Mammographic density. A marker of treatment outcome in breast cancer?

Dalene Skarping, Ida

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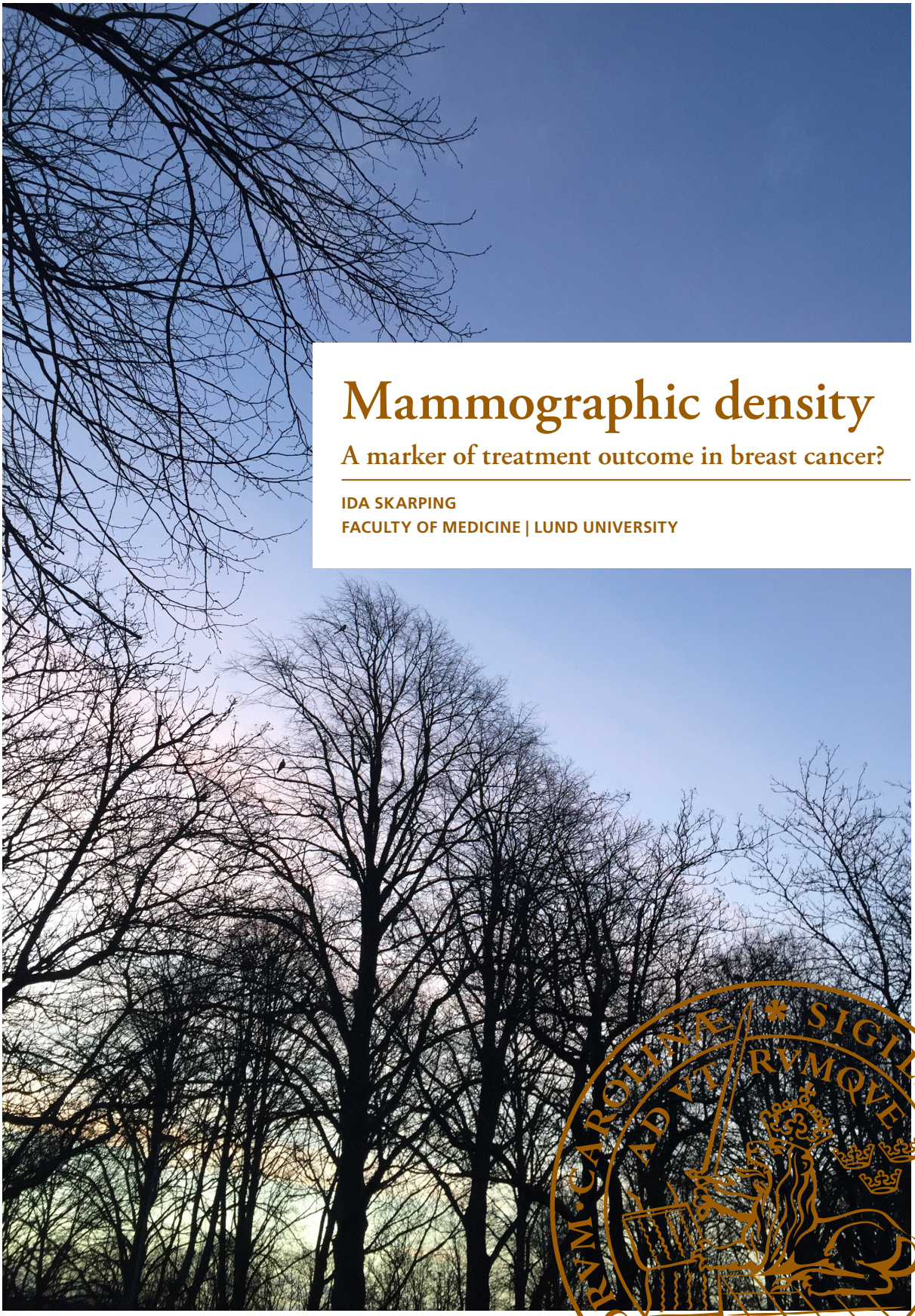

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Mammographic density:
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A marker of treatment outcome in breast cancer?

Ida Skarping

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Abstract

Background: Breast cancer is the most common cancer in women worldwide. Personalized cancer treatment requires predictive biomarkers, including image-based biomarkers. Mammographic density (MD) is a risk factor for developing breast cancer (BC). To study MD might be a feasible way to clarify the role of both potential risk factors and risk-reducing factors for BC, with statins among the latter. MD might also serve as a treatment predictive marker. During neoadjuvant chemotherapy (NACT), it is possible to radiologically evaluate treatment response and compare the results to whether pathological complete response (pCR) was accomplished or not.

Aims: The biological explanation for the link between MD and BC seems to be highly complex. We aim to address this association from a different point of view; understanding if pharmaceuticals can cause a decrease in MD, thereby studying how MD can serve as a predictive biomarker, advantageously tested in the neoadjuvant setting.

Methods: Paper 1: More than 41,000 women attending BC screening completed a questionnaire covering BC risk factors and various baseline characteristics, as included in the prospective national study cohort, KARMA. Information on medication use was derived from national registers.

Paper 2: The retrospectively gathered regional study cohort, NeoMon, consists of more than 300 BC-patients receiving NACT. MD was assessed according to the Breast Imaging-Reporting and Data System (BI-RADS) 5th Edition. Patient and tumor characteristics were retrieved from medical charts.

Papers 3 and 4: In the prospective study, NeoDense, BC patients receiving NACT (N = 200), underwent mammography, breast tomosynthesis (N = 156), and ultrasound at baseline, and after 2 and 6 cycles, respectively. MD was measured with Volpara™. The different imaging modalities' capacities in terms of evaluating response to treatment and their relation to pCR were studied.

Results: Paper 1: After a multivariable adjustment, we found no association between statin use and absolute dense volume. Statin users reporting ever using hormonal replacement therapy (HRT) had a larger absolute dense volume than the non-statin users ever using HRT.

Paper 2: Logistic regression models, with multiple adjustment factors, showed that in comparison to patients with non-dense breasts (BI-RADS a), patients with denser breasts had a lower chance of accomplishing pCR, most prominently seen in premenopausal patients.

Paper 3: A total of 74% of patients decreased their absolute dense volume during NACT. The likelihood of accomplishing pCR following NACT was independent of volumetric MD at diagnosis and change in volumetric MD during treatment.

Paper 4: Early radiological responders had 2–3-times higher chance of pCR than early radiological non-responders. Post-NACT, mammography, ultrasound, and tomosynthesis could accurately estimate tumor size (within a 5 mm margin compared to pathological evaluation) in 43–46% of all tumors. The diagnostic precision in predicting pCR was similar between the three modalities; however, tomosynthesis had slightly higher specificity and positive predictive values.

Conclusions: MD might have a predictive value during NACT; however, future larger studies are needed to understand how MD can be used in the clinical routine. Lack of early radiological response is worrisome, and there might be a need for improved monitoring and changed treatment plans; trials designed to evaluate the efficacy of changing or adding treatment are warranted.

Keywords: Mammographic density, pCR, neoadjuvant chemotherapy, diagnostic precision

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To Ebba and Viktor

Table of Contents

List of original papers 10
Thesis at a glance 11
Populärvetenskaplig sammanfattning (in Swedish) 12
Abbreviations 14

Introduction 15

Background 19

Breast cancer 19
The breast 19
Breast carcinogenesis 20
Risk factors 20
Obesity and inflammation 23
Breast cancer classification – prognostic and predictive factors 26
Breast cancer treatment 28
Breast imaging 33
Mammographic density 38

Aims 53

Overall aims 53
Specific aims 53

Patients and Methods 55

Cohorts 55
Patient and tumor characteristics 57
Patient characteristics 57
Tumor characteristics 58
Neoadjuvant chemotherapy 59
Breast imaging 60
Statistical analysis 62
Ethical considerations 69

Methodological considerations 71

Results and discussion **77**
Paper I 77
Results 77
Discussion 78
Paper II 80
Results 80
Discussion 80
Paper III 81
Results 81
Discussion paper II and III 84
Paper IV 89
Results 89
Discussion 90
Conclusions **93**
Future perspectives **95**
Acknowledgements **97**
References **101**

List of original papers

The thesis is based on the following papers, which are referred to in the thesis by their Roman numerals:

I. Skarping I, Brand J, Hall P, Borgquist S
Effects of statin use on volumetric mammographic density; results from the KARMA study
BMC Cancer, 15:435, 2015

II. Skarping I, Förnvik D, Sartor H, Heide-Jørgensen U, Zackrisson S, Borgquist S.
Mammographic density is a potential predictive marker of pathological response after neoadjuvant chemotherapy in breast cancer.
BMC Cancer, 19(1):1272, 2019

III. Skarping I, Förnvik D, Heide-Jørgensen U, Sartor H, Hall P, Zackrisson S, Borgquist S
Mammographic density changes during neoadjuvant breast cancer treatment: NeoDense, a prospective study in Sweden
The Breast, Vol. 53, p33–41 Published online: June 17, 2020

IV. Skarping I, Förnvik D, Heide-Jørgensen U, Rydén L, Zackrisson S, Borgquist S
Neoadjuvant breast cancer treatment response; tumor size evaluation by various conventional imaging modalities in the NeoDense study
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Thesis at a glance

Paper	Questions	Methods	Results and Conclusions
I "KARMA"	In order to clarify the effect of statins on breast cancer risk, we asked: Is there an association between statin use and volumetric MD?	Over 41,000 women attending breast cancer screening and completing a questionnaire covering breast cancer risk factors and various baseline characteristics were included in the study cohort. Information on statin use was derived from national registers.	After a multivariable adjustment, we found no effect of statin use on absolute dense breast volume. Statin users reporting ever using HRT had a larger absolute dense volume than the non-statin users ever using HRT.
II "NeoMon"	Can MD, assessed with BI-RADS, be a predictive marker of pCR?	The retrospectively gathered regional study cohort consists of over 300 patients receiving NACT. MD of diagnostic mammograms was scored according to BI-RADS 5th Edition. Patient and tumor characteristics were retrieved from medical charts.	Logistic regression models, with multiple adjustment factors, showed that in comparison to patients with low MD (BI-RADS a), patients with denser breasts had a lower OR of accomplishing pCR, most prominently seen in premenopausal patients.
III "NeoDense"	Can MD, assessed with Volpara™, be a predictive marker of pCR? Does MD change during NACT - and to what degree?	Breast cancer patients receiving NACT (N = 207) enrolled in the study underwent imaging evaluation at baseline after 2 and 6 cycles of NACT, respectively. MD was measured with Volpara™ and BI-RADS.	Logistic regression models, with multiple adjustment factors, showed no evidence of MD as a predictive marker with neither Volpara™ nor BI-RADS. A total of 74% of patients decreased their absolute dense volume during NACT.
IV "NeoSize"	What is the association between radiological complete response and pCR post-NACT? How is early radiological response associated with pCR?	The same cohort as in paper III. Detailed information on radiological tumor characteristics at baseline, after 2 and 6 cycles of NACT for mammography, breast tomosynthesis and ultrasound, respectively. Agreement and accuracy (in relation to pathological tumor size) were evaluated with Bland-Altman plots. The ability to correctly identify pCR with conventional breast imaging was evaluated.	Patients with radiological early response had a chance of pCR 2.9-, 2.8-, and 1.8-times higher compared to radiological early non-responders assessed with ultrasound, breast tomosynthesis and mammography, respectively. Post-NACT imaging was accurate (within a 5 mm margin), in terms of tumor size estimation by imaging in relation to pathological tumor evaluation, in 43–46% of all tumors. Early radiological non-responding patients may be considered for changed treatment plans.

Abbreviations: BI-RADS: Breast Imaging-Reporting and Data System; HRT: hormonal replacement therapy; MD: mammographic density; NACT: neoadjuvant chemotherapy; OR: odds ratio; pCR: pathological complete response

Populärvetenskaplig sammanfattning (in Swedish)

Bröstcancer är den vanligaste cancerformen hos kvinnor globalt så väl som i Sverige. Uppskattningsvis kommer var nionde svensk kvinna att drabbas av bröstcancer under sin livstid. Antalet fall av bröstcancer ökar i Sverige. Överlevnaden vid bröstcancer är mycket god, och blir allt bättre. Den genomsnittliga 5-års överlevnaden är 92% och 10-års överlevnaden är 86%. Bröstcancer utan dottersvulster (metastaser), är därmed att betrakta som botbar.

Bröstcancer är inte bara en sjukdom, utan ett samlingsbegrepp för många tumörer med olika egenskaper t.ex. i form av känslighet för kvinnligt könshormon, östrogen. För att varje patient ska få skräddarsydd behandling, ge eller avstå från extra och starkare behandlingar till patienter utifrån risk för återfall, är det viktigt med markörer som kan hjälpa oss att identifiera patienternas riskprofil. Vanligtvis ges cellgiftsbehandling efter operationen och syftar i första hand till att ta bort mikroskopisk cancersjukdom som kan finnas kvar i bröstet och på andra ställen i kroppen efter att man opererat bort brösttumören. Ett alternativ är att göra tvärtom – ge cellgiftsbehandling först och därefter operera, så kallad neoadjuvant behandling. På så sätt går det att mäta tumörens storlek före, under och efter behandlingen och säkerställa att behandlingen hjälper mot just den tumören hos just den kvinnan.

I Sverige har vi ett screeningprogram för bröstcancer där alla kvinnor i åldern 40–74 år regelbundet bjuds in att ta röntgenbilder av brösten, en så kallas mammografiundersökning. I Sverige upptäcks ca 60% av alla bröstcancrar genom screeningprogrammet. Bilddiagnostik av brösten spelar en stor roll när man fattar beslut om behandling av patienter. De vanligaste metoderna för bröstdiagnostik är mammografi och ultraljud.

Förutom tumören, så kan själva bröstet i sig se ut på olika sätt i en mammografibild. Bröst som innehåller mycket körtelvävnad är vitare på en mammografibild medan fettrika bröst är mörkare på en mammografibild. Det vita, eller relationen mellan vitt och svart i bilden är det som kallas mammografisk täthet eller brösttäthet. Hög (mammografisk) brösttäthet är en etablerad riskfaktor för att utveckla bröstcancer och för att drabbas av återfall om man en gång har haft bröstcancer. Studier har visat att kvinnor vars brösttäthet minskar under anti-hormonell behandling har lägre risk för att få tillbaka bröstcancer jämfört med kvinnor vars brösttäthet inte minskar.

Genom att studera hur olika mediciner påverkar brösttäthet, t.ex. statiner som är en kolesterolsänkande medicin med anti-inflammatoriska egenskaper, kan man indirekt studera hur dessa mediciner påverkar risken att utveckla bröstcancer. Med anledning av detta finns det även förutsättningar att undersöka om cellgiftsbehandling påverkar brösttätheten. Avseende cellgifter vill vi även undersöka om brösttäthet är relaterad till behandlingseffekt.

Det övergripande målet med denna avhandling, bestående av fyra delarbeten, är att skapa djupare förståelse för hur läkemedel, primärt cellgifter, påverkar brösttätheten. Vi har studerat om brösttäthet är förknippat med behandlingseffekt av cellgifter i den neoadjuvanta behandlings-situationen. Vi har även undersökt hur tumörstorleken förändras under cellgiftsbehandling med tre olika bilddiagnosiska metoder: mammografi, ultraljud och bröst-tomosyntes, en 3D-mammografi av brösten. Vi undersökte hur väl man med olika bilddiagnostiska metoder kan förutspå vilka patienter som kommer att ha så god effekt av cellgifterna att ingen tumör finns kvar när alla behandlingar är givna och det är dags för operation. Här följer en kortfattad sammanfattning av de fyra delarbetena.

Delarbete 1 bygger på en stor nationell studie av kvinnor utan bröstcancer som genomgick mammografiundersökning. Genom utförliga frågeformulär samt datauttag från svenska läkemedelsregister, hade vi tillgång till detaljerad information om kvinnornas användning av statiner. Våra resultat kunde inte påvisa några starka samband mellan mängd tät bröstvävnad och användande av statiner.

I **delarbete 2 och 3** tittade vi på sambandet mellan brösttäthet och behandlingseffekt av cellgifter. I **delarbete 2** samlade vi in information om ca 300 kvinnor som tidigare fått neoadjuvant cellgiftsbehandling i Skåne. Vi gjorde en visuell täthetsbedömning av mammografibilder från diagnostillfället och undersökte om det fanns ett samband mellan brösttäthet och om tumören hade försvunnit helt efter avslutad behandling, så kallad komplett respons. Vi fann att kvinnor med mycket täta bröst, speciellt kvinnor som ännu ej kommit i klimakteriet, hade en lägre sannolikhet att tumören var helt borta efter cellgifterna jämfört med kvinnor med mindre täta bröst.

Strax över 200 kvinnor med bröstcancer aktuella för neoadjuvant cellgiftsbehandling tillfrågades om de vill vara med i NeoDense-studien i samband med att de fick sin bröstcancerdiagnos. I **delarbete 3** undersökte vi hur kvinnornas brösttäthet, mätt med ett automatiserat datorprogram för brösttäthet, vid start av cellgiftsbehandling, under och strax efter avslutad behandling, var relaterat till behandlingseffekt i tumören. Vi såg att brösttätheten minskade under behandling för en stor andel av kvinnorna, men vi kunde inte hitta något samband mellan brösttäthet och behandlingseffekt i tumören.

I **delarbete 4** undersökte vi om bilddiagnostik av brösten redan under pågående behandling kunde förutspå om tumören svarade så bra på behandlingen att den försvann helt. Vi jämförde tre olika sorters bilddiagnostik (mammografi, bröst-tomosyntes och ultraljud av bröstet). Vi undersökte hur överensstämmande den bilddiagnostiska tumörstorleken var med den av patologen uppmätta tumörstorleken i den bortopererade bröstvävnaden. Vi såg att om en tumör inte markant minskade i storlek redan under de första två omgångarna med cellgifter så var det låg sannolikhet att tumören skulle försvinna helt efter alla sex omgångar.

Abbreviations

ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
BI-RADS	Breast Imaging-Reporting and Data System
BMI	Body Mass Index
BRCA	Breast Cancer Gene
CC	Craniocaudal
CI	Confidence Interval
DCIS	Ductal Carcinoma *In Situ*
EC	Epirubicin and Cyclophosphamide
ER	Estrogen Receptor
FEC	Fluorouracil, Epirubicin and Cyclophosphamide
HER2	Human Epidermal growth factor Receptor 2
HRT	Hormonal Replacement Therapy
MLO	Mediolateral Oblique
MRI	Magnetic Resonance Imaging
NSAID	Non-Steroidal Anti-Inflammatory Drugs
OR	Odds Ratio
pCR	pathological Complete Response
PR	Progesterone Receptor
TNM	Tumor, Node, Metastases
VBD%	Volumetric Breast Density percentage

Introduction

Breast cancer is the most commonly diagnosed cancer form in women, accounting for nearly one in four female cancer cases worldwide[1]. Every year, more than two million women are diagnosed with breast cancer[1], and one in eight women in the western world is affected by breast cancer during their lifetime[2,3]. Breast cancer incidence in Sweden is increasing (Figure 1), and in 2016, 7558 women were diagnosed with breast cancer, representing 29% of all cancer cases in Swedish women[4]. However, mortality rates have improved; the 5- and 10-year survival rates have increased steadily, reaching, 92% and 86% in 2016, respectively[4]. Early breast cancer is thus considered to be curable, in contrast to advanced, metastatic disease[5].

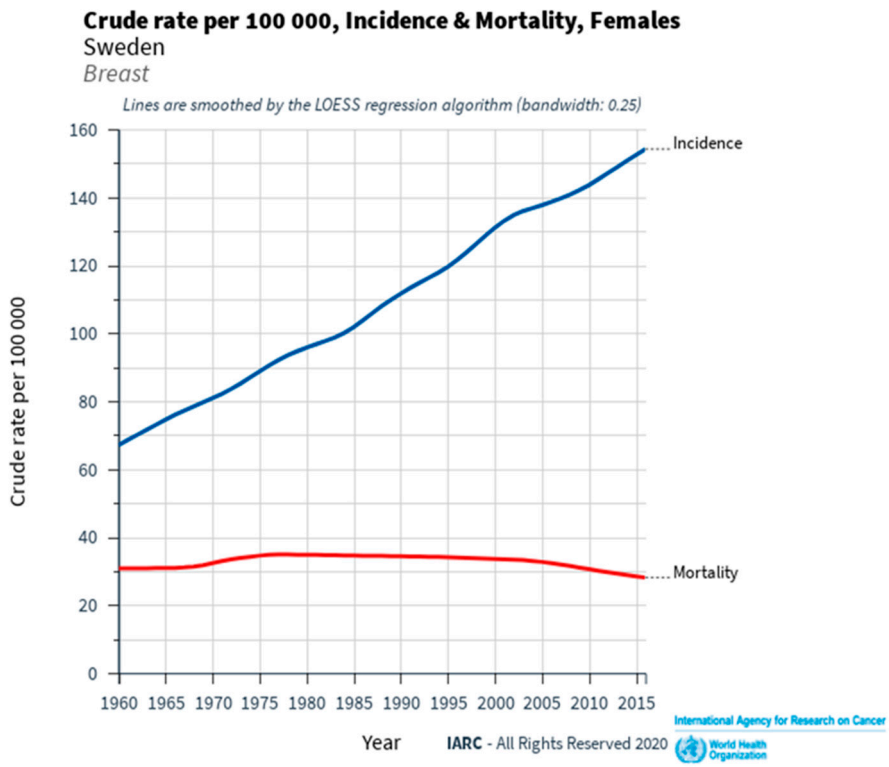

Figure 1. Incidence and mortality of breast cancer in Sweden[6,7] © International Agency for Research on Cancer

Breast cancer is a heterogeneous disease, with different subtypes having a diverse natural history and responding differently to treatment[8]. The categorization of subgroups can be based on the histology of the tumor[5], e.g., ductal or lobular carcinoma, on their pattern of gene expression (the intrinsic system[9]), or – most often used in the clinic – a surrogate intrinsic system[10] based on the immunohistochemical expression of a few key points. Reasons beyond the improved long-term survival are multifactorial: earlier detection through screening programs[11], and improved adjuvant treatment according to molecular subtype[12]; a beneficial shift from focusing on tumor burden to directing treatment towards the actual biology of the cancer[5].

Risk factors for developing breast cancer can traditionally be divided as either non-modifiable factors (e.g., gender, age, height, genes) or modifiable factors (lifestyle factors, weight)[13], the latter category having the potential to be modified by preventive measures. Mammographic density can be considered a partly modifiable factor. After female gender, age, and breast cancer gene (BRCA) mutation status, mammographic density, reflecting the amount of radiodense tissue (fibroglandular) and the radiolucent tissue (fat) on an X-ray of the breast (mammogram)[14], is considered to be the most important risk factor[15,16]. It has been suggested that almost 30% of premenopausal and 15% of postmenopausal breast cancers can be attributed to high mammographic density alone[17]. Since mammographic density is such a major risk factor for breast cancer[18], to study mammographic density might be a feasible way to clarify the role of both potential risk factors and risk-reducing factors for breast cancer.

There are three levels of prevention: primary prevention (prevent disease before it occurs), secondary prevention (reduce the impact of disease that has already occurred), and tertiary prevention (reduce severity and sequelae)[19]. Early detection (secondary prevention) and personalized treatment (tertiary prevention) is the best strategy for a better cancer outcome. However, a large challenge lies in finding strategies to prevent breast cancer occurrence (primary prevention)[20-22]. Statins, blocking a rate-limiting step in the mevalonate pathway, are cholesterol-lowering drugs with proven anti-proliferative and anti-inflammatory properties in cancer[23]. Epidemiological studies have shown conflicting results regarding the role of statins in prohibiting breast cancer occurrence[24-26], but more certainty exists regarding statins and tumor progression[27-30]. An alluring concept is to address potential risk-reducing factors for breast cancer by studying its association with mammographic density, considered as an intermediate in breast cancer etiology. In this thesis, we studied the association between statins and breast cancer risk by studying mammographic density.

A clinical treatment decision, preferably a multidisciplinary one[31,32], is based on tumor characteristics (i.e., stage and molecular subtype[33]) and the individual

patient's personal wishes, prerequisites, and potential comorbidities. Breast cancer treatment is based on two pillars: locoregional treatment (surgery and radiotherapy) and systemic treatment (chemotherapy, endocrine therapy and targeted therapy). Often breast cancer patients considered operable undergo primary surgery, and depending on clinical, pathological, and molecular risk factors[34], are followed by adjuvant systemic treatment and locoregional radiotherapy. The adjuvant treatment aims at eradicating any remaining cancer cells.

When the concept of neoadjuvant treatment, i.e., systematic chemotherapy before surgery, was introduced in the 1970s[35], the primary aim was to downstage locally advanced inoperable tumors and make them operable[36]. Subsequently, the indication has broadened, enabling breast-conservatory surgery, and now neoadjuvant chemotherapy is widely used for both large tumors and also smaller tumors with other risk factors[36,37]. In the neoadjuvant setting, one can study the tumor's response to given treatment *in vivo*, both clinically and – of great interest to this thesis – by imaging.

Imaging of the breast is dominated by mammography and ultrasound, although other modalities, such as magnetic resonance imaging (MRI), are also widely used. Recently, breast tomosynthesis, a 3-dimensional X-ray technique, has become more common[38]. For metastatic screening, i.e., cancer staging, computed tomography of the chest, abdomen, and pelvis is used in the vast majority of cases[39]. For patients treated with neoadjuvant chemotherapy, radiological diagnostics help evaluate treatment response *in vivo*, and imaging should help clinicians make an as early and accurate informed treatment decisions as possible.

This thesis focuses on radiological characteristics, predominantly mammographic density, and its association with treatment response to neoadjuvant chemotherapy. Also, we want to deepen the knowledge of mammographic density and its association with systemic treatment with pharmaceuticals (statins).

Background

Breast cancer

The breast

The female breast consists mainly of fat, connective tissue, and glandular tissue, and the proportion of the components differs individually and over time. The structure of the mammary gland is similar to a tree. Most distal in the tree-like structure is the alveoli, consisting of two layers; facing the lumen is the milk-producing and secreting cells (luminal epithelial cells) and basal to this layer, the (basal) myoepithelial cells, responsible for contraction and thus transportation of milk into the ducts and out of the nipple of the lactating breast[15]. The alveoli unite to form lobules, which in turn is a component of one of the 15–20 lobes that each breast contains. A thin continuous basement membrane surrounds the myoepithelial cells of the lobules, lobes, and ducts[15]. The functional unit of the breast (milk-producing and milk-secreting) is the terminal duct lobular unit, consisting of the lobule, and the intra- and extra lobular terminal ducts (Figure 2).

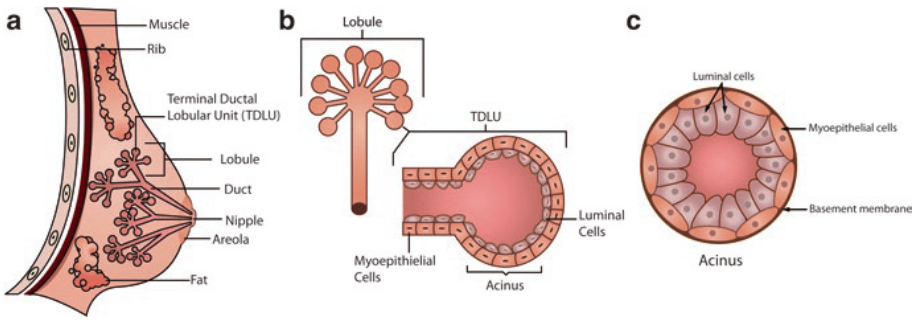

Figure 2. a) Schematic cross-section of a breast b) Illustration of the terminal duct lobular unit (TDLU) c) Details of a single acinus (alveolus). Reprinted from "Breast Cancer and its Precursor Lesions", Chapter 2, Patricia A Thomas, Humana Press, Totowa, NJ, 2011, with permission © 2011, Springer Nature.

The lobular and ductal structure of the mammary gland is surrounded by stromal connective tissue with the main cellular component being collagen synthesizing fibroblasts and adipocytes. Interspersed lies blood vessels, neuronal cells, and

various kinds of immune cells. The major differences in breast volume between women are mainly due to the individual differences in the amount of fat and connective tissue[15] and not in the lobular/ductal tissue. Conveniently, the two cell types found in the alveoli – luminal epithelial cells and basal myoepithelial cells – have distinguished immunohistochemistry[40].

Breast carcinogenesis

Tumor development is a complex multi-step biological process during which normal cells are transformed into tumor cells. The transformation is believed to follow a chronological development starting with premalignant atypical hyperplasia followed by pre-invasive carcinoma *in situ* (e.g., ductal carcinoma *in situ* (DCIS) and lobular carcinoma *in situ*) and lastly, invasive carcinoma (i.e., cells are capable of crossing the cell membrane enabling metastatic spread)[41]. This is, however, perhaps an oversimplification; DCIS might not progress into invasive carcinoma, and some invasive tumors might develop directly of normal-appearing epithelial cells[42]. The ten hallmarks of cancer, the first six presented in 2000 by Hanahan and Weinberg[43], and a decade later update with four additional hallmarks[44], are general biological principles contributing to tumorigenesis for cancer in general. The six original hallmarks that enable tumor growth and dissemination are: sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis[43]. The two enabling characteristics/hallmarks are: genome instability and mutation, and tumor-promoting inflammation and lastly, and the two latest emerging hallmarks are reprogramming of energy metabolism and evading immune destruction[44].

Almost all breast cancers are carcinomas (sarcomas account for < 1% of all breast malignancies[45]), developing from the epithelial cells lining the ductal tree in the breast, the functional unit of the breast[5]. A histological classification is based on the tumor's structural organization, e.g., whether the tumor has risen from the ducts (ductal carcinoma) or the lobules (lobular carcinoma) of the breast[5,8]. The majority of breast cancers are ductal carcinomas (also called invasive carcinoma of no special type) (70–75%), followed by lobular carcinomas (12–15%)[46]. The other 18 subtypes are rare (0.5–5%) and demonstrate specific characteristics, e.g., tubular, cribriform, mucinous, medullary, and apocrine carcinoma[5,46,47].

Risk factors

Risk factors for breast cancer can be grouped as either non-modifiable (e.g., hereditary predisposition, age, female gender)[16] or modifiable (e.g., weight, alcohol consumption, age first birth, number of pregnancies, breast-feeding, hormonal

replacement therapy (HRT), oral contraceptives)[48-54], the latter having the potential of being affected by preventive actions. It is estimated that ~20% of breast cancers can be attributed to modifiable risk factors[5].

Of all breast cancer, <1% occur in men[5], and the female gender is – together with age – the most important risk factors for breast cancer[16]. In Sweden, the highest incidence is seen in women aged 60–69 years[37]. Previously, the typical incidence curve of breast cancer showed a constant increase with age, with a less steep slope around/after menopause, called the Clemmensen's hook[55], interpreted as the overlapping of two separate curves corresponding to pre- and postmenopausal breast cancer. Later this theory has been dismissed in several papers, and the current incidence curves by age do not mirror this theory[55,56].

After gender, age, and BRCA mutation carriership, mammographic density is considered to be one of the strongest risk factors for breast cancer[15,16]. For a detailed description, please refer to the section "Mammographic density" on page 38.

High socioeconomic status and high level of education increase breast cancer risk, partly due to exogenous hormone use and reproductive factors[57,58]. Alcohol is an established risk factor for breast cancer[48], each 10 g of alcohol (~1 drink) consumed daily will lead to a 7–10% increase in breast cancer risk[5,59,60]; one contributing explanation might be the higher levels of estrogen and androgen seen in women consuming alcohol[61]. In terms of tobacco smoking and the risk of breast cancer, the literature has long shown no convincing support for an association between smoking and increased risk of breast cancer[16]. However, there is now some evidence for a moderate increase in the risk of breast cancer in women who smoke tobacco[62].

The breasts are radiosensitive organs, and previous radiation is a risk factor for breast cancer[16]. Knowledge about radiation-related breast cancer risk originates mainly from epidemiological studies of patients exposed to medical radiation and of the Japanese atomic bomb survivors[63]. The risk increases linearly with dose, and radiation is most harmful at a young age; the risk is estimated to be minimal for women exposed after the menopausal ages[63].

The previous history of benign breast disease, a heterogeneous entity encompassing numerous of histological subtypes, is a risk factor for breast cancer[16,64]. The risk is almost doubled for women with proliferative changes without atypia and 3–5-fold increased for women with atypical hyperplasia[64].

Genetic factors

Approximately 10% of breast cancers are considered inherited and associated with a family history[5]. In patients with a personal and/or family history suggestive hereof, a specific gene is identified in <30% of cases[65]. The rare, but highly penetrant genes BRCA1 and BRCA2 are the most common breast cancer susceptibility genes[66], and

female carriers of mutations in these genes have a lifetime risk of breast cancer of 50–85%[65,67,68]. Around 2–3% of the hereditary breast cancer cases are due to moderate penetrant genes, e.g., CHEK2, ATM and PALB2, and carriers of these genes have around a twofold increase in risk[65]. Through the basis of genome-wide studies, multiple single-nucleotide polymorphisms have been identified and reported to be associated with a minor increase in breast cancer risk[69].

Reproductive factors

Early age of menarche, late menopause, and nulliparity are all aspects that increase the lifetime exposure of endogenous hormones and are associated with increased risk of breast cancer[49]. For each birth, the relative risk of breast cancer is reduced by 7%[50]. The vulnerability of the breast epithelium is considered to be highest between menarche and first childbirth since the glandular tissue of the breast has not yet undergone the further differentiation associated with pregnancy and lactation[53,70] and postponing childbirth is estimated to increase the relative risk of breast cancer with 3% each year[50]. Breastfeeding is protective of breast cancer, with a 4% relative risk reduction per year of breast feeding[50]. In both premenopausal and postmenopausal women, systemic levels of sex hormones are positively associated with the risk for breast cancer[71-73]. Not only endogenous hormones are associated with breast cancer risk, hormonal replacement therapy, especially the combined preparations containing both estrogen and progestogen[51] (a synthetic compound that mimics the physiological effects of progesterone), increases the risk for breast cancer. Also, the use of oral contraceptives has been shown to slightly increase the risk of premenopausal breast cancer[53,54]. In terms of breast cancer subtypes, reproductive risk factors are mainly associated with estrogen receptor (ER) positive breast cancer[74].

Risk models and preventive measures

A wide range of breast cancer risk prediction models have been presented, both models that estimate the risk of developing breast cancer and models that estimate the risk of carrying a high risk-gene/genes[75]. One of the earliest models, and also the most widely validated model for clinical use, is the Gail model, based on hormonal variables (age at menarche, age first live birth), pathologic variables (number of prior breast biopsies), and hereditary variables (first-degree relatives with breast cancer)[75]. The Tyrer-Cuzic model can be used in the general population but is most useful in high-risk populations since it gives an estimation of mutation carrier status as well as the risk of breast cancer development[75]. Recognizing the large impact of mammographic density on the risk of developing breast cancer, many later developed risk prediction models include mammographic density in the models[75].

For women with a high risk of developing breast cancer, preventive measures can be undertaken[76,77]. Individualized screening, reducing risk factors, and taking chemoprevention are three different strategies[76]. Apart from the use in the adjuvant setting, selective estrogen receptor modulators, such as tamoxifen, and aromatase inhibitors, such as anastrozole, reduce the risk of developing (ER-positive) breast cancer when used in the primary preventive setting[78]. A significant proportion of breast cancers can be assigned to high mammographic density, thus indicating that interventions to reduce mammographic density might have the potential to eliminate a large proportion of breast cancers in the population[17,77]. Recently, the intervention trial "KARISMA 2"[79] closed for inclusion and the data are currently being analyzed. By identifying the lowest dose of tamoxifen that reduces mammographic density and at the same time keeping the side effects as mild as possible, the "KARISMA 2" trial aims at identifying the optimal dose for tamoxifen when used in the prevention of breast cancer. The results of the "KARISMA 2" trial will be key for the study design of "KARISMA 3", a study that will be including women at high risk of breast cancer. Other biological pathways in dense breast tissue are also being explored, such as the extracellular matrix proteins osteopontin[80], decorin, and lumican[81].

In Sweden, known BRCA1 and BRCA2 mutation carriers are offered clinical oncogenetic consultation, yearly breast imaging (breast-MRI), and discussion regarding prophylactic bilateral mastectomy[37]. It is also important to offer regular gynecological consultations and discussions regarding prophylactic salpingo-oophorectomy[37].

Obesity and inflammation

Adipocytes are the major component of adipose tissue (also containing, e.g., connective tissue, nerve cells, stromovascular cells, and immune cells)[82]. Dysfunction in the energy balance leads to overweight (body mass index (BMI) ≥ 25) that can evolve into obesity (BMI ≥ 30). Adipose tissue is not a passive energy storage reservoir; on the contrary, adipose tissue is a highly metabolic active endocrine organ[83]. Besides releasing metabolic substrates (free fatty acids, cholesterol, glycerol, and triglycerides), a wide range of adipokines are released from adipose tissue, especially estrogen (increased aromatase enzyme activity in adipose tissue that converts androgens into estrogen), adiponectin, and leptin[82]. Adipokines are biologically active factors, including enzymes, hormones, growth factors, and inflammatory cytokines produced by the adipose tissue[84]. The local fat in the breast, the black or radiolucent part of a mammogram, contributes considerably to the relative mammographic density estimates (please refer to the section "Mammographic density" on page 38).

The association between obesity and breast cancer is complex and is dependent on menopausal status and histological subtype[52]. Postmenopausal obesity is predominantly associated with increased risk for hormone receptor positive breast cancer, but not HR or triple-negative breast cancer[52,85]. Contrasting this, obesity is associated with a decreased risk of premenopausal breast cancer[52,85]. However, some studies have shown an association between obesity and increased risk of HR negative, basal-like and triple-negative breast cancer in premenopausal women[52,85]. The association between HR- and triple-negative breast cancer in postmenopausal is less clear, and two large meta-analyses have reported null associations[86,87], while others have reported moderate association[88] or inverse association[89].

Obesity-associated comorbidities such as metabolic syndrome[90], diabetes mellitus type II[91], and hypercholesterolemia[92] are associated with increased risk for breast cancer, particularly HR-positive breast cancer[52]. Physical activity is shown to play a protective role against breast cancer[93-95], and this association might partly be explained by a reduction of endogenous sex hormones in physically active postmenopausal women[94].

The worldwide prevalence of overweight (BMI $\geq$ 25) is approximately 40%[96] and is considered responsible for almost 7% of all breast cancers[97]. Furthermore, obese (BMI $\geq$ 30) breast cancer patients have a poorer prognosis than their normal-weight counterparts regardless of menopausal status[98]. In 2004, Calle and Kaaks presented three possible mechanisms explaining the link between adiposity and cancer: sex hormone metabolism, insulin and insulin-like growth factor signaling, and adipokine dysregulation[99] (Table 1). Related to the adipokine system, systemic subclinical chronic inflammation has become apparent as an additional important link between obesity and cancer[84]. Recent evidence from experimental and translational research have provided mechanistic insights to the role of the obesity-, insulin resistance- and adipokine- triad in breast cancer[52] (Table 1).

Table 1. Summary of links between adiposity and breast cancer.

Mechanism	Simplified explanation	Pharmaceutical	Implications
Sex hormone metabolism	Aromatase in adipocytes converts androgens to estrogens, resulting in increased levels of estrogen and consequent stimulation of estrogen-dependent tumors[84].	Aromatase inhibitors, Tamoxifen	Used as chemoprevention for breast cancer[78].
Insulin and IGF signaling	Obesity-related insulin resistance results in elevated levels of insulin and growth-promoting signaling. Insulin-IGF hypothesis: Elevated levels of bio-active IGF promote the development and progression of tumors[100].	Metformin	Inhibit breast cancer cells in vitro[101] Associated with a better outcome in patients with breast cancer[102,103]. Conflicting results regarding metformin and incidence of breast cancer[104-106]
Adipokines and inflammation	Obesity is associated with chronic subclinical inflammation. Increased leptin, decreased adiponectin and increased inflammatory cytokine secretion (C-reactive protein (CRP), tumor necrosis factor α, interleukin-1β (IL-1β), IL-6 and IL-18)[84].	NSAID/Aspirin	Protective role of aspirin and NSAID in breast cancer survival[107]. A moderate to no decrease in breast cancer incidence in aspirin users[108,109].
Mevalonate pathway	Importance in cholesterol metabolism. Metabolites of the mevalonate pathway also have apoptotic, anti-proliferative, and inflammatory-inhibitory effects[23].	Statins	Please, read the section "Statins" below

Abbreviations: NSAID: non-steroidal anti-inflammatory drugs, IGF: insulin-like growth factor

Statins

Statins are frequently prescribed cholesterol-lowering drugs, inhibiting 3-hydroxy-3-methyl-glutaryl co-enzyme A reductase and thus blocking a rate-limiting step in the mevalonate pathway[23]. Metabolites from the mevalonate pathway have, in addition to their importance in the cholesterol metabolism, apoptotic, anti-proliferative and inflammatory inhibitory effects[23]. Given these properties, there has been scientific interest in statins from an oncological point of view. Pre-clinical studies have reported anti-carcinogenic properties of statins[110,111]. Regarding statins in breast cancer prevention, i.e., primary prevention, studies have shown conflicting results[24-26]. However, on the recurrence side, epidemiological studies of patients from Scandinavia have shown a lower risk of breast cancer-related death[27-29] and breast cancer recurrence[30] among statin users, i.e., a large amount of evidence of statins in the secondary preventive setting. Today data thus suggest that statins

inhibit the progression of existing cancers (affecting the phenotype), rather than preventing cancer initiation (affecting the malignant genotype).

Breast cancer classification – prognostic and predictive factors

Different classifications schemes are used to categorized breast cancer according to different criteria (e.g., grade, stage, and additional histopathological characteristics) and different purposes, i.e., prognostic (associated with risk of recurrence and natural history of the disease[112]), predictive (prognosis after a certain intervention/treatment[112]), or comparing groups of patients in clinical trials[113]. Treatment decisions are based on evidence-based clinical guidelines, i.e., The Swedish Breast Cancer Group (http://www.swebcg.se/).

Staging system

The tumor node metastases (TNM) system[114] of malignant tumors is widely used for cancer staging, estimating the extent of the cancer burden. Patients are categorized into four prognostic groups (I- IV) based on the three parameters: the extent of the primary tumor (T), cancer affected regional lymph node (N), and distant metastases (M). The parameters have different prognostic value in different cancers, e.g., for breast cancer, the size of the primary tumor (along with several other factors such as lymph node involvement) is a key factor, whereas for colorectal cancer, the size is less important, and the depth of invasiveness is a more important prognostic feature[115]. Invasive breast cancer size is divided into four groups: T1: ≤ 20 mm; T2: 21–50 mm; T3: > 50 mm; and T4: skin or muscular involvement irrespective of size[115]. The number of involved lymph nodes is categorized as follows: no positive nodes, 1–3 positive nodes, 4–9 positive nodes and ten or more positive nodes; the N-stage is also dependent on the localization of the pathological lymph nodes[115]. The M-stage is dichotomous; with apparent distant metastases or not[115]. The staging can be based on clinical pre-surgery parameters ("c" as a designator) or information from surgery ("p" as a designator). In the case of neoadjuvant treatment, the designator "yp" is used for post-chemotherapy staging[115]. In order to adhere to the knowledge of cancer biology and better reflect the prognosis for patients, in the 8th and the latest version of the TNM-staging system ("AJCC Prognostic Stage Group"), in addition to the traditional TNM-variables, non-anatomical factors such as tumor grade and tumor receptor status (human epidermal growth factor receptor 2 (HER2), ER, and progesterone receptor (PR)) have been included in determining the prognostic stage group[114].

Tumor grade

The Nottingham histological grade[116] is an international widespread grading system, also used in Sweden. Grading is a measure of differentiation, simply put, how abnormal the tumor cells and tumor tissue looks under the microscope in comparison to normal epithelial cells[117]. The Nottingham histological grading system, categorizing tumors in three categories (I-III), is based on numerical scores of three morphological features: tubule formation, nuclear pleomorphism, and mitotic count[116].

Molecular subtypes

On the molecular level, breast cancer is a heterogeneous disease, diverse in both their natural history and their responsiveness to treatment[8,9,118]. The intrinsic system, developed by Perou and Sørlie in 2000[9,119], is a way of categorizing breast cancer according to the combination of multiple genetic alterations. At least four subtypes of breast cancer were identified: luminal A, luminal B, HER2-enriched and basal-like subtype. Using a standardized assay testing for 50 genes, prediction analysis of microarray (PAM50)[120,121], making it easier to decide on intrinsic subtype. Due to the high cost, clinical decision-making is often based on immunohistochemistry and fluorescence *in situ*[121] rather than the intrinsic subtype based on PAM50. A surrogate system based on a few surrogate key points (ER, PR, HER2, and Ki67)[122] (Figure 3) was developed and incorporated into international guidelines in 2011[10]. The appropriate cut-off for high vs. low Ki67 fluorescence is debated[123,124]. Lately, tumor grade in addition to Ki67, have been utilized in order to better discriminate between the subtypes Luminal A-like and Luminal B-like[37,125].

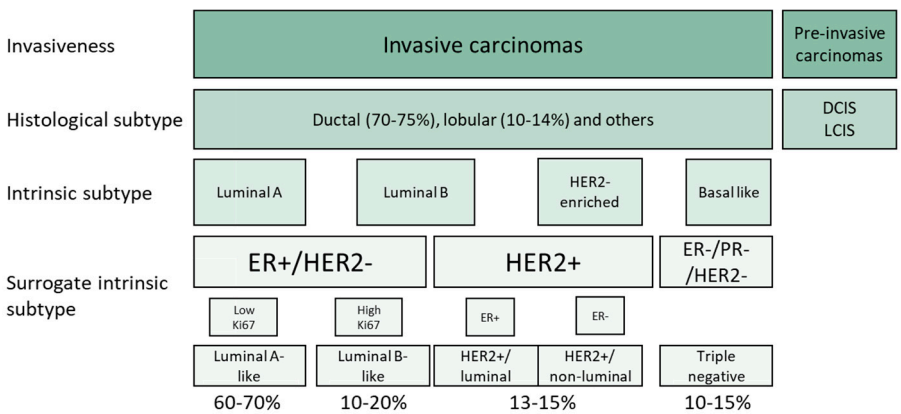

Figure 3. Illustrating different levels of breast cancer classification and the distribution of different subtypes[5,126]. Ductal carcinoma is also called carcinoma of no special type (NST). The overlap between the intrinsic and surrogate intrinsic subtypes is not complete (e.g., basalness and triple negativity are not synonyms, although the overlap is approximately 80%[127]), as visualized in the figure. Also, the clinically defined HER2-overexpressing (HER2+) group is heterogeneous; approximately half occurring in the HER2-enriched group, another part in the luminal group[128].

Predictive factors - neoadjuvant treatment

Accomplishing pathological complete response (pCR) after neoadjuvant chemotherapy for breast cancer is considered a surrogate marker for long term survival and is the Food and Drug Administration (FDA)-approved as an endpoint in clinical studies[129]. Different rates of pCR are to be expected depending on breast cancer subtype[129] (Table 2); in general, tumors with the worst prognostic factors have the best pCR rates[130]. It is implicated that pCR is not associated with prognosis for less aggressive tumors[131], such as luminal A tumors[130]. High Ki67 is associated with higher pCR rates[132].

Other factors associated with better response to neoadjuvant chemotherapy is: younger age[133] and lower BMI[134,135]. In one study, menopausal status was not associated with pCR[134].

More predictive markers are warranted, and one of the aims with this thesis is to address whether mammographic density holds predictive value in the neoadjuvant setting. Immunological biomarkers, e.g., tumor-infiltrating lymphocytes, have been shown to be predictive markers to neoadjuvant chemotherapy[136]. Another approach for finding predictive markers has been to investigate tumor gene expression, and some of the profiles relevant in the adjuvant setting seem to predict pCR in the neoadjuvant setting[137].

Table 2. pCR rate following neoadjuvant chemotherapy for different subtypes of breast cancer

Subtype	pCR-rate
All	18% (def: no invasive cancer in breast nor axilla, remaining DCIS is permitted)
"Luminal A-like"*	7.5%
"Luminal B-like"**	16.2%
Triple-negative	33.6%
HER2+/non-luminal	Without trastuzumab: 35.7%, 30.2% With trastuzumab: 72.4%, 50.3%
HER2+/luminal	Without trastuzumab 17.6%, 18.3% With trastuzumab: 45.7%, 30.9%

*Hormone-receptor-positive, HER2-negative grade 1/2 **Hormone-receptor-positive, HER2-negative, grade 3
Ref: 131,138

Breast cancer treatment

Early breast cancer, in contrast to advanced (metastatic) disease, is considered curable with modern multidisciplinary management[5]. The two main pillars of breast cancer care are locoregional treatment and systemic treatment. Histological and molecular characteristics of the tumor influence the clinical treatment decision. Two major categories of classifications systems help clinicians worldwide: tumor burden expressed according to the TNM-system[113] and the more recent biology focused

classifications such as the intrinsic system[9] (gene expression) and the surrogate intrinsic system[10] (based on immunohistochemistry and fluorescence *in situ*).

Surgery

Most women with breast cancer have some type of surgical removal of the tumor as part of their treatment[139], and for women with early-stage breast cancer, primary surgery, alone or in combination with radiotherapy, is considered to cure the disease[140]. There are mainly two different types of surgical approaches: breast-conserving surgery if the tumor can be radically removed with good cosmetic results or mastectomy, i.e., for large and multifocal cancers[37]. Breast-conserving surgery followed by radiotherapy has been a standard treatment regimen for a couple of decades[141], and patients undergoing this treatment have similar survival rates as patients undergoing mastectomy[142,143].

Clinical lymph node status has low sensitivity and specificity[144], and the addition of imaging (ultrasound) improves the numbers for the correct staging of the axilla[145]. Sentinel lymph node biopsy, a concept that involves identifying the first draining lymph node/nodes from the tumor with the help of a radioactive isotope and blue dye injected near the tumor[146,147], further improves correct staging of the axilla and is a widely practiced method for the staging of the axilla[148] with high accuracy[149]. In clinical routine, when there are no cytology-verified lymph node metastases in the axilla, sentinel node biopsy is a standard procedure to pathologically detect micro- and macro-metastases[37]. For breast cancer patients with pathology verified axillary lymph node metastases, axillary lymph node dissection is the standard treatment[37,150]. There is a high risk of arm morbidity associated with axillary dissection[151], and, if considered safe, the omission of this procedure would be beneficial to the patient.

There has been a shift in the clinical management of the axilla, from extensive axillary surgeries to more reliance on effective adjuvant therapies[145,148]. However, whether patients with macro-metastases in the sentinel node can be spared of axillary surgery and treated systemically alone, still remains uncertain[148]. For neoadjuvant treated patients, the timing for the sentinel node biopsy is controversial[152]. Sentinel node biopsy is considered reliable when performed prior to neoadjuvant chemotherapy[37,153], and in case of a negative sentinel node biopsy prior to neoadjuvant chemotherapy, axillary dissection after completion of neoadjuvant chemotherapy was omitted (provided good tumor response to neoadjuvant chemotherapy)[37,153]. However, more recently, several studies support the use of sentinel node after neoadjuvant chemotherapy and consider it safe to omit axillary dissection for sentinel node-negative (no macro- or micro-metastases and no isolated tumor cells[154]) patients[155-157].

Radiotherapy

Radiotherapy, administrated after both mastectomy and breast conservatory surgery, reduces the rate of local relapse and breast cancer-related death[158,159]. Whole breast irradiation is the standard treatment for the optimal outcome for patients undergoing breast-conserving surgery[33]. Current international guidelines recommend regional radiotherapy (regardless of breast-conserving surgery or mastectomy) in the case of ≥ 4 positive lymph nodes in the axilla and in the case of 1–3 positive lymph nodes with adverse prognostic factors[33]. However, results from the prospective MRC/EORTC SUPREMO trial (which assesses the value of regional radiotherapy to intermediate risk patients with 1-3 positive lymph nodes) might change current recommendations[160]. Mostly, post-mastectomy radiotherapy is given to the chest wall for primary tumors >5 cm[33]. In case of neoadjuvant treatment and according to Swedish national recommendation, postsurgical radiotherapy is given loco-regionally unless: the initial sentinel node biopsy was negative, then the regional radiotherapy can be omitted; and radiotherapy can be omitted altogether if the initial sentinel node biopsy was negative, initial tumor size < 5 cm and a mastectomy have been performed[37]. The most severe but rare side effects of radiotherapy are cardiovascular events (a relative increase of 7.4% per Grey)[161] and lung cancer[162].

Systemic treatment – chemotherapy

To eliminate any remaining cancer cells, a systemic treatment is recommended to high risk (of recurrence) patients conditional on clinical, pathological and molecular features[34]. Different biomarkers are used as surrogates for these features to estimate the prognosis and to predict treatment response.

In a chemotherapy regimen consisting of different cytotoxic drugs, synergistic effects are seen, and the breast cancer survival rates improve[163]. Adjuvant chemotherapy for breast cancer is shown to reduce the 10-year mortality rate by one third[163]. According to the latest St Gallen Conference, for ER-positive and node-negative tumors, an alkylator- and taxan-based regimen is often recommended, whereas, for the higher risk tumors, an anthracycline-based regimen is recommended[33]. In Sweden, the current recommended adjuvant and neoadjuvant chemotherapy regimen is: Epirubicin$(E)_{90}$Cyclophosphamide$(C)_{600}$ alternatively fluorouracil$(F)_{500}E_{100}C_{500}) \times 3 \rightarrow$ Docetaxel$_{80\text{-}100} \times 3$ every third week[37]. Side effects associated with chemotherapy can be seen as acute or as late side effects and are partly drug-specific and dose-related. Examples of severe acute side effects are neutropenia, and infections[164] and severe long term effects are, for example, cardiac toxicity, secondary leukemia, cognitive function impairment, and neurotoxicity[165]. It is therefore important to identify subgroups of patients were chemotherapy safely can be omitted; commercial gene assays (e.g., MammaPrint[166] and OncotypeDx[167]) that help identify these patients are now incorporated in several guidelines[46,168].

Neoadjuvant treatment

The indication for neoadjuvant chemotherapy, i.e., chemotherapy before surgery, for breast cancer has broadened, and an increasing number of patients receive chemotherapy before, instead of after, surgery. There is no proven difference in recurrence rate or overall survival between the two treatment strategies[169], and the advantages with neoadjuvant treatment in terms of being able to monitor the tumor's response to given treatment *in vivo* makes this an appealing strategy. In accordance to international guidelines, national and local guidelines recommend that the following patients should be offered neoadjuvant chemotherapy: patients with large tumors (T3/T4), patients with positive lymph node status in the axilla, or patients with tumors larger than 20 mm and simultaneously, other risk factors, e.g., triple-negative tumor or HER2-overexpressing tumors[33,37]. Another important indication for neoadjuvant chemotherapy is the possibility for surgical conversion from mastectomy to partial mastectomy[170,171], subsidizing the use of neoadjuvant chemotherapy.

Currently, with the introduction of the important concept of salvage adjuvant chemotherapy (recommending additional (adjuvant) chemotherapy, according to ypTN-staging) has been added to the list of benefits of neoadjuvant chemotherapy[172-174].

Endocrine treatment

In ER-positive breast cancer, estrogen is the main driver for tumor development. In premenopausal women, the ovaries are the main producers of estrogen, whereas, for postmenopausal women, estrogen is produced in adipose tissue and muscles through the conversion of androgens produced in the adrenal glands[175]. It is historically known that breast cancer prognosis improved by lowering the patients' estrogen levels, by oophorectomy or later by radiating the ovaries[37]. Modern endocrine treatment functions by targeting the ER-pathway[175]. Tamoxifen is a selective ER modulator, a competitive inhibitor that blocks the downstream signaling in the ER-pathway[176], and thus works irrespectively of menopausal status.

Aromatase is a cytochrome P450 enzyme that catalyzes the conversion of androgens to estrogen, and thus, aromatase inhibitors are mainly used in patients with no ovarian estrogen production, i.e., postmenopausal women[177]. According to current national guidelines, adjuvant endocrine treatment for at least 5 years is recommended to patients with ER-positive tumors, with the exception of very low-risk tumors (tumors $\leq$ 10mm, axillary node-negative, and Luminal A-like) where the systemic treatment may be omitted after a discussion with the patient[37]. Results from the ATLAS study showed that patients with ER-positive disease have a further reduction in recurrence (3.7%) and mortality (2.8%) when continuing tamoxifen treatment for 10 years instead of stopping after 5 years[178]. International guidelines recommend women with lymph node-positive disease to continue adjuvant

endocrine therapy for 10 years[179]. At present, in Sweden, only patients with high-risk ER-positive tumors (axillary lymph node metastases, HER2-overexpressing or other risk factors) are recommended 10 years of endocrine treatment[174]. Importantly, and an individualized discussion regarding the balance between the benefits and the drawbacks of prolonged treatment is warranted.

Non-adherence or discontinuation is, as with many medications used chronically, a considerate problem with endocrine treatment; studies indicate up to 49% non-adherence rate after 5 years of follow up[180]. Side effects of tamoxifen treatment are menopausal symptoms such as hot flushes, increased risk of thromboembolic complications, and endometrial hyperplasia[127]. Aromatase inhibitors are associated with fewer adverse health outcomes than tamoxifen[181]. Still, aromatase inhibitors users are at increased risk for osteoporosis and cardiovascular events than non-users[181]. However, many patients treated with aromatase inhibitors suffer from musculoskeletal symptoms, vaginal side effects, and cognitive symptoms[182].

Targeted therapy

For patients with HER2-overexpressing tumors, chemotherapy, in combination with HER2-targeted therapy, is offered[46,174]. The addition of trastuzumab to chemotherapy dramatically improved the overall survival for HER2-overexpressing early breast cancer; the hazard ratio was 0.66; 95% confidence interval (CI) 0.57 to 0.77 in favor of the combined therapy[183]. Trastuzumab and pertuzumab are both humanized monoclonal antibodies that target the HER2-protein[184,185]. They do, however, bind to different domains[185], and indeed, even if only marginally, the addition of pertuzumab to chemotherapy and trastuzumab improved the survival rates for HER2-overexpressing breast cancer (estimated 3 years rate of disease-free survival: 94.1% in the pertuzumab group vs. 93.2% in the placebo/standard treatment group)[184]. In the adjuvant setting, double HER2-blockade with trastuzumab and pertuzumab is, in contrast to the neoadjuvant setting, not routinely recommended in Sweden[37]. However, many patients with HER2-overexpressing tumors are treated with neoadjuvant therapy and thus receive double HER2-blockade[37]. One major drawback with anti-HER2 treatment is its cardiac toxicity[186], and cardiac function is monitored for patients receiving these drugs[37,186].

Bisphosphonates

Bisphosphonates are osteoclast inhibitors and have been successfully used in the metastatic breast cancer setting, with a shown reduction in skeletal-related events[187]. In 2016, the Swedish national guidelines broadened the indication for bisphosphonates, now a treatment added to the adjuvant treatment arsenal for early postmenopausal breast cancer, mainly to patients with lymph node metastases or other high-risk factors of recurrence[37]. Results from a meta-analysis showed an

absolute risk reduction of 2.2% for bone metastases and 3.3% for breast cancer mortality in postmenopausal women[188].

Breast imaging

Radiological imaging of the breast is dominated by a traditional, two-dimensional x-ray of the breast, a mammogram. Other imaging modalities such as ultrasound, breast tomosynthesis, and MRI are also widely used. In addition to imaging, clinical examination and biopsy are the cornerstones of the concept of triple-assessment, considered as the gold standard when a patient is presenting with clinical symptoms in the breast or is selected from screening mammography due to suspicious findings. Several reports support that triple assessment has an accuracy reaching 100%[189,190].

Mammography and breast tomosynthesis

In mammography, the x-ray photons move through the breast and are absorbed by an image detector and transferred to a digital signal that creates the image we know as the mammogram[191]. The inherent differences in x-ray attenuation between the different components of the breast are used in image acquisition[191]. The craniocaudal (CC) and mediolateral oblique (MLO) views are both used in screening, and in clinical examinations, the lateromedial view is acquired in addition to the other two views. Repeated imaging with mammography during neoadjuvant chemotherapy treatment is seen in Figure 4.

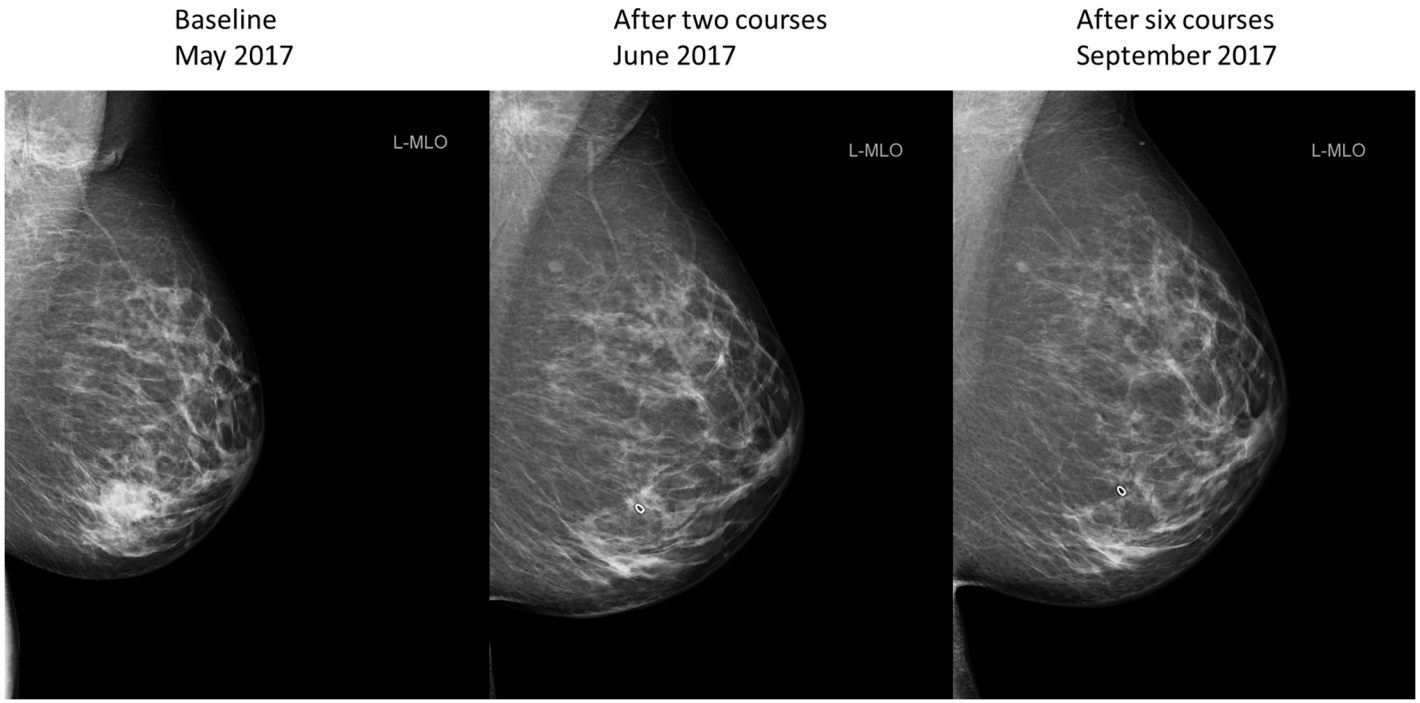

Figure 4. Mammography at three different time points during neoadjuvant chemotherapy for breast cancer in a premenopausal patient. In the first mammogram, the tumor was estimated to be 27 mm; in the mammogram, in the middle, the tumor has been coil-indicated, and the tumor estimate was 8 mm, and in the last mammogram, the tumor is no longer visible, radiological complete response. The mammographic density was scored according to BI-RADS (5th Edition) as BI-RADS b. For this patient, pCR was accomplished. © Region Skåne, SUS

Breast tomosynthesis, a 3-dimensional x-ray technique, utilizes an x-ray source that moves along an arc of the excursion, and thin slices are reconstructed with the aim to minimize the influence of overlapping breast tissue[38,192]. In comparison to mammography, breast tomosynthesis has been shown to improve accuracy, as well as lesion conspicuity and localization[38]. Many studies have evaluated the radiation dose for breast tomosynthesis when performed in addition to mammography, which, obviously, substantially increases the radiation dose[193]. However, instead of performing both a breast tomosynthesis and a 2D mammogram, a synthetic 2D reconstructed image can be used in addition to breast tomosynthesis, and thus limit the additional radiation dose that a "double" examination entail[194]. The radiation dose for breast tomosynthesis is similar to a conventional mammogram[193,195]. The average effective dose from two-view mammography equals approximately two months of natural background radiation[196]. One recently published meta-analysis showed no significant difference in diagnostic sensitivity (detection) between breast tomosynthesis alone and the combination of breast tomosynthesis with mammography[38]. A tumor depicted with both mammography and tomosynthesis is shown in Figure 5.

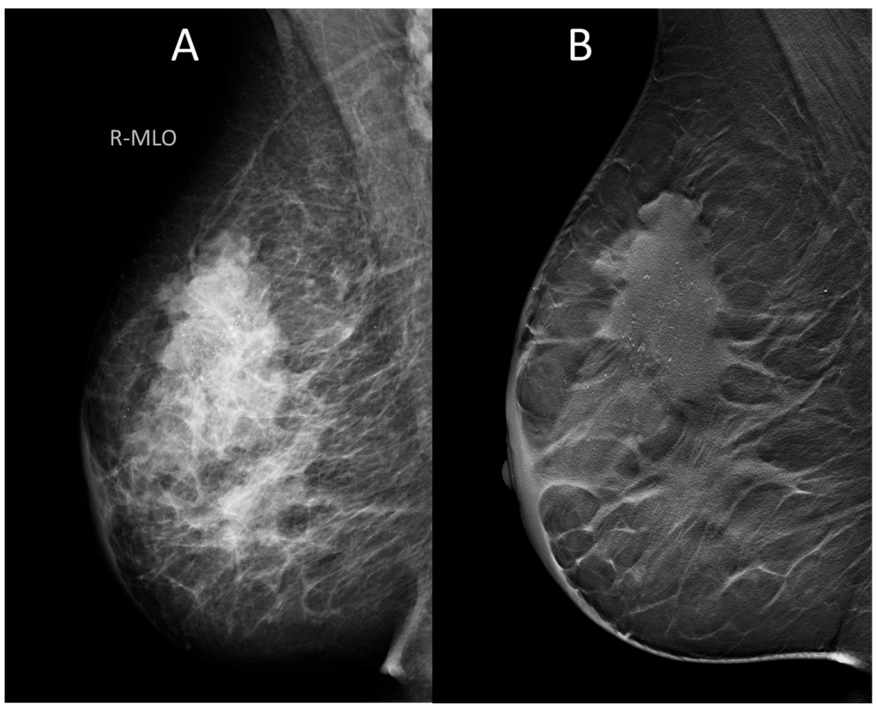

Figure 5. Comparison of imaging of the same breast depicted with (A) mammography and (B) tomosynthesis. Breast imaging at baseline before neoadjuvant chemotherapy for breast cancer. A premenopausal woman with heterogeneously dense breast (BI-RADS c (5th Edition) as assessed by the clinical radiologist at the time of the examination). © Region Skåne, SUS

Other breast imaging modalities

In ultrasound, high-frequency sound waves are generated in a transducer and are partially reflected from tissue interfaces[197]. Thus, the technique behind the image generation in ultrasound is very different from mammography. The combination of ultrasound and mammography increases the sensitivity and the diagnostic accuracy[198], especially in younger women and/or women with dense breasts[198]. However, for high-risk women (e.g., high mammographic density and BRCA mutation carriers), MRI (with or without mammography) is the standard modality for follow-up in breast cancer screening in many places[199,200]. In addition to detection and follow up of breast lesions, ultrasound is important for image-guided breast and axillary biopsies. In addition, ultrasound is the first-hand choice for axillary imaging[201].

Recent technical developments with the introduction of automatic breast ultrasound, and computer-aided detection of breast ultrasound, thus partly overcoming some of the drawbacks with the modality[198].

Contrast-enhanced MRI has become an additional tool in breast imaging. In comparison to the other imaging methods, MRI has the highest sensitivity for detecting breast cancer, but the specificity is lower compared to mammography (the specificity of MRI ranges between 83% and 98%)[202]. The specificity of MRI is improved by adding specialized MRI-sequences[203]. The supplemental use of mammography increased the sensitivity and decreased the specificity, whereas adding ultrasound only decreased the specificity (no supplemental value)[202]. MRI is useful for screening in very high-risk populations, e.g., BRCA mutation carriers[204]. Also, MRI is being evaluated as a supplemental screening method in women with high mammographic density and normal results on screening mammograms[205]. The role of MRI in preoperative staging is debated; either improving surgical treatment or merely leading to more mastectomies[206]. It is suggested that MRI can detect additional malignancy in 12–13% of the cases[207], and a Swedish multi-centre study showed higher rates of MRI-associated conversions (from breast-conserving surgery to mastectomy), however, the final numbers of mastectomies was the same (with or without MRI), thus the number of reoperations was lower when using MRI pre-operatively[208]. A meta-analysis by Houssami *et al.*, did not show improved surgical treatment; higher rates of mastectomies (and of contralateral prophylactic mastectomy), although not associated with reduced risk of recurrence[209], and no favourable effect on margins/incomplete excision or re-operation rates[206]. MRI is also used for monitoring treatment response in the breast of breast cancer patients receiving neoadjuvant chemotherapy[207,210,211].

Contrast-enhanced mammography, offering a morpho-functional approach, is gaining increased clinical utility. The technique includes an algorithm generating a subtracted image, highlighting sites of contrast enhancement in the breast, i.e.,

hypervascular lesions such as cancer[212]. Contrast-enhanced mammography is challenging MRI as superior method in many important breast cancer settings, e.g., pre-surgical staging, post-operative surveillance, option for recalled suspicious findings, and neoadjuvant chemotherapy response monitoring[213]. A comparison between breast imaging modalities is seen in Table 3.

Table 3. Comparison between breast imaging modalities.

	Radiation dose	Advantages	Disadvantages
Digital mammography	Screening: ~3 mGy[193,196]	Easy for storage High throughput of patients Short reading time for radiologists	Sensitivity in screening around 80%[38] (higher for non-dense breasts, Table 6, page 50).
Breast tomosynthesis	Screening: ~3 mGy[193]	Sensitivity in screening around 90%[38]. Lower false-positive rates[214] Lower recall rates when baseline recall rates are relatively high[215]	Longer reading time than for mammography[216], though with the prospects of improving[217] Larger data volumes for storage compared to mammography
Ultrasound	None	High degree of patient acceptability[198] High sensitivity in younger women and/or dense breasts[198] No radiation	Operator dependent Time-consuming Less suitable for saving and interpretation afterward High false-positive rate[199]
MRI	None	High sensitivity[207] No radiation	High cost Low availability Long examination time Lower specificity[207] Gadolinium contrast: nephrogenic systemic fibrosis and gadolinium retention[218]
Contrast-enhanced mammography	Approximately 20-80% more than a digital mammogram[219,220] (low energy contrast-enhanced images are considered sufficient, lowering radiation dose[212])	Higher sensitivity and specificity in clinical and screening setting compared to digital mammography[221-223] Lower cost than MRI[224].	Iodine contrast Higher radiation dose More time-consuming than mammography.

Screening

In Sweden, women aged 40–74 years are invited to participate in breast cancer screening with mammography every 18–24 months[37]. Ever since the introduction of the mammography screening programs, there has been a debate over the benefits and harms[225]. The estimated reduction in breast cancer mortality in women attending screening is 20–40%[225-228]. The major harm by screening is overdiagnosis, i.e., finding cancer that would otherwise not have come to attention during that woman's lifetime[225]. The best method to estimate overdiagnosis is debated. However, two expert panels propose that a valid estimate of overdiagnosis for a population invited

to screening is around 10%[200,229]. The specificity of the screening program is high 94–97%[230], a prerequisite to avoid false-positive and the associated negative psychological consequences[225]. False-positive readings (the mammogram looks abnormal even though no cancer is actually present) are also troublesome, and the rates vary with both patients (e.g., more frequent in younger women due to lower specificity of mammography in dense breasts and a lower cancer incidence in younger ages) and radiology-related factors[231]. The cumulative proportion of false-positive readings in women regularly attending screening every second year over two decades is estimated to be 20% in a European study[232] and are considerably higher in the United States[231]. In Sweden, approximately 60% of all breast cancers are screening-detected[4].

Mammographic density

Breast density is an imaging parameter, most often assessed with mammography – mammographic density, although breast density can be assessed with other imaging modalities as well. In the following chapter, mammographic density will be addressed, but most of the reasoning is also applicable to breast density in its more general meaning. For an overview of the histological and radiological aspects of mammographic density, please refer to Figure 7 on page 48.

Mammographic density on a cellular level

Mammographic density reflects the relationship between radiodense tissue, i.e., fibroglandular tissue, and radiolucent tissue, i.e., fat, in a mammogram. Note that mammographic density is an imaging parameter mirroring the entire breast/breasts, and does not refer to tumor stiffness[233]. Mammographic density is a strong determinant of risk for all breast cancer precursors[234] as well as invasive breast cancer, with up to a 4–6 fold increase in breast cancer risk for women with extremely dense breast (>75% dense tissue) compared to women having non-dense breast (<5% dense tissue)[18]. Studies of both surgical breast specimens and mammograms have shown that higher mammographic density was associated with greater amounts of stroma (including collagen) and marginally larger amounts of epithelium[235-238] and a greater total nuclear area with higher mammographic density[237]. The differences in amounts of epithelial cells are seemingly smaller than the difference in the stroma between high and low-density breast tissue[238], possibly explaining the inconsistent results regarding epithelial cells and mammographic density reported in the litterature[239]: reports of an association between high mammographic density and proportion of epithelial cells[237,240-242], as well as studies finding no such association[243,244]. Fibroblasts, stromal cells responsible for synthesizing products of the extracellular matrix, and their capacity to differentiate into adipocytes[15], make

them important in the biological understanding of mammographic density. Also, it is indicated that the phenotype of fibroblasts varies depending on mammographic density[239]. The collagen structure, with a more organized linearized collagen in high-density tissue[238], varies between high and low dense breast, and abnormal expression of extracellular matrix proteins, with increased stiffening of the microenvironment, may cause cancer-promoting structure[15]. A study of breast tissue from rats showed less linearized collagen and a lower degree of stromal stiffness in parous rats compared to nulliparous rats[245]. Perhaps this is a contributing factor to the lower risk of breast cancer[246] seen in parous women compared to nulliparous women. An initial study by McConnel *et al.* demonstrated that, on top of collagen remodelling info large stiff fibers, the upregulation of periostin and collagen XVI, molecules associated with collagen organization, proliferation and cell invasiveness, is seemingly a feature of high-mammographic density peri-ductal regions[247]. These molecules are, thus, possibly markers of breast cancer risk and not only a marker of breast cancer and metastasis[247]. On the molecular level, systemic circulating levels of mitogens (insulin-like growth factor I[236,248-250]), regulators of growth factors (tissue inhibitor of metalloproteinase 3[236]), and mutagens (malondialdehyde[251-253]) have been linked to increased mammographic density.

Breast tissue with high mammographic density has greater amounts of the stromal matrix regulatory protein tissue inhibitor of metalloproteinase 3[236], regulating stromal matrix, activating growth factors, effector of early stage tumorigenesis, as well as player in the process of metastasis[254]. Also, the repression of transmembrane receptor CD36 (not limited to a certain cell type but generally expressed in stromal components), controlling both adipocyte content and matrix accumulation, has been associated with both high mammographic tissue and breast cancer stroma[255]. The repression of CD36, controlling several stromal elements, creates a pro-oncogenic stromal state; the broad impact of CD36 makes it an attractive therapeutic target[255]. CD36-nonexpressing fibroblasts exhibit changes towards characteristics of cancer-associated fibroblasts (CAFs)[255]. Thus, high mammographic density is associated with "activated" fibroblasts as illustrated in Figure 6, a desmoplastic/pro-tumorigenic phenotype, with some of the properties of cancer-associated fibroblasts. The research surrounding cancer-associated fibroblasts is vast since they are key players, involved in many of the hallmarks of (breast) cancer (e.g., immune suppression, angiogenesis, altered metabolism, tumor cell survival, migration, and metastases), by creating/supporting a pro-oncogenic milieu[256,257]. Relevant to this thesis, are the findings that the expression of CD36 is increased by statin, aspirin, and corticosteroids (dexamethasone)[258-260].

DeFilippis *et al.* further explored mechanism behind CD36-repression and concluded that telomere malfunction and activin A expression in epithelial cells repress CD36 expression in the neighbouring fibroblasts[261]. Epithelial cells in tissue with high mammographic density had more basal DNA damage, and a different

DNA damage response/signaling, shorter telomeres, and higher activin A secretion, in comparison to epithelial cells from low-density tissue[261]. Without knowing if the initial event occur in the epithelial or stromal compartment (or in both), these findings further highlight the importance of interactions between epithelial and stromal cells and present findings of a difference in this important cross-talk between high and low breast density tissue.

A study published in 2016[262] established that the cancer cells "remember" the environment they were in, as was also suggested by DeFilippis *et al.*[261] two years earlier. When MDA-MB-231 cells (triple negative breast cancer epithelial cells) were grown on top of the extracellular matrix derived from healthy fibroblasts, a significantly lower proliferation rate was observed, in comparison to the proliferation of their counterparts grown on stiffer surfaces. Interestingly, when these cells were moved from fibroblast-derived matrices to stiffer surfaces, they retained their lower proliferation rate. In other words, the growth-inhibitory effects of the healthy fibroblast-derived extracellular matrix on the breast cancer cells persisted[262].

Cells sense the stiffness of the surrounding microenvironment through pulling on the extracellular matrix and/or the nearby cells. Forces are transmitted through the cytoskeleton by processes between cells and extracellular matrix, cell-to-cell adhesions, or intracellular contractility, the latter being mediated by Rho GTPases[263]. Thus, Rho GTPases allows cells to sense matrix stiffness and respond to mechanical signals. There may be different levels of Rho GTPases between low and high density breast tissue[77] (Figure 6), of interest since breast cancer cells seems to proliferate in response to stiff matrices, partly mediated by Rho GTPases[263].

The association between circulating sex hormone levels and mammographic density in postmenopausal women is complex: obese women have higher estradiol levels through increased aromatase activity in adipose tissue[264]; on the other hand, obese women have in average lower mammographic density. It has been suggested that mammographic density may represent the influence of circulating estrogen; however, associations of endogenous estrogen levels with mammographic density in postmenopausal women have been inconsistent, including null, positive, and inverse association[265-270]. However, it has previously been suggested that, in the premenopausal period, an etiologically important exposure period, with, on average, higher levels of sex hormones, high levels of sex hormones might be associated with premenopausal mammographic density[271]. Investigating a group of young premenopausal women, no association between breast density (MRI-based) and circulating levels of estrogen was found[272]. Recent results from a sub-study within the KARMA cohort presented by Gabrielson *et al.*, including over 1000 women, showed that several sex hormones were associated with mammographic density (using the automated software STRATUS[273]) in the following way: positive

association with progesterone, 17OH-progesterone, oesterone sulphate, prolactin, and SHBG, and negative association with 11-deoxycortisol[274]. However, *change* in mammographic density was primarily associated with androgens. The authors further presented a non-linear association between sex hormones and mammographic density, possibly contributing to the diverse results from previous studies[274]. In terms of receptor expression, no differences have been found between the expression of ER and PR between high- and low-density breast tissue[238,241].

Mammographic density changes naturally during a woman's life with age and hormonal events (e.g., pregnancy, breastfeeding, and HRT)[275,276]. The breast of a premenopausal woman undergoes both expansion and involution during a menstrual cycle, though, with only quite small/negligible changes in mammographic density[277]. On average, mammographic density declines with age and the normal breast development in a postmenopausal woman includes lobular involution (physiologic atrophy of breast, the replacement of glandular tissue with adipose tissue)[70]. Although involution and mammographic density are correlated[278-280], complete involution is not a guarantee for non-dense breast. In a study by Ghosh *et al.*[241], surprisingly, 52% of the women with complete involution had dense breasts and 23% of the women with no involution had non-dense breasts. However, epithelia are only a minority of the dense tissue; the dominating part is the stroma. In addition, the first step in lobular involution is the dense stroma, further complicating the association between age, lobular involution and mammographic density and, also, reflecting the importance of the timing of mammography assessment[281]. An accelerated decline is often seen during the perimenopausal period, corresponding to up to two decades of aging[282]. Identifying why mammographic density remains high for some postmenopausal women, might be a fruitful approach towards improved understanding of the association between mammographic density and breast cancer risk, with possible targets for intervention.

Different amounts of fat and connective tissue mainly explain the differences in breast volume between women[15]. The local fat in the breast is thought to play a protective role against breast cancer[283]. Although a higher BMI (through raised circulating metabolites and adipokines), subsequent increase postmenopausal breast cancer risk, local adipose tissue in the breast *per se* may reduce breast cancer risk by diminishing the possibilities of hazardous interactions in the breast between epithelial cells and the stroma[238]. However, findings from pre-clinical studies highlight that obesity is associated with increased adipose tissue fibrosis[284] (also locally in the breast) and extracellular matrix stiffness that promote breast tumorigenesis[285]. For a deepened discussion, please refer to section "Absolute or percent dense tissue" on page 51.

In conclusion, the interaction between the different components of the breast seems to be highly complex[14] and targeting different aspects of the stroma may have an impact on breast cancer treatment[286].

A schematic image of differences between high and low-density breast tissue is seen in Figure 6. Histological and radiological aspects of mammographic density are illustrated in Figure 7 on page 48.

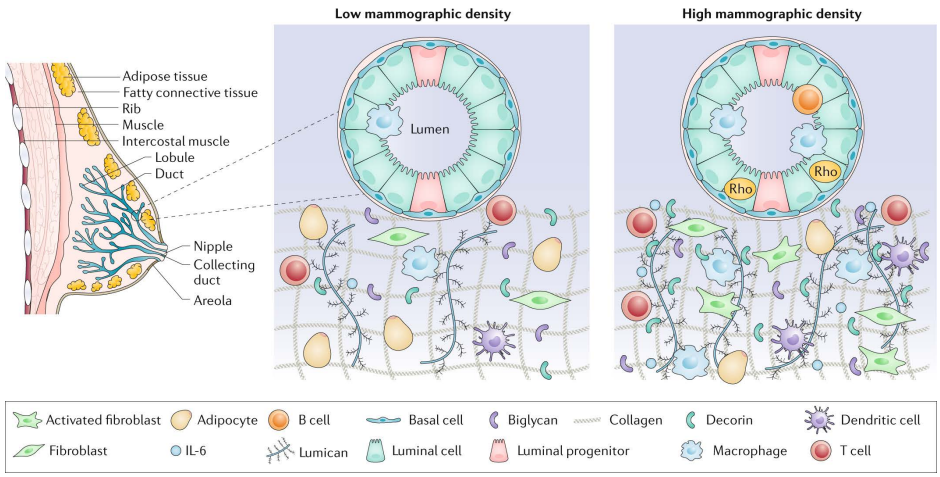

Figure 6. Biological differences between low and high mammographic density breast tissue. Reprinted from "Key steps for effective breast cancer prevention." *Nat Rev Cancer.*, 2020, Britt *et al.*, with permission © 2020, Springer Nature.

Inflammation and mammographic density

Studies indicate that high mammographic density reflects a pro-inflammatory environment[239,287]. High-density normal breast tissue showed a higher number of CD45+ and CD68+ immune cells in comparison to less dense breast tissue[238,287] and presented a pro-inflammatory cytokine profile; higher levels of interleukin-6, interleukin-8[287], and higher levels of osteopontin, a key protein in the inflammatory response[80]. In addition, the angiogenic vascular endothelial growth factor (VEGF) seems to be more abundant in dense tissue in comparison to non-dense breast tissue[287,288]. Immune cell infiltration is seen in DCIS as well as in invasive breast cancer[289]. A Swedish study on the microenvironment in healthy breast tissue and breast cancer tissue, respectively, showed a similar profile of inflammatory proteins in the microenvironment in dense breast tissue and breast cancer, namely a pro-inflammatory microenvironment in both cases[290]. In summary, these findings indicate dense breast tissue as a possible site for local inflammation.

The use of non-steroidal anti-inflammatory drugs (NSAID) has been associated with reduced risk for breast cancer[109]. A biological mechanism is presented through which aspirin might exercise its breast density- and breast cancer risk-reducing

effects: suppression of cyclooxygenase-2 (possibly increased in high mammographic density tissue[291]), lowering prostaglandin production, and in so doing, reducing the carcinogenic activity in breast tissue. The pro-inflammatory microenvironment in dense breast tissue further supports the association between NSAID and reduced risk of breast cancer. There are only a few studies investigating the association between NSAID, including aspirin, and mammographic density, the literature showing none[292-294] or an inverse (i.e., lower density in aspirin-users)[295] association between NSAID and mammographic density. Thus, it is plausible that NSAID exerts its anti-carcinogenic effect through a different pathway than mammographic density.

The masking effects

High mammographic density, besides being a risk factor for breast cancer, reduces the potential to detect a malignancy on a mammogram, known as the "masking effect"[296-299]. As a result of this effect, the sensitivity of mammograms is dependent on mammographic density. It is well known that mammography has lower cancer sensitivity in dense breasts. Studies of a large Danish screening cohort[300] showed an overall sensitivity of mammography (invasive cancer or DCIS) of 72% (95% CI, 68–76) and a specificity of 98% (95% CI, 97–98). Sensitivity decreased with increasing mammographic density; sensitivity for Breast Imaging-Reporting and Data System (BI-RADS) was 78% (95% CI, 69–85) and sensitivity for BI-RADS d was 47% (95% CI, 30–65)[300] (also, please refer to Table 6 on page 50).

Different approaches are available in order to circumvent the masking effect: young women (in average having higher mammographic density) might benefit from more frequent screening[174], and other complementary imaging modalities might be used for normal screening findings in high-density breasts[301] (please refer to section "Other breast imaging modalities" on page 36 and "Legislation" on page 51).

Risk factors associated with mammographic density

Paradoxically, with increasing age, mammographic density decreases and the incidence of breast cancer increases[282]. The Pike model[302], a model of cumulative exposure of hormones during a woman's life leading to breast tissue aging and increased breast cancer risk, might explain this apparent paradox[303]. Data from a large Dutch breast cancer screening cohort indicate a slight increase in mammographic density across birth cohorts[304]. Mammographic density has a strong heritable component, heritability accounting for 60% of the variation[305].

In terms of hormonal and reproductive factors, on average mammographic density decreases with every live birth[306]. A steep decline is often seen during menopause[306]. On the contrary, both nulliparity[306] and higher age at first birth[307] are associated with higher mammographic density. While breastfeeding reduces the risk of breast cancer, the association between breastfeeding and mammographic density is less

clear[308]. An interesting approach towards understanding the association between reproductive history and risk of breast cancer was used within the KARMA project: exploring the association between tissue composition and hormone receptors and breast cancer risk factors[309]. Among many interesting findings, they found that breastfeeding was associated with the increased epithelial area later in life, but not associated with changes in the stroma[309]. Also, the amount of local adipose tissue in the breast was inversely associated with breastfeeding, even further complicating the translation to mammographic density. HRT increases mammographic density[310,311], whereas the association between oral contraceptive use and mammographic density seems to be mainly unexplored[312]. However, one study reported no association between oral contraceptive use and mammographic density[307].

Mammographic density is inversely associated with BMI[275,313]. There seems to be no effect of smoking and physical activity on mammographic density[314,315], whereas a small or no increase in mammographic density was seen in users of alcohol[316,317].

High mammographic density is associated with an increased risk of DCIS[318,319]. Mutations in BRCA1 or BRCA2 do not seem to be associated with mammographic density[320].

In recent years, mammographic density change in association to breast cancer risk has been investigated with interesting results: many risk factors for breast cancer associated with baseline mammographic density are not associated with change in mammographic density (except from age, physical activity, and BMI)[276,321].

A summary of risk factors/protective factors of breast cancer and their association with mammographic density is seen in Table 4. Mammographic density and its association with prognosis is briefly addressed in the section “Mammographic density - breast cancer risk and prognosis” on page 46.

Table 4. Summary of associations between risk/risk-reducing factors of breast cancer and mammographic density.

	Risk of breast cancer	Mammographic density
Higher age	↑[16]	↓[282,303]
BRCA1, BRCA 2	↑[66]	No effect[320]
Higher socioeconomic class	↑[58]	↑[322]
Alcohol use	↑[59,60]	No effect[323] or a small ↑[307,316]
Smoking	Some evidence a slight ↑[62]	No effect[316]
Physical activity	↓[95]	No effect[315,316]
Previous benign breast disease	↑[64]	
Radiation	↑[16]	(Radiotherapy: probably no effect[324,325])
HRT	↑[51]	↑[310]
Parity	↓[50]	↓[306]
Breastfeeding	↓[50]	Unclear[308]
Premenopausal obesity	↓ HR-positive breast cancer[52]	↓[275]
	↑ HR-negative, basal-like and triple-negative breast cancer[52]	
Postmenopausal obesity	↑ HR-positive breast cancer[97]	↓[275]
	No association or minimally ↓ for ER-negative and triple-negative breast cancer[85]	
Height	↑[326]	↑[326]
Diabetes Mellitus II	↑[97,327]	No effect[328]
Postmenopausal systemic estrogen levels	↑[72,73]	No effect[269,270] or a ↑[274]
Premenopausal systemic estrogen levels	↑[71]	No effect[272] or a ↑[274]
Aspirin	↓[109]	No effect[293]
Metformin	Conflicting results[104-106]	Probably no effect[328]
Statins	Probably no effect[24-26]	No effect[329]
Tamoxifen, aromatase inhibitors	↓[78]	↓[330]
Subclinical inflammation	↑[84]	↑[289]

Mammographic density and tumor characteristics

In general, scientific inconsistency exists regarding the association between tumor characteristics and mammographic density[331]. In a large meta-analysis of 16 studies, most studies found no association between HR status or molecular subtype and mammographic density[332]. Among the studies finding an association, there was a predominance forwards higher density being associated with larger, ER-positive, axillary lymph node-positive cancers[332]. A large pooled analysis with data from over 3000 breast cancer cases and over 7000 controls, showed an association between mammographic density and all breast cancer subtypes across all ages, but with a more pronounced association between high mammographic density and large tumors and positive axillary lymph nodes[331]. For women < 55 years old, they found an association between high mammographic density and ER-negative tumors[331]. The authors, Bertrand *et al*., conclude that high mammographic density may be

important in tumor aggressiveness, especially in younger women[331]. A meta-analysis from 2012 showed that mammographic density is a marker of breast cancer risk overall; the magnitude of association was similar between mammographic density and ER-positive and ER-negative tumors, and the association did not differ by HER2 status[333]. A Canadian study from 2019 showed no association between mammographic density and tumor receptor status[334]. The association between nodal status and mammographic density was explored in another study; it was hypothesized that the high extracellular matrix in high-density breasts could influence tumor spread; however, they found no association between mammographic density and spread to axillary lymph nodes in the nearly 2000 patients included in the analyses[335]. An Australian study found that high mammographic density was associated with larger tumors and positive lymph node status (but no associations with ER, PR, and HER2 status of the tumor)[336], and the authors, Krishnan *et al.*, propose that their findings suggest that dense breast tissue play a role in faster tumor growth and spreading to the lymph nodes that would not be accounted for by the delay of cancer detection due to masking[336].

The association between mammographic density and the proliferative marker Ki67 in normal breast tissue remains controversial[81]. One study found increased stromal Ki67 expression in breasts with high mammographic density, while other studies found no association[238,337,338]. Analyzing breast core biopsies from healthy women, Ghosh *et al.* found no association in the expression of Ki67 between dense and non-dense tissue[241]. Investigating Ki67 in tumors in relation to mammographic density in nearly 2000 patients, Heusinger *et al.* found no significant differences in mammographic density between women with tumors of low versus high Ki67 values[339]. Aiello *et al.* neither found any association between mammographic density and Ki67 in breast cancer tissue[266].

In conclusion, the associations between mammographic density and tumor characteristics, especially HR-status, seems to be ambiguous. Giving the incoherent results from the literature, in my opinion, the associations are probably not very large and, therefore, possible of borderline clinical significance.

Mammographic density - breast cancer risk and prognosis

Only female gender, age and BRCA mutation status seem to be associated with a higher risk of breast cancer than high mammographic density[15,16]. Despite mammographic density being related to hormonal events in a woman's life, high mammographic density is, as it seems, equally associated with ER-negative and ER-positive breast cancer[333]. The clinical association is well-studied, but the biological links are less clear. Breasts with high mammographic density have greater proportions of the epithelium, stroma, and collagen, and less fat, than in low-density breasts[241], and the properties of these components, single-handedly or in combination, may support the acquirement of mutations in the epithelial cells[340].

High mammographic density is also associated with various kinds of benign breast disease[341]. The increased risk of breast cancer in women with high mammographic density is most likely due to a cumulative "exposure" to (a larger amount of) epithelium and various hormonal factors in the microenvironment and a pro-inflammatory milieu[238]. One alluring hypothesis is presented by Abrahamsson *et al.*; if endothelial atypia arises in dense breast tissue, the pro-inflammatory microenvironment would be accommodating these cells to continue carcinogenesis, forming a carcinoma[290]. The complex interplay between the epithelia and the stromal cells, possibly dissimilar in high and low breast density tissue, might also be of great importance in breast carcinogenesis[261].

Mammographic density is associated with an increased risk of local recurrence and of a second primary breast cancer[342-344]. The association between mammographic density and breast cancer survival has been studied with various designs, outcomes and conclusions[345-349]. Biases in many studies are associated with mode of detection and the masking effect related to dense breasts. In a study investigating the potential of pre-operative mammographic density as a prognostic marker for breast cancer, it was shown that high mammographic density was associated with improved survival, mainly in patients older than 50 years, and, when split according to subtype, only in the HR-positive/HER2-negative subtype[350]. The study authors, Hwang *et al.*, believe that their results could partly be explained by clinico-pathological factors (younger age, lower BMI, positive hormone receptor status in high mammographic density patients), treatment factors (more active treatment in high mammographic density patients), and other factors (socio-economical and lifestyle factors)[350]. The predictive value of mammographic density in an adjuvant setting is mainly studied for endocrine treatment and not for chemotherapy.

In conclusion, even though there is strong evidence of mammographic density as a risk factor for breast cancer, mammographic density may not necessarily be associated with the risk of death from breast cancer once it has occurred.

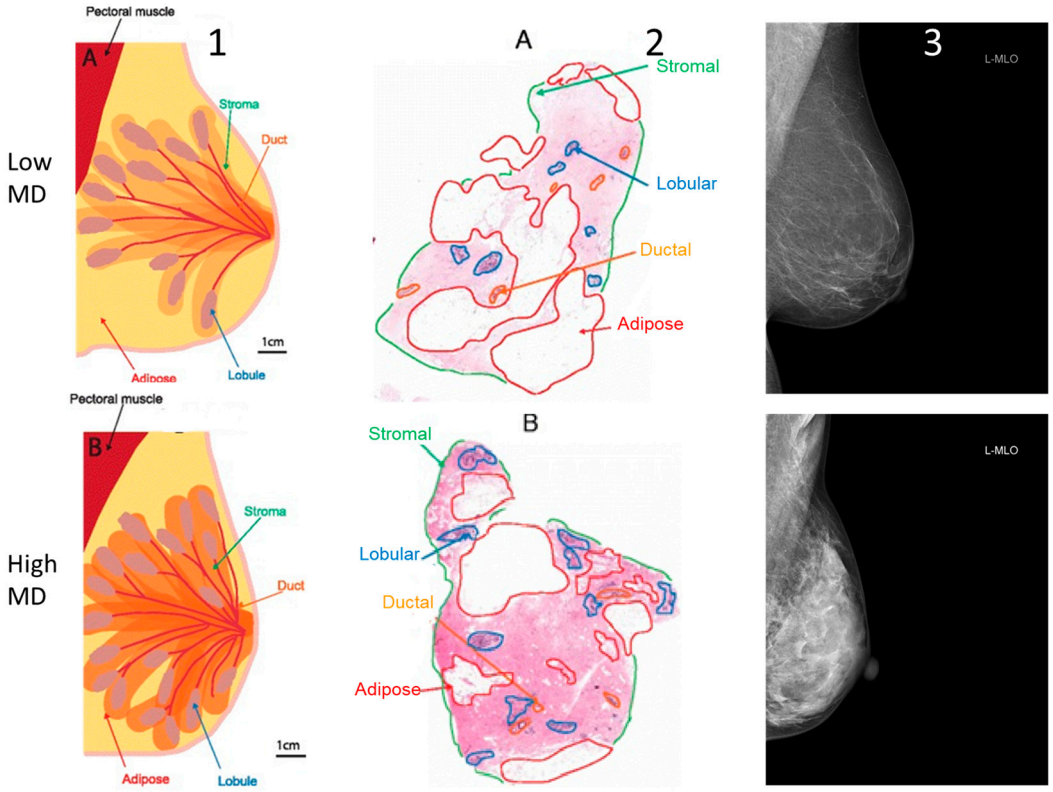

Figure 7. Low and high mammographic density (MD). 1. Schematic picture of breast anatomy. 2. Histology of breast tissue. Paraffin-embedded tissue section, stained with hematoxylin and eosin. 3. Mammograms. Modified image by Sherratt *et al.*, "Raised mammographic density: causative mechanisms and biological consequences", *Breast Cancer Research*, 2016. Permission to reprint under the terms of CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). Mammograms © Region Skåne, SUS and courtesy of Hanna Sartor[351].

Assessment of mammographic density

It is of great importance that mammographic density can be assessed accurately both clinically and in a research setting[352], and many methods, with their respective advantages and limitations, are available. Mammographic density assessment-methods can broadly be classified according to the mode of assessment (visual, semi-automated, fully-automated), whether they are area or volume-based, or whether they are qualitative or quantitative[352]. Mammographic density was originally assessed with area-based methods, and, in an attempt to increase the precision, volumetric methods were introduced. In Table 5, a summary of the assessment methods is presented.

Table 5. Mammographic density assessment methods

Mode of assessment			Method	Output
Visual	Area-based	Parenchymal patterns	Wolfe patterns[353]	4 categories
			Tabar[354]	4 categories
		Qualitative	BI-RADS[355]	4 categories
		Semi-quantitative	Boyd categories[240]	6 categories
			Visual analog scale[356]	Continuous percent
Semi-automated	Area-based	Quantitative	Cumulus[357]	Continuous percent
			Madena[358]	Continuous percent
Fully-automated	Area-based	Qualitative	DenSeeMammo[359]	4 categories
		Quantitative	AutoDensity[360]	Continuous percent
			ImageJ[361]	Continuous percent
			STRATUS[273]	Continuous percent
			Libra[362]	Continuous percent
			MedDensity[363]	Continuous percent
	Volumetric	Quantitative	BD_{SXA}[364]	Continuous percent
			CumulusV[365]	Continuous percent
			Quantra[366]	Continuous percent
			Spectral Density[367]	Continuous percent
			Volpara[368]	Continuous percent

One of the most clinically used qualitative methods of mammographic density assessment is BI-RADS[355]. The 5th version of BI-RADS aims at improving mammographic density assessment by taking into account the masking effect[369].

BI-RADS has four categories[355]: a: “the breast are almost entirely fatty”, b: “there are scattered areas of fibroglandular density”, c: “the breasts are heterogeneously dense, which may obscure small masses” and d: “the breasts are extremely dense, which lowers the sensitivity of mammography” (Figure 8).

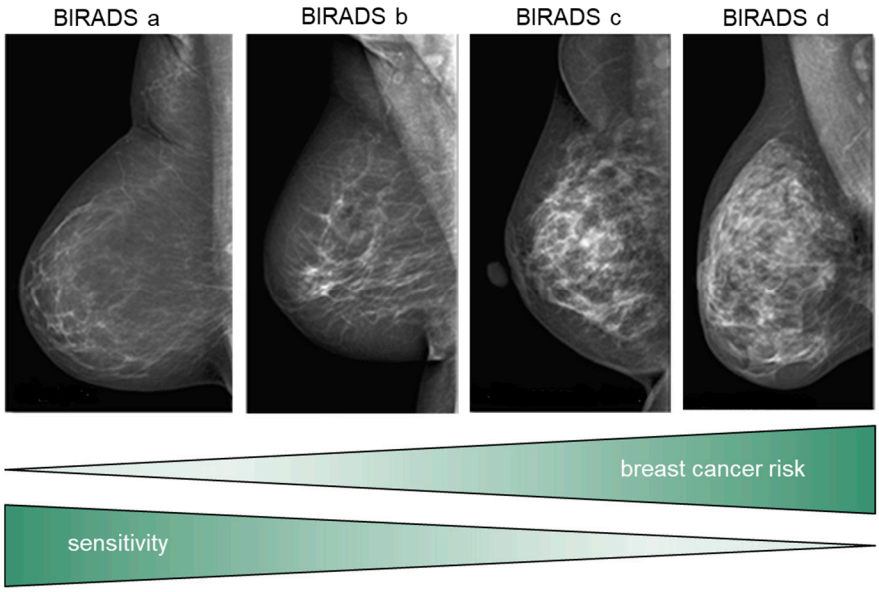

Figure 8. The appearance of mammograms, BI-RADS a-d. Modified image by Kwan-Hoong Ng and Susie Lau, “Vision 20/20: Mammographic breast density and its clinical applications”, *Medical Physicist*, 2016 Permission to reprint under the terms of CC BY 3.0 (https://creativecommons.org/licenses/by/3.0/)

One often-quoted guide of the distribution of mammographic density among women in the mammography practices in the United States (the screening population), using BI-RADS, is: a = 10%, b = 40%, c = 40% and d = 10%[355,370], although other studies have shown a larger proportion of BI-RADS a[300,371] (Table 6).

Table 6. Summary of breast density distribution in the screening population and the sensitivity of mammography (invasive cancer/DCIS) according to BI-RADS classification.

	Percentage of the screening population			**Sensitivity**		
BI-RADS	Posso *et al.*, EJRad[371]	Von Euler-Chelpin *et al.*, Breast Cancer Res[300]	Spauge *et al.*, J natl Cancer Inst[372]	Posso *et al.*, EJRad[371]	Von Euler-Chelpin *et al.*, Breast Cancer Res[300]	Carney *et al.*, Ann Intern Med[373]
	N=177,164	N=54,997	N=764,507	N=177,164	N=54,997	N=329,495
a	24.7%	28%	13.3%	89.2%	78%	87%
b	54.7	40%	43.3%	79.4%	75%	
c	14.0%	27%	35.9%	75%	68%	
d	6.6%	5%	7.4%	67.9%	47%	62.9%

Volpara™ is a Food and Drug Administration (FDA)-approved fully-automated software tool to calculate volumetric density[368]. It is validated against MRI[374,375] and is considered robust during changes in imaging conditions such as vendor[376,377]. Volpara™ uses the 2-dimensional digital mammogram, and with the pre-defined assumption of breast anatomy and information of breast thickness from the image, it creates an artificial volume[368]. The software measured mammographic density in both absolute (absolute dense and absolute non-dense volume) as well as in relative terms (percent dense volume).

Absolute or percent dense tissue

It is debated whether absolute dense tissue or percent dense tissue is a stronger breast cancer risk factor[18,378]. Some studies support increased risk of breast cancer with larger amounts of dense tissue, i.e., more fibroglandular tissue at risk[234], leaning towards absolute dense tissue as the stronger risk factor. Other studies advocate higher risk for percentage density[378,379], an estimate dependent on both dense and non-dense tissue (i.e., mostly adipose tissue), implying that either is non-dense tissue inversely associated with risk, i.e., is a protective factor, or the ratio itself reflects a biological mechanism in breast cancer etiology[234,378]. Regarding non-dense tissue in the breast, i.e., predominately adipose tissue, it is proposed to decrease breast cancer risk by diminishing the possibilities of harmful interactions in the breast between epithelial cells and the stroma[238]. However, results from pre-clinical studies also highlight hazardous properties of fat, including local breast fat[284,285] (please refer to page 41 under the heading "Mammographic density on a cellular level"). Also, the positive association between lobular involution (occurring with aging and during/after pregnancy[70]), associated with decreased breast cancer risk and non-dense tissue[278] could, as an expression for lobular involution, additionally contribute to the inverse association between non-dense tissue and breast cancer risk[378]. It is noteworthy that inconsistency exists in the field; it has, contradicting other studies, been suggested that fat in the breasts increases breast cancer risk[380].

In conclusion, there is no consensus on the most useful measure of mammographic density. As an indicator of breast cancer, perhaps relative measures are preferred; however, absolute density measures might be a better choice when investigating the causes and determinants of breast density[381]. Also, breast density can be assessed with other imaging modalities such as breast-MRI, tomosynthesis or automated whole breast ultrasound (ABUS)[382].

Legislation

Starting from patient advocacy groups in Connecticut, the United States in 2009, a legislative change made it mandatory for the radiologists to notify the patients of their breast density as well as the possible need for complementary imaging[383], the

"Are you dense?"-campaign (https://www.areyoudense.org/). Several states have followed; in 2015, 19 American states had passed the corresponding laws[383], and in 2018, more than 70% of American states had a density reporting law[384]. Since over 40% of US women aged 40 to 74 years are estimated to have dense breasts (BI-RADS c or d)[372], as a consequence of the legislation, breast cancer screening practices have changed; more complementary screening with a substantial increase in follow-up ultrasound[385] and MRI[386,387]. The uncertainty regarding the appropriate next step in breast cancer screening and whether breast density alone is sufficient to warrant further examinations, might have contributed to the temporary decrease in the percentage of mammograms reported as dense immediately after enactment of the density legislation, seen in a large American register-based study of 4 million mammograms[388]. Today in Sweden, mammographic density is considered and reported in the diagnostic setting, but not used or reported in screening[174].

Aims

Overall aims

The biological link between mammographic density and breast cancer is highly complex. The overall aim of this thesis is to address this association from a different point of view; to understand if pharmaceuticals, mainly chemotherapy, can cause a decrease in mammographic density, and thereby study how mammographic density can serve as a predictive biomarker, predominantly tested in the neoadjuvant setting.

Specific aims

Paper I

To investigate the effect of statin on breast cancer risk by studying the association between volumetric mammographic density and statin use in a large screening based cohort of healthy women.

Paper II

To investigate the association between mammographic density assessed qualitatively at diagnosis and the pCR-rate after neoadjuvant chemotherapy for breast cancer in a retrospective cohort.

Paper III

To investigate if mammographic density changes during neoadjuvant chemotherapy for breast cancer in a prospective cohort. Also, to investigate if mammographic density assessed both qualitatively and quantitatively, can serve as a predictive marker of pCR.

Paper IV

To investigate the association between early radiological response by three conventional breast imaging modalities and pCR. In addition, to investigate the agreement between these modalities, and the accuracy of predicting pathological residual tumor burden by imaging.

Patients and Methods

Cohorts

The papers in the thesis are based upon three different cohorts described below: "KARMA", "NeoMon" and "NeoDense"-cohort.

KARMA-cohort (paper I)

The Karolinska Mammography (KARMA)-project[389] for the risk prediction of breast cancer is a multicentre Swedish study and consists of 70,876 women who had either a screening or clinical mammography performed at different study sites. All mammograms were stored in their raw digital format and an extensive patient questionnaire including more than 250 questions covering main breast cancer areas such as reproductive health, medication, substance use, diseases and treatments, heredity aspects, quality of life, physical activity, and diet, were filled in by the study participants. For paper I, only women performing a screening mammogram were included. Exclusion criteria are shown in Figure 9. Finally, a total of 41,102 women made up the study population for paper I and were part of the statistical analyses.

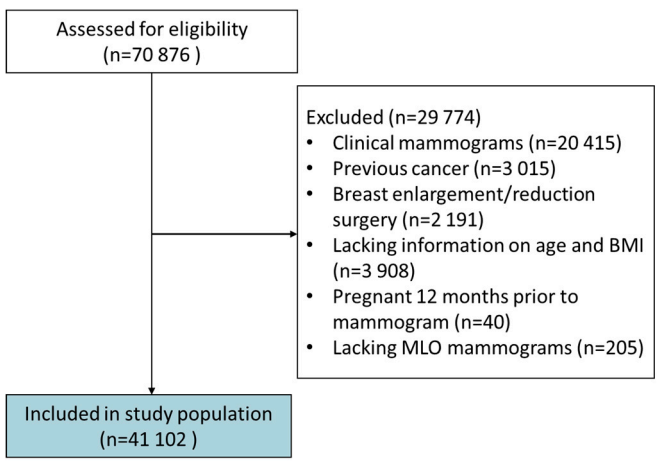

Figure 9. Patients included in paper I, "Karma". MLO: medio-lateral oblique

NeoMon-cohort (paper II)

The consecutive retrospectively gathered NeoMon cohort consists of patients receiving neoadjuvant chemotherapy (alone or in combination with HER2 targeted therapy) for breast cancer from January 2005 to June 2016 at Skåne University Hospital, Sweden. A total of 419 patients were identified through a search in a national cancer database (INCA) (search terms neoadjuvant treatment and breast cancer). In accordance with a regional ethical committee decision, all living patients were asked for consent at the time of the study. Only a minority (N = 8) did not wish to participate: the others were subsequently included in the database. Deceased patients (N = 23) were identified through cross-referencing with the Swedish population registry and were included without consent. Exclusion criteria are shown in Figure 10. Finally, a total of 302 patients were included in the study population and were part of the statistical analyses.

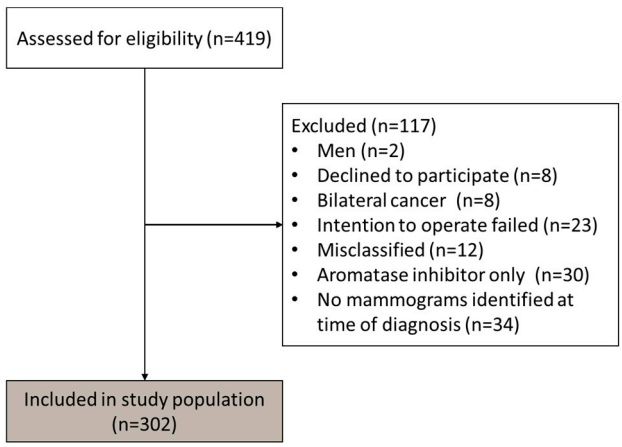

Figure 10. Patients included in paper II, "NeoMon".

NeoDense-cohort (papers III and IV)

During 2014–2019, neoadjuvant treated breast cancer patients at the University Hospital of Skåne, Sweden were prospectively included in the study cohort. Patients were simultaneously enrolled within the SCAN-B trial (Clinical Trials ID NCT02306096, https://www.scan-b.lu.se/), and patients gave informed consent with the possibility of different levels of study participation. The clinical oncologist included patients at the time of breast cancer diagnosis. The reasons for not completing the study are shown in Figure 11. Finally, a total of 200 patients were part of the statistical analyses in paper III, and 202 patients were part of the statistical analyses in paper IV, respectively.

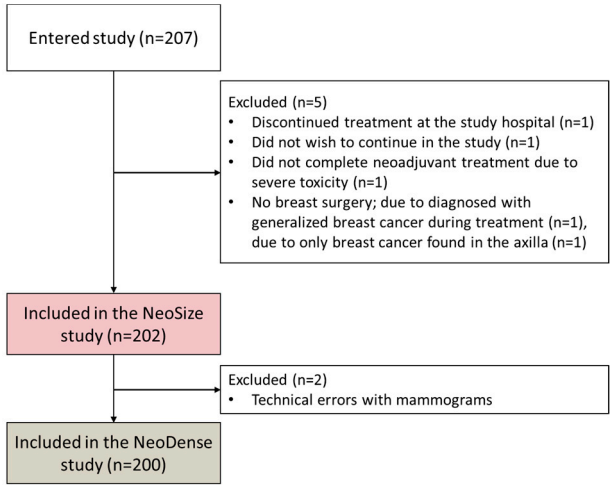

Figure 11. Patients included in paper III, "NeoDense" and paper IV, "NeoSize".

Patient and tumor characteristics

For descriptive statistics in the form of baseline characteristics, but also for adjustment of potential confounders, a wide variety of patients and tumor characteristics were collected/abstracted for all three cohorts.

Patient characteristics

Karma-cohort

Upon inclusion in the Karma study (https://karmastudy.org/), each woman filled in a web-based questionnaire covering: breast cancer risk factors, including a family history of breast cancer. Also, a wide range of baseline characteristics anthropometrics, habits regarding alcohol and smoking, level of education, reproductive and hormonal factors (age at menarche, number of births and age at first birth, menstruation status, use of oral contraceptives, and HRT).

Data were also retrieved through linkage with Swedish national registers (inpatient care, prescriptions of pharmaceuticals, cancer, and causes of death).

NeoMon-cohort

Data were manually retrieved from digital medical charts.

NeoDense-cohort

At the time of diagnosis/study inclusion, each patient filled in a questionnaire with question covering anthropometrics, lifestyle, hormonal events (menarche, age at first birth, number of children, menstruation status, current and former use of oral contraceptives and HRT), alcohol and smoking habits, history of breast disease, and use of prescribed drugs.

Tumor characteristics

In papers II–IV, tumor characteristics were derived from clinical pathology reports. A summary is presented in Table 7.

Estrogen and progesterone receptor

There is not yet a global consensus among clinicians and scientists regarding what cut-off level should be used classifying a tumor as ER/PR positive or negative. The American Society of Clinical Oncology and the College of American Pathologists recommends that tumors are considered positive when at least 1% of tumor nuclei are stained positive in the specimen[390]. Objections to this have been that tumors with low-ER (1–9% staining cells) show more clinic-pathological and molecular similarities with ER-negative tumors[391,392]. Since our study cohorts partly pre-dated the recommendation of using 1% as a cut off[390], different local clinical guidelines were used for reporting receptor positivity during the study´s time span of eleven years. We used the current Swedish guidelines[174] of 10% cut-off for both ER and PR in order to dichotomize receptor status the same way for each study participant.

Ki67

There is no doubt that Ki67 carries strong prognostic information[393]. Regarding Ki67 as a predictive marker, some uncertainty exists for the adjuvant setting[394,395], whereas the data that support Ki67 as a predictive marker in the neoadjuvant setting are stronger[396-398]. Ki67 is used in order to discriminate luminal A-like from luminal B-like[10], but there is a matter of controversy regarding the optimal cut-off, due to interlaboratory variations[399] and different discriminatory cutoffs are proposed in the literature[123,124]; the consensus of the 12th St Gallen conference was that Ki67<14% should be considered as "low"[10], however, 2 years later the panel voted for a threshold of 20%[400]. Several groups have later demonstrated that 20%, the cut-off used in paper II, is a reliable cut-off of Ki67 to stratify patients with luminal-like breast cancer[124,401,402] and identifying high-risk patients[403].

It is suggested that laboratory-specific cut-offs for Ki67 are used and the tumors later categorized as low, intermediate or highly proliferative[404], a methodology used in the NeoDense-cohort (paper III and IV).

pCR

In NeoMon and NeoDense, pCR was defined according to current guidelines[405] in the following way: the absence of any residual invasive cancer in the resected breast and all sampled regional lymph nodes. However, in NeoSize, our main focus was on the radiological response in the breast only, and we, therefore, used a modified definition of pCR not accounting for the existence of lymph node metastases, "pCR_{breast}".

Table 7. Patient and tumor characteristics.

	Paper I "Karma"	**Paper II "NeoMon"**	**Paper III "NeoDense"** **Paper IV "NeoSize"**
Patients characteristics	Web-based questionnaire (by the patient)	Medical charts (by the researcher)	Questionnaire and medical charts (by patient and researcher)
Menopausal status	According to a detailed questionnaire on menstruation status, when lacking information postmenopausal when ≥55 years	According to medical charts, when lacking information considered as postmenopausal when ≥55 years	According to a detailed questionnaire on menstruation status, when lacking information postmenopausal when ≥55 years
BMI	Self-reported length and weight	Derived from medical charts	Self-reported length and weight
Tumor characteristics	Not applicable		
ER/PR		*Positive: >10% Negative: ≤10%	Positive: ≥10% Negative: <10%
Ki67		High: >20% Low: ≤20%	Low, intermediate or high. Local laboratory-specific cut-offs.
HER2		Positive if the tumor was assessed as 3+ with immunohistochemistry and/or amplified with *in situ* hybridization	Positive if the tumor was assessed as 3+ with immunohistochemistry and/or amplified with *in situ* hybridization
pCR		No invasive cancer in neither breast nor axilla	NeoDense: No invasive cancer in neither breast nor axilla NeoSize: No invasive cancer in the breast. "pCR_{breast}"

*No patient had ER/PR=10%; thus, this slightly different cut-off has not influenced the comparison of the results.

Neoadjuvant chemotherapy

The standard neoadjuvant chemotherapy treatment contained three series of FEC/EC followed by three series of taxanes (docetaxel or paclitaxel) and, in the case of HER2-overexpressing tumor, combined with HER2-blockade. A summary of the received treatment of the study participants in papers II–IV is presented in Table 8.

NeoMon-cohort

A total of 96% of the patients received neoadjuvant chemotherapy containing taxanes. Of the 95 patients with HER2-overexpressing tumors, a total of 97% (N =

92) received a single anti-HER2 treatment (trastuzumab), while the remainder (N = 3) received no anti-HER2 treatment.

NeoDense-cohort

Ninety-seven percent of the patients received standard treatment (paper III: 194 out of 200, and paper IV 196 out of 202), five of the patients received a taxane-only chemotherapy-regimen, and one patient received EC only. Among the patients with HER2-overexpressing tumors, 94% (paper III: 45 of 48 for and paper IV: 46 out of 49) received a double HER2-blockade whereas the remainder (N = 3) received only trastuzumab.

Table 8. Neoadjuvant chemotherapy in the NeoMon and NeoDense-cohort.

	Treatment	**Paper II "NeoMon"**	**Paper III "NeoDense"**	**Paper IV "NeoSize"**
All	FEC/EC + taxane	240	194	196
	Taxanes	50	5	5
	EC/FEC	8	1	1
	Other	4	-	-
		N=302	N=200	N=202
HER2+	Single HER2-blockade	92	3	3
	Double HER2-blockade	-	45	46
	No HER2-blockade	3	-	-
		N=95	N=48	N=49

Breast imaging

A summary of breast imaging features of papers I–IV is presented in Table 9.

Karma (paper 1)

Raw-data ("for processing") mammograms were saved. Volpara^{TM} was used for mammographic density assessment, and we used the average score from both breasts (of the MLO view) in our statistical models.

NeoMon (paper II)

Processed mammograms were collected from the Picture Archiving and Communication System and were retrospectively evaluated by a radiologist (HS) and categorized according to BI-RADS 5th Edition, accounting for all available views in both breasts. The reviewing radiologist (HS) was blinded to all patient and tumor characteristics besides the name and birthdate of the patient.

NeoDense (paper III)

In cooperation with Karolinska Institutet, using their already existing server-solution for women in the large KARMA-study, we saved all raw data mammograms as well as processed mammograms from the examinations as part of the study. Breast tomosynthesis images were saved in a separate server. In real-time, the clinically assigned radiologist filled in a study-specific form with detailed information on bilateral cancer, multifocal cancer, tumor size, and axillary lymph node status by imaging as well as categorized each patient according to BI-RADS 5th Edition. At the time of data-management, using the automatic software Volpara^{TM}, density analysis was carried out. The CC and MLO-views in both breasts and the contralateral healthy breast only, respectively, were used. Both volumetric breast density percentage (VBD%), a continuous variable calculated as the ratio of absolute dense tissue volume to total breast volume, and the absolute dense tissue volume were calculated.

NeoSize (paper IV)

The same study-specific forms, as in paper III, were used. Patients were categorized according to radiological response as follows: complete radiological response representing no visible tumor and a partial radiological response indicating a $\geq 30\%$ decrease in the (largest) diameter of the largest foci (modified after RECIST criteria, version 1.1.[406]); otherwise, they were classified as radiological non-responders. The concept of early radiological response refers to the radiological response after two cycles of neoadjuvant chemotherapy compared to baseline (early radiological responders: $T1 \leq 0.7 \times T0$).

The tumor size assessments were based on three-view mammograms, ultrasound, and one-view (MLO), wide-angle digital breast tomosynthesis.

Table 9. Summary of imaging features.

	Paper I "Karma"	Paper II "NeoMon"	Paper III "NeoDense"	Paper IV "NeoSize"
Breast imaging modality	Mammography	Mammography (ultrasound for tumor size)	Mammography (ultrasound for tumor size)	Mammography, breast tomosynthesis, and ultrasound
Main radiological feature	Mammographic density	Mammographic density	Mammographic density	Tumor size (largest diameter)
Are raw image data available?	Yes	No	Yes	Yes
Method of mammo-graphic density assessment	Volpara™	BI-RADS	Volpara™ BI-RADS	Not applicable
View	MLO	All available views	Three views (only CC, MLO for density analysis)	Mammography: Three views Tomosynthesis: MLO
Density assessment in both breasts?	Yes	Yes	Volpara™: Analyses with contralateral and bilateral breasts, respectively. BI-RADS: contralateral breast	Not applicable
Multifocal BC	Not applicable	No data, largest foci used in the analyses	N=25 on mammography, only the largest foci in analyses	N=25 on mammography, only the largest foci in analyses
Bilateral BC	Not applicable	Exclusion criteria, N=8. Both breasts are included in density assessment	Bilateral BC (N=4), the breast with the single largest foci included	Bilateral BC (N=4), the breast with the single largest foci included

Statistical analysis

Statistics is essential to all medical research. Through the art of collecting, organizing, and presenting data, we can estimate associations and test hypotheses. Statistics is used to formally manage the data, taking several levels of variation into account. In this section, statistical terms and concepts are explained, and in the end, Table 12 on page 68 summarizes the statistics used in each paper of this thesis.

Normal distribution

Many statistical methods/tests are based on the assumption of the normal distribution, referring to a symmetrical bell-shaped distribution where the mean, median and mode values are identical. When these assumptions are met, a parametric test can be used (e.g., the Student T-test), and otherwise, a non-parametric test can be used (e.g., Mann-Whitney or Kruskal-Wallis test). Also, the non-normally distributed variable can be transformed in a number of ways to

approximate the normal distribution. Logarithmic transformation (e.g., for the mammographic density measurements in paper I) can sometimes be used to approximate the normal distribution. In order to make the results easier to comprehend after such logarithmic transformation, when reporting the results from log-transformed variables, they are often transformed back (anti-logs or exponentiation), resulting in a geometric mean. After the exponentiation of the corresponding 95% confidence interval as well, the values will not be symmetric about the geometric mean on the natural scale. The geometric mean is thus a special type of average, using the product of the values, and is often defined as the n^{th} root of the product of n numbers.

In paper I, differences in categorical or categorized clinical variables were tested with the $\chi 2$ test, and for continuous variables, the Student's T-test was used. In paper IV, the $\chi 2$ test was used when analyzing pCR-rate by radiological early response.

Level of significance

The *p*-value is a value between zero and one, and is defined as the probability of obtaining a result at least as extreme as in the study if the null-hypothesis (there is no difference between the groups) is true. Often the significance level is arbitrarily set at $\alpha = 0.05$, implying a 5% risk of falsely rejecting the null hypothesis when it is true (type I error). Reporting a point estimate and a confidence interval is preferred over *p*-values since they tell us not only if the difference is significant, they give an estimation of the direction, magnitude, and uncertainty of the effect, enabling interpretation of clinical relevance.

ANOVA (Analysis of Variance) and ANCOVA (Analysis of Covariance)

In paper I, we used ANCOVA to address the question of an association between statin use and volumetric breast density. Simply put, ANCOVA is a statistical model that combines ANOVA with regression. ANOVA is used to compare the means of two or more groups (and requires at least one continuous variable and one nominal variable). Instead of an ANOVA, one could use multiple t-tests between the different groups, but we would then run into difficulties with each test having its own α-level and error compounds with each test, causing α-inflation[407]. ANOVA is a variability ratio expressing the association between variance between each subgroup and the general mean and the variance within each subgroup ("variance between"/"variance within"). Given that the ANOVA test is significant, and that we have three or more groups, one would need follow-up tests called *post hoc* tests, e.g., Bonferroni Tukey, Newman-Keuls and Scheffé, since ANOVA does not tell where in the difference lies[407].

Subgroup analyses and interaction

In a study, we often want to investigate if the observed relation is different across subgroups (e.g., is there a difference for premenopausal vs. postmenopausal patients). It might be interesting to look for heterogeneity of an effect, i.e., is an effect modified by the value of another variable. This is what the term interaction refers to and can commonly be interpreted as synergy, or the opposite, between variables. In a statistical model (e.g., logistic regression or ANCOVA) with many confounders, the potential interactions are plentiful. Even if we do not believe that the included variables are truly independent of one another, in order to steer away from "significance fishing", we should abstain from such exploratory testing. On the contrary; if we have previous knowledge or suspicious of a particular interaction, we should rightly include it in our model, as we did in paper I with statin and HRT. I believe models should be pre-specified; interaction terms in statistical models can often be (statistically) insignificant because few patients will have both/all conditions. Also, statistical and biological interaction are two different things, and the focus should be on what is biologically important.

Logistic regression

The term regression refers to a statistical model displaying the association between a dependent variable (outcome) and independent (explanatory) variables. Logistic regression is a common method when the outcome is (often) a binary variable, e.g., pCR in paper II and III, and gives an odds ratio (OR) of the outcome with a confidence interval, comparing levels of the independent variables. Although not applicable in this thesis, multinomial logistic regression can be used with ordinal or nominal outcome[408]. In logistic regression, we can control, or adjust for a number of potential confounders, e.g., HRT or ER-status in papers II and III, i.e., we can build a model where we ideally remove the effect of the confounders from the result.

Diagnostic precision

A diagnostic test is usually a test (or procedure) to classify individuals as positive ("sick") or negative ("healthy"). The perfect test would correctly identify all sick persons as sick (100% sensitivity) and correctly identify all healthy persons as healthy (100% specificity); conditions seldom met in clinical tests. There is always a trade-off between sensitivity and specificity, and often we can decide on which of the two is prioritized, e.g., deciding on a cut-off for when a laboratory test value is considered pathological. In clinical practice, when facing a test result in a patient and deciding on course of action, it is of interest to answer the questions: "What is the likelihood that this patient has the disease given that the test result is positive?" (positive predictive value) and "What is the likelihood that this patient does not have the disease given that the test result is negative?" (negative predictive value). Positive and negative predictive value depends on the sensitivity, specificity and

notably, the prevalence in the population. If the prevalence of a disease is very low, e.g., breast cancer in a mammography screening population, a large proportion of women with a positive test result will actually be healthy (the positive predictive value will not be near 1). In paper IV, we presented test characteristics for three conventional radiological imaging modalities in terms of the association between radiological complete response (test variable) and pCR post-neoadjuvant chemotherapy (gold standard) (Table 10).

Table 10. Association between rCR (test variable) and pCR (gold standard).

	pCR	**Non-pCR**
rCR	True positive (A)	False positive (B)
Non-rCR	False negative (C)	True negative (D)

Sensitivity: $A/(A+C)$, Specificity: $D/(B+D)$, positive predictive value: $A/(A+B)$, and negative predictive value: $D/(C+D)$

Correlation and agreement

When comparing two tests with one another and no gold standard or "truth" is available, other methods are needed. Correlation and agreement are two related but different statistical terms. A perfect linear association between x and y ($y = x$, $y = 0.75x$) will both have a perfect correlation ($R^2 = 1$), but their agreement will not be the same (Table 11). Possible ways of assessing agreement are the paired t-test (parametric) or Wilcoxon's matched-pairs signed-rank test (non-parametric); calculating the slope of the trend line in a scatterplot, or as done in paper IV, a Bland-Altman plot. Kappa or Cohen's Kappa is a statistic model ranging from -1 to 1, measuring the agreement between classifiers, which accounts for agreement by chance. The interpretation of a kappa (κ)-value is as follows: complete agreement κ = 1, a level of agreement that would be expected by chance alone then κ = 0, and last, less agreement than would be expected from chance alone, κ < 0[409].

Table 11. Correlation and agreement.

	Correlation	**Agreement**
Equation	**Pearson's correlation (R^2)**	**Paired t-test**
$y = x$	1	No significant difference
$y = 0.75x$	1	Significant difference

Bland-Altman plot

In paper 4, several Bland-Altman plots were presented, and this is a short guide of how to interpret these graphs. The advantage of Bland-Altman-plots is that it graphically shows systematic differences between the variables. Bland-Altman plots are used when comparing two measurements, x and y, that are supposed to be the same[410]. In a Bland-Altman plot, the difference ($y = a - b$) between two methods are plotted against the mean value ($x = (a+b)/2$) in a scatter plot (Figure 12).

The 95% limits of agreement and the mean difference summarize how far apart measurements by the two methods are[411]. The plot highlights differences, e.g., if one of the methods systematically over- or underestimates a result, compared to the other and if (dis)agreement is dependent on high or low mean values[412].

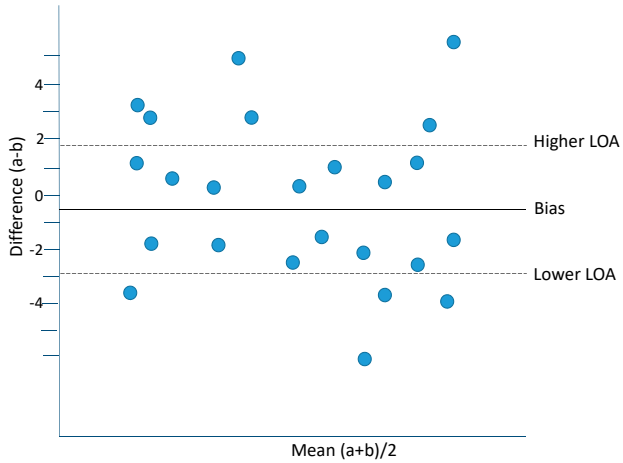

Figure 12. Schematic picture: Bland–Altman plot with limits of agreement (LOA).

If one of the methods is the reference method, "gold standard", one can use these values instead of the mean. However, this is debated, since a plot of the difference against a "gold standard" will always show a relation between difference and magnitude when there is none[411]. In paper IV, we, therefore, chose to have the mean of the two methods at the x-axis, even when comparing radiological tumor size against pathological tumor size.

Missing data

In epidemiological and clinical studies, missing data is almost unavoidable. It is important to account for missing data and how they are dealt with. Exclusion of cases with missing data can exclude a substantial proportion of cases, and thereby compromise conclusions from clinical studies due to decreased power and less precision. There is a risk of bias due to missing data since there might be a systematic reason for the missingness. The reasons for missing data are often classified as: "missing completely at random", "missing at random", and "missing not at random"[413]. When data is "missing at random", but not "completely at random", analyzing complete cases only might bring bias into the results. Different statistical strategies have, therefore, been developed to handle missing data. One way is to replace missing values with what is assumed best- and worst-case scenarios. This, however, creates a (too) wide range of estimates, and another method is multiple imputation.

In paper I, missing data was assumed to be "missing at random", and multiple imputation was used. Multiple imputation is the process of replacing missing data with replaced values several times, thus making multiple copies of the completed dataset. Each dataset can then be analyzed, and the results can be aggregated; the imputation itself is a statistical technique for sampling values of the variables based on associations with other variables in the dataset[414]. In paper II, analysis of complete cases only was done, and since all data came from medical charts and none was self-reported, we assumed variables to be missing "completely at random". In paper III and IV, the number of missing data was very small and here too, only analyses of complete cases were done.

Confounders

In papers I–III, a number of predefined confounders were accounted for. A confounder is a "third factor" that might be partly or wholly responsible for an observed association between the exposure and the outcome[415], and in data analyses, researchers want to adjust for such variables. It is problematic defining a confounder; an often used definition of a confounder is a variable being associated with both the exposure, and the outcome (conditional of the exposure) in the population and is not in the causal pathway between the two[416]. However, this definition is not complete; and more comprehensive definitions are proposed[417], though out of scope for this thesis. Decisions on included confounding variables should not be based on statistical grounds only (e.g., a factor change the estimate by more than 10% or stepwise selection) since statistics cannot: 1) Draw conclusions on temporality, or 2) Differ between a confounder and a mediator[415]. Prior knowledge should influence the choice of confounders[416]. In epidemiology, to eliminate a false association, one can adjust or stratify on common causes[416]. Stratifying means that we produce subgroups within which the confounder does not vary and then evaluate the association between exposure and outcome within each stratum[418]. Several techniques can be helpful when accounting for confounding; however, it is still challenging to fully exclude the impact of remaining confounding, especially since confounding may be unknown to the researcher and/or difficult to identify/measure[419]. Lastly, lacking consensus of potential confounding variables in epidemiological studies might lead to inconsistent results and reduced reproducibility[419].

In papers I–III, confounding variables were chosen based on *a-prior* knowledge (and practice in adjacent studies) of plausible biological associations. For example, in the field of mammographic density, age, BMI, parity, and menopausal status are standardly considered important confounders[420].

Table 12. Statistics of paper I-IV - in summary

Paper	Study design	Methods	Outcome measure	Independent variable ("exposure")	Subgroup analyses	Confounders	Missing values
I "KARMA"	Cross-sectional	Characteristics of statin users: $\chi 2$ for categorial, T-test continuous ANCOVA to examine the association between statin use and mammographic density	Volumetric breast density	Statin	Statin class and statin duration	Four models with an increasing number of adjustment factors. BMI, density confounders (hormonal, lifestyle factors), co-medication	17% had missing values in one or more covariates, multiple imputation (missing at random)
II "NeoMon"	Retrospective cohort study	Logistic regression models: estimate OR (95%CI) for association BI-RADS and pCR.	pCR (dichotomous)	BI-RADS 5th Edition	Menopausal status	Three models with an increasing number of adjustment factors: crude, minimally and fully adjusted.	Only complete cases in the logistic regression models
III "NeoDense"	Prospective cohort study	Logistic regression models: estimate OR (95%CI) for association BI-RADS and VBD%, respectively, and pCR.	pCR (dichotomous)	BI-RADS 5th Edition VBD% (static and dynamic)	Menopausal, ER, and axillary lymph node status	Three models with an increasing number of adjustment factors. Crude, minimally and fully adjusted.	Only complete cases in the logistic regression models
IV "NeoSize"	Prospective cohort study	Bland-Altman-plots, test characteristics, kappa statistics, $\chi 2$	pCR (dichotomous), remaining invasive focus	Imaging tumor size, radiological complete response	None	Not applicable	Only patients with visible tumor with all three modalities post-treatment included in statistics for test characteristics

Ethical considerations

The studies were performed in accordance with the Helsinki declaration and its later amendments and were approved by the Regional Ethics Committees.

Paper I was approved by the Regional Ethics Committee in Stockholm, and paper II-IV were approved by the Regional Ethics Committee in Lund (Dnr 2014/13, 2014/521 and 2016/521).

Methodological considerations

Errors in estimation can either be random or systematic; the latter is often referred to as bias. Validity is most easily explained as the opposite of bias; an estimate with little bias is a valid estimate. In analogy, precision is the opposite of random error. Accuracy comprises precision and validity[421].

Study design

All papers, as part of this study, are observational studies. Paper I is based on a prospective cohort, and the nature of this particular study is cross-sectional with its inherent limitations, most notably the lack of temporal associations. Paper II is a retrospective cohort study, meaning that the cohort was identified retrospectively, and data were abstracted at the time of the execution of the study. However, it is worth mentioning that (most of) the data are still prospectively collected by the clinician in that we went through medical charts rather than asked someone (patient/relative/doctor) what the patient's, e.g., BMI was back then. Paper III and IV are based on the same cohort and are prospective cohort studies (Figure 13). An advantage with the prospective design is the pre-defined questions and collection of specific data hereafter. On the other hand, it is more time-consuming and requires long durations of follow-up[422].

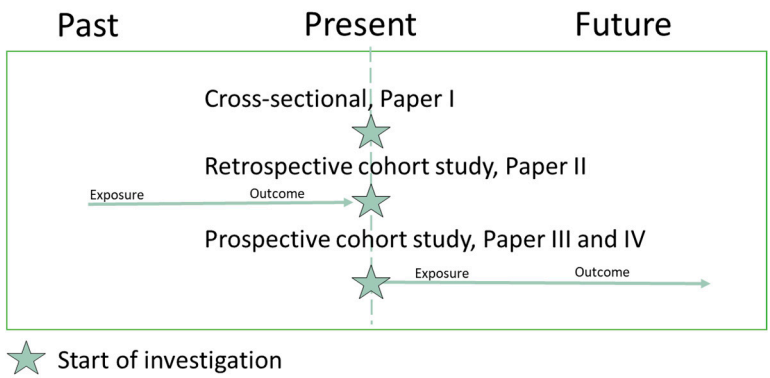

Figure 13. Study design of papers I–IV.

p-value and Type I/II errors

Finding differences/effects between study groups through statistical tests is essential to medical research. What is more important, though, is to know if our finding is applicable in the underlying population; are our results clinically useful? A way of reducing random error, and thereby increase precision, is by making the study population larger. Statistical inference, meaning drawing statistical conclusions about some population-based on studies of a sample from that population, is done by calculating the probability that chance alone might have accounted for the study results. In this context, three statistical concepts are important; effect/point estimate, confidence intervals and *p*-value.

In null hypotheses significance testing, we start our statistical analyses by specifying the null hypothesis, typically assuming that there is no difference between groups. We then apply our different statistical methods in order to test if the null hypothesis can be rejected.

A *p*-value is a number between 0 and 1 and measures how consistent the study result is with the null-hypothesis and answers the question: What is the probability of observing an effect as strong, or stronger, like the one we found in our results, if every model assumption was correct[423], including the null-hypotheses? The degree of acceptable certainty, the level of significance, is often arbitrary set at $\alpha = 0.05$.

There has been a shift in the scientific world with a more critical approach towards "statistical significance"[423]. The main critic is the dichotomization as a statistically significant result or not, and the conclusion of "no difference/association" is based on a *p*-value being larger than a certain threshold (e.g., 0.05)[424]. A shift towards confidence intervals and estimation[424,425] is an improvement, although thoughtful interpretation is warranted. Therefore, in papers II–IV, we were very restrictive with presenting *p*-values. In Paper I, however, multiple *p*-values are presented, and it is possible that, if the paper was to be re-written today, less focus would have been on the *p*-values and more on the effect sizes.

When performing hypothesis testing, there is always a risk of two errors to occur: types I and II errors. Type I error is related to the level of significance (α) and happens when one wrongly rejects a true null-hypotheses. Simply put, a type I error is a "false positives" - the test shows a statistically significant difference even though there is no difference. Another way to look at it, type I error can be thought of as *overreacting* to results that are actually caused by chance alone. Type II errors, on the other hand, are "false negatives". It happens when one wrongly accepts a false null-hypotheses. Type II errors can thus be thought of as *underreacting.* The probability of type II error is denoted β and depends on the power of the study (β = 1- power).

Statistical significance is not the same as clinical significance, and in many epidemiological studies, it is sometimes implausible that there is no effect at all; the question is whether the effect is clinically meaningful and that the estimates are precise enough to make you feel confident enough to draw firm conclusions. In paper I, the main conclusion "no evidence of an overall effect of statin use on absolute dense volume"[329], based on a *p*-value of 0.06, is, in my current opinion, a bit too categorical, and this reasoning *per se* sets grounds for a potential type II error. On the other hand, the finding/conclusion of a modification effect by HRT (*p*-value 0.03) could, in theory, correspondingly be a type I error. For further details, please refer to section "Effect size", on page 79. You can never for sure know when you run into a type I or type II error; however, a safe way to avoid them is to abstain from null hypothesis significance testing.

The power of a study is the likelihood that it will detect an effect as statistically significant when there is actually an effect to be detected. If a study is under-dimensioned (low statistical power), we might reject results as null even if there is an important finding, and possibly even more important: the lower the power, the higher the risk that the statistically significant effects are overestimated[426]. I believe this phenomena "effect inflation/exaggeration" as well as type I and II errors, are avoided in paper II–IV; we have most certainly not found true/exact effect estimates, but we have presented the actual estimates, and as such, we have not reduced our results to the overly simplistic statement that there is an/no association.

Statistical power should be seen as a tool when planning a study, and should not be used when interpreting test-results[423]. A small difference (effect size) in groups with large variability is harder to find/detect as a statistically significant difference, in comparison to larger differences, thus, requiring a larger sample size if statistical significance is the goal. Power estimations have several drawbacks; mainly, it is connected with the dichotomous outcome statistical significance, not recognizing continuous findings/measures. As an alternative, study size can be based on desired precision (i.e., a width of a confidence interval)[427].

In epidemiological, registry-based, studies, such as paper 1, we often have very little control over the number of patients, and power calculation *per se* is not applicable. However, the concept can help us decide whether it is worthwhile conducting the study or not. In paper II, all patients meeting the inclusion criteria were included (after consent), and hence no power calculation was performed. As for the cohort in paper III and IV, the number of patients included was a balance between power and time; the 200 patients were collected during several years, and longer inclusion time was not possible in order to conduct the study within the framework of this PhD. One can ask if it is correct to abstain from research just because there are not very many patients – and how will this affect research on rare disorders and/or study sites with a relatively small number of patients.

Validity

Validity can be divided into internal validity (study sample vs. target population) and external validity (vs. a more general population - generalizability). Confounding, selection bias and information bias, three forms of systematic errors, are all violations of the internal validity. Systematic errors, in contrast to random error, do not disappear when the sample size is increased[421]. Confounding is already accounted for in the previous chapter ("Confounders" page 67), and here the other two forms of bias will be discussed.

Selection bias may occur when there is a systematic difference between a characteristic of study participants and those who are not part of the study. It is well known that people with better health status are more prone to participate in studies. In paper I, there are two steps of selection bias: only women attending screening (or a clinical mammogram) could be included in the cohort; and women then visiting the mammography units actively chose to participate in the KARMA cohort. We expect that women in the KARMA cohort generally have higher education and are more likely to have a family history of breast cancer in comparison to women invited to a screening in Sweden[389]. In paper II, the patients were retrospectively identified through the Swedish cancer register. It is mandatory by law for healthcare to report to this register. Patients were informed by mail and asked to communicate if they did not wish to participate (N = 8). Thus, in paper II, the selection bias is assumed to be negligible. In papers III and IV, only patients judged to be able to give informed consent and fully understand the oral and written (only in Swedish) information provided to them. This procedure thus excluded patients not speaking Swedish, predominantly immigrants with a lower socioeconomic status.

Inaccurate information collection of study participants is called information bias. In paper I, the study participants independently filled in a web-based questionnaire with its inherent limitations and strengths, e.g., self-reported alcohol consumption, especially high consumption[428], might not be as accurately/truthfully answered as the number of births. Other data came from national registers (such as the Swedish Prescribed Drug Register) and are therefore believe to be associated with as little information bias as possible in the cohort. In paper II, only one researcher made a thorough review of the patients' medical charts, and data was filled in according to pre-specified algorithms. Also, in paper II, only one radiologist evaluated all the BI-RADS categorizations. In papers III and IV, the patients filled in a paper questionnaire with detailed information on anthropometrics, hormonal events, prescribed drug use, personal medical history, and family history of breast cancer. Information on tumor characteristics was retrieved from clinical pathology charts. Radiological data was retrieved from study-specific forms, filled in by the clinically assigned radiologist at each examination. The different radiologists were at group meetings instructed on how to fill in the forms. However, since it is a question of individual judgment of the findings by the radiologist, information bias regarding

the radiological data is inevitable, with the exception of the volumetric density assessment as it was performed using the fully-automatically software Volpara™. For secure data entry, the Research Electronic Data Capture[429] application was used. Only one researcher (IS) entered data to the database, except for the pathology parameters, for which it was two researchers following the same algorithms for data entry.

Paper I is based on a national, multicentre cohort, and the external validity to the Swedish population in screening-age (40–74 years old) is believed to be acceptable (selection bias, as previously mentioned, slightly reduces the external validity). Papers II–IV are based on cohorts from one-site (Skåne University Hospital) only, thus reducing the external validity. However, the oncological treatment for these patients followed the national guidelines, and there are no major reasons for questioning the generalizability to a Swedish national level. Hence, I do not believe that the (biased) selection resulted in (much) biased estimates. I believe that the conclusions of the associations between exposure and outcome would not be different if I could convince everyone to participate in the studies. Thus, although the populations under study are selected, I believe estimates from the populations still are valid/generalizable. Breast cancer is a global concern with different patients- and tumor characteristics across the world[5,430], as well as differences in access to (early) diagnostics and treatment in different parts of the world, and therefore the global generalizability of our results remains to be elucidated.

Lastly, but most importantly, when evaluating the validity of a study, the statistical associations and the clinical relevance must both be carefully interpreted[431]. A clinically meaningful effect size (or clinically meaningful difference/minimally important change), refers to a change in the outcome that is considered "worthwhile" for the clinician/patient or would result in a change in treatment decisions[432].

An overview of strengths and limitations in each paper is presented in Table 13.

Table 13. Strengths and limitations in papers I–IV.

Paper	Strengths	Limitations
I "KARMA"	The large, well-characterized cohort with detailed patient information through an extensive web-based questionnaire. Reliable registry information on pharmaceuticals. Access to information on statins sold and dispensed by prescription at pharmacies, not only prescribed statins. Access to raw mammograms enabling volumetric density with Volpara™: measurement and analysis of both dense, non-dense, and percent dense volume. Statistical stringency: Analyses followed a pre-specified statistical plan; the main objective was set up before analysis. Multivariable analyses are adjusting for other known risk factors. Multiple imputation was used for missing data.	A cross-sectional study limits the ability to account for any temporal association between statins and mammographic density. No available data on statin dosage. Due to the population-based prescription register, not reaching full coverage until 2005, we could not study the long-term effects of statins.
II "NeoMon"	Detailed patient and tumor information retrieved from electronic medical charts. Statistical stringency: The analyses followed a pre-specified statistical plan, and the main objective was set up before analysis. Multivariable analyses are adjusting for other known risk factors. One person created the database, hence minimal collecting bias. One experienced radiologist made all the BI-RADS classifications blinded to all other data.	Mammograms were restricted to processed images, with its limitations. Complete cases only in logistic regression. Few patients within the extreme BI-RADS categories (a and d), creating small reference groups. No subgroup analyses are based on breast cancer subtype. A single-site study, reducing external validity.
III "NeoDense"	Prospective well-defined cohort Detailed patient information through patient questionnaires and search in electronic medical charts. Extensive tumor information from pathology reports. Assess to raw mammograms enabling volumetric density assessment using the fully-automated software Volpara™. Statistical stringency: The analyses followed a pre-specified statistical plan, and the main objective was set up before analysis. Multivariable analyses are adjusting for other known risk factors. A very small number of missing values.	Mammograms were retrieved from three different vendors, and no subgroup analyses were made based on vendor. No subgroup analyses are based on breast cancer subtype due to a limited number of patients. A single-site study, reducing external validity.
IV "NeoSize"	Same as paper III. However, statements of density measures and multivariable adjustments are not applicable.	Same as in paper III, also: No analyses of inter-observer variability Only one-view tomosynthesis, no MRI.

Results and discussion

In this chapter, the main results of each paper are presented and discussed. For more detailed results and discussion, please refer to each paper, respectively.

Paper I

Results

Statin-users were more likely to be older, postmenopausal, to have a higher BMI, and have a smoking history compared to non-users. Also, they more often reported co-medication use (i.e., HRT, metformin, and aspirin-use).

Statin users had a lower percent dense volume than non-users (p-value < 0.001); this was interpreted as due to the influence of a larger absolute non-dense volume in statin users (p-value < 0.001). Therefore, in paper I, the emphasis was on the absolute dense volume.

In terms of absolute dense volume, we found a near-significant association between higher dense volume and statin use, after multivariable adjustment, including concomitant use of low-dose aspirin and metformin (p-value = 0.06). The effect size is seen in Table 14. Also, we found no effect of statin class (lipophilicity) or treatment duration.

Table 14. Absolute dense volume in relation to statin use in paper I.

		Geometric mean (95 % CI)			
		Model 1	**Model 2**	**Model 3**	**Model 4**
Absolute dense volume (cm^3)	Statin use				
	No	56.8 (56.6–57.1)	57.1 (56.8–57.3)	57.1 (56.8–57.3)	57.0 (56.8–57.3)
	Yes	60.7 (59.7–58.5)	57.6 (56.7–58.5)	57.5 (56.7–58.4)	58.0 (57.0–59.0)
	p value	<0.001	0.31	0.32	0.06

Our results showed a positive association between statin use and absolute dense volume in women who had ever used HRT ($P_{interaction}$= 0.03).

Discussion

Metabolites of the mevalonate pathway have apoptotic and anti-proliferative properties[23], hence the vast interest of statins in oncological research. Lower risk of breast cancer death and breast cancer recurrence has been shown in statin users (i.e., tumor progression)[27-30]. However, no associations have been shown between statins and the risk of developing breast cancer (i.e., tumor initiation)[24-26]. The current understanding is that statins do not have primary preventive properties in breast cancer. This, however, was less clear when this study was published in 2015. Our findings are in line with this current understanding, and perhaps paper I contributed a little to this knowledge.

The concept of paper I was to address a potential breast cancer risk-reducing effect of statins by studying its effect on an intermediate factor in breast cancer initiation – mammographic density – instead. Simply put, we wanted to study statins use in the primary prevention setting, but as a substitute, we studied statins association with mammographic density.

Considering statins anti-inflammatory, apoptotic and anti-proliferative properties[23], our pre-study hypothesis was that statin users would have lower mammographic density than non-users. Although not reaching the pre-defined level of significance, if anything, we found the opposite: higher mammographic density in statin-users compared to non-users. A previous population-based study[433] of women attending breast cancer screening showed no association between statin use and change in breast density (BI-RADS) between two successive mammographic screenings; however, when patients using HRT were excluded, the study indicates a possible increase in breast density with statin use, which also was in the opposite direction of their hypotheses. In three small studies, no association was found between breast density (area based-percentage breast density, BI-RADS, and a volumetric density method (Cumulus), respectively) and statin use[434-436].

Returning to the discussion of CD36-repression in high dense breast tissue (page 39 under the heading "Mammographic density on a cellular level"), the finding of an upregulation of CD36 by statins[260], further adds to the biological reasoning of a possible links between mammographic density and statin use.

HRT interaction term

Since a previous study showed that (long term) use of HRT influenced the association between statins and breast cancer[437], we included a multiplicative interaction term between statin and HRT in our adjusted models. HRT is widely known to increases mammographic density[310] and is an established risk factor for breast cancer[438,439], a risk that seems to be more pronounced in women having low levels endogen estrogen prior to HRT treatment[440]. One study, though only in 50 patients, showed that estrone sulfate levels were lower in women using statins[434].

Hence, women with statin use could theoretically have lower levels of estrone sulfate, and this might explain the observed larger absolute dense volume in statin users who also reported HRT use in our study. An alternative explanation might be found in the underlying indication for statins: hypercholesterolemia. A metabolite of cholesterol, 27-hydroxycholesterol, has been linked to ER-positive breast cancer[441], indicating a cross-talk between cholesterol and estrogen. Thus, the larger absolute dense volume in women using/used both statins and HRT could be due to the effect of HRT on hypercholesterolemia and not statins itself. However, our findings in the subgroup of study participants using HRT are not easily comparable to the results found in a previously referenced study[433] investigating the change in breast density during statin use, since they only report an increase in breast density with statin use when excluding HRT-users, and do not account for the effect of HRT on mammographic density in statin users. Also, the value of the comparison is limited since they categorized breast density according to BI-RADS, and small differences may not be apparent, and in paper I, an automatic volumetric method was used.

Effect size

Given the opportunity to further analyze the results from paper I while writing this thesis, I believe the effects sizes merit deepened attention. For example, there was a positive association between statin use and absolute dense volume in women who had ever used HRT (*p*-value = 0.01). The effects sizes were 53.0 (52.5–53.6) vs. 55.2 (53.7–56.7), the point estimates deviate from one another with 4%. A valid question is: Is this a clinically meaningful difference? For the non-significant association (*p*-value = 0.06) between absolute dense volume and statin use, the differences are even smaller (57.0 (56.8–57.3) vs 58.0 (57.0–59.0)). When interpreting the differences, the limits of the method, i.e., the measuring error with Volpara™, needs to be considered. In my opinion, the difference in absolute dense volume in women using/not using statin among the HRT-users, is of borderline clinical significance. An even smaller difference in absolute dense volume depending on statin use is seen, and is, in my opinion, not a clinically significant difference, especially since the direction of the association is opposed to the pre-study hypothesis based on biologically plausible associations.

In conclusion, there might be a complex association between statin use and mammographic density, and it is possible that an association is masked when combined with characteristics of statin users, such as high BMI, old age, or concomitant use of HRT. Since mammographic density is an important risk factor for breast cancer, potential undesirable effects in women using HRT and statins merits further investigation.

Paper II

Results

The NeoMon cohort consists of 302 patients treated with neoadjuvant chemotherapy from January 2005 to June 2016 in Skåne, Sweden. The number of patients with mammographic density BI-RADS a (N = 16), b (N = 120), c (N = 140), and d (N = 26), with most patients in the intermediate groups. A total of 19% (N = 57) of the patients accomplished pCR following neoadjuvant chemotherapy. At baseline, prior to chemotherapy, patients accomplishing pCR had to a higher degree smaller tumors, positive axillary lymph node status, high Ki67, negative ER/PR status, and/or HER2-overexpressing tumors, in comparison to patients not accomplishing pCR.

In our logistic regression models, using BI-RADS a as the reference, the likelihood of accomplishing pCR decreases with increasing mammographic density. In Table 15, the results for the whole cohort and the premenopausal patients, separately, are presented.

Table 15. Association between mammographic density and pCR (logistic regression) in paper II.

	BI-RADS	N	N of cases	OR (95% CI)
All patients				
Model 3*	a	11	4	(ref)
	b	92	22	0.32 (0.07–1.50)
	c	108	17	0.30 (0.06–1.45)
	d	17	1	0.06 (0.01–0.56)
Premenopausal patients only				
Model 3	a	4	2	(ref)
	b	27	2	0.07 (0.00–1.38)
	c	71	13	0.15 (0.01–1.67)
	d	13	1	0.03 (0.00–0.76)

*Model 3, adjusted for: age, BMI, menopause, pregnancies, HRT, ER, PR, Ki67, HER2, and tumor size at diagnosis

Discussion

Why are women with a high mammographic density less likely to accomplish pCR following neoadjuvant chemotherapy?

There might be several answers to this question. On a tissue level, a high mammographic density is associated with a proliferative and pro-inflammatory milieu due to a complex interaction between mitogens and mutagens[238,306]. The interaction between tumor cells and stromal cells was stressed in a gene expression study, reporting that increased stromal gene expression predicted resistance to

neoadjuvant chemotherapy[442]. Also, there is a higher proportion of both epithelial and stromal cells, and breast cancers develop from the former[18]. This might all be a part of the rationale between the higher risk of breast cancer[18] and a higher risk of local recurrence of breast cancer [344] for women with high mammographic density in comparison to women with low mammographic density.

We believe that these same mechanisms, responsible for the association between breast cancer development and mammographic density, are responsible for the poorer response to neoadjuvant chemotherapy in high mammographic density breasts. Not only tumor characteristics, but also host factors are considered predictive factors. Mammographic density, a breast-imaging parameter, is thought to be a simplified marker of the microenvironment on a tissue level. Also, in the discussions part for paper II, we have hypothesized of a higher tissue grade in dense breasts[443,444], obstructing the transportation of the administrated drug to the tumor in the site, contributing to the poorer response to treatment. Drug delivery in solid tumors is a complicated dynamic physiochemical process[445] and a detailed description is beyond the scope of this discussion. However, the matrix of a solid tumor, such as a breast tumor, might physically obstruct drug delivery[445], and since the matrix of dense breast tissue share some properties of tumor matrix (please refer to page 39 under the heading "Mammographic density on a cellular level"), drug delivery obstruction is possibly applicable to high mammographic density matrix as well. Also, cancer-stromal (especially fibroblast) interactions seems to be important in the acquirement of chemotherapy resistance[446,447], if this process could perhaps be associated with the predictive value of mammographic density is only speculative.

Paper III

Results

The prospectively gathered NeoDense cohorts consist of 207 patients, of whom 200 patients were part of paper III. A total of 74% of the patients had a decrease in absolute dense volume during neoadjuvant chemotherapy (Figure 14).

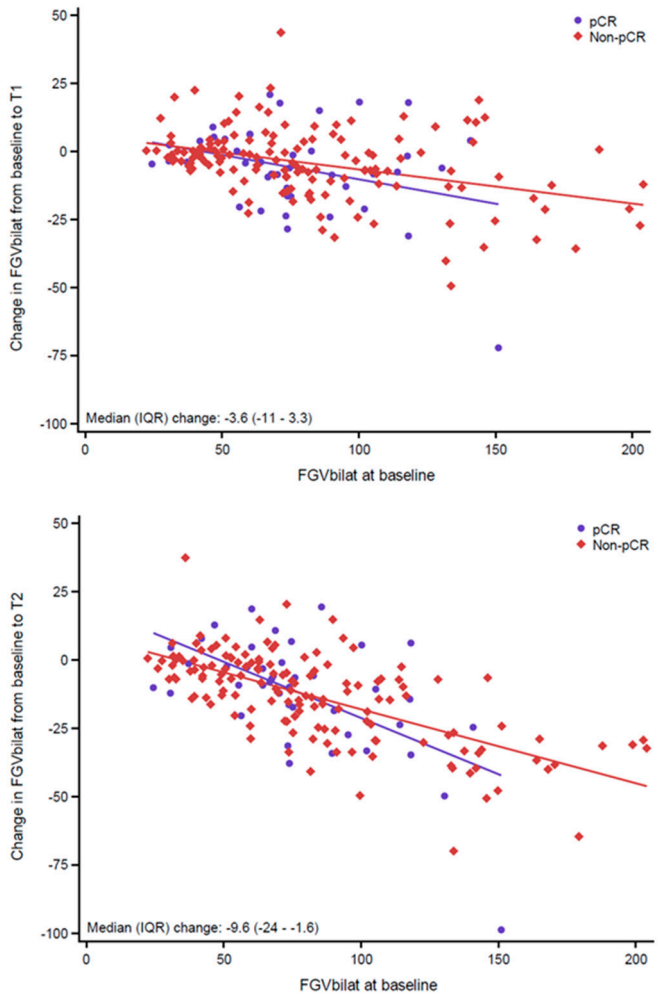

Figure 14. Change in volumetric density during neoadjuvant chemotherapy[448]. Modified by Skarping et al., "Mammographic density changes during neoadjuvant breast cancer treatment: NeoDense, a prospective study in Sweden", *The Breast*, 2020. Permission to reprint under the terms of CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

No association was seen between neither volumetric mammographic density (assessed with Volpara™) nor mammographic density assessed qualitatively with BI-RADS, and pCR in multivariable-adjusted logistic regression models (Table 16 and 17).

Table 16. Association between VBD% and pCR in paper III.

All patients	N	N of cases	Model 3* OR(95% CI)	Model 3 adjusted for $VBD\%_{bilat}$ at baseline (T0) OR(95% CI)
OR correspond to a 0.5 unit change in VBD%				
Static T0	188	42	1.01 (0.97-1.06)	
Static T2	187	43	1.01 (0.97-1.06)	
Dynamic T0-T1	180	41	0.96 (0.87-1.06)	0.97 (0.89-1.07)
Dynamic T0-T2	181	41	0.99 (0.91-1.08)	1.00 (0.92-1.09)
Dynamic T1-T2	181	42	1.02 (0.93-1.12)	1.05 (0.95-1.16)**

*Model 3, adjusted for: age, BMI, menopause, parity, HRT, ER, Ki67, HER2, axillary node status and tumor size at diagnosis
**adjusted for $VBD\%_{bilat}$ at T1

Table 17. Association between BI-RADS and pCR (BI-RADS c as reference) in paper III.

All patients		N	N of cases	Model 3* OR (95% CI)
BI-RADS	a	9	3	1.56 (0.43 - 5.70)
	b	72	19	1.49 (1.45 - 1.52)
	c	87	16	ref
	d	27	6	2.37 (1.15 - 4.88)

*Model 3, adjusted for: age, BMI, menopause, parity, HRT, ER, Ki67, HER2, axillary node status and tumor size at diagnosis

The same results are presented using BI-RADS a as reference (Table 18.), in order to enable an easier comparison between paper II and III.

Table 18. Association between BI-RADS and pCR (BI-RADS a as reference) in paper III.

All patients		N	N of cases	Model 3* OR (95% CI)
BI-RADS	a	9	3	ref
	b	72	19	0.95 (0.22 - 4.10)
	c	87	16	0.64 (0.15 - 2.70)
	d	27	6	1.52 (0.21 - 11.0)

*Model 3, adjusted for: age, BMI, menopause, parity, HRT, ER, Ki67, HER2, axillary node status and tumor size at diagnosis

Discussion paper II and III

Change in mammographic density during neoadjuvant chemotherapy

Temporal association (repeated density measurements over time; dynamic measurements) between mammographic density and a certain intervention is important, since a decrease in mammographic density is considered advantageous in terms of both risk and recurrence[449,450]. In the case of tamoxifen treatment, a previous study showed a reduction in mammographic density at 18 months[451]. On the other hand, despite the proven effect as a risk-reducing agent both in primary[452] and secondary breast cancer prevention[453], studies exploring the association between change in mammographic density following aromatase inhibitors have shown conflicting results regarding consistent density change[330,451]. A relatively large longitudinal cohort study investigating the effect of adjuvant endocrine treatment on volumetric mammographic density showed an annual decrease in VBD% (measured with Volpara^{TM} and Quantra^{TM}) of 1–2% among premenopausal women (tamoxifen treatment) and 0–1% for postmenopausal women (both tamoxifen and AI, with a larger reduction in tamoxifen-users in comparison to AI-users)[330]. The same question (volumetric density change during tamoxifen treatment) being addressed in a small MRI-based study, showed a median reduction of 5.8% VBD% during a mean of 17.5 months[454]. In terms of adjuvant radiotherapy, based on two studies, radiotherapy does not seem to be associated with change in mammographic density[324,325].

Two small studies (N < 45 in both studies), investigating the change in breast density measured with MRI during neoadjuvant chemotherapy, showed an 11–13% reduction in percent (MRI) breast density[455,456]. Studies[324,325,457] have shown a decline in mammographic density during adjuvant chemotherapy, although only one of them[325] quantified the decline (approximately -3 percentage point in percent mammographic density).

For premenopausal breast cancer patients, permanent chemotherapy-induced amenorrhea, i.e., chemotherapy-induced menopause, is considered to be a positive prognostic indicator[458,459]. In the NSABP B-30 trial[460], the majority of premenopausal breast cancer patients receiving adjuvant chemotherapy had at least a 6-months long period of amenorrhea. The resumption of menstruation was age-dependent, and for the youngest patients (< 40 years old), almost half of the patients started to menstruate again[460]. It is reasonable to believe that the corresponding proportions are about the same for the patients in our neoadjuvant treated cohort since the patients were treated with the same combination of chemotherapy agents as in the referenced study[460]. Mammographic density in women´s breasts changes along with age and hormonal events, such as pregnancy, breastfeeding, and menopause[275]. A steep decline in mammographic density is seen in the perimenopausal period, and the reduction occurring around the menopausal change

can be compared to 15–20 years of aging[282]. Chemotherapy-induced lobular atrophy and other processes in the benign breast tissue[461] might also influence the mammographic density change during chemotherapy treatment. Also, in lobular involution, before the final replacement of epithelia with adipose tissue, the dense stroma is an intermediate step, highlighting the importance of timing of the density assessment[281]. In light of this, it is difficult to single out the underlying biological explanation for the small decline in mammographic density seen in our study.

Mammographic density in a breast cancer cohort compared to the general population

In our cohort, only 4.5% of the patients were categorized as BI-RADS a. The proportion of American women of breast screening age categorized as BI-RADS a ranges from 8% to 20% in different age categories[372], and in a Swedish screening population, the proportion of patients categorized as BI-RADS a was 15%[462]. In a nationwide large Dutch screening cohort (median age 60 years (IQR 54 to 66)), the mean VBD% (Volpara™) was 7.25[322], and in a large Swedish screening cohort (mean age 58 years, range 40–76 years), the median VBD% (Volpara™) was 7.2[463]. In our study, the median VBD% of both breasts was 11.0 (IQR 7.5–17.1), and the median VBD% of the contralateral healthy breast was 10.2 (IQR 6.7–16.3) at baseline in our neoadjuvant treated breast cancer cohort (median age 53.1 years (IQR 45.9–62.5 years)). Hence, our cohort has many more patients with higher mammographic density relative to the general population, and this could partly be due to our cohort being younger than the general screening populations in the previously referenced studies. High breast density is a known risk factor for developing breast cancer[18] and, thus, it might seem safe to assume that a breast cancer cohort, on average, will have a higher breast density in comparison to a healthy cohort (the screening population). Several case-control studies have shown a higher mammographic density in breast cancer patients compared to their healthy controls[49,464,465]. However, the association is multifactorial with some paradoxical association with mammographic density and breast cancer, e.g., with increasing age, the risk of breast cancer increases and mammographic density decreases, explained by the Pike model[302]. Also, high BMI is associated with lower percent mammographic density[466], with different associations between increasing BMI and the risk of breast cancer depending on menopausal status[17,466,467]. Hence, assuming a breast cancer cohort to have a higher average mammographic density compared to a healthy cohort based on their breast cancer diagnosis alone might be a premature conclusion.

Volpara™

When investigating a relatively small temporal change in density, the software used must deliver consistent density readings in order to deduce that the perceived change is, in fact, not related to measurement error. Since Volpara™ is shown to be in

agreement with itself between serial exams[468,469], the software is believed to fulfill this demand for robustness. It has been indicated that Volpara™ underestimates mammographic density relative to MRI, and this is most pronounced in high-density breasts[374,470]. In these studies, it seems that the underestimation by Volpara™ is mainly an issue in extremely dense breasts; the divergence is most apparent, starting at 30% breast density on MRI. A small systematic underestimation would not affect our Δ-mammographic density-measurements and would, thus, not influence the results of our logistic regression models. Also, in our cohort, only 1.5% of the patients had VBD% over 30, and 4.8% of the patients had VBD% over 25; thus, our Volpara™-output is considered robust for our material.

Why the inconsistent results in paper II and III?

In Paper II, we found an association between mammographic density and pCR, whereas no such association was seen in Paper III. In this paragraph, possible explanations are discussed, and Table 19 gives a summary of the differences.

Since the number of patients included in the statistics was fairly similar, the different results could not be explained as a matter of different power in the studies. The distribution of pre- and postmenopausal patients was similar, although, of the patients categorized as BI-RADS d, 69% of the patients were premenopausal in paper II, whereas only 59% were premenopausal in paper III. Patients in paper II received their chemotherapy during 2005–2016, and patients in paper III were treated during 2014–2019. The recommendations for chemotherapy were rather similar across this period of time, although a change in the recommendation of HER2-blockade therapy; none of the patients with HER2-overexpressing tumors received double HER2-blockade in paper II, whereas a majority of patients with HER2-overexpressing tumors received double HER2-blockade in paper III. However, during this time period, there was probably a shift in the indication for neoadjuvant chemotherapy. Since information on the indication for neoadjuvant treatment lacks in both paper II and III, one could only hypothesize on the differences. One would assume that a larger proportion of patients in paper II, in comparison to patients in paper III, received neoadjuvant treatment in a downstaging purpose; however, this is not reflected in the only marginally larger tumors seen in paper II. However, in paper II N=10 patients presented with inflammatory breast cancer at the time of diagnosis and no tumor size was assessed, whereas for paper III, only one of the patients presented with inflammatory breast cancer and for this tumor, the radiologist could still give a size estimation. A larger proportion of patients in paper III, in comparison to patients in paper II, had axillary lymph node metastases, most likely due to indication for neoadjuvant breast cancer treatment.

No major differences were seen in the ER and PR status of the patients' tumors between paper II and III. There were different pCR-rates for BI-RADS d in paper II

(6%) and paper III (22%). Patients with BI-RADS d in paper II were more often premenopausal, current smokers and never users of HRT and oral contraceptives, in comparison to patients with BI-RADS d in paper III. Also, patients with BI-RADS d in paper II had smaller tumors, more often HER2-overexpressing tumors and less often highly proliferative tumors and a lower rate of axillary node positivity, in comparison to patients with BI-RADS d in paper III. Intuitively, larger tumors and a higher rate of axillary lymph node positivity should be correlated with lower pCR rates, in contrast to our results. In paper III, all patients categorized as BI-RADS d were highly proliferative (Ki67>20%), and a high Ki67 score is known to be associated with better response to chemotherapy[396-398]. One hypothesis contributing to the explanation of the different results seen in paper II and III is that high mammographic density might be associated with high proliferative tumors (Ki67), although with inconsistent results in the previous studies[238,337-339], which in turn is associated with better response to chemotherapy[396], thus diluting the association between mammographic density and pCR.

Table 19. Comparison of papers II and III.

			Paper II	**Paper III**
Number of patients	All		N=302	N=200
	BI-RADS d		N=26	N=27
Included in logistic regression models	All		N=228	N=195
Year of chemotherapy treatment			2005-2016	2014-2019
Mammographic density		BI-RADS a	5%	5%
		BI-RADS b and c	86%	82%
		BI-RADS d	9%	14%
Rate of pCR of patients included in logistic regressions models	All		19%	23%
	BI-RADS d		6%	22%
Patient characteristics	All	Age (median, IQR)	53 (44-63)	53 (46-63)
		BMI (median, IQR)	25 (23-28)	26 (22-29)
		Premenopausal	45%	48%
		Current smoking	17%	10%
		Ever hormone replacement therapy	15%	9%
		Ever oral contraceptives	57%	76%
	BI-RADS d	Age (median, IQR)	44 (37-54)	47 (43-60)
		BMI (median, IQR)	23 (21-26)	24 (22-26)
		Premenopausal	69%	59%
		Current smoking	15%	4%
		Ever hormone replacement therapy	4%	11%
		Ever oral contraceptives	46%	78%
Tumor characteristic	All	ER-positive	63%	60%
		PR-positive	48%	52%
		Tumor size (mm) median (IQR) (average between mammography and ultrasound if both values were known)	32.5 (23.3-45.0)	29 (20.0-37.5).
		HER2-overexpression	31%	24%
		High Ki67 (>20%)	68%	92%
		Axillary node positivity	65%	72%
	BI-RADS d	ER-positive	77%	70%
		PR-positive	58%	59%
		Tumor size (mm) median (IQR) (average between mammography and ultrasound if both values were known)	30 (20-40)	39 (23-42)
		HER2-overexpression	35%	22%
		High Ki67 (>20%)	58%	100%
		Axillary node positivity	58%	89%

For this table, the values for tumor size for paper III has been recalculated and is here presented as the mean of two imaging moadlites and not the largest of the two as in the original study.

Paper IV

Results

A total of 202 patients from the NeoDense-cohort were included in paper IV. The median tumor size at baseline was similar between the three modalities: 30 mm (IQR 20–40), 28 mm (IQR 19–35), and 28 mm (IQR 21–36), as assessed with mammography, ultrasound, and tomosynthesis, respectively. At diagnosis, 71% of the patients had pathology/cytology verified lymph node metastases to the axilla. The largest proportion of non-measurability (i.e., the radiologist could not give a size estimate of the tumor), as well as un-detectability, were seen for mammography, although the differences were small (Table 20).

Table 20. Tumor size at baseline and numbers of non-measurability and un-detectability in paper IV.

Modality		
Mammography	Tumor size, mm, median (IQR)	30 (20 - 40)
	No detectable tumor (%)	11 (5.4)
	Tumor size not assessable (%)	10 (5.0)
	Test not performed (%)	1 (0.5)
Tomosynthesis	Tumor size, mm, median (IQR)	28 (21 - 36)
	No detectable tumor (%)	6 (3.0)
	Tumor size not assessable (%)	6 (3.0)
	Test not performed (%)	50 (24.8)
Ultrasound	Tumor size, mm, median (IQR)	28 (19 - 35)
	No detectable tumor (%)	2 (1.0)
	Tumor size not assessable (%)	5 (2.5)
	Test not performed (%)	0 (0)

According to the visual assessment of Bland-Altman plots, at baseline, the agreement, between the modalities was similar, although tomosynthesis vs. ultrasound had the smallest mean difference. At the later time points, after cycle 2 and after cycle 6, respectively, mammography and tomosynthesis showed the best agreement with one another. Ultrasound seemed to underestimate tumor size in comparison to both mammography and tomosynthesis, at the later time points.

The accuracy of tumor size estimation by imaging modality, separately, in relation to pathology within 2- and 5-mm margins, respectively, was slightly lower for tomosynthesis in comparison to mammography and ultrasound (Table 21).

Table 21. Accuracy of radiological modalities post-neoadjuvant chemotherapy (in relation to the invasive focus on pathology) in paper IV.

Measure	N*	Median (IQR) size, mm	Accurate within 2 mm, N (%)	Accurate within 5 mm, N (%)
Invasive focus	202	10 (0 - 19)		
Mammography	182	13 (0 - 24)	57 (30)	88 (46)
Tomosynthesis	141	13 (7 - 23)	39 (27)	63 (43)
Ultrasound	195	10 (0 - 18)	61 (31)	92 (46)

*Tumors with size not assessable as well as tumors with size assessed as 0 at baseline were excluded, the latter since they were considered undetectable at baseline.

For the patients being categorized as early radiological responders by mammography, 35% accomplished pCR_{breast} post-treatment, in comparison to only 19% of the patients categorized as early radiological non-responders. The corresponding proportions of pCR_{breast} among early radiological responders for tomosynthesis and ultrasound were slightly higher; 44% and 42%, respectively (Table 22).

Table 22. pCR rate by radiological early response in paper IV.

After cycle 2	Radiologic non-responders	Radiologic responders	Relative chance* of pCR	95% CI
Mammography	23 (19%)	23 (35%)	1.8	1.1 - 3.0
Tomosynthesis	14 (16%)	23 (44%)	2.8	1.5 - 5.2
Ultrasound	16 (14%)	35 (42%)	2.9	1.6 - 5.2

*Relative chance (of the favourable outcome pCR) is used as a synonym to relativ risk.

Discussion

Our results show that being categorized as an early radiological responder is associated with a 2–3-times higher chance of accomplishing pCR in comparison to being categorized as an early radiological non-responder. Although current guidelines recommend re-evaluation of neoadjuvant chemotherapy only in case of tumor progression and intolerable toxicity during treatment[37], we believe the concept of response-guided neoadjuvant treatment[471], i.e., an adaptive treatment plan according to individual response, should be considered. We suggest an improved clinical awareness of early radiological non-responders. Also, we recognize the difficulties with early radiological non-responders, still accomplishing pCR, since these patients could be subjected to (unnecessary/unfavorable) treatment modification.

In paper IV, we made a distinction between undetectable and non-measurable tumors. A non-measurable tumor is a tumor that is detectable with that particular modality, although the radiologist could not make a size estimation. The undetectable tumors, on the other hand, was not visible at all, and, e.g., in a screening-setting, these tumors would have been missed, and the reply to the woman

would be that everything looked fine. It could be argued that undetectable and non-measurable tumors should be grouped together since they, in clinical practice, can be hard to separate from one another. However, we believe that a non-measurable, although visible, the tumor could be interpreted as a radiological non-responder, whereas the undetectable tumors were omitted from the statistics. Coherently, in the evaluation of radiological complete response as an indicator for pCR after completion of neoadjuvant chemotherapy, only patients with a visible (although not necessarily measurable) tumor in all three modalities at baseline were included in the statistics.

Pathological evaluation after neoadjuvant chemotherapy

In paper IV, we used pCR (or lack thereof) and the largest remaining invasive focus as a pathological outcome. However, there are several other systems for evaluating response to neoadjuvant chemotherapy. In general, most systems have a category corresponding to pCR and a category with little/none response; the largest difference lies in the categorization of partial response. In Table 23, some of the systems for pathological evaluation are listed along with a brief comment.

Previous studies assessing pathological response to neoadjuvant chemotherapy in relation to radiological response have used different definitions of pCR; similar to our current study DCIS was accepted in the pCR group in some studies[472-478], whereas patients with remaining DCIS did not qualify for a pCR categorization in other studies[479-482]. Still, other studies have not specified how DCIS was handled[483]. Also, in the case of non-pCR different pathological measurements was used in comparison to radiological measurements: the largest remaining invasive focus[472,473,481,484,485], the extent[480], the largest diameter of the tumor bed (rather than the largest cellular focus)[474], and the sum of all invasive components as well as DCIS components[479].

When evaluating neoadjuvant chemotherapy response, two different approaches can be used: absolute assessment of the residual tumor or the relative assessment of tumor response (relative pre-treatment tumor characteristics and imaging results). Parameters as the ypTNM stage belong to the former in which the largest invasive focus of residual tumor defines the ypT category according to the 8th Edition of the American Joint Committee on Cancer System[486]. In 2003, the Miller-Payne system was presented, a histological prognostic 5-point grading system focusing on reduction in tumor cellularity as a response to chemotherapy[487]. One shortcoming with the pCR concept, as is used in this and most other studies, is that it does not reflect the different levels of partial responses as does the Miller-Payne grading system (Grade 1-4 categorized as partial response and grade 5 corresponding to pCR). The literature heavily supports the use of pCR as a surrogate marker for long-term survival for breast cancer patients treated with neoadjuvant chemotherapy[169,405,488], even though many studies support the use of the Miller-

Payne system as a predictive tool[487,489,490], the literature is not as established. International guidelines recommend reporting information needed to determine pCR in the clinical pathology reports[46,405], and the reporting, according to Miller-Payne System, is not a clinical established routine in Sweden[37], hindering the clinical interpretation. Also, the Miller-Payne system does not take into account potential axillary lymph node metastases[491].

Table 23. Examples of systems for pathological evaluation of response to neoadjuvant chemotherapy.

System	Features
AJCC system[492]	TNM stage: Tumor size and lymph node status. Not include changes in cellularity, i.e., continuous carcinoma equates microscopic foci scattered in a tumor bed of the same size[493].
The Miller Payne System[487]	Tumor cellularity Not include the response in the lymph node.
Sataloff System[494]	pCR includes “near-total therapeutic effect”[493]
RCB System[495]	Residual cancer burden: an algorithm using: residual invasive carcinoma cellularity distributed over the tumor bed, the number of lymph nodes with metastases, and the size of the largest metastasis. Available free Residual Cancer Burden Calculator at MD Anderson[496].
Honkoop Classification[497]	Two categories: minimal residual disease (either no residual tumor or scattered foci of tumor microscopically) and gross residual disease (either macroscopic tumor or diffuse infiltration microscopically)
The Kuerer classification[498]	three categories of response: no evidence of residual tumor, <1 cm3 of residual tumor macroscopically and >1 cm3 of residual tumor macroscopically.

Conclusions

Paper I

We found no association between statin use and volumetric mammographic density in terms of absolute dense volume.

Paper II

For patients receiving neoadjuvant chemotherapy, the higher mammographic density at baseline (assessed with BI-RADS), the lower likelihood of accomplishing pCR following treatment, an association more pronounced in premenopausal patients.

Paper III

A large proportion of the patients decreased their mammographic density during neoadjuvant chemotherapy. We found no association between mammographic density, assessed with both Volpara^{TM} and BI-RADS, and the likelihood of accomplishing pCR following neoadjuvant chemotherapy.

Paper IV

Our results show that predicting residual tumor size after neoadjuvant chemotherapy is challenging using mammography, ultrasound, and tomosynthesis. Early radiological non-response is worrisome, and these patients might need improved monitoring and changed treatment plans.

Future perspectives

In order to offer future breast cancer patients the best care, both over-treatment – associated with unnecessary complications and toxicity – and under-treatment – leading to relapse – should be avoided. Personalized treatment requires more predictive biomarkers, there among imaging biomarkers.

Deepened knowledge of mammographic density and its biological link to breast cancer initiation, progression, as well as, response to treatment is needed. How can we optimally reduce the risk of breast cancer by modulating mammographic density? Current breast cancer preventive medicines, such as the anti-estrogen tamoxifen, are associated with severe side-effects and providing non-toxic alternatives, either lowering the dose until tolerable side-effects (i.e. as explored in the KARISMA trial) or finding new targets, are obviously needed. Through in-depth studies of molecular/cellular differences between breast tissue from low and high mammographic density areas and similarities between (benign) breast tissue from high mammographic density areas and breast cancer tissue, respectively, many possible targets have already been identified and, undoubtedly, more will be explored in the future. Risk assessment models already exists, but needs to be improved. Algorithms considering risk of specific breast cancer subtypes are needed since it is likely that future preventive medications will, at least partly, target a specific breast cancer subtype. It is also of great importance that the risk-assessment models, possibly followed by risk-reducing recommendations/medications, are applied to women at a young age, when there is still time to reduce the risk of breast cancer in an effective way. A personalized preventive approach based on information from a blood sample, a "liquid biopsy", or a minimally invasive breast tissue biopsy, is an appealing idea, probably not available in the near future, but perhaps not decades away either. A translational approach is warranted.

Mammographic density is currently not routinely used in Sweden, neither in the screening-setting nor in the breast cancer treatment setting. In paper I and III, Volpara^{TM}, a software using raw-data mammograms, was used. However, in order to be an efficient imaging biomarker in the clinic, a software, of many who already exists at the time of writing, operating on processed mammograms, is preferred. Also, guidelines, enabling easy and standardized interpretation, with appropriate levels/cutoffs of mammographic density for the clinicians to relate to are needed.

It is my understanding that the concept used in paper I, using mammographic density as an intermediate marker for breast cancer risk, could be further explored in studies investigating breast cancer risk and its association with risk/protective factors. It would be interesting to investigate the association between mammographic density and a large number of dietary products as well as pharmaceuticals.

As a result of the findings in paper II and III regarding mammographic density as a predictive marker for treatment response during neoadjuvant chemotherapy, one can conclude that larger multicenter studies are required in order to make the results useful in the clinic. The NeoDense-cohort will be the subject of future studies, next ahead of a study with longer follow-up time investigating the association between mammographic density (with one additional time point, 1 year after treatment) and breast cancer-related events. It would be interesting to further explore the predictive value of mammographic density in the adjuvant setting, adjusting for many confounders, and with breast cancer events as the outcome measure.

Paper IV enlightens both the difficulties with conventional imaging during neoadjuvant treatment, but also the need for greater awareness of early radiological non-responders. In order to evaluate the clinical efficacy of changing or adding treatment for early radiological non-responders, prospectively conducted trials are warranted. In my opinion, more advanced *functional* imaging, e.g. MRI and, predominantly ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET), but also positron emission tomography with other radiotracers, should be considered more often in Swedish clinical practice in order to offer state-of-the-art treatment.

Since breast cancer is a heterogeneous disease, whenever possible, subgroup analyses based on breast cancer subtype should be performed. This, however, requires larger studies and should be accounted for in future study designs.

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