Mammographic density in relation to breast cancer Tumor characteristics, mode of detection, and density assessments

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Tumor characteristics, mode of detection, and density assessments

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LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Abstract

Mammographic density reflects the composition of the breast tissue and can be measured by different methods. Mammography has a lower sensitivity in women with dense breasts, and women with dense breasts have a higher incidence of breast cancer than do women with non–dense breasts. Furthermore, there has been an increased interest in improving the measurement of mammographic density.

The aim of this thesis was to study how mammographic density relates to breast cancer in terms of mammographic tumor features, pathological tumor characteristics, and mode of detection. An additional aim was to assess the agreement between two methods of measuring mammographic density.

In Papers I-III, we used 826 breast cancer cases from the population-based, prospective Malmö Diet and Cancer Study. Our findings imply that the spiculated mammographic tumor feature was related to invasiveness, and ill-defined mass was related to large tumor size, regardless of the mode of detection and mammographic density. Second, higher mammographic density was associated with larger tumor size, as well as axillary lymph node involvement in invasive breast cancer. Furthermore, in screening detected breast cancer, higher mammographic density was associated with lower histological grade, although the evidence for this was weak. Finally, our findings in clinically detected breast cancer, but not in cancers detected during screening, imply that higher mammographic density was associated with estrogen receptor-negative and triple-negative breast cancers.

In Paper IV, we used 8,889 mammography examinations from the Malmö Breast Tomosynthesis Screening Trial. There was substantial agreement between the Breast Imaging-Reporting and Data System (BI-RADS) score from different radiologists and moderate agreement between the BI-RADS score and the fully automated volumetric assessment (Volpara software) of mammographic density.

This thesis shows that some of the mammographic tumor features and the pathological tumor characteristics in breast cancer tend to differ with mammographic density and the mode of detection. Further, there was moderate agreement between a fully automated volumetric assessment and the radiologists' qualitative classification of mammographic density.

Key words: breast cancer, screening, mammography, mammographic tumor features, mammographic density, mode of detection, pathological tumor characteristics

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Mamma, vi forskar också. På dagis. Om dyngbaggar.

- Sten & Dag

Contents

List of original papers

This thesis is based on the following papers, which will be referenced to in the text by their Roman numerals.

Paper I: Do mammographic tumor features in breast cancer relate to breast density and invasiveness, tumor size, and axillary lymph node involvement?

Sartor H, Borgquist S, Hartman L, Olsson Å, Jawdat F, Zackrisson S.

Acta Radiologica 2015 May;56(5):536-544

Paper II: Do pathological parameters differ with regard to breast density and mode of detection in breast cancer? The Malmö Diet and Cancer Study

Sartor H, Borgquist S, Hartman L, Zackrisson S.

The Breast 2015 Feb;24(1):12-17

Paper III: Mammographic density in relation to tumor biomarkers, molecular subtypes, and mode of detection in breast cancer

Sartor H, Zackrisson S, Elebro K, Hartman L, Borgquist S.

Cancer Causes & Control 2015 Jun;26(6):931-939

Paper IV: Measuring mammographic density: Comparing a fully automated volumetric assessment versus European radiologists' qualitative classification

Sartor H, Lång K, Rosso A, Borgquist S, Zackrisson S, Timberg P.

Submitted

Abstract

Mammographic density reflects the composition of the breast tissue and can be measured by different methods. Mammography has a lower sensitivity in women with dense breasts, and women with dense breasts have a higher incidence of breast cancer than do women with non–dense breasts. Furthermore, there has been an increased interest in improving the measurement of mammographic density.

The aim of this thesis was to study how mammographic density relates to breast cancer in terms of mammographic tumor features, pathological tumor characteristics, and mode of detection. An additional aim was to assess the agreement between two methods of measuring mammographic density.

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This thesis shows that some of the mammographic tumor features and the pathological tumor characteristics in breast cancer tend to differ with mammographic density and the mode of detection. Further, there was moderate agreement between a fully automated volumetric assessment and the radiologists' qualitative classification of mammographic density.

Populärvetenskaplig sammanfattning (in Swedish)

Bröstcancer är kvinnans vanligaste cancer och ungefär en av tio kvinnor i Sverige insjuknar i bröstcancer. Bröstcancer är en sjukdom med olika ansikten; en knöl i bröstet eller omöjlig att känna, begränsad till bröstet eller spridd i kroppen, botbar eller dödlig. Vilken kvinna får vilken cancer?

I Sverige har vi ett screeningprogram där kvinnor regelbundet undersöks med en röntgen av brösten, en så kallad mammografi. Målet med screeningprogrammet är att upptäcka och behandla bröstcancer tidigt för att kunna förbättra prognosen. Bröstet kan ha olika utseenden på mammografin. Ett fettrikt bröst gör röntgenbilden av bröstet svartare. Ett tätt bröst, som innehåller mycket stödjevävnad och körtelceller, gör röntgenbilden av bröstet vitare. Detta kallas för mammografisk täthet. Man har tidigare visat att kvinnor med mammografiskt täta bröst har en högre risk för bröstcancer jämfört med kvinnor som har mammografiskt fettrika bröst. Det täta bröstet gör att själva tumören – som också blir vit på röntgenbilden – blir svår att upptäcka, men det är också något med tätheten i sig som ökar risken för bröstcancer hos kvinnor med täta bröst. Även brösttumören har olika utseenden på mammografin. Den kan t.ex. vara strålig, rund eller bara en liten oregelbundenhet i bröstvävnaden.

Målet med denna avhandling var att undersöka ledtrådarna i röntgenbilden ytterligare. Vi ville ta reda på om olika sorters mammografiska tumörutseenden förekom oftare i täta eller i fettrika bröst. Vi ville också ta reda på om mammografisk täthet och olika tumörutseenden på röntgenbilden var förknippade med olika faktorer hos brösttumören. Vi ville också jämföra två olika sätt att mäta mammografisk täthet i röntgenbilden.

För att undersöka detta använde vi oss i de tre första arbetena av en stor befolkningsstudie i Malmö som heter Malmö Kost Cancer studien inkluderande 17,035 kvinnor, varav 826 kvinnor insjuknade i bröstcancer mellan 1991-2007. Dessa kvinnor har en mammografi och tumörprov ifrån tillfället då de insjuknade. Det finns också information om huruvida tumören blev upptäckt på röntgenbilden genom screeningprogrammet (screeningupptäckt) eller genom att kvinnan själv kände tumören i sitt bröst (kliniskt upptäckt).

I det fjärde arbetet använde vi oss av Malmö Breast Tomosynthesis Screening Trial. Kvinnorna i denna studie gjorde två olika röntgenundersökningar av brösten; en vanlig mammografi och en tomosyntes (en slags 3D skiktröntgen av bröstet). För en del av dessa mammografier var röntgenbildens råmaterial sparat och detta råmaterial kan användas till att göra vissa mätningar. Volpara är en mjukvara som kan mäta den mammografiska täthen på sådant råmaterial. När läkare mäter mammografisk tähet tittar de på röntgenbilden och skattar den mammografiska tätheten enligt en fyra-gradig skala som kallas BI-RADS (Breast Imaging-Reporting and Data System).

I det första arbetet såg vi att vissa mammografiska tumörutseenden var vanligare i täta än i fettrika bröst. T.ex. var tumörer som innehöll kalk eller var svåravgränsade vanligare i täta bröst, i jämförelse med tumörer som var välavgränsade eller stråliga som var vanligare i fettrika bröst. Vi hittade också att stråliga tumörer oftare var invasiva tumörer och att svåravgränsade tumörer oftare var stora tumörer än de välavgränsade tumörerna, även efter att hänsyn tagits till den mammografiska tätheten och på vilket sätt tumören blev upptäckt på.

Avseende mammografisk täthet såg vi i det andra och tredje arbetet att högre mammografisk täthet var kopplat till större tumörstorlek, spridning till lymfkörtlar och bland screeningupptäckta tumörer möjligen också till lägre histologisk grad. Bland de kliniskt upptäckta tumörerna, såg vi ett samband mellan högre mammografisk täthet och östrogenreceptor-negativa tumörer samt så kallade trippel-negativa brösttumörer, en extra allvarlig typ av bröstcancer.

Sammantaget förefaller det mammografiska tumörutseendet och de olika karaktäristika hos brösttumören i vissa fall att skilja sig åt beroende på mammografisk täthet och brösttumörens upptäcktssätt.

I det fjärde arbetet såg vi att överensstämmelsen mellan läkarnas och mjukvarans sätt att mäta mammografisk täthet var måttlig. En anledning till detta kan vara att mjukvarans gränsvärden är satta utifrån amerikanska läkares sätt att mäta mammografisk täthet. En anpassning till europeiska förhållanden skulle möjligen kunna förbättra överensstämmelsen.

Röntgenbilden av bröstet bär på mycket information, information som inte används fullt ut i klinisk praxis idag, såsom mammografiskt tumörutseende, mammografisk täthet och brösttumörens upptäcktssätt. Vi hoppas att resultaten från den här avhandlingen ska kunna bidra med ökad kännedom kring en del av informationen i röntgenbilden, så att på sikt fler parametrar ifrån röntgenbilden kan användas rutinmässigt i bröstcancervården.

Kanske ska kvinnor undersökas och tas omhand på olika sätt utifrån hur hennes bröst och eventuella brösttumör ser ut på röntgenbilden? Mer forskning på området behövs, men vi tror att informationen i röntgenbilden har möjlighet att göra prevention, diagnostik och behandling av bröstcancer ännu bättre.

Abbreviations

ALNI	Axillary Lymph Node Involvement
AR	Androgen Receptor
BI-RADS	Breast Imaging-Reporting and Data System
BMI	Body Mass Index
CC	Craniocaudal
CIS	Cancer *in situ*
DBT	Digital Breast Tomosynthesis
ER	Estrogen Receptor
HER2	Human Epidermal growth factor Receptor 2
HRT	Hormone Replacement Therapy
IHC	Immunohistochemistry
MBTST	Malmö Breast Tomosynthesis Screening Trial
MDCS	Malmö Diet and Cancer Study
ML	Mediolateral
MLO	Mediolateral Oblique
MRI	Magnetic Resonance Imaging
PR	Progesterone Receptor
SNP	Single Nucleotide Polymorphism
VBD	Volumetric Breast Density
VDG	Volpara Density Grade
WHO	World Health Organization
TMA	Tissue Micro Array
TNBC	Triple-Negative Breast Cancer

1 Introduction

A picture is worth a thousand words.

- Frederick R. Barnard

The radiographic picture of the breast, i.e., the mammogram, is the basis for this thesis. Mammography plays a central role in breast cancer care as it is the gold standard for breast imaging in the clinical setting and in population-based screening. Most women in the developed world have a mammogram at some point during their lifetime, which makes information from mammograms readily available in a female population. Breast cancer is the most common female cancer, representing a third of all cancers in females in Sweden (1). There are several risk factors for breast cancer including age, family history, and hormone replacement therapy (HRT) (2). In this thesis we focus on another interesting risk factor, the mammographic density. The risk of breast cancer is increased by four to six times in women with very dense breasts compared to those women with fat-involuted and less dense breasts (3). The breast composition determines the mammographic appearance of the breast because of differences in how epithelium, fat, and stroma attenuate x–rays. A high mammographic density corresponds to a breast composition with a high proportion of epithelium and breast stroma and results in a whitish image on a mammogram (4), which may obscure a breast tumor whose image also is whitish. The probability of detection of breast cancer during mammography is also related to factors such as the tumor growth rate and mammographic tumor features (5-7). It is well known that high mammographic density decreases the sensitivity of mammography (8-10), but there may also be a biological relationship between the mammographic density and the development and progression of breast cancer (11). Hence, mammographic density carries information related to both risk and prognosis (3, 12) and may therefore have a prominent role in individualizing care of breast cancer patients (11).

This thesis aims to further clarify issues related to mammographic density, such as its relation to mammographic tumor features, pathological tumor characteristics (e.g., invasiveness, tumor size, axillary lymph node involvement (ALNI), and hormone receptors), and the use of a new method of measuring mammographic density. Increased knowledge concerning mammographic density may further define its role in the future care of breast cancer patients.

2 Background

2.1 The breast

The breast is the organ of milk production that appears at about the 5th week of embryonic life. Developmentally, the breast can be seen as a modified sweat gland. The development of the female breast accelerates during puberty and continues to change with age and different phases in a woman's life. Breast development is predominantly under the influence of estrogen and progesterone. The breast consists of 15-25 glandular lobes that are covered and separated from each other by fibrous connective tissue and adipose tissue. Within the lobes, there are smaller units called lobules. The terminal duct lobular unit is the milk-producing and milk-secreting unit of the breast (Fig. 1). Small ducts from each of the terminal duct lobular units form a lactiferous duct in each of the 15-25 lobes, which merge and open on the surface of the nipple (13).

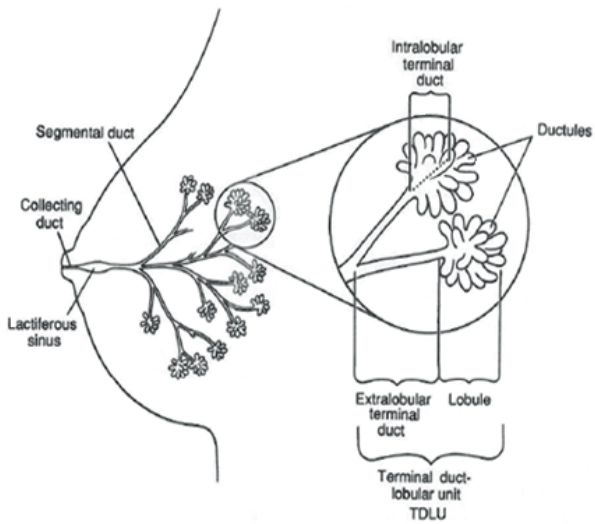

Fig. 1. The female breast.
Reprinted from Breast Care: A Clinical Guidebook for Women's Primary Health Care Providers. Hindle, William H. New York, NY: Springer-Verlag; 1999, with kind permission from Springer Science and Business Media.

2.2 Breast cancer

2.2.1 Epidemiology of breast cancer in Sweden

Incidence and prevalence

Breast cancer represents 31% of all female cancer cases, which makes it the most common form of female cancer (1). In 2013, 9123 cases of invasive cancer and 1464 breast cancer *in situ* (CIS) were reported in Sweden (1). The age-standardized incidence of breast cancer in women has increased by 1.4% annually for the last 20 years and 2.0% for the last 10 years (1). The increase in incidence has been most pronounced for women in the age group 60-69 years (14, 15) (Fig. 2). In 2013, the age-standardized incidence of breast cancer was 132/100,000 (15) (Fig. 3), and the number of women living with breast cancer was estimated to be 99,874 (15). The increased incidence is thought to be due to a combination of a true increase and an effect of the screening program, which detects more breast tumors.

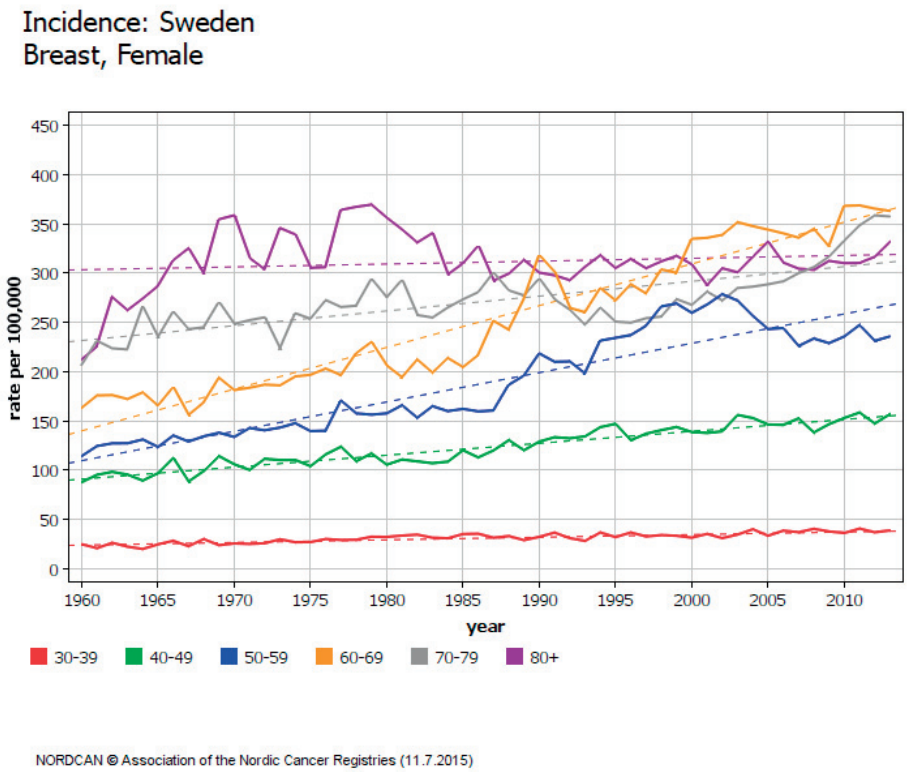

Fig. 2. Incidence in different age groups (15).

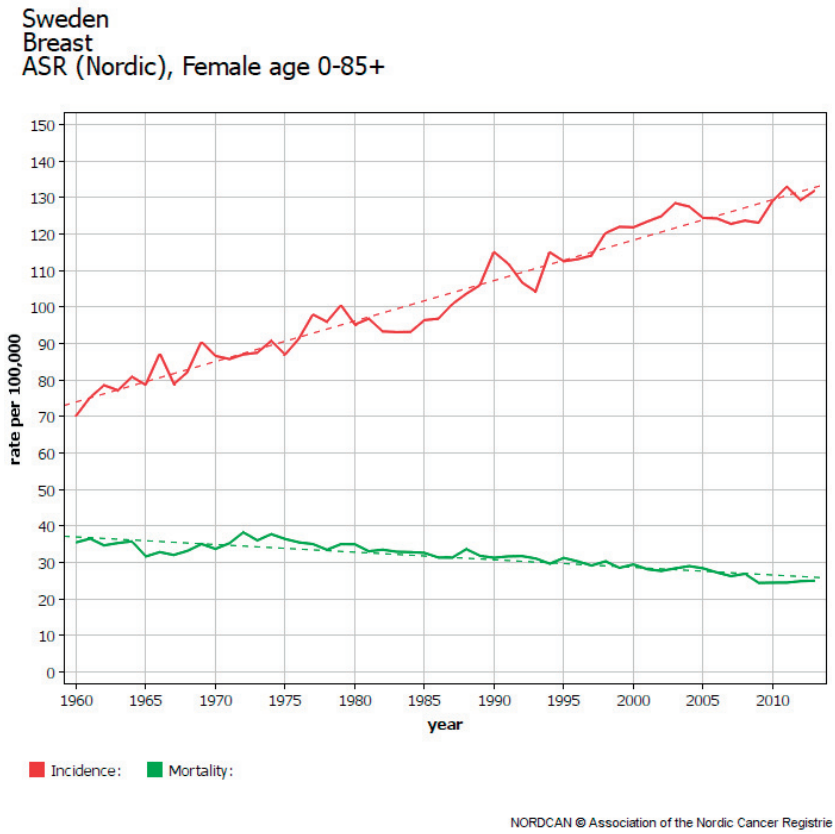

Fig. 3. Incidence (red) and mortality (green). Age standardized according to the Nordic standard population (15).

Mortality and Survival

Even though breast cancer incidence is increasing, breast cancer mortality has been decreasing in Sweden for the past decades (Fig. 3) (15). This trend in mortality is probably due to a combination of early detection through the screening program corresponding to diagnosis at an earlier stage and improved prognosis, along with improvements in treatment strategies.

Both the 5-year relative survival and the 10-year relative survival have increased substantially since the 1960s. The 5-year relative survival was 88% in 2013, and the 10-year relative survival was 80% (Fig. 4) (15).

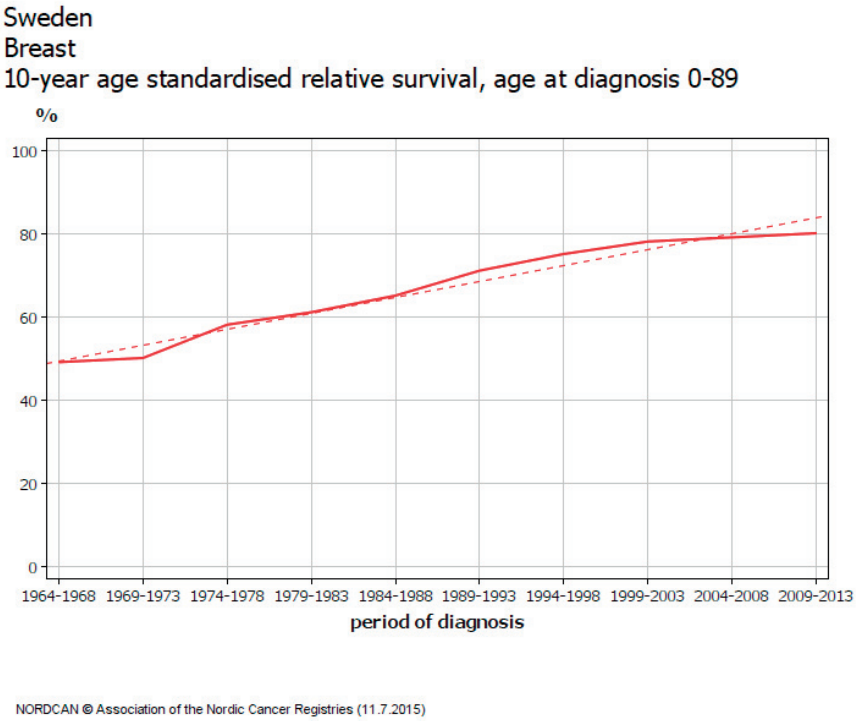

Fig. 4. Time trends in 10 year relative survival (15).

Global differences

Breast cancer incidence is higher in more developed areas of the world than in less developed areas, ranging from 96/100,000 in Western Europe to 27/100,000 in Middle Africa and Eastern Asia in 2012 (16). It is proposed that the different incidences across ethnic groups could be based on differences in hereditary factors and, perhaps to an even greater extent, environmental factors. In studies of migrants from Japan to Hawaii, the migrants assumed the breast cancer rate in the host country within one or two generations, highlighting the importance of environmental factors (2).

2.2.2 Risk factors for breast cancer

Age and socioeconomic status

Together with female sex, increasing age is the strongest risk factor for breast cancer. Further, women with a higher socioeconomic status have a higher breast cancer incidence than do women with a lower socioeconomic status (2). This difference is presumed to be due to a higher attendance in breast cancer screening

programs (in women with higher socioeconomic status) and differences in reproductive patterns (17, 18).

Genetic factors

Breast cancer is a partly heritable trait, and women with first degree relatives with breast cancer have an increased risk of developing breast cancer (2). Ten percent of all breast cancers are thought to be caused by genetic factors. Mutations in the well-known high-penetrance genes *BRCA 1* and *BRCA 2* are known to increase the risk of breast cancer by 10-30 times compared to the general female population (19). In addition, there are a few uncommon genes with intermediate penetrance that are known to increase the risk of breast cancer by 2-3 times. Furthermore, there are common single nucleotide polymorphisms (SNP) and genes with low penetrance that are associated with a minimal increase in breast cancer risk (19).

Reproductive and hormonal factors

A woman's risk of breast cancer is strongly related to several reproductive and hormonal factors. Early age at menarche, older age at menopause, and nulliparity are all reproductive factors associated with an increased risk of breast cancer (2, 20). In other words, the longer time period over which a woman has her menstrual cycles, the higher is the breast cancer risk. In addition, the longer a women breastfeeds, the more she is protected against breast cancer: there is a 4% decreased relative risk of breast cancer for every additional year of breastfeeding (21). There is also evidence that breast cancer risk is positively associated with both endogenous (22) and exogenous estrogen in the form of HRT, especially when the HRT comprises a combination of estrogen and progesterone (23).

Dietary factors

The risk of breast cancer can be reduced by avoiding weight gain in adult life and by seeking a normal body mass index (BMI) (24, 25). There is limited evidence that consumption of total fat or other dietary factors (e.g., soy products or dairy products) affect the risk of breast cancer (26). A high consumption of alcohol has been shown to increase the risk of breast cancer (26), but smoking has not been convincingly shown to be associated with breast cancer (2).

Previous benign breast disease

Previous benign breast disease, such as atypical hyperplasia, has been shown to increase the risk of breast cancer (2).

2.2.3 Pathological tumor characteristics

Carcinogenesis is the process by which normal cells are transformed via genetic and cellular changes into cancer cells. Most cancers share six common hallmarks that contribute to the transformation of a normal cell into a cancer cell, as described by Hanahan and Weinberg (Fig. 5) (27). In the breast, the cancer cell is thought to be derived from the terminal duct lobular unit (28). The complex interplay between several factors (such as genetic factors, hormones, growth factors, and environmental factors) affects the genesis, growth, and progression of breast cancer (26).

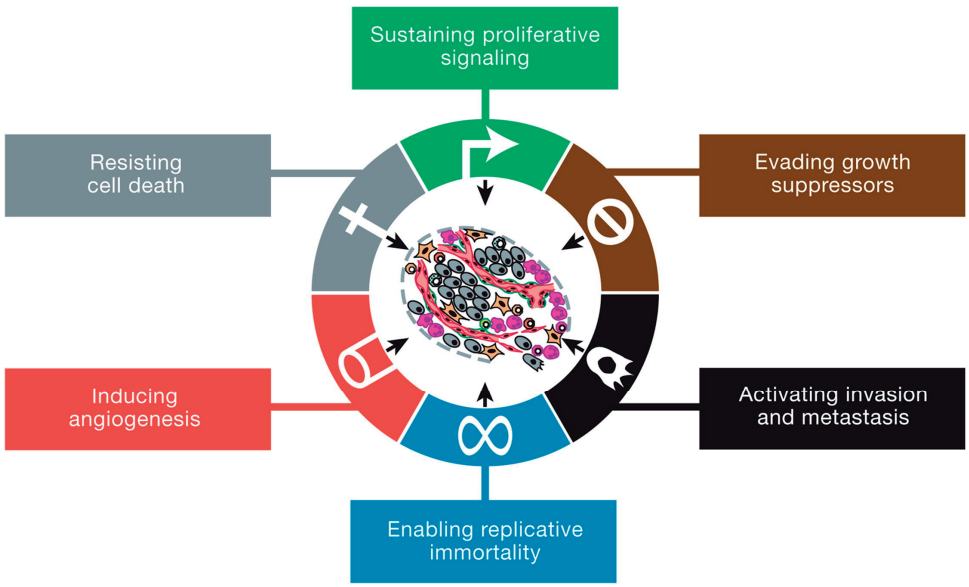

Fig. 5. The Hallmarks of Cancer. Reprinted from the Cell, Vol 144 (5), Hanahan D & Weinberg RA. Hallmarks of Cancer: The Next Generation, Pages No. 646-674, Copyright (2011), with permission from Elsevier.

Pathological tumor characteristics

The breast tumor is classified as a CIS or an invasive cancer. A CIS has preserved integrity of the cellular basal membrane. Ductal CIS is now more commonly diagnosed because of the screening program, as it frequently presents with easily detectable calcifications on mammography (29).

In Sweden, the following prognostic and/or predictive factors are used in daily clinical practice: tumor size, axillary lymph node status, histological grade, and the expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and the cell proliferation marker Ki67 (14).

The most important prognostic factors in breast cancer are stage according to the TNM classification (T:tumor size, N:axillary lymph nodes, M:distant metastases) and histological grade (I-III) (14, 30-32). Histological grade is based on mitotic count, tubular formation, and the degree of nuclear atypia (32). Furthermore, breast cancers are classified according to the World Health Organization (WHO) into different histological types, with ductal (40-85%) and lobular cancer (5-15%) being the most common types of invasive breast cancer.

The ER, which binds estrogen, is a positive prognostic marker. Women with breast tumors that express high levels of ER have a survival advantage compared to women with hormone receptor-negative tumors. Approximately 80-85% of all breast tumors express the ER, and the majority of ER-positive tumors also express the PR. In addition, the ER and PR are predictors of response to endocrine treatment (31, 33). The androgen receptor (AR) is expressed in 70-90% of breast tumors and has been highlighted as a novel positive prognostic marker, a predictive marker for response to endocrine therapy, and also a target in more innovative treatment strategies (34-36). The prognostic factor HER2 is important in cell growth and differentiation. The gene is amplified in 15-30% of all breast cancers and is also associated with more aggressive tumor behavior. HER2 is also a predictive factor, and the receptor can be targeted with anti-HER2 treatment (37). Lastly, the proliferation marker Ki67 adds information on cell proliferation and tumor aggressiveness, in that high expression of Ki67 is associated with a higher risk of relapse and a worse survival (38). In addition, Ki67 may be valuable to discriminate grade II tumors both in terms of prognosis and in the selection of patients for adjuvant medical treatment (39).

Molecular subtypes

In order to achieve further prognostic information for different types of breast cancer, which can be useful in individualizing treatment, Sorlie *et al.* defined breast cancer subtypes based on gene expression profiling (40). For practical purposes, the subtypes can be approximated by clinic-pathological data according to the St. Gallen International Breast Cancer Conference surrogate definition of molecular subtypes (41). The subtype has both prognostic and therapeutic predictive value, with the Luminal A subtype having the most favorable prognosis compared to other subtypes (Luminal B, HER2 positive, and triple-negative breast cancer (TNBC)). Women with TNBC have a prognosis worse than those with other subtypes of breast cancer. TNBC represents 15-20% of all breast cancer and is associated with poor survival, higher frequency of relapses, and insensitivity to endocrine as well as to anti-HER2 treatment (42).

2.2.4 Triple diagnostic

The triple diagnostic is the gold standard of breast cancer diagnostics. It has a sensitivity of almost 100% (43). The triple diagnostic consists of a clinical examination of the breast, a breast imaging modality (mammography and often also ultrasound), and a needle biopsy for cytopathological diagnosis. In case of dubious or discrepant findings in one or more of the modalities, the suspicious finding should be treated as a malignancy or further diagnostic evidence should be sought, e.g., by using other imaging modalites.

2.3 Breast imaging

2.3.1 Mammography

Mammography is the gold standard for breast imaging in the clinical setting and in population-based screening. Mammography utilizes the inherent x-ray attenuation that differs between fat-involuted, fibroglandular, and tumor tissue. This difference results in a contrast difference between the structures in the breast. Epithelium and stroma (fibroglandular tissue) appear white (radio-opaque) because of higher attenuation of x-rays than in fat-involuted tissue, which appears black (radiolucent). The x-rays transmitted through the breast are absorbed by a detector and converted to digitized signals that form the image (29). For analog mammography images, the x-rays affect a photographic film to different degrees, which creates the image (29). Radiation is a well-known risk factor for breast cancer (2). However, the radiation dose from mammography is very low, around 1-2 mGy per image, which corresponds to a very low risk of radiation-induced breast cancer (44). There are no absolute contraindications for mammography. The routinely used views in screening programs are the medio-lateral oblique (MLO) and the cranio-caudal (CC) view. When performing a diagnostic mammogram, a medio-lateral (ML) view is added along with additional special projections as necessary, e.g., magnification views or spot views.

2.3.2 Mode of detection

The breast tumor can be screening-detected or clinically detected, i.e., a woman experiencing symptoms from her breasts, most commonly a lump. Some of the clinically detected cancers are so-called interval cancers, i.e., cancers detected by the woman between screening examinations. High mammographic density, mammographic tumor features, tumor growth rate, and aspects of interpretation and image quality all affect the proportion of interval cancers (7, 9). The interval cancer

can be “false”, i.e., cancer in patients with a prior false negative screening mammogram. Or, the interval cancer can be “true”, i.e., highly proliferative tumors that truly arise between two screening examinations. The mode of detection has been shown to be associated with certain tumor characteristics and prognosis of breast cancer: clinically detected cancers (including interval cancers) are associated with more severe tumor characteristics (e.g., larger tumor size and higher histological grade) (45) and a worse prognosis than screening-detected cancers (45-47). Furthermore, a previous study reported differences in associations between higher mammographic density and decreased breast cancer survival depending on the mode of detection, with stronger associations for clinically detected cancers (12).

2.3.3 Examples of other breast imaging modalities

Ultrasound

Ultrasound is routinely used in the clinical setting. For women younger than 30 years, or women who are pregnant or lactating, ultrasound could be considered the method of choice. Because of physical differences in image generation between the two techniques, ultrasound is a valuable addition to mammography. First, the ultrasound can distinguish a cystic lesion from a solid lesion, indicating severity of the lesion. Second, because ultrasound generates cross-sectional images, ultrasound can visualize breast tissue free from overprojection. This aspect is helpful in women with dense breasts and makes it possible to confirm or exclude the presence of a suscpicious lesion detected by mammography. Lastly, ultrasound is used for image guidance for biopsies of non-palpable suspicious lesions. Mammography and ultrasound together have a higher sensitivity than either of the imaging modalities alone (48, 49). However, an additional ultrasound has also been shown to be associated with more false-positive findings (49-51). According to two recent reports, there is currently no sound evidence supporting routine use of an additional ultrasound to screen women having an average risk of breast cancer (e.g., women with dense breasts) and a negative result on a mammogram (51, 52). Ultrasound is also severely operator-dependent and more time consuming than mammography, making it less suitable in a screening situation.

Magnetic Resonance Imaging (MRI)

Breast MRI has a high sensitivity and is currently primarily used for women with a very high risk of breast cancer due to cancer susceptibility genes. MRI could also be valuable in patients for whom the results of mammography/ultrasound and biopsy are inconclusive or who have dubious findings in terms of suspected malignancy and tumor multifocality or due to previous surgery (53). As for ultrasound, MRI is also reader-dependent in terms of specificity and creates more

false-positive findings in combination with mammography than does mammography in combination with ultrasound (49).

Digital Breast Tomosynthesis (DBT)

DBT has been developed as a three-dimensional mammographic technique with the aim of reducing the adverse effect of overlapping breast tissue (54). DBT images can be acquired in any of the conventional digital mammography projections. The accuracy of DBT has been shown to be superior to that of digital mammography (55) and two-view DBT in combination with two-view digital mammography has been shown to increase cancer detection compared to two-view digital mammographic screening (51, 56, 57). The use of one-view DBT or DBT alone in screening has recently been investigated and has been reported to result in an increased breast cancer detection rate, albeit with a somewhat increased, but still low, rate of recalled women from the screening program (58). In Malmö, DBT is currently used in selected clinical cases and for research purposes.

2.3.4 Mammographic screening

The harms and benefits of mammographic screening have been debated over decades. Based on a meta-analysis on 11 randomized trials, the Independent UK Panel on Breast Cancer Screening nevertheless, concluded that screening corresponded to a relative risk reduction in breast cancer mortality of 20% (59). In addition to the randomized trials, more recent and robust observational studies are also considered to provide sound evidence regarding mammographic screening, especially considering the fact that the aforementioned randomized trials were conducted more than 20 years ago (51). The International Agency for Research on Cancer (IARC) recently confirmed the effectiveness of the mammographic screening program. Their analysis was based on previous randomized trials and also considered high-quality observational studies (51).

History of mammographic screening

Population-based mammographic screening was implemented in Sweden in 1986 and was gradually introduced throughout the country (60). The implementation was based on evidence from the randomized studies in the United States, Canada, and Sweden (59, 61). The Malmö Mammographic Screening Trial (MMST) started in 1976 (62). The randomized setting of the MMST was kept until the implementation of the screening program in Malmö in 1990. The age groups invited to screening have changed over time. In Malmö, the age groups invited were as follows: 50-69 years during 1990-1996, 50-74 years during 1997-2008, and 40-74 from 2009 and onwards at 1.5- to 2-year intervals. In Malmö, until 2008, the screening interval was defined by mammographic density, with a shorter interval (1.5 year) for dense breasts. From 2009 onwards, the screening interval was defined by age, with a

shorter interval for women under 55 years of age. In Sweden today, all women between 40-74 years are invited to screening at 1.5- to 2-year intervals in accordance with guidelines from the National Board of Health and Welfare. In 2014, the screening attendance rate in Malmö was 77%, which was slightly lower than the screening attendance rate of 82% in the areas of Skåne combined (personal communication Unilabs).

Evaluation of mammographic screening

Several factors must be considered when evaluating a screening program. The illness should be highly prevalent and severe and have a long sojourn time (i.e., the preclinical time from when a tumor is possible to diagnose to when it would have been detected clinically in the absence of screening), all of which are applicable to breast cancer (63, 64). The mammographic screening program has been shown to reduce breast cancer specific mortality by 20% (59), which is the primary goal of the screening program. The sojourn time for breast cancer is long, 2-4 years, and in general with a longer interval for older women (65).

The validity of the screening test in terms of sensitivity (true positive/true positive+false negative) and specificity (true negative/true negative+false positive) must be high. For mammography, the sensitivity has been reported to be 71-96% (66). However, with younger age and high mammographic density, the sensitivity could be as low as 30-48% (9, 48). With decreasing sensitivity, the proportion of interval cancers increases. Ideally, the proportion of interval cancers should be low (<30%) to indicate an effective screening program (67). In 1990-1999, interval cancers constituted 16% of the first-time breast cancers in the population invited to screening in Malmö (68). The specificity of mammography is high, 94-97% (66). This high specificity is crucial as false-positive results leading to further diagnostic evaluations are associated with negative psychological consequences (66).

An often debated drawback of the screening program is over-diagnosis, i.e., detection of tumors that would not have otherwise caused symptoms. The rate of over-diagnosed breast tumors has been estimated to 11-19 %, with the lower end of the range relevant for the invited population, and the higher end of the range for women participating in the screening program (59).

Bias in mammographic screening

Randomized trials provide a more trustworthy base than do observational studies when it comes to investigating and evaluating the effect of mammographic screening on mortality. Non-randomized studies investigating mammographic screening are associated with several types of bias that must be considered (69). Selection bias is a result of women attending the screening program being different from women who are not attending, which may create both a better or decreased survival in women participating in the screening program. Length bias refers to the tendency of the tumors detected by screening to be of indolent character, and lead

time bias refers to the spuriously increased survival in women with tumors detected by screening, which is merely due to earlier detection of the tumor.

2.3.5 Mammographic tumor features

The mammographic growth pattern of the breast tumor varies; however, certain distinct mammographic tumor features have been described (Fig. 6). To different degrees, several factors (the breast stroma, the mammographic density, and overlapping breast structures) all interfere and contribute to the mammographic features of the tumor (6, 70, 71). A well-defined mass may represent a benign lesion such as a cyst or a lymph node; however, if the mass is suspicious, ultrasound is warranted to differentiate against a malignant mass. Some tumors provoke a reactive fibrosis in the tumor and the surrounding tissues, which may render an ill-defined or a spiculated tumor border (72). The majority of the spiculated tumors are malignant; however, they are associated with a good prognosis (73). An ill-defined mass on mammography has been associated with prognostically unfavorable tumor factors such as a high histological grade or large tumor size (74). Malignant calcifications tend to vary in size, shape, and density (72). There has been conflicting reports regarding associations between calcifications and survival: some studies report an association between calcifications and poor prognosis (75, 76), while others report no such association (73, 77). Asymmetric densities and architectural distortions can be seen as a slight disruption of the normal architecture of the breast without a dominating mass and may be difficult to detect. Especially lobular cancer may present with such an ambigous mammographic tumor feature (72).

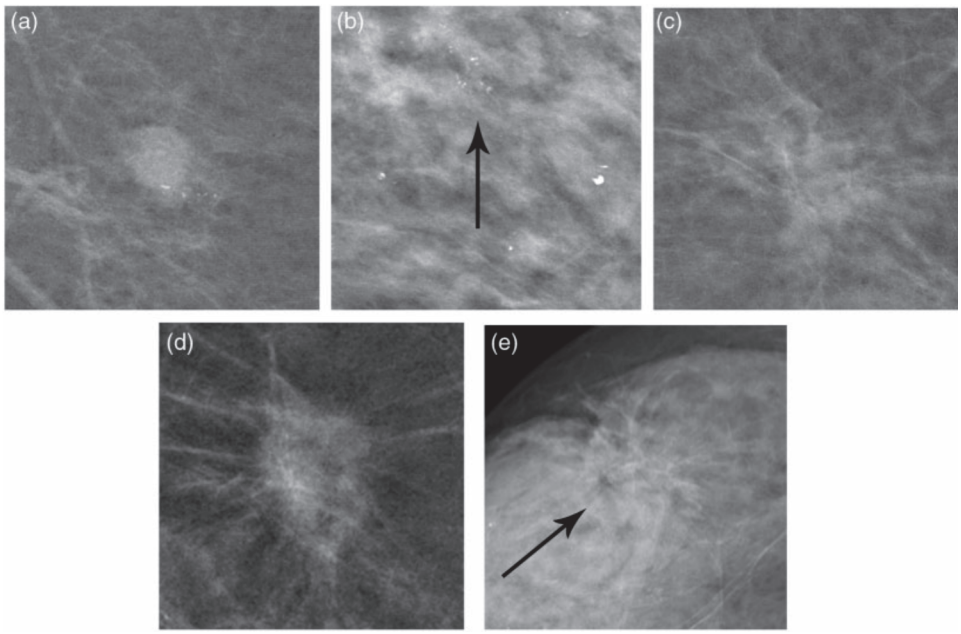

Fig. 6. Examples of some mammographic tumor features: (a) distinct mass, (b) calcifications, (c) ill-defined mass with slight retraction, (d) spiculated appearance, and (e) architectural distortion (referred to in the study as the mammographic tumor feature tissue abnormality) (78).

2.3.6 Mammographic density

Association with breast cancer

It is well known that mammography has a lower sensitivity in women with high mammographic density and that women with high mammographic density have a 4-6 times higher risk of breast cancer than do women with non–dense breasts (3, 9, 79) (Fig. 7 and 8). The relationship between high mammographic density and breast cancer was initially thought to be due entirely to the masking effect, i.e., dense breast tissue masking the breast tumor, leading to delayed detection (a breast tumor often has the same x-ray attenuation as dense tissue) (80). However, there is now evidence of an association between mammographic density and breast cancer in addition to the masking effect (81), as demonstrated by consistent associations in studies of prevalent cancer and of screening-detected cancer in which the tumor is detected in the presence of the masking effect (79, 82, 83). Further, the association between mammographic density and breast cancer has been consistent in cohort studies with as much as 10 years of follow-up, in which time the masking effect would diminish (79). There have been conflicting results regarding the association between mammographic density and survival; two large studies found no association (84, 85), but a recent study found that in women with breast cancer, very low

mammographic density predicted a decreased survival (86). Furthermore, a recent study of the breast cancer patients in the Malmö Diet and Cancer Study (MDCS) showed that high mammographic density at diagnosis may be associated with decreased breast cancer-specific survival, with a stronger association in clinically detected breast cancers (12).

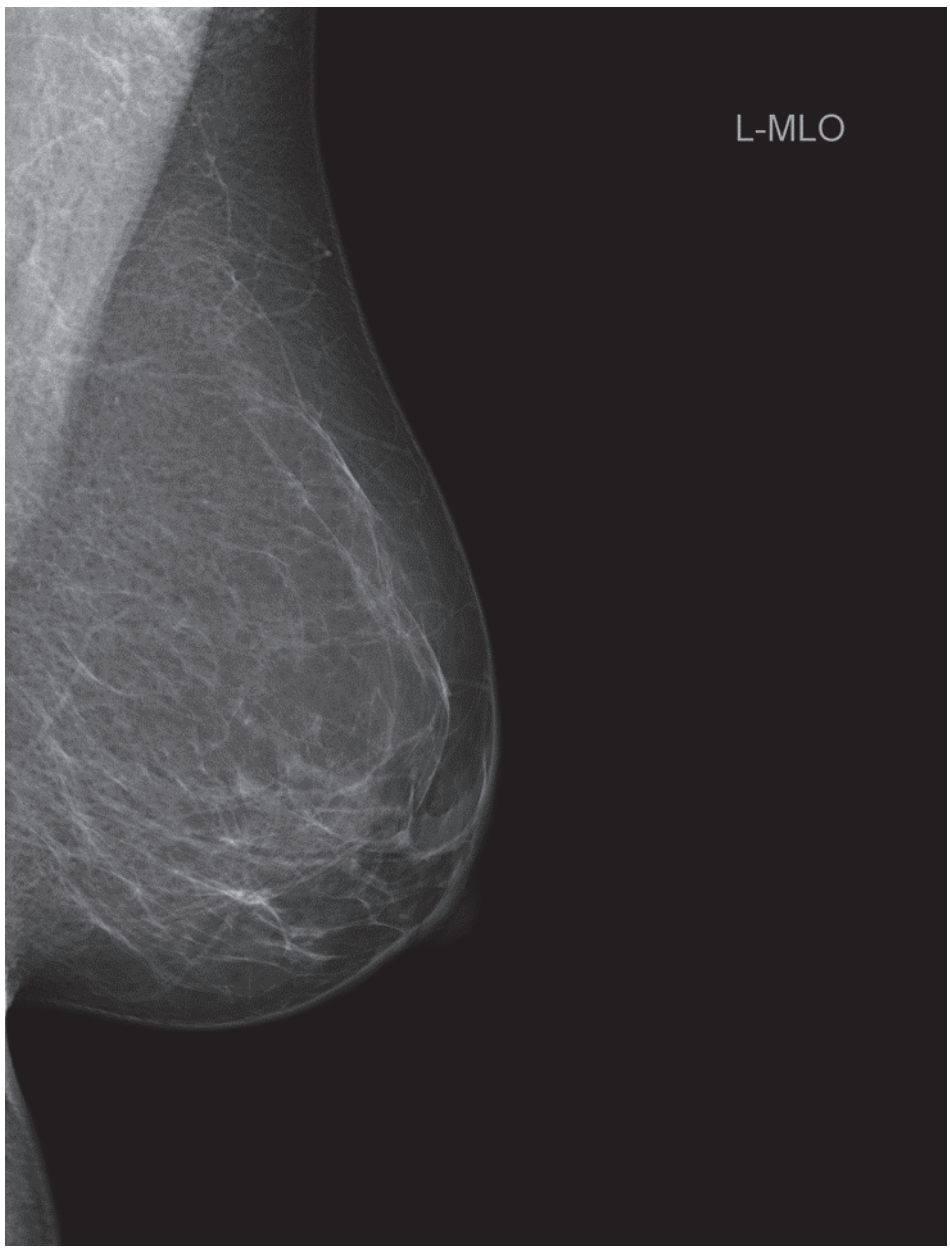

Fig. 7. Example of a fat-involuted breast in an MLO-projection.

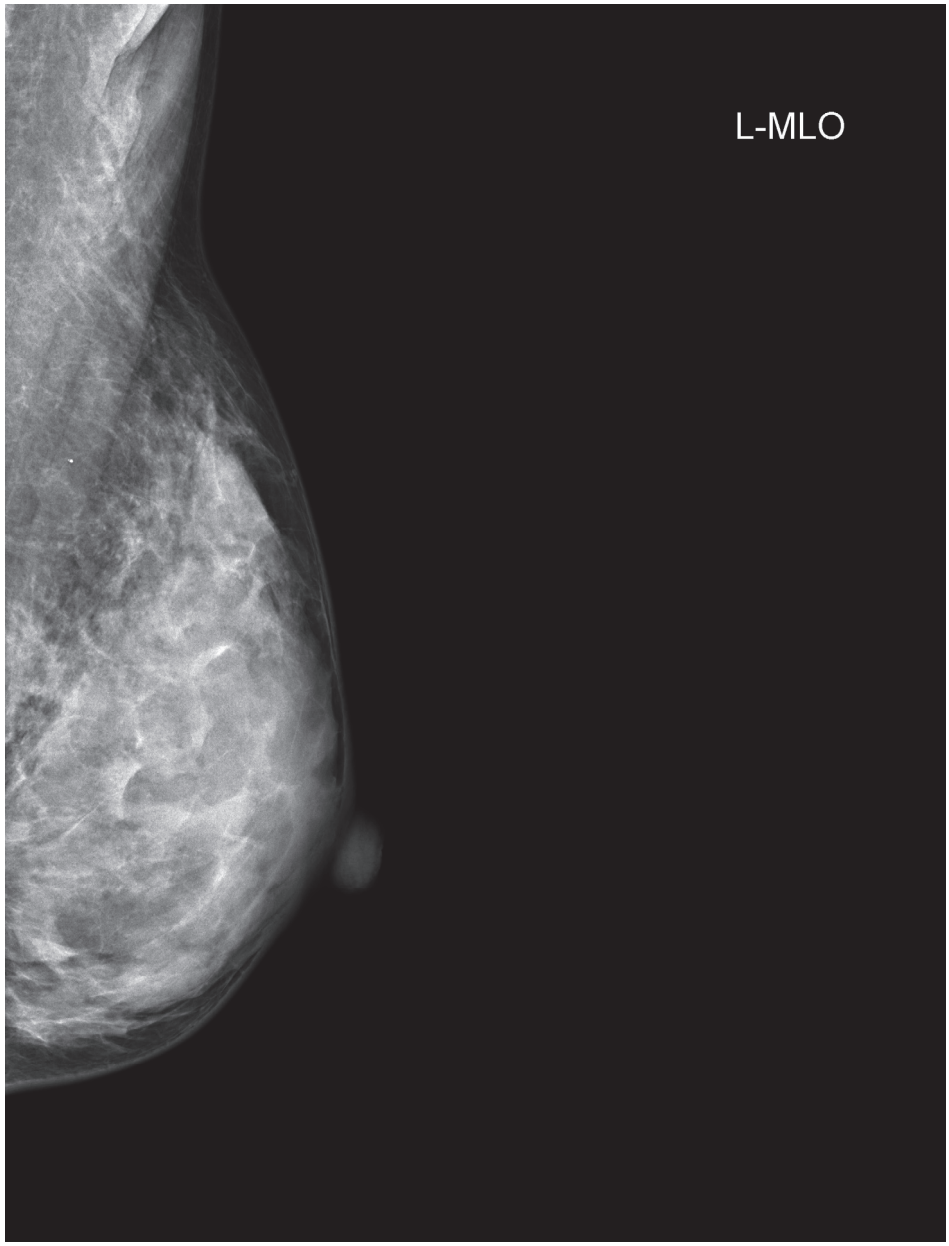

Fig. 8. Example of a very dense breast in an MLO-projection.

Factors associated with mammographic density

Genetic factors have been shown to be important in mammographic density, with heritability accounting for 60% of the variation in density (87). Furthermore, around 10% of the common SNPs associated with breast cancer risk are also associated

with mammographic density (11). In addition, mammographic density is known to be higher in Asian populations than in Caucasian populations (88) and higher in urbanized areas than in non-urbanized areas (89). Mammographic density decreases with increasing age, especially during and after menopause (90). The decreasing mammographic density with increasing age might seem contradictory, as increased age is associated with increased breast cancer risk. However, it has been proposed that the cumulative exposure of breast tissue to different hormone levels during a woman's life (which leads to breast tissue aging), rather than the chronological age, is related to breast cancer risk (91, 92). BMI is inversely associated with mammographic density in that high BMI is associated with a large non-dense area of the breast, the non-dense area being the fat deposit site (93). Furthermore, mammographic density is associated with parity, as demonstrated by a decrease in mammographic density for every live birth (94). Regarding blood levels of endogenous hormones and growth factors, most studies have not found any convincing associations with mammographic density (94). HRT is known to increase mammographic density, especially for women using combined progestogen and estrogen therapy (95, 96). However, estrogen-only HRT has also been found to be associated with increased mammographic density (95). Tamoxifen treatment (i.e., endocrine treatment) has been shown to reduce mammographic density (97), and it has also been shown that women whose mammographic density decreased during treatment had better breast cancer-specific survival than did women whose mammographic density did not decrease (98).

Mammographic density on a tissue level

Breast tissue from mammographically dense areas differs histologically from tissue from non-dense areas, with greater proportions of both epithelial and stromal tissues in dense areas (4, 99). The stromal tissue may be of substantial importance because both epithelial benign and malignant cells interact with the surrounding stroma in cancer initiation, growth and progression (100-102). The link between mammographic density and breast cancer risk is complex and not yet fully understood. However, a recent review by Huo *et al.* suggested possible biological mechanisms involving stromal cells and proteins (such as fibroblasts, immune cells, and collagen) (11). Further studies are warranted to elucidate this relationship.

Assessment of mammographic density

Both qualitative and quantitative methods of measuring mammographic density have shown an association between high mammographic density and breast cancer risk (3, 103).

Qualitative measurements

The first classification of mammographic density and parenchymal pattern were suggested by Wolfe in 1976 (104) and was followed by classifications by Tabár (105) and Boyd (81). Today, the most often clinically used qualitative classification

of mammographic density is the Breast Imaging-Reporting and Data System (BI-RADS) classification (106). The BI-RADS classification has four categories; BI-RADS 1 is an almost fat involuted breast (<25% fibroglandular tissue), BI-RADS 2 is a breast with scattered fibroglandular densities (25-50% fibroglandular tissue), BI-RADS 3 is a heterogeneously dense breast (51-75% fibroglandular tissue), and BI-RADS 4 is an extremely dense breast (>75% fibroglandular tissue). Previous studies on inter-observer variability of BI-RADS scores have reported kappa values of 0.43–0.77 (107-111), where a kappa value of 1 would represent perfect agreement.

Quantitative measurements

In order to more objectively depict mammograpic density and to reduce inter-observer variability, quantitative measurements have been developed (112).

The software Cumulus is an example of a quantitative area-based measurement of mammographic density in digitized analog films or digital images (113). It is a computer-assisted thresholding technique with an operator setting two thresholds to separate the breast from the background and to separate dense from non-dense tissue. Cumulus is currently considered to be the gold standard for measuring quantitative mammographic density (114).

Because the breast and the dense breast tissue are three-dimensional, fully automated volumetric density assessments have been developed with the intent to more accurately depict mammographic density and to further reduce inter-observer variability. It has been proposed that volumetric breast density may add knowledge and improve future models for risk estimation and screening stratification (114). The Volpara software is an example of a fully automated volumetric density measurement (115). Volpara measures the x-ray attenuation in relevant parts of the breast and relates it to a region in the breast considered to only contain adipose tissue (assuming an even breast thickness). Volpara then produces a fibroglandularity content map of the breast that allows for estimation of breast density measurements. The volumetric breast density refers to the percentage of breast density, computed by dividing the fibroglandular tissue volume by the breast volume.

3 Aims

3.1 Overall aim

The overall aim of this thesis was to study how mammographic density relates to breast cancer in terms of mammographic tumor features, pathological tumor characteristics, and mode of detection.

An additional aim was to assess the agreement between two methods of measuring mammographic density.

3.2 Specific aims

Paper I

The aim of Paper I was to investigate if mammographic tumor features were associated with mammographic density and pathological tumor characteristics in breast cancer.

Paper II

The aim of Paper II was to investigate the associations between mammographic density and clinically established tumor characteristics in breast cancer, with emphasis on mode of detection.

Paper III

The aim of Paper III was to investigate the associations between mammographic density and tumor biomarkers, including molecular subtypes, in screening- and clinically detected breast cancer.

Paper IV

The aim of Paper IV was to assess the agreement of mammographic density by a fully automated volumetric method with the radiologists' classification according to BI-RADS.

4 Materials and Methods

In God we trust; all others must bring data.

-W. Edwards Deming

4.1 Databases

The Malmö Diet and Cancer Study (MDCS) (Paper I-III)

The MDCS is a population-based, prospective cohort study whose primary object was to investigate a possible relationship between diet and cancer (116). The study started in 1991 and enrolled participants up until 1996. It included 28,098 participants, of whom 17,035 were women. This corresponded to a participation rate of 40% (117). Entire birth cohorts were invited; the invited women were born between 1923 and 1950. In addition to base-line variables (anthropometric measures, blood samples, and an extensive questionnaire including data on socio-demographics, reproductive factors, life-style, medication, and health status), the breast cancer cases have been identified and the associated pathological variables have been added to the database. The MDCS cohort is continuously updated with new cancer cases and causes of death through record-linkage to national registries held by the National Board of Health and Welfare. The screening attendance rate in the MDCS ranged from 87.6% to 94.5% during the study period (118). The MDCS has been described in detail previously (116, 117, 119). Papers I-III were approved by the Ethical Committee at Lund University (Dnr 652/2005 and Dnr 166/2007).

Malmö Breast Tomosynthesis Screening Trial (MBTST) (Paper IV)

The MBTST is a prospective, one-arm, single-institution study with the aim of investigating the use of one-view DBT (MLO) alone compared to two-view digital mammography (CC and MLO) in a population-based screening program in Malmö, Sweden (www.clinicalTrials.gov; NCT01091545). A random sample of women eligible for the ordinary screening program in Malmö were invited to participate in the MBTST. Women were chosen from the population-based screening registry in order to achieve a representative sample of the population in terms of age distribution (40-74 years). The MBTST was finalized in March 2015, at which point it included 15,000 women. Raw data from the digital mammography examinations

were saved on a dedicated server from February 8, 2012 and onwards. The MBTST was approved by the Regional Ethical Review Board at Lund University (Dnr 2009/770) and the local Radiation Safety Board at Skåne University Hospital in Malmö. Results from the first part of the MBTST have been recently described (58).

4.2 Study populations

In Papers I, II, and III, study populations were created from the MDCS. Between 1991 and 2007, 826 incident breast cancer cases were identified in women in the MDCS. Because recurrent breast cancer may differ from incident breast cancer in terms of risk factors and biomarkers, women with a history of breast cancer at baseline (n=576) were excluded in Papers I, II, and III. Of the 826 incident breast cancer cases, 15 women with bilateral tumors were excluded because of the difficulty of retrospectively evaluating information on mammography data and breast tumor characteristics for these cases. Papers I and II included both CIS and invasive breast cancer, but only invasive breast cancer cases were included in Paper III. Furthermore, cases without sufficient tumor tissue for the tissue micro array (TMA)-analyses were excluded in Paper III. For women diagnosed with breast cancer, the median time between inclusion in the MDCS and breast cancer diagnosis was 7.6 years. The study populations in Papers I, II, and III are illustrated in Fig. 9.

In Paper IV, the study population was created from the MBTST. This present study was based on the digital mammography images with available raw data from the screening examinations from February 8, 2012 up until March 11, 2014. The study population included examinations from both women without breast cancer (n=8,789) and women with breast cancer (n=100) during the study period. The final study population of 8,889 examinations had 8,880 examinations with BI-RADS scores, 8,531 examinations with Volpara values, and 8,522 examinations with both Volpara values and BI-RADS scores (7,939 examinations with Volpara values and BI-RADS scores from the first radiologist). The study population in Paper IV is illustrated in Fig. 10.

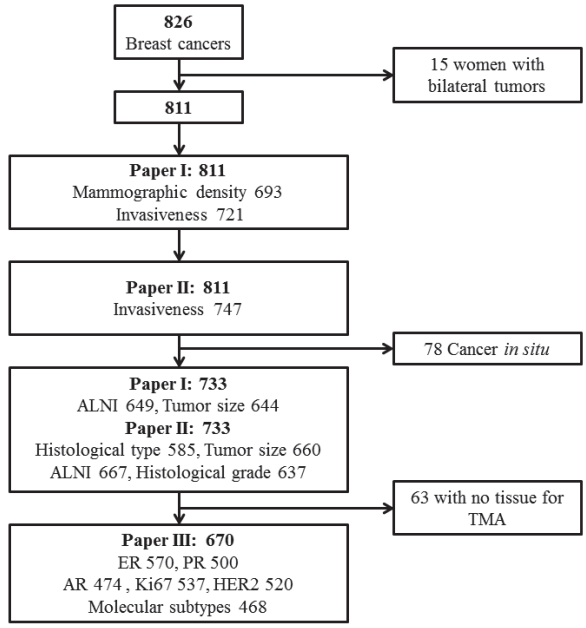

Fig. 9. The Malmö Diet and Cancer Study. Flowchart illustrating study population, exclusions, and subgroups of Paper I, II, and III. Cases available for analyses will differ due to differing numbers of cases with missing values in analyses with mammographic tumor features (Paper I) and mammographic density (Papers II and III).

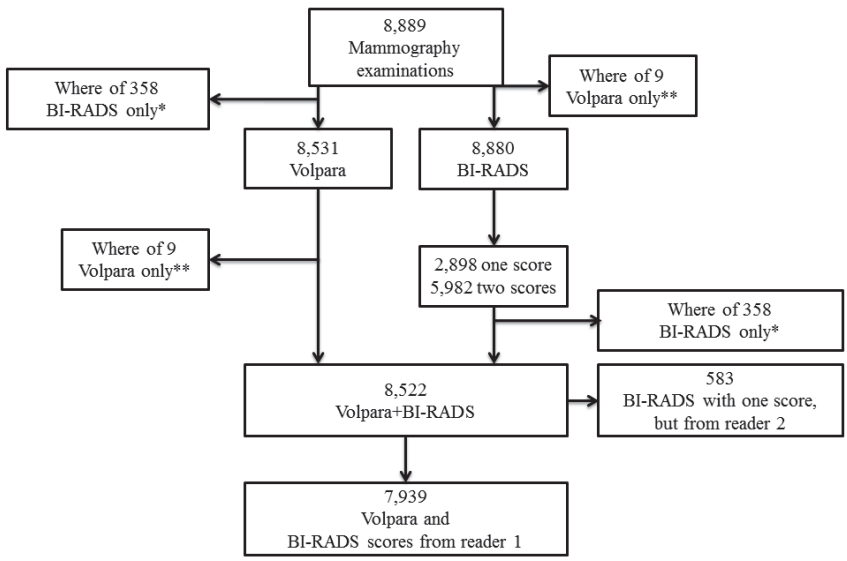

Fig. 10. The Malmö Breast Tomosynthesis Screening Trial. Flowchart illustrating study popuation, exclusions, and subgroups in Paper IV. *This represents the same examinations. 1 not included in Volpara file, 22 breast implants, 335 missing Volpara-values. **This represents the same examinations. 9 examinations without BI-RADS scores.

4.3 Mammographic information (Papers I-III)

In Papers I-III, mammographic information was assessed from the radiology report from the mammogram closest to the date of diagnosis. The initial evaluation was made by experienced radiologists at the Department of Breast Radiology, Malmö, Sweden. A research protocol was established to register the following information from the radiology reports: the mode of detection, mammographic density, and mammographic tumor features.

The mode of detection was defined as screening (including opportunistic screening) or clinical (i.e., cancers in women with symptoms in the breasts, including interval cancers). For seven cases, information regarding the mode of detection (screening vs. clinical) was missing. The clinical cases included at least three images per breast (CC, MLO, and ML views). Additional special projections, e.g., magnification views and spot views, were added when needed. The screening cases had one set of screening mammograms (CC and MLO) and additional images from the diagnostic work-up at the recall, usually including an ML view and special views of the affected breast.

4.3.1 Mammographic tumor features

The most dominant mammographic tumor feature (defined as the most easily perceived abnormality) was assessed using the radiology report from the diagnostic mammogram. The most dominant mammographic tumor feature was then defined according to a classification by Luck *et al.* (5): mass (well-defined, partly ill-defined or ill-defined/diffuse), spiculated mass, architectural distortion or asymmetric density. Microcalcifications were categorized as either comedo-type or non-specific calcifications. For the statistical analysis, the following categories were used: distinct mass (well-defined or partly ill-defined), ill-defined mass (ill-defined/diffuse), spiculated appearance, calcifications (comedo-type or non-specific calcifications), and tissue abnormality (architectural distortion or asymmetric density). For those cases where information from the reports on mammographic tumor feature was uncertain (one fifth of the cases), the images were re-read by one breast radiologist and categorized accordingly. Cases where no report and/or image could be located (n=90) were classified as having missing data.

4.3.2 Mammographic density

During the initial evaluation of the diagnostic mammogram, mammographic density was qualitatively evaluated based on both breasts and all views. Three categories were routinely reported: “fat involuted”, “moderately dense” and “dense”. The

classification can be regarded as a modification of BI-RADS categorization of breast composition; “fat involuted” corresponds to BI-RADS 1 (almost fat-involuted), “moderately dense” to BI-RADS 2+3 (scattered fibroglandular densities and heterogeneously dense), and “dense” to BI-RADS 4 (extremely dense). For those cases where information on mammographic density was missing (about one third of the cases), mammograms were retrospectively re-read by one breast radiologist and one trained, supervised resident in radiology. Cases where no report and/or image could be located (n=64) were classified as having missing data.

4.4 Mammographic information (Paper IV)

4.4.1 Qualitative assessment of mammographic density

The 8,880 examinations from the MBTST with BI-RADS scores where prospectively classified according to BI-RADS as part of the initial screening reading procedure by at least one of the two readers. The following BI-RADS categories for mammographic density were used: BI-RADS 1, almost fat-involuted (<25% fibroglandular tissue); BI-RADS 2, scattered fibroglandular densities (25–50% fibroglandular tissue); BI-RADS 3, heterogeneously dense (51–75% fibroglandular tissue), and BI-RADS 4, extremely dense (>75% fibroglandular tissue). Nine examinations were not evaluated with BI-RADS (Volpara only). Of the 8,880 examinations (with BI-RADS scores), 2,898 had one score, and 5,982 had two BI-RADS scores (reader 1/reader 2). The scores were performed by five breast-radiologists with more than 10 years’ experience in breast radiology.

4.4.2 Volumetric breast density assessment

The 8,531 examinations with two-view digital mammography raw data were assessed with the fully automated volumetric breast density measurement software Volpara (version 1.5.11, Matakina Technology, Wellington, New Zealand) (115). Breast density was measured both as a continuous variable (volumetric breast density (VBD)) and as an ordinal variable with four grades (Volpara density grade (VDG)). The VDG thresholds have been based on performance data from American radiologists (115). Because of the lack of digital mammography raw data (n=281), breast implants (n=49), or software-failure (n=5), 335 examinations were not included in Volpara analyses. Examinations with previously known breast implants were excluded because the software has known difficulties in correctly measuring volumetric breast density in these images (n=22).

4.5 Pathological tumor characteristics (Papers I-III)

Tumor tissue was collected and stored in the biobank at the Department of Pathology, Skåne University Hospital, Malmö, Sweden. Pathological tumor data such as histological tumor type, pathological tumor size, histological grade, invasiveness, and ALNI were assessed from clinical notes and pathology reports.

4.5.1 Tissue Micro Array (TMA)

Invasive tumors with sufficient tumor tissue were examined by TMA, from which information was used for Paper III. The previously studied immunohistochemistry (IHC) markers included ER, PR, AR, Ki67, and HER2 (120, 121). To construct the TMA, two cores 0.6mm (1991-2004) or 1.0mm (2005-2007) in size were retrieved from each tumor and arranged in a recipient TMA block. The TMA blocks were cut into 4μm sections and processed automatically for IHC analyses. Dichotomized variables were used for ER, PR, AR, and Ki67; samples with 10% or fewer stained nuclei were considered negative (or low regarding Ki67), and those with more than 10% stained nuclei were considered positive (or high regarding Ki67), in accordance with current Swedish clinical guidelines for ER and PR and previous MDCS studies for AR and Ki67 (120, 121). All arrays (ER, PR, HER2, and Ki67) were evaluated independently twice by the same investigator. In the case of a discrepancy, a third evaluation was performed by the same investigator. In the case of a heterogeneity between the two cores, the evaluation was based on the core with the highest expression. The AR arrays were evaluated independently twice, and a third examination was performed in the case of a discrepancy. In the case of heterogeneity of AR expression between the two cores, the decision was based on visual assessment of the two cores' total tumor area pooled together. HER2 was classified as negative or positive based on protein expression and immunohybridization, as described previously (121).

Antibody	Clone	Source	Dilution
ER	6F11	Ventana, US	Prediluted
PR	16	Ventana, US	Prediluted
Ki67	MIB-1	Dako, Denmark	1:200
HER2	Z4881	Zymed, US	1:100
AR	AR441	Thermo Scientific, US	1:200

Fig. 11. Antibodies used in Paper III.

4.5.2 Molecular subtypes

The molecular subtypes (based on IHC) were defined according to a modified version of The St. Gallen International Breast Cancer Conference surrogate definition of subtypes (41). The subtypes were defined as follows: Luminal A: ER-positive and/or PR-positive and low Ki67 (≤10%), Luminal B: ER-positive and/or PR-positive and high Ki67 (>10%), HER2: all HER2-positive tumors regardless of ER/PR/Ki67 status, TNBC: ER-negative, PR-negative, and HER2-negative regardless of Ki67 status.

4.6 Statistical analysis

All statistical analyses were carried out using SPSS Statistics for Windows (Version 20-22 IBM Corp., Armonk, NY, USA) (Papers I-III) and Stata v13 (StataCorp LP, Texas, USA) (Paper IV).

4.6.1 Brief description of statistical analyses used

Kruskal-Wallis and Mann-Whitney tests

The Kruskal-Wallis and Mann-Whitney tests are rank-based, non-parametric methods of comparing the distribution between two (Mann-Whitney) or more (Kruskal-Wallis) groups in a sample. Dunn's method (122) uses the ranking from the full sample for pairwise comparisons of groups (using the Mann-Whitney test).

Logistic regression

For binary outcomes, odds ratios (ORs) and confidence intervals (CIs) can be modeled using logistic regression. Logistic regression compares the odds of having the outcome given the exposure to the odds of having the outcome without the exposure and allows adjustment for possible confounders.

Multinomial and ordinal regression

Multinomial and ordinal regression are extensions of logistic regression, which allows the use of categorical outcomes with more than two groups. Ordinal regression is used when there is an ordering between the categorical outcome

values, whereas multinomial regression does not presuppose an order between the possible outcomes.

Kappa-analysis

A kappa-analysis can be used to assess the agreement between two categorical variables. The kappa value takes the agreement that would occur by chance into account. If the variables are ordered, the weighted kappa can be used. This weighted kappa method weighs the scores differentely depending on how far apart the scores are. By convention, values of <0.0, 0.00–0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80 and 0.81–1.00 are respectively indicative of poor, slight, fair, moderate, substantial, and almost perfect agreement (123).

Bonferroni correction

Bonferroni correction helps to prevent potential mass-significance in analyses with multiple comparisons. After Bonferroni correction, each individual hypothesis is tested at a statistical significance level of 1/x times what it would be if only one hypothesis were tested, where x is equal to number of hypotheses being tested. Bonferroni correction is the most conservative method to correct for multiple testing and may result in reduced power to detect differences.

4.6.2 Statistical analyses for Papers I-IV

Paper I

Differences in mammographic density in relation to mammographic tumor features were analyzed using the Kruskal-Wallis test, followed by pairwise Mann-Whitney tests using Dunn's method (122). The p-values of the 10 pairwise tests were presented with and without Bonferroni correction. Associations between mammographic tumor features (five categories) and pathological tumor factors (binary outcomes) were analyzed using logistic regression. The models were adjusted for age at diagnosis (linear), mode of detection (binary), and mammographic density (linear on three levels), as these factors could potentially influence both the mammographic tumor features and the studied breast tumor characteristics.

Papers II and III

In Papers II and III, mammographic density was treated as a linear variable (on three levels); thus, the OR should be interpreted as the increased odds per step in mammographic density. Possible associations between mammographic density and binary outcomes (invasiveness, ALNI, histological type, and tumor biomarkers) were analyzed using logistic regression. For ordinal outcomes (tumor size and histological grade), ordinal regression was used. The classification in molecular

subtypes was treated as an unordered categorical outcome, and the association with mammographic density was analyzed in a multinomial regression model.

In all regression models in Papers II and III, adjustments were made for age at diagnosis (linear), mode of detection (binary), BMI at baseline (linear), and (in paper III) HRT at baseline (binary (no HRT/HRT)). All analyses were further stratified for the mode of detection (screening-/clinically detected). In sensitivity analyses, mammographic density (three categories) (in Papers II and III), and age at diagnosis (four categories: 45-49, 50-59, 60-69, ≥70) (in Paper III) were entered as categorical variables.

Regarding the ordinal regression, the proportional odds assumption was studied using a parallel lines test, and if a non-proportionality was indicated (i.e., $p<0.05$) the separate logistic regressions were analyzed, and the ORs were compared. To report consistent measures across outcomes and subgroups, the ORs from the ordinal regression were still reported, which should be interpreted as an average OR across the cut points in the outcome.

Paper IV

Weighted kappa and 95% CI were calculated for the estimation of interobserver variability for examinations with two BI-RADS scores. In analyses with BI-RADS and Volpara, the BI-RADS score from reader 1 was used. Agreement between VBD (continous variable) and BI-RADS scores was analyzed descriptively. Kappa values for comparison between VDG (ordinal variable) and BI-RADS scores were calculated rendering both a separate kappa for each reader (reader vs. Volpara) and a pooled kappa (all readers vs. VDG) (124). Examinations from women with breast cancer (n=100) were included in all of the analyses except for additional sensitivity analyses.

5 Results

Paper I

The aim of Paper I was to investigate if mammographic tumor features were associated with mammographic density and pathological tumor characteristics in breast cancer.

Trends with regard to mammographic density differed among the different mammographic tumor features (Kruskal Wallis $p<0.001$). Tumors presenting as an ill-defined mass, calcifications or tissue abnormality were more common in dense breasts than tumors presenting as a distinct mass or with a spiculated appearance as the dominant mammographic tumor feature, which were more common in fat involuted breasts.

Tumors with a spiculated appearance were more likely to be invasive cancers than tumors presenting as a distinct mass (OR_{adj} 5.68 (CI 1.81-17.84)). In invasive cancers, tumors presenting as an ill-defined mass (OR_{adj} (3.16 (1.80-5.55)) or tissue abnormality (OR_{adj} 4.05 (1.41-11.64)) were more often large (pathological tumor size >20 mm) than tumors presenting as a distinct mass. In invasive cancer, the mammographic tumor features did not differ according to ALNI ($p=0.277$). However, tumors presenting as an ill-defined mass or a spiculated appearance tended to be ALNI positive more often than tumors whose dominant mammographic feature was a distinct mass.

Paper II

The aim of Paper II was to investigate the associations between mammographic density and clinically established tumor characteristics in breast cancer, with emphasis on mode of detection.

There was an indication of lobular cancer being more frequent than ductal cancer in denser breasts (OR_{adj} 1.25 (0.90-1.72)). Mammographic density was associated with tumor size; there was in general strong evidence of larger tumors in denser breasts (all modes of detection: OR_{adj} 1.59 (1.26-2.01), screening-detection: OR_{adj} 1.50 (1.09-2.06), clinical detection: OR_{adj} 1.76 (1.23-2.51)). There was moderate evidence of ALNI-positive cancer being more frequent than ALNI-negative cancer in denser breasts (OR_{adj} 1.32 (1.00-1.74)). There was even stronger evidence of ALNI-positivity in screening-detected cancers (OR_{adj} 1.69 (1.11-2.56)). There was weak evidence of an inverse relationship between mammographic density and

histological grade in screening-detected cancers; the higher the mammographic density, the lower the histological grade was (OR_{adj} 0.73 (0.53-1.02)).

Paper III

The aim of Paper III was to investigate the associations between mammographic density and tumor biomarkers, including molecular subtypes, in screening- and clinically detected breast cancer.

Higher mammographic density was associated with ER-negative tumors in clinically detected breast cancer (OR_{adj} 1.93 (1.04-3.59)). There was an indication that higher mammographic density was associated with AR-negative tumors in clinically detected breast cancer (OR_{adj} 1.77 (0.80-3.93)).

There was no overall indication of heterogeneity in the OR for mammographic density across subtypes (p=0.17). However, higher mammographic density was associated with TNBC (Luminal A as reference) (OR_{crude} 1.70 (1.02-2.84)). In adjusted analyses, the evidence of an association between higher mammographic density and TNBC was slightly weaker (OR_{adj} 1.64 (0.94-2.86)). However, in clinically detected tumors there was moderate evidence of an association between higher mammographic density and TNBC (OR_{adj} 2.44 (1.01-5.89)).

Sensitivity analyses using mammographic density (Papers II and III) and age (Paper III) as categorical variables instead of linear variables, did not change the results.

Paper IV

The aim of Paper IV was to assess the agreement of mammographic density by a fully automated volumetric method with the radiologists' classification according to BI-RADS.

There was substantial agreement between BI-RADS scores, with a weighted kappa of 0.77 (0.76-0.79)). There was a spread of VBD values across each BI-RADS category which might be considered to indicate poor agreement; if these two methods of mammographic density measurement were in agreement, there would be only a certain range of VBD values in each BI-RADS category. There was moderate agreement between VDG and BI-RADS, with a pooled kappa for all five radiologists of 0.55 (0.53-0.56). Excluding the examinations from women with breast cancer (n=100) did not change the results of the sensitivity analyses.

6 Discussion

6.1 Methodological considerations

There are three essential alternative explanations for a statistical association: chance, bias, and confounding. The influence of chance can be evaluated with a test of statistical significance. Bias is when systematic errors lead to misclassifications of exposure and/or outcome. Confounding occurs when the exposure and outcome have common causes. Random errors affect the study precision, and systematic errors and confounding affects the validity of the study.

6.1.1 Study design

In Papers I-III, data from the population based, prospective MDCS was used. The percentage of foreign-born women in the MDCS was lower than in the city of Malmö in general, and the educational level of the participants in the MDCS was slightly higher. These factors may have limited the representativeness of Papers I-III. After inclusion, participants in the MDCS had a higher incidence of breast cancer but a lower breast cancer mortality than did non-participants, which may imply a higher proportion of screening-detected tumors and a greater concern for ones health in participants (119). However, the studied radiological and pathological factors in Papers I-III were commonly distributed, and we thus believe that internal comparisons should not be affected to any large extent by the possible selection bias of perhaps more health-conscious individuals in the MDCS.

In Paper IV, we used data from the MBTST. The population in the MBTST was a random sample of the screening population representative of the female population in the screening ages 40-74 years in the city of Malmö, Sweden (58).

6.1.2 Precision

Chance and random errors affect the study precision. Increasing the number of participants is the best way to reduce the influence from random errors and chance. Increasing the number of participants will, however, not reduce the impact of bias (systematic errors).

A statistical test evaluates if the data is consistent with a predefined null hypothesis (i.e., no differences between groups). The statistical test generates a p-value and a CI. A p-value is the certainty with which we say that the observed association (or a more extreme value than the one observed) would appear by chance. The p-value does not evaluate if the association is true; the association could still be a result of systematic errors. Furthermore, the p-value does not evaluate the strength of association. However, the CI includes both the significance and the strength of an association. The commonly used 95% CI means that one can be 95% confident that the "true" value lies within that range.

Type I and type II errors refer to the inaccurate rejection or non-rejection of a given null hypothesis. In this thesis, we examined several analyses with different endpoints and within subgroups which may increase the Type I error (i.e., increasing the possibility of finding false-positive associations). In addition to this, some of the stratified analyses had a low number of cases, which decreases the possibility of detecting any associations within subgroups, hence possibly increasing the number of Type II errors (i.e., increasing the possibility of finding false-negative associations).

6.1.3 Validity

Misclassification of exposures

No formal assessment of intra- or interobserver variability was performed for the estimation of mammographic density and mammographic tumor features in Papers I-III, which is a limitation. Mammographic tumor features were classified into categories originally defined by Luck *et al.* (5). Classification of mammographic tumor features varies between studies, although the major groups, such as spiculation or calcification, are usually similar between classifications. We believe the classification used in Paper I to be specific enough to distinguish between the major types of mammographic tumor features. Previous studies investigating inter-observer variability of BI-RADS have reported kappa values of 0.43–0.77 (107-111). The radiologists at the Department of Breast Radiology in Malmö were consistent during the MDCS study period, which assured reliability over time. In Paper IV, 5,982 screening examinations were double-read by in general the same radiologists who qualitatively estimated mammographic density in Papers I-III. The agreement between radiologists was substantial (weighted kappa of 0.77 (0.76-0.79), Paper IV), which provides support for the qualitative estimation of mammographic density used in Papers I-III.

There was a change from analog to digital mammography at the Department of Breast Radiology in 2004, so Papers I-III are based on both analog and digital mammography images. A previous study reported no effects on the results related

to the mode of acquisition when using a qualitative mammographic density measure such as BI-RADS (125).

Misclassification of outcomes

In Papers I-III, information on invasiveness was available for all cases, and very few cases had missing data on pathological tumor size. In Papers I and II, 129 cases had missing information on ALNI, most likely because the pre-operative evaluation indicated no need for axillary dissection. Cases who had a tumor size of ≤ 20mm and who had no distant metastases at diagnosis, or had a CIS, were then re-classified as ALNI-negative. Eight cases retained the missing data classification. Without reclassification, there would be a risk of selection bias because most of these cases probably had ALNI-negative cancers. All cases diagnosed between 1991 and 2004 were re-evaluated according to WHO classification (histological tumor type) and Elston and Ellis (histological grade) by one senior breast pathologist (120). For the cases diagnosed between 2005 and 2007, information was retrieved from clinical notes and pathology reports. The tumor biomarkers used in Paper III were analyzed using the established TMA technique, in which the use of two cores (each sized 0.6mm-1mm) have been shown to be highly representative of the tumor (126). The St. Gallen criteria have currently set the cut-off for Ki67 at 14% (41), although we have used a cutoff of 10% for Ki67 in Paper III, in line with previous studies within the MDCS (120, 121). The 10% cut-off is considered acceptable, as the optimal Ki67 cut-off is still under debate (127-129). The classification of molecular subtypes (based on IHC) has been presented in different ways (41, 130), and previous studies have used somewhat different classifications (131-133). The modified classification used in Paper III is overall in line with current clincal practice in Southern Sweden.

In Paper IV, two methods of measuring mammographic density were compared (Volpara software vs. radiologist). Breast tumors are known to possibly affect the sorrounding breast tissue and thereby perhaps also the mammographic density. Therefore, examinations from women with breast cancer were excluded in additional sensitivity analyses, which did not change the results. Unfortunately, we did not have consistently registered information on previous breast surgery, use of HRT, or reproductive information, all of which are factors known to affect the mammographic density (11). However, because these factors are not expected to affect the two modes of assessment differently, analyses of agreement between them should not be affected to any large extent. Further, mammographic density is known to be higher in urbanized areas (as Malmö) than in non-urbanized areas (89), but this factor is also not expected to affect the two modes of assessment differently, though it may limit the representativeness of this study. For a few cases in Paper IV (n=10), the BI-RADS and VDG scores were discrepant over several categories (BI-RADS 1 vs VDG 4 and vice versa). However, when those examinations were examined more closely, the BI-RADS scores were believed to be due to human

labeling errors. Hence, human labeling errors might be an issue for some of the examinations in Paper IV.

Confounding

In Papers I-III, confounding factors were identifed and adjusted for on the basis of already established and potential factors that influence mammographic tumor features, mammographic density, and tumor characteristics.

Mode of detection may be associated with a tumor presenting with certain characteristics such as larger size and lymph node positivity. Further, the mammographic density and mammographic tumor features may be associated with the tumor being screening-detected or clinically detected. Because of the known relationship between mammographic density, BMI, and HRT (11) adjustments were made for BMI at baseline (in Paper II) and for BMI and HRT at baseline (in Paper III). It would have been preferable to adjust for BMI at diagnosis, as that is the time point closest to the diagnostic mammogram; unfortunately, no information regarding BMI had been registered at diagnosis. Although the largest weight changes in women usually appear with menarche, pregnancy, and menopause (most MDCS women were postmenopausal), weight changes over time cannot be excluded (134). Information relating to HRT at diagnosis was available for some patients, but there was a considerable fraction of cases with missing data, making it less suitable for the analyses. Hence, by instead using information relating to HRT at baseline, the fraction of women who used HRT may be both higher and lower, as the MDCS women may have both initiated and terminated HRT after inclusion. Thus, even when adjustments were made for BMI and HRT (at baseline), there could still be some residual confounding effects. In addition, it would have been appropriate to adjust for both BMI and HRT also in Papers I and II. However, retrospective sensitivity analyses for the main unstratified analyses in Papers I and II, with the inclusion of adjustment for BMI and HRT at baseline, did not in general change the results considerably.

6.2 Main findings and interpretation

Mammographic density, tumor characteristics, and mode of detection

The distribution of mammographic tumor features differed across mammographic density categories, with more tumors presenting as an ill-defined mass or calcifications in denser breasts. The findings in Paper I might be explained by the tendency of mammographic density to mask the mammographic tumor feature in dense breasts, which creates differences in features (6, 70). The distribution of mammographic tumor features may also be related to an epithelial-stromal interaction between the breast tumor and surrounding dense breast tissue while the

stroma contributes both to the mammographic density (4) and to the mammographic tumor feature (71).

Furthermore in Paper I, there was an association between spiculated tumor appearance and pathological invasiveness, as well as between ill-defined mass, tissue abnormality and large tumor size. These findings were consistent after adjustment for mammographic density and mode of detection. These results may imply a true relationship between certain mammographic tumor features and the studied pathological tumor characteristics, not related to mammographic density or the mode of detection. This is consistent with previous studies showing a relationship between mammographic tumor features and pathological factors (5, 73, 74, 76) as well as prognosis (73, 75, 76, 135). However, further studies are needed to determine whether the mammographic tumor features are useful and should have an impact on early clinical descision-making.

In Paper II, we found that higher mammographic density at diagnosis was associated with larger tumor size and ALNI positivity in invasive breast cancer, which is consistent with results from previous studies (6, 136-138). Larger tumor size and ALNI positivity in denser breasts are tumor characteristics that are considered related to delayed diagnosis, i.e., due to reduced sensitivity of mammography in denser breasts (6, 137). However, the association of more ALNI positivity with denser breasts was stronger in screening-detected cancers than in clinically detected cancers. This may suggest that even when the tumor is screening-detected, which is considered to be associated with a better prognosis (46), the women could still be disadvantaged by having breasts with higher mammographic density. In addition, in screening-detected cancer, higher mammographic density was also associated with lower histological grade, however the evidence for this was weak. One possible explanation for the association between higher mammographic density and lower histological grade may be that tumors in fat-involuted breasts develop more quickly because the tissue environment is more permissive to higher-grade, highly proliferative tumors (136, 139). In addition, a recent report confirmed the association between high mammographic density and lower histological grade and did also report an association between very low mammographic density and decreased survival (86).

Another possible interpretation of the findings in Paper II is that the combination of higher mammographic density, larger tumor size, ALNI-positivity, and (in screening-detected tumors) lower histological grade relates to lobular cancer (140-142), which was present in a slightly higher proportion in denser breasts than in fat-involuted breasts.

In Paper III, higher mammographic density at diagnosis was associated with ER-negative breast cancer including TNBC in clinically detected breast cancers. No association was found between mammographic density and any of the tumor biomarkers in screening-detected cancer. In a previous meta-analysis, high

mammographic density was associated with both ER-negative and ER-positive breast cancer (143). However, in studies in which the analyses were stratified by the mode of detection, diverging results were found (136, 139, 144). Interval cancers, which are categorized as clinically detected cancers, have been shown to occur more often in breasts with high mammographic density and to more often be highly proliferating ER-negative tumors (9, 145). The observed higher frequency of TNBC in mammographic denser breasts in Paper III, may be a contributing factor to the association between increased mammographic density and poorer survival, especially in clinically detected cancer (12), as women with TNBC have a worse prognosis (42). The association between higher mammographic density and TNBC may partly be explained by the often easily overlooked mammographic tumor features of TNBC (5, 132); the features may in turn be a result of the epithelial-stroma interaction discussed in Paper I. The easily overlooked tumor features can further reduce the sensitivity of mammography in breasts with higher mammographic density, which may delay diagnosis.

It is interesting to note that some of the associations between mammographic density and tumor characteristics differed with the mode of detection which, to the best of our knowledge, has not been frequently studied.

Perhaps the tumor microenvironment in denser breasts promotes the growth rate and the metastatic potential of the tumor (11, 100-102). The combination of a possible true biological relationship between higher mammographic density and aggressive tumor characteristics with the masking effect by higher mammographic density would give women with dense breasts a double disadvantage.

The combined findings in Papers I, II, and III highlight the importance of considering mammographic tumor features, mammographic density and mode of detection in mammography image interpretation.

Agreement of mammographic density assessments

In Paper IV, the agreement between BI-RADS scores was substantial, meaning that the radiologists evaluated the mammographic density in a similar manner. The agreement between VDG and BI-RADS was moderate, which has been previously described (146-148). One explanation for this lower degree of agreement may be that the BI-RADS scores were performed by European radiologists (149), while the VDG thresholds have been based on American radiologists' assessments (115). There could be additional explanations for the lower degree of agreement between Volpara and BI-RADS. First, BI-RADS scores are set based on processed images, while Volpara analyses are performed on raw digital mammography data. Second, VBD is measured on a continuous scale, and BI-RADS scores are evaluated on an ordinal scale of four groups. Third, both Volpara and the radiologist estimate the proportion of dense breast tissue; however, the radiologist also takes into account the possibility that the mammographic density masks the breast tumor. This

masking effect, however, may not always represent an actual increased amount of dense breast tissue.

Further studies investigating fully automated volumetric density assessments in different populations are needed to ensure accurate reflection of mammographic density. In addition, we need to further analyze the differences between the software's and the radiologists' interpreations of mammographic density.

7 Conclusions

Some of the mammographic tumor features and the pathological characteristics of breast tumors tend to differ with mammographic density and the mode of detection. Furthermore, there was moderate agreement between a fully automated volumetric assessment and the radiologists' qualitative classification of mammographic density.

With these papers, we aimed to deepen the knowledge of relationships between mammographic density and various breast tumor characteristics as well as measurements of mammographic density. Both mammographic density and the mode of detection may have a prognostic role in breast cancer, which stresses the potential benefit of considering them both in the interpretations of mammograms. Currently, neither of these factors are included in clinical decision-making but perhaps it might eventually become so. Additional studies are needed to address the biological explanations behind the impact of mammographic density and also to determine how to make the best use of mammographic density in the clinical setting.

8 Future perspective

”Radiologists have inside information”

It is of great importance to identify prognostic factors that may help us differentiate and individualize treatment of breast cancer. Mammographic density is an easily accessible parameter, which may be of great use in breast cancer care. But we need to know more about its benefits and limitations. What would be the perfect use of a comprehensively understood and consistently measured mammographic density? I think we need further studies to reach that perfect understanding, especially studies regarding the biological background behind mammographic density and its various relationships with tumor characteristics that may have a possible prognostic impact. But just as breast cancer and its causes have many faces, the answer to understanding and making the best use of mammographic density is probably multi-faceted. It would be interesting if clearer mechanisms tying dense breast tissue to breast cancer development were found. It would be exciting to use imaging to depict aspects of mammographic density other than pure volume. It would be helpful to have a consistent method of measuring mammographic density that could then be used to stratify women in different ways with respect to imaging modality, screening interval and/or risk prediction. And finally, for women with breast cancer, mammographic density could perhaps aid in the effort to offer women individualized care.

The image of the breast holds so much valuable information. Even if image modalities, modes of measurements, and/or studied breast tumor characteristics change over time, the image of the breast remains an early documentation of the breast and the breast tumor, emphasizing its role in the treatment of breast cancer as well as in future research.

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