we estimated that women using HRT have a 36% lower rate of lymph node spread than non-users. The 95% confidence interval ranged from a reduction of 58% to a reduction of 8%. Of the 1631 women used for estimating the association between HRT use and rate of lymph node spread, 1373 women had complete data on screening history and mammographic density. Based on these we estimated that tumour in women taking HRT, on average, grow at a 15% reduced growth rate, compared to tumours in women not taking HRT. However, at $\alpha = 0.05$, this was not statistically significant ($p = 0.16$).

We also illustrated the potential consequences of false negative screens by plotting the probability for lymph node positivity at later time points for women who were lymph node negative at screening detection, and by plotting the probability for lymph node negativity at earlier time points for women who were lymph node positive at symptomatic detection.

Study IV

Title:
Lymph node metastases in breast cancer: investigating associations with tumour characteristics, molecular subtypes, and polygenic risk score using a continuous growth model

Background & aims:
Women who are diagnosed with breast cancer, within the Swedish health care system, are given treatment partly based on measurements of tumour grade, estrogen receptor (ER) status, progesteron receptor (PR) status, and human epidermal growth factor receptor 2 (HER2) status. These factors, together with each woman's age and Ki67 status can be used to estimate a tumour's molecular subtype (67).

The purpose of our study was to investigate association between rate of breast cancer lymph node spread and grade, estrogen receptor status, progesteron receptor status, a decision tree derived PAM50 molecular subtype, and a polygenic risk score.

Empirical results:
Using data on a total of 10950 women from CAHRES and ST01-08, we modelled the rate of metastatic breast cancer lymph node spread as a function of clinical factors.

We modelled the association between lymph node spread and grade in two different ways. Modelling the association on a continuous scale, we estimated the rate ratio, associated with a unit increase in grade, to be 1.51 (95% confidence interval: $1.34, 1.69$). The corresponding p-value was smaller than 10^{-16}. Modelling the association between grade and lymph node spread on a discrete scale, using grade 1 as reference, the rate ratio and 95% confidence interval for grade 2 tumours was 1.59 $(1.20, 2.06)$. The corresponding estimate for grade 3 tumours was 2.32 $(1.73, 2.99)$. The p-value for this model was smaller than 10^{-16}.

Compared to ER negative breast cancer, ER positive breast cancer was associated with a rate ratio and corresponding 95% confidence interval of 0.61 $(0.47, 0.76)$. The estimated p-value was $1.1 \cdot 10^{-4}$. Similarly for PR positive breast cancer, the rate ratio and corresponding 95% confidence interval was estimated as 0.65 $(0.52, 0.78)$. The p-value was estimated as $1.2 \cdot 10^{-4}$.

In the analysis of molecular subtypes, HER2-enriched breast cancer was associated with a rate ratio and corresponding 95% confidence interval of 1.83 $(1.05, 4.18)$, compared to luminal A breast cancer. The p-value for association between molecular subtype and rate of lymph node spread, based on a likelihood ratio test with 3 degrees of freedom, was $7.2 \cdot 10^{-4}$.

We did not find any convincing evidence that the polygenic risk score is associated with the rate of lymph node spread.

Chapter 7

Future directions

In the breast cancer literature, the effectiveness of screening has mainly been investigated based on randomised clinical trials and simulation studies. Randomised clinical trials are expensive and resource intensive, but have the potential to give clear cut and reliable results. Statistical models, on the other hand, are cheap and reusable once developed. Furthermore, simulation programs can be developed using data from a number of different sources, such as clinical trials, expert opinion, and observational screening data. Unfortunately, because of complex interactions between the biological processes leading to breast cancer death, realistic models are very hard to develop. A complete model of breast cancer needs to incorporate sub-models for treatment, disease onset, disease progression, and screening.

The aims of my thesis have been to develop statistical models of breast cancer disease progression and screening, based on observational breast cancer screening data, and to apply these models to patient data in order to draw clinically relevant conclusions. In this thesis, we have been able to develop new statistical theory for continuous growth models, and new models of metastatic breast cancer lymph node spread. We have also shown how these models can be used in practice to study the interaction between clinical factors and tumour progression. A natural next step for these models would be to incorporate them into a larger framework that also models disease onset, distant metastatic spread, or breast cancer treatment/survival.

Related directions that I am interested in pursuing are: to translate the models of breast cancer tumour growth and metastatic lymph node spread into analogous models of melanoma tumour growth and metastatic lymph node spread, and to develop continuous models of the natural history of liver cancer caused by hepatitis virus. In the second case, exponential tumour growth could be analogous to exponential proliferation of the virus, and symptomatic debut could correspond to onset of liver cancer. In some countries, testing for hepatitis infection is part of general health care practice for injecting drug users, in a similar way that mammography screening is common practice for women.

References

[1] Globocan I. Fact Sheet: Cancer Incidence and Mortality Worldwide; 2018.

[2] Bergman O, Jacobson L, Jaresand M, Nilsson S. Cancerfondsrapporten 2018. Cancerfonden; 2018.

[3] Altobelli E, Rapacchietta L, Angeletti P, Barbante L, Profeta F, Fagnano R. Breast cancer screening programmes across the WHO European region: differences among countries based on national income level. International journal of environmental research and public health. 2017;14(4):452.

[4] Lidbrink EK, Törnberg SA, Azavedo EM, Frisell JO, Hjalmar ML, Leifland KS, et al. The general mammography screening program in Stockholm: organisation and first-round results. Acta Oncologica. 1994;33(4):353–358.

[5] Socialstyrelsen. Screening för bröstcancer: rekommendation och bedömningsunderlag. Sweden. 2019;.

[6] Socialstyrelsen. Allmanna råd från Socialstyrelsen: Mammografiscreening. (General guidelines from the National Board of Health and Welfare: Mammography screening). Sweden. 1986;3.

[7] Tabar L, Gad A, Holmberg L, Ljungquist U, Group KCP, Fagerberg C, et al. Reduction in mortality from breast cancer after mass screening with mammography: randomised trial from the Breast Cancer Screening Working Group of the Swedish National Board of Health and Welfare. The Lancet. 1985;325(8433):829–832.

[8] Hanahan D, Weinberg RA. The hallmarks of cancer. cell. 2000;100(1):57–70.

[9] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. cell. 2011;144(5):646–674.

[10] Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science. 1994;p. 66–71.

[11] Clamp A, Danson S, Clemons M. Hormonal risk factors for breast cancer: identification, chemoprevention, and other intervention strategies. The Lancet Oncology. 2002;3(10):611–619.

[12] Michailidou K, Lindström S, Dennis J, Beesley J, Hui S, Kar S, et al. Association analysis identifies 65 new breast cancer risk loci. Nature. 2017;551(7678):92.

[13] Feigelson HS, Jonas CR, Teras LR, Thun MJ, Calle EE. Weight gain, body mass index, hormone replacement therapy, and postmenopausal breast cancer in a large prospective study. Cancer Epidemiology and Prevention Biomarkers. 2004;13(2):220–224.

[14] on Hormonal Factors in Breast Cancer CG, et al. Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. The Lancet. 1996;347(9017):1713–1727.

[15] Smith-Warner SA, Spiegelman D, Yaun SS, van den Brandt PA, Folsom AR, Goldbohm RA, et al. Alcohol and breast cancer in women: a pooled analysis of cohort studies. Jama. 1998;279(7):535–540.

[16] Rosner B, Colditz GA, Willett WC. Reproductive risk factors in a prospective study of breast cancer: the Nurses' Health Study. American journal of epidemiology. 1994;139(8):819–835.

[17] on Hormonal Factors in Breast Cancer CG, et al. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50 302 women with breast cancer and 96 973 women without the disease. The lancet. 2002;360(9328):187–195.

[18] Monninkhof EM, Elias SG, Vlems FA, van der Tweel I, Schuit AJ, Voskuil DW, et al. Physical activity and breast cancer: a systematic review. Epidemiology. 2007;18(1):137–157.

[19] Duffy SW, Morrish OW, Allgood PC, Black R, Gillan MG, Willsher P, et al. Mammographic density and breast cancer risk in breast screening assessment cases and women with a family history of breast cancer. European Journal of Cancer. 2018;88:48–56.

[20] Hunter DJ, Willett WC. Diet, body size, and breast cancer. Epidemiologic reviews. 1993;15(1):110–132.

[21] Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. Epidemiologic reviews. 1993;15(1):36.

[22] Berry DA, Cronin KA, Plevritis SK, Fryback DG, Clarke L, Zelen M, et al. Effect of screening and adjuvant therapy on mortality from breast cancer. New England Journal of Medicine. 2005;353(17):1784–1792.

[23] Uhry Z, Hédelin G, Colonna M, Asselain B, Arveux P, Rogel A, et al. Multi-state Markov models in cancer screening evaluation: a brief review and case study. Statistical methods in medical research. 2010;19(5):463–486.

[24] Duffy SW, Chen HH, Tabar L, Day NE. Estimation of mean sojourn time in breast cancer screening using a Markov chain model of both entry to and exit from the preclinical detectable phase. Statistics in medicine. 1995;14(14):1531–1543.

[25] Chen T, Duffy S, Day N. Markov chain models for progression of breast cancer. Part I: tumour attributes and the preclinical screen-detectable phase. Journal of Epidemiology and Biostatistics. 1997;(2):9–23.

[26] Chen T, Duffy S, Tabar L, Day N. Markov chain models for progression of breast cancer. Part II: prediction of outcomes for different screening regimes. Journal of Epidemiology and Biostatistics. 1997;(2):25–35.

[27] Chen HH, Yen AMF, Tabár L. A stochastic model for calibrating the survival benefit of screen-detected cancers. Journal of the American Statistical Association. 2012;107(500):1339–1359.

[28] Gøtzsche PC, Nielsen M. Screening for breast cancer with mammography. Cochrane Database Syst Rev. 2011;1.

[29] Cox B, Sneyd MJ. Bias in breast cancer research in the screening era. The Breast. 2013;22(6):1041–1045.

[30] Marmot MG, Altman D, Cameron D, Dewar J, Thompson S, Wilcox M. The benefits and harms of breast cancer screening: an independent review. British journal of cancer. 2013;108(11):2205.

[31] Gøtzsche PC, Jørgensen KJ. Screening for breast cancer with mammography. Cochrane database of systematic reviews. 2013;(6).

[32] Plevritis SK, Munoz D, Kurian AW, Stout NK, Alagoz O, Near AM, et al. Association of Screening and Treatment With Breast Cancer Mortality by Molecular Subtype in US Women, 2000-2012. JAMA. 2018;319(2):154–164.

[33] Hunink MM, Weinstein MC, Wittenberg E, Drummond MF, Pliskin JS, Wong JB, et al. Decision making in health and medicine: integrating evidence and values. Cambridge University Press; 2014.

[34] McCabe C, Claxton K, Culyer AJ. The NICE cost-effectiveness threshold. Pharmacoeconomics. 2008;26(9):733–744.

[35] Svensson M, Nilsson F. TLV: s betalningsvilja för nya läkemedel har analyserats: Kostnadseffektivitet och sjukdomens svårighetsgrad avgörande för subvention-Cancerläkemedel får kosta mer. Läkartidningen. 2016;113(28-30).

[36] Williams A. Cost-effectiveness analysis: is it ethical? Journal of medical ethics. 1992;18(1):7–11.

[37] Herdman M, Gudex C, Lloyd A, Janssen M, Kind P, Parkin D, et al. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). Quality of life research. 2011;20(10):1727–1736.

[38] Mäkelä P. Alcohol-related mortality by age and sex and its impact on life expectancy: Estimates based on the Finnish death register. The European Journal of Public Health. 1998;8(1):43–51.

[39] Virchow R. Vorlesungen über Pathologie. vol. 1. Hirschwald; 1862.

[40] Noguchi S, Aihara T, Koyama H, Motomura K, Inaji H, Imaoka S. Discrimination between multicentric and multifocal carcinomas of the breast through clonal analysis. Cancer. 1994;74(3):872–877.

[41] Carter CL, Allen C, Henson DE. Relation of tumor size, lymph node status, and survival in 24,740 breast cancer cases. Cancer. 1989;63(1):181–187.

[42] Clayton F, Hopkins CL. Pathologic correlates of prognosis in lymph node-positive breast carcinomas. Cancer. 1993;71(5):1780–1790.

[43] van Ravesteyn NT, Van Den Broek JJ, Li X, Weedon-Fekjær H, Schechter CB, Alagoz O, et al. Modeling ductal carcinoma in situ (DCIS): An overview of CISNET model approaches. Medical Decision Making. 2018;38(1_suppl):126S–139S.

[44] Chen TH, Kuo H, Yen M, Lai M, Tabar L, Duffy S. Estimation of sojourn time in chronic disease screening without data on interval cases. Biometrics. 2000;56(1):167–172.

[45] Taghipour S, Banjevic D, Miller A, Montgomery N, Jardine A, Harvey B. Parameter estimates for invasive breast cancer progression in the Canadian National Breast Screening Study. British journal of cancer. 2013;108(3):542–548.

[46] Weedon-Fekjær H, Vatten LJ, Aalen OO, Lindqvist B, Tretli S. Estimating mean sojourn time and screening test sensitivity in breast cancer mammography screening: new results. Journal of Medical Screening. 2005;12(4):172–178.

[47] Duffy SW, Agbaje O, Tabar L, Vitak B, Bjurstam N, Björneld L, et al. Overdiagnosis and overtreatment of breast cancer: estimates of overdiagnosis from two trials of mammographic screening for breast cancer. Breast Cancer Research. 2005;7(6):1.

[48] Gunsoy N, Garcia-Closas M, Moss S. Estimating breast cancer mortality reduction and overdiagnosis due to screening for different strategies in the United Kingdom. British journal of cancer. 2014;110(10):2412–2419.

[49] Bartoszyński R, Edler L, Hanin L, Kopp-Schneider A, Pavlova L, Tsodikov A, et al. Modeling cancer detection: tumor size as a source of information on unobservable stages of carcinogenesis. Mathematical biosciences. 2001;171(2):113–142.

[50] Plevritis SK, Salzman P, Sigal BM, Glynn PW. A natural history model of stage progression applied to breast cancer. Statistics in medicine. 2007;26(3):581–595.

[51] Hanin L, Yakovlev A. Multivariate distributions of clinical covariates at the time of cancer detection. Statistical methods in medical research. 2004;13(6):457–489.

[52] Weedon-Fekjær H, Lindqvist BH, Vatten LJ, Aalen OO, Tretli S. Breast cancer tumor growth estimated through mammography screening data. Breast Cancer Research. 2008;10(3):R41.

[53] Weedon-Fekjær H, Tretli S, Aalen OO. Estimating screening test sensitivity and tumour progression using tumour size and time since previous screening. Statistical methods in medical research. 2010;19(5):507–527.

[54] Spratt JA, Von Fournier D, Spratt JS, Weber EE. Decelerating growth and human breast cancer. Cancer. 1993;71(6):2013–2019.

[55] Abrahamsson L, Humphreys K. A statistical model of breast cancer tumour growth with estimation of screening sensitivity as a function of mammographic density. Statistical methods in medical research. 2016;25(4):1620–1637.

[56] Abrahamsson L, Czene K, Hall P, Humphreys K. Breast cancer tumour growth modelling for studying the association of body size with tumour growth rate and symptomatic detection using case-control data. Breast Cancer Research. 2015;17(1):1–11.

[57] Shwartz M. An analysis of the benefits of serial screening for breast cancer based upon a mathematical model of the disease. Cancer. 1978;41(4):1550–1564.

[58] Isheden G, Abrahamsson L, Andersson T, Czene K, Humphreys K. Joint models of tumour size and lymph node spread for incident breast cancer cases in the presence of screening. Statistical Methods in Medical Research. 2019;28(12):3822–3842.

[59] Tan S, Van Oortmarssen GJ, De Koning HJ, Boer R, Habbema JDF. The MISCAN-Fadia continuous tumor growth model for breast cancer. MONOGRAPHS-NATIONAL CANCER INSTITUTE. 2006;36:56.

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

[61] Rosenberg LU, Granath F, Dickman PW, Einarsdóttir K, Wedrén S, Persson I, et al. Menopausal hormone therapy in relation to breast cancer characteristics and prognosis: a cohort study. Breast Cancer Research. 2008;10(5):R78.

[62] Eriksson L, Czene K, Rosenberg L, Humphreys K, Hall P. The influence of mammographic density on breast tumor characteristics. Breast cancer research and treatment. 2012;134(2):859–866.

[63] Wedrén S, Lovmar L, Humphreys K, Magnusson C, Melhus H, Syvänen AC, et al. Oestrogen receptor α gene haplotype and postmenopausal breast cancer risk: a case control study. Breast Cancer Research. 2004;6(4):R437.

[64] Michailidou K, Beesley J, Lindstrom S, Canisius S, Dennis J, Lush MJ, et al. Genome-wide association analysis of more than 120,000 individuals identifies 15 new susceptibility loci for breast cancer. Nature genetics. 2015;47(4):373.

[65] Holm J, Humphreys K, Li J, Ploner A, Cheddad A, Eriksson M, et al. Risk Factors and Tumor Characteristics of Interval Cancers by Mammographic Density. Journal of Clinical Oncology. 2015;p. JCO–2014.

[66] Michailidou K, Hall P, Gonzalez-Neira A, Ghoussaini M, Dennis J, Milne RL, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nature genetics. 2013;45(4):353.

[67] Holm J, Eriksson L, Ploner A, Eriksson M, Rantalainen M, Li J, et al. Assessment of breast cancer risk factors reveals subtype heterogeneity. Cancer research. 2017;77(13):3708–3717.

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