Mammographic breast density (MBD) is a topic of broad and current interest both in the literature and the lay press. While most focus is usually placed on MBD’s effect on screening sensitivity and its role as a breast cancer risk factor, this thesis looks at MBD from a different angle, its role as a breast cancer prognostic factor. This study concluded that very low MBD is indeed an independent prognostic factor for breast cancer, and has a strong reciprocal relationship with high expressions of Hyaluronan and its synthesizing enzymes.
Mammographic Breast Density, Tumour Characteristics and the Expression of Hyaluronan as Prognostic Surrogate Markers for Breast Cancer
AMRO MASARWAH

Mammographic Breast Density, Tumour Characteristics and the Expression of Hyaluronan as Prognostic Surrogate Markers for Breast Cancer

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Mammographic Breast Density, Tumour Characteristics and the Expression of Hyaluronan as Prognostic Surrogate Markers for Breast Cancer

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ABSTRACT

Breast cancer constitutes a major worldwide health issue; it is the most common cancer and leading cause of cancer related deaths in women all around the world. Nowadays, tumour size, lymph node status, hormone receptor status, tumour grade and HER2 status are considered as the strongest and most important prognostic factors correlating with patient outcomes. Finding newer and potentially more accurate prognostic factors could help to better understand breast cancer as a disease and successfully personalize and tailor future treatment regimens.

Mammographic breast density (MBD) is an established risk factor for the development of breast cancer and women with breast density in the upper quartile have a 4 - 6 fold increased risk for developing breast cancer compared to those within the lower quartile. Hyaluronan (HA) is a glycosaminoglycan widely found in different tissues; it is one of the molecular markers and effectors with a strong influence on the progression of breast cancer and by itself is considered as a prognostic factor.

The aim of this thesis was to investigate MBD in terms of its prognostic value as opposed to its more usual and better recognized role as a risk factor, the secondary aim was to assess the potential association between mammographic density and mammographic tumour features with hyaluronan and its synthesis. For the purpose of this study, 139 consecutive HER2 positive invasive breast cancer patients who were operated in Kuopio University Hospital during the years 2002–2008, were matched with an equal number of HER2 negative patients, i.e. there was a total of 278 patients examined in these analyses. The first study was intended to examine the prognostic value of mammographic breast density and mammographic tumour features and their relationship with the established prognostic factors. The second study aimed to assess the potential association between HA and its synthesizing enzymes with that of MBD and other mammographic and tumour characteristics in an attempt to uncover any potential underlying relationship that would help connect those two previously separated and not previously evaluated aspects of breast cancer biology. The third study examined the possible additional value of very low mammographic breast density (VLD), HER2, ER and PR statuses in a patient group within the matched Nottingham Prognostic Index (NPI) categories and whether the addition of any of those factors could serve to improve its prognostic capabilities.

The first study concluded that very low MBD is indeed an independent prognostic factor for breast cancer, and is associated with higher tumour grades and predicted worse survival, even after correcting for all possible confounders. The second study further elicited the value of low MD and discovered the existence of a strong reciprocal relationship between low breast density and high expressions of HA and its synthesizing enzymes. Moreover, a dramatic reduction in patient survival was found when HA abundance was combined with low breast density. In the third study, VLD and HER2 positivity were found to be prognostic factors for breast cancer, independent of the NPI. Furthermore, it was possible to incorporate those factors into a newly coined prognostic index called the Kuopio-Nottingham Prognostic Index (KNPI), which has a higher predictive power than the original NPI.

National Library of Medicine Classification: WP 870, WP 815, WB 142, QU 83
Medical Subject Headings: Breast cancer; Mammography; Density; prognosis; Radiology; Oncology; Hyaluronic acid.
Masarwah, Amro
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TIIVISTELMÄ

Rintasyöpä on merkittävä terveysongelma, sillä se on naisten yleisin syöpä ja johtava syy naisten syöpäpätkuolemiin maailmanlaajuudessa. Tärkeimmät rintasyövän ennustetekijät ovat kasvaimen koko ja erilaistumisas, leviäminen kainaloimusolmukoisiin, kasvaimen hormonireseptorit ja HER2-positiivisuus. Uusien, aiempaa tarkempiin ennusteellisten tekijöiden kehittäminen olisi merkityksellistä, sillä tulokksilla saattaa olla tulevaisuudessa merkitystä rintasyöpäpotilaan yksilöllisen hoidon suunnittelussa.

Rinnan tiiviiden yhteys rintasyövän riskiin on hyvin tiedossa. Naisilla, joilla on erittäin tiivis rauhaskudosrakenteen on 4-6-kertainen riski sairastua rintasyöpään. Hyaluronaani on soluväliaineen sokerimolekyyli, joka esiintyy normaalisti lähes kaikissa kudoksissa, ja sen aineenvaihdunta edistää syövää edistäen syövän edistäen syövän työntymistä.


Tulokset osoittivat, että hyvin matala rintarauhaskudoksen tiiviys oli itsenäinen rintasyövän ennustetta huonontava tekijä (sekä tautivapaa että kokonaiselinaika), ja matalaan tiiviitteen liittyi myös kasvainten suurempi aggressiivisuus. Tilastollinen merkitsevyys säilyi myös monimuunnuttaja-analyysissä, jossa huomioitiin mm. potilaiden ikä, menopausaalin valinnat ja obesiteetti. Toisessa osajulkaisussa osoitettiin, että jos rinnan tiiviys oli <25 %, hyaluronaanin korkea pitoisuus sekä syöpäalasta että stroomassa korreloivat tilastollisesti rintasyövän huonoon ennusteeseen ja oli senäinen riskitekijä riippumatta HER2-positiivisuudesta. Huomioitavaa on, että huorakuvan korkea pitoisuus sekä syöpäalasta että stroomassa korreloivat rintasyövän huonoon ennusteeseen ja oli senäinen riskitekijä riippumatta HER2-positiivisuudesta kanssa sisällyttimyhin uuteen ennustemallii (Kuopio-Nottinghain prognoositinesis indeksi) jonka prediktiviinen ennusteellinen kyky osoittautui paremmaksi kuin alkuperäisessä mallissa.

Yleinen Suomalainen asiasanasto: Rintasyöpä; Mammografia; Tiiviys; ennusteet; radiologia; syöpätaudit; hyaluronaani.
"I almost wish I hadn’t gone down that rabbit hole — and yet — and yet — it’s rather curious, you know, this sort of life!"

-Alice in Wonderland
Acknowledgements

This study is part of the Cancer Imaging project and based on research carried out in the Departments of Clinical Radiology and Oncology, University of Eastern Finland and Kuopio University Hospital, between the years of 2013-2016.

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you were always more than happy to offer your expert advice and help in any possible way. To Professors Markku Tammi and Raija Tammi, who in spite of being extraordinarily busy, they took time to hear me and guide me through my second article. I was honored by your contribution and critical review and it has been an absolute pleasure working with both of you. To Professor Veli-Matti Kosma, your professional input and your expert help with this thesis is deeply appreciated.

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To my parents, Nazih and Muna, to my brothers, Tareq and Sa’ad, thank you for showing me love in its rarest form, showing me what it feels like, and how it can push you to overcome life’s greatest challenges. One couldn’t have wished for a better family and for a better upbringing, all that I have accomplished and any success that I may enjoy in the future will be built on the support and belief that you have installed in me, this thesis is for you.
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Amro Masarwah  
Kuopio, Finland  
May 2016
List of the original publications

This dissertation is based on the following original publications:


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### Abbreviations

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<th>Abbreviation</th>
<th>Description</th>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADC</td>
<td>apparent diffusion coefficient</td>
<td>HER2</td>
<td>human epidermal growth factor receptor 2</td>
</tr>
<tr>
<td>BCDDP</td>
<td>Breast Cancer Detection Demonstration Project</td>
<td>HR</td>
<td>hazard rate</td>
</tr>
<tr>
<td>Bhabc</td>
<td>biotinylated HA-binding complex</td>
<td>ICC</td>
<td>intraclass correlation coefficient</td>
</tr>
<tr>
<td>BI-RADS</td>
<td>Breast Imaging Reporting and Data System</td>
<td>K-NPI</td>
<td>Kuopio-Nottingham prognostic index</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
<td>LOD</td>
<td>low density</td>
</tr>
<tr>
<td>CEUS</td>
<td>contrast-enhanced ultrasound</td>
<td>MBD</td>
<td>mammographic breast density</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
<td>MID</td>
<td>mixed density</td>
</tr>
<tr>
<td>c-index</td>
<td>concordance index</td>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>CISH</td>
<td>chromogenic in situ hybridization</td>
<td>NPI</td>
<td>Nottingham prognostic index</td>
</tr>
<tr>
<td>CMF</td>
<td>cyclophosphamide, methotrexate, 5-fluorouracil</td>
<td>NSAID</td>
<td>non-steroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>DBT</td>
<td>digital breast tomosynthesis</td>
<td>PACS</td>
<td>picture archiving and communication system</td>
</tr>
<tr>
<td>DCIS</td>
<td>ductal carcinoma in situ</td>
<td>PR</td>
<td>progesterone receptor</td>
</tr>
<tr>
<td>DFS</td>
<td>disease Free Survival</td>
<td>SEER</td>
<td>Surveillance Epidemiology and End Results</td>
</tr>
<tr>
<td>ECM</td>
<td>extra-cellular matrix</td>
<td>tdROC</td>
<td>time-dependent receiver</td>
</tr>
<tr>
<td>EGFR</td>
<td>epidermal growth factor receptor</td>
<td>TdROC</td>
<td>operating characteristic curve</td>
</tr>
<tr>
<td>ER</td>
<td>estrogen receptor</td>
<td>TNM</td>
<td>Classification of Malignant Tumours (tumour, lymph nodes, Metastasis)</td>
</tr>
<tr>
<td>EUSOMA</td>
<td>The European Society of Breast Cancer Specialists</td>
<td>US</td>
<td>ultrasound</td>
</tr>
<tr>
<td>GLcA</td>
<td>glucuronic acid</td>
<td>VLD</td>
<td>very low density</td>
</tr>
<tr>
<td>GlcNAc</td>
<td>N-acetyl-glucosamine</td>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>HA</td>
<td>hyaluronan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAS</td>
<td>hyaluronan synthase</td>
<td></td>
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</tbody>
</table>
1 Introduction

Mammographic breast density (MBD) refers to the appearance and relative amount of radio-opaque breast parenchyma in a mammogram; it is a topic of interest in both the medical literature and the lay press. Historically, Wolfe et al. (1) were the first to describe mammographic density and classify it into different patterns. Furthermore, Wolfe (2) was the first to describe an association between mammographic breast patterns and the risk of breast cancer. Since then, numerous newer methods have been proposed for assessing mammographic density and several studies have been published, not only validating those earlier findings but also trying to provide a biological explanation. Nowadays, increased mammographic breast density is universally accepted to be a strong independent risk factor for the development of breast cancer (3, 4). Nonetheless, a surprisingly small number of studies have focused on MBD from a prognostic point of view, with those who did so, delivering conflicting and often inconclusive results.

Hyaluronan (HA) is a large glycosaminoglycan widely found in different tissues; it possesses several roles e.g. as a molecular marker and as an effector on breast cancer progression (5). Three isoenzymes called hyaluronan synthases (HAS1-3) in the plasma membrane are responsible for the synthesis of HA (6). In human breast cancer, a high level of HA is associated with epidermal growth factor receptor 2 (HER2) positivity and CD44 (the principal transmembrane cell surface receptor for HA) positive stromal cells, and it is considered as an independent factor for a poor prognosis, along with hyaluronan synthases which contribute to the accumulation of HA in breast cancer (7, 8).

This doctoral dissertation focuses on mammographic breast density as a prognostic factor for patient outcome in patients with newly diagnosed breast cancer. For the first time, the relationship between mammographic characteristics and density are compared with hyaluronan and its synthetizing enzymes in a bid to reveal underlying biological mechanisms and thus enhance our understanding of cancer as a disease. Furthermore, this dissertation incorporates MBD into the well-known Nottingham Prognostic Index (NPI) to form a new prognostic system in an effort to provide a gateway for these results to be applied in clinical practice.
2 Review of the Literature

2.1 BREAST CANCER SUBTYPES AND PREVALENCE

2.1.1 History of breast cancer
Due to its visible and progressive symptoms, breast cancer was amongst the first cancers ever to be described in history. The earliest recorded cases of breast cancer can be traced as far back as 1600 BC in ancient Egypt. The ancient Egyptian “Edwin Smith” papyrus described 8 cases of “untreatable” breast fungating ulcers; and this represents the first historical attempt to actually look at cancer in a scientific manner. Mastectomy as a surgical treatment for breast cancer has been performed as early as 548 AD, but the linking of axillary surgery to original breast surgery was not done until the 17th century when the first successful radical mastectomy was performed. Nowadays, breast cancer is considered as the most common cancer and the commonest cause of cancer related deaths amongst women all around the world (9, 10).

2.1.2 Breast cancer statistics
According to the latest international cancer statistics report, 1,676,600 new cases were diagnosed in the year 2012 and 521,900 women died of breast cancer in the same year (10). Breast cancer alone accounts for 25% of all cancer cases and 15% of all cancer deaths among females. The more developed countries account for about one-half of all breast cancer cases and 38% of deaths. The international variations in breast cancer incidence rates reflect differences in the availability of early detection as well as risk factors.

Over the course of their lifetime, about every eighth woman in the USA will develop invasive breast cancer. An estimated 231,840 new cases of invasive breast cancer are expected to be diagnosed in women in the U.S. during 2015, along with 60,290 new cases of non-invasive (in situ) breast cancer. According to the Nordcan project statistics (11), an average of 4462 women were diagnosed annually in Finland for the years 2009-2013 which means that breast cancer accounted for 30% of all cancers suffered by Finnish women. By the end of the year 2013, there were 62,103 women living with breast cancer in Finland, representing a prevalence of 2241 per 100,000 of the population. According to the Finnish cancer registry, 695 women were diagnosed with breast cancer in the Kuopio region (North, East and South Savo, North Karelia and Central Finland) in 2013, i.e. 29.5% of the 2355 women diagnosed with cancers in that year. This marks a 14.7% increase from the 606 cases of breast cancer diagnosed in the previous year, and is a record high for breast cancer in the Kuopio region.

The vast majority of cancers in Finland (85.6%) are diagnosed in women over the age of 50. The highest incidence rate has been recorded in patients aged between 60 and 65 (18.0%). About 5-10% of breast cancers can be linked to inherited paternal gene mutations. The remaining 85% of breast cancers occur in women with no close family history of breast cancer. These occur due to genetic mutations resulting from the normal aging process and life in general, rather than heredity.
2.1.3 Breast cancer survival rates

The relative survival of breast cancer varies from region to region. According to the Finnish cancer registry (http://www.cancer.fi/syoparekisteri/en), one- and five-year relative survival rates of breast cancer patients diagnosed in Finland in 2005–2012 and followed up between 2010–2012 was 97% and 90%, respectively.

According to the American Cancer Society (12), breast cancer five-year survival rate for women aged 15 or older is 89% in the United States, 82% in Switzerland, and 80% in Spain. Survival rates are generally lower in the developing countries than in Europe and North America, with rates as low as 38.8% in Algeria, 36.6% in Brazil, and 12% in Gambia (13, 14).

The stage at diagnosis is the most important prognostic variable. For instance, according to the Surveillance Epidemiology and End Results SEER cancer statistic (15), the relative 5 year survival for breast cancer was 89%. The rates in table 1 below come from the same SEER database, and show the relative survival of patients by stage.

<table>
<thead>
<tr>
<th>Stage</th>
<th>5-year Relative Survival Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>I</td>
<td>100%</td>
</tr>
<tr>
<td>II</td>
<td>93%</td>
</tr>
<tr>
<td>III</td>
<td>72%</td>
</tr>
<tr>
<td>IV</td>
<td>22%</td>
</tr>
</tbody>
</table>

2.1.4 Breast cancer subtypes

The majority of breast malignancies have epithelial origins and are therefore categorized as carcinomas. Although breast carcinomas are often discussed as a single homogenous disease, they are actually a diverse group of lesions that differ in both their microscopic appearance and biologic behaviour.

Breast cancers can be grouped into either in situ carcinomas or invasive carcinomas according to their ability to metastasize and infiltrate adjacent tissues. Moreover, based on histopathologic properties, both of the aforementioned types can be divided into subtypes (16). The in situ carcinomas of the breast could be either ductal or lobular (the latter is included in lobular neoplasia) while invasive breast carcinomas are more complex and consist of several histologic subtypes.

The estimated percentages according to the SEER database of the National Cancer Institute are as follows (15):

- Infiltrating ductal – 76%
- Invasive lobular - 8%
- Ductal/lobular – 7%
- Mucinous (colloid) - 2.4%
- Tubular - 1.5%
- Medullary - 1.2%
- Papillary – 1%
- Other subtypes account for fewer than 5% of cases
2.1.4.1 Ductal carcinoma in situ (DCIS)
DCIS is a neoplastic intraductal lesion; it is characterized by an increase in cellular atypia, epithelial proliferation, and a strong predilection to progress to invasive breast carcinoma (17). The terminal ductal lobular unit (TDLU) is the site where DCIS originates, and it also the site from where it extends into the lobular glands’ epithelium and spreads inside the ductal structures. Since the start of the widespread screening programs, there has been a considerable increase in DCIS detection. An estimated 10-30% of all detected malignancies in current screening programs are DCIS (18, 19).

2.1.4.2 Invasive carcinoma of no special type
With 70-80% of all invasive lesions, infiltrating ductal carcinoma is the most common type of invasive breast cancer. According to the World Health Organization (WHO) classification, it is a “heterogenous group of tumours that fails to exhibit sufficient characteristics to achieve classification as a specific histological type” and “it comprises the largest group of malignant breast tumours” (17, 20). The 5-year relative survival of the patients with ductal carcinoma is 79% (20).

2.1.4.3 Invasive lobular carcinoma
Infiltrating lobular carcinomas are the second most common type of invasive breast cancer, accounting for about 5 to 10 percent of invasive lesions, and often presents as bilateral or multifocal lesions (21, 22). Invasive lobular carcinoma is difficult to define in both its macroscopic and mammographic form, when viewed in the microscope, its growing pattern exhibits strands of tumour cells that are thread-like and loosely dispersed throughout the fibrous stroma (16, 23).

2.1.4.4 Other types of invasive carcinoma
Several other types of invasive carcinomas such as invasive cribriform, tubular and mucinous carcinomas are usually well-differentiated histopathologically and are detected by mammography as either spiculated masses (tubular and cribriform carcinomas) (24, 25) or well delineated masses (mucinous carcinoma) (17, 26). These tumours have favourable 10-year outcomes in a range between 90-100% (27, 28). Carcinomas with medullary features are an overlapping group of tumours with a medullary appearance, in the WHO classification of breast tumour 4th edition, the use of older names such as medullary carcinomas, atypical medullary carcinomas and invasive carcinomas of no special type are discouraged. They are often well-defined both clinically and in imaging studies (17). Inflammatory carcinoma is a rare and particularly aggressive form of breast carcinoma with a 5-year survival ranging between 18% to 41% (20, 29, 30).

2.2 TRADITIONAL RISK AND PROGNOSTIC FACTORS FOR BREAST CANCER

2.2.1 Risk factors
Breast cancer occurs 100 times more frequently in women than in men, in the United States approximately 2000 cases of male breast cancer are diagnosed annually while 200,000 are diagnosed in women (9, 10, 31). Moreover, a positive family history of breast cancer increases the risk according to the type of the relative affected, age at which the relative developed the disease and the number of relatives affected (32), the risk is increased to about twofold for women with one affected first-degree relative and threefold in women with two such relatives (33). Age is also one of the strongest risk factors associated with cancer development, with the risk increasing with increasing age. The probability of a woman developing breast cancer in the USA according to age is as follows: (34)
• Birth to age 39 – 0.49% (1 in 203 women)
• age 40 to 59 – 3.76% (1 in 27 women)
• age 60 to 69 – 3.53% (1 in 28 women)
• age 70 and older – 6.58% (1 in 15 women)
• Birth to death – 12.29% (1 in 8 women)

Obesity (body mass index, BMI ≥30 kg/m²) is associated with an overall increase in both breast cancer morbidity and mortality. However, this risk of breast cancer that is related to BMI seems to be dependent on the patient’s menopausal status. A higher body mass index (BMI) and/or perimenopausal weight gain have been consistently associated with a higher risk of breast cancer among postmenopausal women (35, 36). On the other hand, increased BMI is associated with a lower risk in premenopausal women (37).

Only 5-10% of breast cancers are directly caused by inherited breast cancer susceptibility genes such as BRCA1, BRCA2, TP53, CDH1, LKB1, CHEK2, BRIP1, PTEN, ATM, and PALB2 i.e. it is rare that one encounters a specific genetic mutation predisposing to breast cancer (17, 38).

The most common mutations implicated in breast cancer are in the BRCA1 and BRCA2 genes. On average, women with a BRCA1 mutation have a 55-65% lifetime risk of developing breast cancer. For women with a BRCA2 mutation, the risk is 45%. Breast cancer that is positive for the BRCA1 or BRCA2 mutations tends to develop more often in younger women. An increased ovarian cancer risk is also associated with these genetic mutations. In men, BRCA2 mutations are associated with a lifetime breast cancer risk of about 6.8%; BRCA1 mutations are a less frequent cause of breast cancer in men. PALB2 mutations are an important cause of hereditary breast cancer, and it is suggested that PALB2 mutations overlap with BRCA2 mutations in terms of breast cancer risk (39).

Several reproductive factors are associated with an increased breast cancer risk. Early age at menarche is associated with a higher risk of breast cancer (40). Women with menarche at or after 15 years of age were less likely to develop estrogen receptor/progesterone receptor positive breast cancer compared with women who experienced menarche before the age of 13 years (40). Women with menarche at or after age 15 years also had a 16% decreased risk of hormone negative breast cancer. Nulliparity also increases the relative risk of breast cancer by 1.2 to 1.7, and it is postulated to increase the risk synergistically with increased BMI (31, 41). Advanced age at the time of the first pregnancy is also associated with an increased risk (42). Infertility has a controversial association with breast cancer risk, some epidemiological data link anovulatory disorder infertility with a decreased risk while others observed no association or a slight increase in risk (43-45). Increased levels of endogenous estrogen also increase the risk of breast cancer (46) and elevated androgen levels are linked with an increased risk of postmenopausal and premenopausal breast cancer (47).

Women with mammographically dense breast tissue in the upper quartile (≥75 percent density), have a four to six times the risk of developing breast cancer compared with women of similar age in the lowest quartile (fatty breasts, density <25%) (3, 4, 48, 49).

The risk of developing invasive breast cancer in the contralateral breast is increased by a personal history of ductal carcinoma in situ (DCIS) or invasive breast cancer (50). The risk associated with a positive family history of breast cancer is strongly affected by the number of female first-degree relatives with cancer and with the age at which they were diagnosed. The risk is increased almost twofold if a woman had one affected first-degree relative and threefold if she had two affected first-degree relatives, while the risk is threefold higher if the relative was diagnosed before the age of 30 and 1.5 fold if diagnosed after the age of 60 (33). Several lifestyle factors such as alcohol consumption (51), smoking (52), diet (53) and radiation exposure are also associated with an increased relative risk of breast cancer.

On the one hand, the literature generally shows no association between previous or current oral contraceptive use and the risk of breast cancer later in life (54, 55). On the other hand, there is evidence that the risk of developing breast cancer is increased in
postmenopausal women using hormonal replacement therapy and increases with the increasing duration of use (56, 57).

2.2.2 Protective factors
Factors that might protect from breast cancer, independent from those mentioned in the previous section have also been described. For instance, breastfeeding’s protective effect has been described in several case-control and cohort studies, the magnitude of its impact depends on several factors including the duration of breast feeding and the confounding effect of parity (58-60). One hypothesis for the mechanism of this effect is that breastfeeding delays the re-establishment of ovulatory cycles, and it has been estimated that a 4.3% reduction in relative risk occurs with every 12 months of breastfeeding (59).

Exercise and physical activity are also linked with a decrease in relative risk (61). A review of nine prospective studies that included 11,656 women found that the postmenopausal breast cancer risk was decreased by 12% for each 5 ng/mL increase in vitamin D (25(OH)D) levels between 27 and <35 ng/mL, with no further reduction for 25(OH)D levels greater than 35 ng/mL (62). Moreover, non-steroidal anti-inflammatory drug (NSAID) use has been linked with an average 12% reduction in breast cancer relative risk, with ibuprofen (21%) showing the greatest protective effect (63).

2.2.3 Prognostic and predictive factors
Although closely related and often mistaken for each other, it is important to make a distinction between what is considered a prognostic and what is considered a predictive factor. By definition, a prognostic factor has the ability to provide information on the clinical outcome independent of therapy at the time of diagnosis. Such factors are often indicators of invasion, growth, and metastatic likelihood. In contrast, a predictive factor is the one that is able to provide insights into the likelihood of a positive response to a specific modality of treatment (64).

For women with newly diagnosed early breast cancer, several factors are utilized to help determine prognosis. Both young and old age (<35 and >65) are associated with a worse prognosis, with differing impacts amongst the differing breast cancer subtypes (65). Tumour size is recognized as an important prognostic factor and is defined as the largest diameter of the primary tumour (66, 67). The five-year breast cancer survival rates in the SEER (Survival, Epidemiology and End Results) program ranged from 91 percent for size =<2 cm to 80 percent for sizes of >2 to 5 cm and 63 percent for size >5 cm (66).

Tumour size is correlated with nodal involvement but the prognostic value of the two factors is independent. For patients who are without metastasis, the five-year survival rates for those who present with localized versus regional disease (i.e., pathologic node involvement) are 99% and 84%, respectively (68). In the SEER program which involved 25,000 cases, the five-year relative survival was 96%, 86% and 66% percent for patients who were pathologically node-negative, had one to three nodes involved, or greater than four nodes involved, respectively (66).

With regards to pathological factors, tumour stage by itself is considered to be a prognostic factor. The tumour node metastasis (TNM) staging system for breast cancer (Table 2) is an internationally accepted system used to determine the disease stage (69). Disease stage is a measure of the extent of the disease (Table 3), which is used to determine prognosis and guide management.
Table 2. Tumour-Node-Metastasis (TNM) classification of breast tumours according to the seventh edition (2010) of the TNM staging system.

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pTX</td>
<td>Primary tumour cannot be assessed</td>
</tr>
<tr>
<td>pT0</td>
<td>No evidence of primary tumour</td>
</tr>
<tr>
<td>Tis</td>
<td>Carcinoma in situ</td>
</tr>
<tr>
<td>pT1mi</td>
<td>Tumour ≤ 1 mm in greatest dimension</td>
</tr>
<tr>
<td>pT1</td>
<td>Tumour 2 cm or less in greatest dimension</td>
</tr>
<tr>
<td>pT2</td>
<td>Tumour more than 2 cm but not more than 5 cm in greatest dimension</td>
</tr>
<tr>
<td>pT3</td>
<td>Tumour more than 5 cm in greatest dimension</td>
</tr>
<tr>
<td>pT4</td>
<td>Tumour of any size with direct extension to chest wall or skin only</td>
</tr>
<tr>
<td>pNX</td>
<td>Regional lymph nodes cannot be assessed</td>
</tr>
<tr>
<td>pN0</td>
<td>No regional lymph node metastases</td>
</tr>
<tr>
<td>pN1mi</td>
<td>Micrometastases (&gt; 0.2 mm and/or &gt; 200 cells, but none &gt; 2.0 mm)</td>
</tr>
<tr>
<td>pN1</td>
<td>Metastases in 1-3 axillary lymph nodes and/or in clinically negative internal mammary nodes</td>
</tr>
<tr>
<td>pN2</td>
<td>Metastases in 4-9 axillary lymph nodes or in clinically detected† internal mammary lymph nodes in the absence of axillary lymph node metastases</td>
</tr>
<tr>
<td>pN3</td>
<td>Metastases in ≥ 10 axillary lymph nodes; or in infraclavicular lymph nodes; or in clinically detected ipsilateral internal mammary lymph nodes in the presence of ≥ 1 positive level I, II axillary lymph nodes; or in &gt; 3 axillary lymph nodes and in internal mammary lymph nodes, with micrometastases or macrometastases not clinically detected; or in ipsilateral supraclavicular lymph nodes</td>
</tr>
<tr>
<td>MX</td>
<td>Distant metastasis cannot be assessed</td>
</tr>
<tr>
<td>M0</td>
<td>No distant metastasis</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastasis</td>
</tr>
</tbody>
</table>
The relationship between the expressions of the estrogen receptor (ER) and progesterone receptor (PR) and prognosis is unfortunately not a straightforward one, and has been a matter of debate for years. Multiple studies have shown that the expression of estrogen and progesterone receptors in the breast cancer tissue correlates with a good outcome with hormonal therapy in the adjuvant setting and even metastatic diseases (70-72). On the other hand, beyond the first five years, the hazard rate (HR) for recurrence is nearly zero percent per year for ER-negative cancers while it remains above zero (of the order of one-half to two percent per year) for those patients with ER-positive disease. Therefore, the prognostic value based on hormone receptor status tends to recede with time (70, 73).

PR appears to have its own prognostic capabilities and acts independently of ER (74, 75). In a multivariate analysis of a large population based cohort study, PR was shown to be independently predictive of disease free survival (HR 1.94) and breast cancer-specific survival (HR 2.12). In contrast to ER, the prognostic significance of PR appeared to increase beyond the sixth year of follow-up.

The human epidermal growth factor receptor 2 (HER2) oncogene encodes for a 185 KD transmembrane glycoprotein receptor with intracellular tyrosine kinase activity (76). It contains an epidermal growth factor receptor (EGFR) which plays an important role in the activation of subcellular signal transduction pathways that control epithelial cell differentiation, growth, and angiogenesis (77-79). HER2 is expressed in a wide range of normal adult and fetal epithelia, playing an important role in growth and development (80). HER2 amplifications or its protein product over-expression are observed in 18-20% of human breast tumours (81, 82).

The recommendations for HER2 testing have been updated by the American Society of Clinical Oncology and the College of American Pathologists (83), and these recommendations have also been adapted and evaluated in other countries (84-86).

- HER2 status should be determined in all patients with invasive breast cancer on the basis of one or more test results.
- HER2-positive status is indicated by evidence of protein overexpression or gene amplification.
- When results are equivocal, reflex testing should be performed with an alternative assay, and repeat testing should be considered if results are discordant with other findings.

<table>
<thead>
<tr>
<th>Stage</th>
<th>T</th>
<th>N</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Tis</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>I</td>
<td>T1</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IIA</td>
<td>T0-1</td>
<td>N1</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IIB</td>
<td>T2</td>
<td>N1</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IIIA</td>
<td>T0-2</td>
<td>N2</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>N1-2</td>
<td>M0</td>
</tr>
<tr>
<td>IIIB</td>
<td>T4</td>
<td>N0-2</td>
<td>M0</td>
</tr>
<tr>
<td>IIIC</td>
<td>T0-4</td>
<td>N3</td>
<td>M0</td>
</tr>
<tr>
<td>IV</td>
<td>T0-4</td>
<td>N0-3</td>
<td>M1</td>
</tr>
</tbody>
</table>

Table 3. Stage according to the Union for International Cancer Control (UICC) classification.
• Laboratories should be able to show high concordance with a validated HER2 test on a large and representative set of specimens, and testing must be performed in an accredited laboratory.

• Providers should recommend HER2-targeted therapies if the patient’s HER2 test result is positive and such treatment is clinically appropriate. Most experts do not recommend HER2-targeted therapy if the HER2 test result is negative and there is no histopathological discordance with HER2 testing. It is recommend to delay the decision to initiate HER2-targeted therapy if the HER2 test result is equivocal. Reflex testing should be done on the same specimen.

• Several methods are used to measure the human epidermal growth factor receptor 2 (HER2) oncogene’s activity; there is no consensus about which is the best method, both in terms of the type of assay used and the optimal way to perform each type of assay, is controversial. The available assays are as follows:
  o HER2 gene amplification by in situ hybridization (ISH) – Fluorescence in situ hybridization (FISH), chromogenic in situ hybridization (CISH), silver-enhanced in situ hybridization (SISH), or differential polymerase chain reaction (PCR).
  o Overexpression of the HER2 protein product – Western blotting, enzyme-linked immunosorbent assay (ELISA), or immunohistochemistry (IHC).
  o Overexpression of HER2 RNA – Northern blotting or reverse transcription PCR (RT-PCR).

The assay for HER2 amplification is nowadays considered as a routine part of the diagnostic work-up of all primary breast cancers (87, 88). HER2 overexpression forecasts an unfavourable prognosis, particularly if patients do not receive HER2-directed agents with chemotherapy (87). Apart from its prognostic role, the main benefit of HER2 testing is its predictive ability in selecting who should receive HER2-directed agents. In the absence of systemic therapy, HER2 overexpression is a marker of poor prognosis in patients with pathologically node-positive (89) and node-negative breast cancer (90). Prognosis vastly improves with trastuzumab treatment (91). In addition, there are data suggesting that HER2 retains prognostic value even in the presence of small tumours of ≤1 cm.

Gene expression studies have managed to identify several distinct breast cancer subtypes that differ markedly in terms of prognosis and in the therapeutic targets they express (92-98). The intrinsic list is the name given to the list of genes that differentiates these subtypes and it is made up of several clusters of genes relating to ER expression (the luminal cluster), HER2 expression, proliferation, and a unique cluster of genes called the basal cluster. Others are being identified as investigators continue to study the genomic data derived from breast cancer specimens.

The intrinsic subtypes segregate into two groups that correspond to expression of hormone receptor-related genes. This segregation is consistent with both the literature and clinical experience showing that ER-positive and ER-negative cancers define biologically distinct phenotypes that may derive from different progenitor cells (95).

Luminal subtypes — luminal A and luminal B express genes associated with the luminal epithelial cells of normal breast tissue and overlap with ER-positive breast (99).

The name “luminal” refers to the similarity between these tumours and the luminal epithelium of the breast. They form the majority of ER-positive breast cancer, and are characterized by the expression of ER, PR, and some other genes associated with ER activation. Despite comprising the majority of ER-positive breast cancers, the luminal A and luminal B subtypes exhibit some important molecular and prognostic differences. The clinico-pathological surrogate definitions of subtypes as adopted by the expert panel of the Saint Gallen international breast cancer conference (100) are summarized in Table 4.

Triple-negative breast cancer is a term that refers to cancers that are low in expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor
receptor 2 (HER2). They account for about 15-20% of all breast cancers and are usually diagnosed in younger women (<40 years) (101, 102). They are usually of a high grade (103) and in comparison to other types of breast cancer, they usually tend to behave more aggressively (104).
<table>
<thead>
<tr>
<th>Intrinsic subtype</th>
<th>Clinico-pathologic surrogate definition</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>'Luminal A-like'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>all of:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ER and PgR positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HER2 negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ki-67 'low'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recurrence risk 'low' based on</td>
<td></td>
</tr>
<tr>
<td></td>
<td>multi-gene-expression assay (if available)</td>
<td></td>
</tr>
<tr>
<td>Luminal B</td>
<td>'Luminal B-like (HER2 negative)'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ER positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HER2 negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>and at least one of:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ki-67 'high'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PgR 'negative or low'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recurrence risk 'high' based on</td>
<td></td>
</tr>
<tr>
<td></td>
<td>multi-gene-expression assay (if available)</td>
<td></td>
</tr>
<tr>
<td>Luminal B</td>
<td>'Luminal B-like (HER2 positive)'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ER positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HER2 over-expressed or amplified</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Any Ki-67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Any PgR</td>
<td></td>
</tr>
<tr>
<td>Erb-B2 overexpression</td>
<td>'HER2 positive (non-luminal)'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HER2 over-expressed or amplified</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ER and PgR absent</td>
<td></td>
</tr>
<tr>
<td>'Basal-like'</td>
<td>'Triple negative (ductal)'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ER and PgR absent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HER2 negative</td>
<td></td>
</tr>
</tbody>
</table>

*Notes*

The cut-point between 'high' and 'low' values for Ki-67 varies between laboratories. A level of <14% best correlated with the gene-expression definition of luminal A based on the results in a single reference laboratory (105).

Similarly, the added value of PgR in distinguishing between 'Luminal A-like' and 'Luminal B-like' subtypes derives from the work of Prat et al. which used a PgR cut-point of $\geq 20\%$ to best correspond to luminal A subtype (106).

Quality assurance programmes are essential for laboratories reporting these results.

'Luminal B-like' disease comprises those luminal cases which lack the characteristics noted above for 'Luminal A-like' disease. Thus, either a high Ki-67 value or a low PgR value may be used to distinguish between 'Luminal A-like' and 'Luminal B-like (HER2 negative)'.

There is an 80% overlap between 'triple-negative' and intrinsic 'basal-like' subtype. Some cases with low positive ER staining may cluster with non-luminal subtypes on gene expression analysis. 'Triple negative' also includes some special histological types such as adenoid cystic carcinoma.
2.3 IMAGING OF THE BREAST

Good coordination between clinicians within several specialties is required in the care of patients suspected of having breast cancer. A successful communicative approach with breast imagers and breast surgeons can help avoid unnecessary biopsies and hasten the diagnosis of tumours (107). A triple assessment should be performed in all suspicious breast abnormalities consisting of clinical examination, breast imaging and tissue sampling (17, 108, 109).

2.3.1 Mammography

A mammogram is a process that involves the exposure of the breast to X-rays with a low energy of around 30 kVp. These X-rays have an attenuation that is based on breast tissue characteristics, then recording devices absorb them as latent images to be analysed for the presence of any abnormal findings (110). Mammography is the single most important imaging method in breast diseases; it is considered as the first line of imaging for diagnostic purposes in women over the age of 30 (111, 112).

2.3.1.1 Screening Mammography

Mammography is usually divided into two parts, screening and diagnostic. In general terms, screening mammography is performed on asymptomatic women to identify malignant breast pathology at an early, potentially more curable, stage.

The importance and impact of screening mammography has been debated fiercely over the past few years. On the one hand, some large randomized studies have shown a reduction of 30% in breast cancer mortality and around a 40% reduction in patients undergoing screening (113-116) with smaller tumour sizes detected at screening resulting in the decrease in mortality rates (117, 118). Screening mammography shows the greatest benefit in women over 50 years of age. Moreover, it has been shown that screening can also significantly decrease mortality rates in women aged 40-49 years (119).

On the other hand, the Cochrane Collaboration (2013) (120) concluded that the trials with adequate randomisation did not find an effect of mammography screening on total cancer mortality, including breast cancer, after 10 years. The authors of the systematic review write: "If we assume that screening reduces breast cancer mortality by 15% and that overdiagnosis and overtreatment is at 30%, it means that for every 2000 women invited for screening throughout 10 years, one will avoid dying of breast cancer and 10 healthy women, who would not have been diagnosed if there had not been screening, will be treated unnecessarily. Furthermore, more than 200 women will experience important psychological distress including anxiety and uncertainty for years because of false positive findings." The authors concluded that the time had come to re-assess whether universal mammography screening should be recommended for any age group.

The guidelines for screening vary from region to region and from country to country. For instance, the American Cancer Society has previously recommended annual mammographic screening and physical examination starting from 40 years of age (121, 122). Those guidelines were updated in 2015 with new recommendations urging women with average risk to start annual screening at the age of 45, later switching to biennial screening after the age of 54 and then continuing screening as long as their overall health is good (123). The Canadian Task Force on Preventive Health Care (124) and the European Union recommend an organized, quality controlled mammography screening programme for women aged 50-69 years at 2-3 years intervals. Women between the ages of 40-49 years should be informed about both the benefits and adverse effects of screening at their age (125).

The year 1987 saw the birth of Finland’s nationwide screening programme which was initially introduced for women aged between 50-59 years and provided at 2 year intervals (126). The participation rates between the years 1987 and 1997 were high (89%) with the recall...
rates and biopsy referrals decreasing substantially in the first three years (127). Screening is currently organized bi-annually for age groups 50-69, estimates of overdiagnosis due to breast cancer screening for both any breast carcinoma and invasive breast carcinoma in Finland have varied from 5% to 7% (128).

2.3.1.2 Diagnostic Mammography
Diagnostic mammography is performed on symptomatic patients, or to work-up an abnormality found on screening mammography (129). The object is to use imaging to typify pathology and arrive at a diagnosis. This is important because diagnoses have outcomes and survival rates. For instance, a diagnosis of a simple breast cyst has few implications and does not affect the patient’s life expectancy. In contrast, a diagnosis of breast cancer has significant implications for the patient and her life expectancy.

The use of supplemental mammographic views and the possibility of performing an ultrasound examination should be used for further characterization of any abnormalities found in screening mammography (130). Several mammographic techniques such as magnification views, spot compression and varied angled views, may serve to characterize lesions in a more precise manner before the final recommendation for management can be made (130).

Interval cancers are cancers that are detected in between negative or normal screening mammograms and they are generally aggressive in nature (131, 132). Moreover, younger women may present with tumours of large size prior to screening age. Therefore, a diagnostic mammogram should always be a part of a patient’s workup even though their last screening mammograms were negative.

In comparison to screening mammography, diagnostic mammography is generally associated with higher sensitivities but lower specificities (133). Higher breast densities and a younger age reduce sensitivity and specificity of diagnostic mammograms.

2.3.1.3 Sensitivity and specificity of mammography
The value of sensitivity of mammography for cancer detection varies widely in the literature with reported ranges from 63% to 98%. The highest sensitivities are found in low density breasts, where sensitivities can reach values of 100% (112, 134). Conversely, dense breasts have sensitivities as low as 30% (112, 134, 135). The density of breast parenchyma is inversely associated with age, therefore causing an increase in mammographic sensitivity as the woman becomes older (136). Due to the presence of dense tissue and the fact that the appearance of cancer in a mammogram mimics the appearance of normal tissue, mammography has a false-negative rate of about 10 percent. Regardless of the previously mentioned facts, mammography remains as the most sensitive imaging method for detecting microcalcifications (137). Most mammographic findings are non-specific permitting only likelihood statements (138). The majority of nonpalpable small carcinomas have mammograms with non-specific changes (119, 139).

2.3.1.4 Mammographic reporting
Several systems have been proposed to better facilitate and standardize reporting. The most commonly used approach is the American College of Radiology (ACR) Breast Imaging Reporting and Data System (BI-RADS®) (140) which was devised to improve the quality of mammographic reporting and interpretations; it was also intended to facilitate communication between radiologists and other physicians. BI-RADS® classifies mammograms into seven different categories from BI-RADS 0 to BI-RADS 6. The zero and one categories are reserved for incomplete examinations and negative findings respectively. Category 2 is for definitely benign and category 3 is for short term follow up. Categories 4 and 5 are suspicious for malignancy and therefore have to be biopsied while category 6 refers to already confirmed malignancies. The most recent BI-RADS® 5th edition classification was released in 2013 and is summarized in Table 5.
Table 5. The assessment categories according to the 5th edition of the ACR BI-RADS® reporting system (140).

<table>
<thead>
<tr>
<th>Category</th>
<th>Management</th>
<th>Likelihood of cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assessment is Incomplete</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Need additional imaging or prior examinations</td>
<td>Recall for additional imaging and/or await prior examinations</td>
<td>Not applicable</td>
</tr>
<tr>
<td><strong>Final Assessment Categories</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Negative</td>
<td>Routine screening</td>
<td>Essentially 0%</td>
</tr>
<tr>
<td>2 Benign</td>
<td>Routine screening</td>
<td>Essentially 0%</td>
</tr>
<tr>
<td>3 Probably Benign</td>
<td>Short interval-follow up (6 month) or continued surveillance</td>
<td>&gt;0% but ≥ 2%</td>
</tr>
<tr>
<td>4 Suspicious</td>
<td>Tissue diagnosis</td>
<td>4a. low suspicion for malignancy (&lt;2% to ≤ 10%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4b. moderate suspicion for malignancy (&gt;10% to ≤ 50%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4c. high suspicion for malignancy (&gt;50% to &lt;95%)</td>
</tr>
<tr>
<td>5 Highly suggestive of malignancy</td>
<td>Tissue diagnosis</td>
<td>≥95%</td>
</tr>
<tr>
<td>6 Known biopsy-proven</td>
<td>Surgical excision when clinical appropriate</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

Most importantly, the BI-RADS-classification provides clinicians with consistent guidelines with which to evaluate different masses, asymmetries, architectural distortions, microcalcifications, and associated findings (e.g. skin and nipple retractions, skin thickening, skin lesions and axillary adenopathy) and provide recommendations for management. The latest BI-RADS® 5th edition lexicon for mammography is shown below in Table 6.

<table>
<thead>
<tr>
<th>Mammography Lexicon</th>
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<tbody>
<tr>
<td>A. entirely fatty</td>
</tr>
<tr>
<td>B. scattered areas of fibroglandular density</td>
</tr>
<tr>
<td>C. heterogeneously dense, which may obscure small masses</td>
</tr>
<tr>
<td>D. extremely dense, which lowers sensitivity of mammography</td>
</tr>
</tbody>
</table>

**Mass**
- **shape**: oval – round – irregular
- **margin**: circumscribed – obscured – microlobulated – indistinct - spiculated
- **density**: fat containing – low – equal – high

**Asymmetry**
- asymmetry – global – focal – developing

**Architectural distortion**
- distorted parenchyma with no visible mass

**Calcifications**
- typically benign
- **morphology**
  - 1. amorphous
  - 2. coarse heterogeneous
  - 3. fine pleomorphic
  - 4. fine linear or fine linear branching
- **distribution**
  - diffuse – regional – grouped – linear – segmental
  - skin retraction – nipple retraction – skin thickening – trabecular thickening – axillary adenopathy – architectural distortion - calcifications

2.3.2 Tomosynthesis
Digital breast tomosynthesis (DBT) is a form of limited-angle tomography (141). Low-dose full field projection images of the breast are obtained from different angles with X-rays passing through the breast from different directions. The projection of structures onto the detector that are closer to the detector will appear to move over a shorter distance between images than the projection of structures that are farther away from the detector (142). The simplest way to synthesize planes through the breast is by instructing the computer to align all of the projection images so that structures in the plane of interest all align precisely.

DBT was developed in an effort to improve on the major success of mammography because the normal structures of the breast can obscure malignant tumours. In addition, the superimposition of structures in the breast can form a summation shadow, which appears to be a lesion when none is present. Both problems complicate the interpretation of conventional 2D mammograms. DBT is an evolutionary technology that builds on the success of 2D mammography. It improves the ability to detect breast cancer while reducing the false-positive, or recall, rate.

A recent review has pointed out issues that must be considered when implementing this new technology into daily clinical practice (143). With the current technology, there are extremely large data files that require PACS (Picture archiving and communication system)
storage, there is an approximately double x-ray dose for the combo-mode of DBT/Digital mammography, there is an estimated double in the interpretation time needed, and there is no approved reimbursement to cover the additional overhead needed to support this new digital mammography platform.

In terms of screening, there is rapid emerging evidence in support of digital breast tomosynthesis. Population screening studies have indicated that adjunct tomosynthesis incremental detection lies in the range of 0.5-2.7 per 1000 screens, with an absolute false recall reduction in the range of 0.8-3.6% (144). Destounis et al. (145) have concluded that tomosynthesis reduces recall rates and increases rates of detection both in the screening and diagnostic setting, however, they have warned against the rapid implementation of this technology due to longer interpretation times, higher costs and increased radiation doses. Lång et al. (146) investigated the performance of one-view digital breast tomosynthesis in a screening setting and claimed that it increased the cancer detection and recall rate significantly, and concluded that one-view tomosynthesis as a stand-alone modality seemed to be feasible.

2.3.3 Ultrasound
For the most part, ultrasonography (US) is considered as an adjunct tool in the breast imaging process. It is usually used to guide percutaneous biopsies and for the evaluation of specific abnormal findings which were detected by either mammography or clinical examination (147, 148). An exception would be in young women below the age of 30 where ultrasonography is used as the primary imaging modality for the evaluation of palpable masses (149, 150).

New breast density notification legislations have been recently introduced in the USA nowadays; women with dense breasts have the choice to undergo further screening with additional imaging modalities, mostly ultrasound (151, 152). Decisions are made in accordance with the severity of the individual patient risk assessment and on a case-by-case basis. In women with dense breast tissue, ultrasound has the ability to detect small, mammographically occult breast carcinomas (149, 150).

Furthermore, one of the major applications of US in the preoperative setting is in the simultaneous evaluation of the axilla. Axillary ultrasonography is used to increase the sensitivity for axillary metastasis detection (153), this sensitivity is further increased by the use of US-guided axillary lymph node sampling with either fine-needle aspiration or core-needle biopsy (154, 155). Contrast-enhanced ultrasound (CEUS) has also been shown to be a feasible procedure that improves breast lesion characterization. Furthermore, CEUS assisted percutaneous axillary sentinel lymph node biopsy can help to exclude the so-called high axillary metastasis burden in predefined US node negative patients and therefore can be recommended in selective patient populations (156, 157).

2.3.4 MRI
Contrast enhanced MRI is the most sensitive of all breast imaging modalities for cancer detection superseding both mammography and ultrasonography with reported sensitivities of up to 98% (158, 159). Its greatest disadvantage is attributable to its low specificity, with values reported as low as 30% (158, 160).

Indications for breast MRI are still controversial, with the European Society of Breast Cancer Specialists EUSOMA guidelines recommending MRI in special situations such as staging before treatment planning e.g. in patients newly diagnosed with invasive lobular cancer; screening of high-risk women; evaluation of response to neoadjuvant chemotherapy; occult primary breast cancer; in nipple discharge if ductography fails for technical reasons or the patient refuses the procedure; characterisation of equivocal findings at conventional imaging (161). Factors that limit the use of MRI include high cost, low specificity which results in a larger number of false positive examinations. When used correctly in the clinical settings in an evidence-based manner, MRI with its high sensitivity is beneficial in earlier
cancer detection compared to the current tests used for breast cancer. Further research efforts will be required to improve the future applications and use of breast MRI (162).

An example of future endeavours is the estimation of apparent diffusion coefficients (ADCs) which assist in the discrimination between malignant and benign lesions, as it has been shown to exhibit lower ADC values for malignant breast lesions (163, 164) and holds a moderate diagnostic ability in identifying axillary metastasis (165).

The results from eight major clinical trials exploring breast MRI as a screening tool have been reviewed and reporting an overall cancer yield of 3% (166). The sensitivity of MRI ranged from 71% to 100% across all studies. Although its reported specificity was variable, the call-back rates and risk of benign biopsies remained high. In general, patients who underwent breast MRI screening had a 10% risk of being called back, and a 5% risk of having a benign biopsy. In Peters et al.’s meta-analysis of 44 studies, pooled weighted estimates of sensitivity and specificity were 0.90 and 0.72, respectively (159).

### 2.4 MAMMOGRAPHIC BREAST DENSITY (MBD)

#### 2.4.1 Definition of MBD
If one views a mammogram, then two major component tissues make up the appearance of the breast: fat and fibroglandular tissue. A mixture of glandular epithelial cells that line the ducts of the breast parenchyma and stroma constitute the fibroglandular tissue. Fibroglandular tissue has higher X-ray attenuations than fat, making fat appear more transparent. Therefore, areas of fat appear dark on radiographs, while fibroglandular associated tissues appear bright and constitute what is called “mammographic density”. The relative amounts of these tissues in breasts can be measured from the differences of brightness in mammograms (167).

#### 2.4.2 Factors which affect MBD
Mammographic breast density is unique amongst breast cancer risk factors in that it changes over time. Genetics is one of the major determinants of density is, there are data demonstrating that inherited genetic factors have a large effect in determining MBD (168). Furthermore, density decreases with age (169), multiparity (170) and menopause (171). Hormonal replacement therapy also has a major effect on density (171, 172) as does pregnancy (173). Dietary factors have also been associated with an increase or decrease in mammographic density (174, 175).

#### 2.4.3 Methods of measurement of MBD
Due to the lack of a standardized and accepted method of assessing breast density, a variety of methods have been proposed for this purpose. They are generally divided into either visual and automated (semi- or fully-automated) or into qualitative and quantitative methods.

##### 2.4.3.1 Visual measurement
The first ever mention of the association between mammographic breast density and cancer risk was made by Dr. John Wolfe back in 1976 (1), he was also the first one to classify breasts into four qualitative parenchymal patterns termed the “Wolfe patterns”.

- **I. N1** – primarily fat tissue; no duct pattern is visible; low risk
- **II. P1** – mostly fat tissue but with some dense areas (ductal prominence) of less than 25% of the total breast; intermediate risk
- **III. P2** – more than 25% of the breast composed of dense tissue along with a noticeable ductal pattern; intermediate risk
- **IV. DY** – primarily homogeneous dense tissue and no conspicuous ductal pattern; radiographically dense; highest risk
Wolfe’s early classification was used in several studies to assess breast cancer risk associated with breast density (176-182). In three cohort studies (180-182), the DY pattern was associated with an increased risk of breast cancer when compared to the N1 pattern. In both case-control and cohort studies, breast density determined by Wolfe’s method has been associated with an increased breast cancer risk (176-182).

Boyd and colleagues proposed an alternative quantitative method that is based on mammographic density percentages measured by radiologists and divided into six unequal interval categories (183, 184).

- **A**: 0%
- **B**: >0-10%
- **C**: >10-25%
- **D**: >25-50%
- **E**: >50-75%
- **F**: >75%

![Figure 1. Boyd classification for mammographic density (A=0%; B=0-10%; C=10-25%; D=25-50%; E=50-75%; F=75%).](image-url)
In the year 1997, Tabár proposed a new qualitative model (185) classifying mammograms into five patterns (I to V) based on histologic-mammographic correlation with a three-dimensional, subgross technique, and on the relative proportion of four “building blocks” (nodular densities, linear densities, homogeneous fibrous tissue, radiolucent fat tissue). Patterns I, II and III are considered low-risk while patterns IV and V as high-risk.

Figure 2. The five categories of the Tabar qualitative model.

- **I**: balanced proportion of all components of breast tissue with a slight predominance of fibrous tissue
- **II**: predominance of fat tissue (fat breast)
- **III**: predominance of fat tissue with retroareolar residual fibrous tissue
- **IV**: predominantly nodular densities
- **V**: predominantly fibrous tissue (dense breast)
Gram et al. (186) reported a poor agreement between the Tabar classification and Wolfe’s patterns when comparing high-risk versus low-risk mammograms. This was most likely because a large proportion (45.6%) of the evaluated mammograms were classified as Wolfe pattern DY (high risk), but as Tabar Pattern I (low risk) (186). The reported discrepancy may be attributable to assessment bias, since the author happened to perform both measurements.

Perhaps the most commonly used classification is the American College of Radiology BI-RADS® classification (140, 187). In the BI-RADS® 4th edition released in 2003 (187), the assignment of the breast composition was based on the overall density resulting in the following continuous quartiles:

1) ACR category 1 (<25% fibroglandular tissue),
2) ACR category 2 (25-50%) 
3) ACR category 3 (50-75%) 
4) ACR category 4 (>75%) 

In the fifth edition of BI-RADS®, the percentage density evaluation ranges were removed (140), but the need for further research into volume-based, reproducible percentage cut points was acknowledged. The use of percentages was discouraged, because in individual cases, it is more important to take into account the chance that a mass can be obscured by fibroglandular tissue than the percentage of breast density as an indicator for breast cancer risk. 

In the BI-RADS® 5th edition released in 2013, the assignment of the breast composition was changed into a, b, c and d-categories followed by a description:

![Figure 3. Breast density composition categories according to the BI-RADS® 5th edition.](image-url)
- **a-** The breast are almost entirely fatty. Mammography is highly sensitive in this setting.
- **b-** There are scattered areas of fibroglandular density. The term density describes the degree of x-ray attenuation of breast tissue but not discrete mammographic findings.
- **c-** The breasts are heterogeneously dense, which may obscure small masses. Some areas in the breasts are sufficiently dense to obscure small masses.
- **d-** The breasts are extremely dense, which lowers the sensitivity of mammography.

Since all of the aforementioned methods suffer from a high-degree of subjectivity, their reproducibility therefore suffers and only low to moderate reproducibility has been reported. The intra-observer intraclass correlation coefficient (ICC) for the Wolfe patterns was reported as 0.68 and the inter-observer ICC as 0.65 (188). The intra-rater reliability of the Tabar patterns was reported in one study as $\kappa = 0.65$ (189), which indicates only good-to-moderate reliability. A study of inter-observer agreement of the BI-RADS$^\circledR$ method reported an overall reliability of $\kappa = 0.43$, with extremely poor agreement for the “extremely dense” category ($\kappa = 0.17$) and highest agreement for the “fatty” category ($\kappa = 0.76$) (190).

### 2.4.3.2 Automated methods

In computerized planimetry, digital mammograms or film mammograms that have been digitized can be utilized. On a computer, a mouse is used to outline the total area of the breast as well as the dense areas and then the respective areas are calculated by the computer, and since the measurement is done partly by the reader and partly by the computer, it is termed as semi-automatic (191, 192). The intra-reader reliability is high, with intra-class correlation coefficients, reported as 0.97 for the non-dense area, 0.82 for the absolute dense area, and 0.93 for percent density (192).

Interactive thresholding is another quantitative method. Using digitized images of the breast, the reader selects a “threshold brightness” to distinguish the breast tissue from the background of the mammogram. Then another “threshold brightness” is chosen that differentiates the dense and non-dense tissue. The computer uses these thresholds to identify both the total area of the breast as well as the areas of density. The number of pixels within these areas is summed to give a measure of the total breast area, the dense area, and the percent density (4, 193, 194). The intra-reader and inter-reader reliability using this computerized interactive thresholding method have been reported to be extremely high, with ICCs >0.90 (194).

Similarly to Wolfe’s method, these quantitative methods have shown an increased association (ORs ranging from 2.0 to 3.8) (200, 206, 218). Finally, studies using both methods have verified these findings and indicate that quantitative methods are more strongly associated with breast cancer risk than with Wolfe’s method (178, 195, 196).

As can be seen, regardless of the method used, breast density has been shown to be a strong risk factor for breast cancer in the general population.

### 2.4.4 Effect of MBD on screening sensitivity

One of the major considerations around mammographic density deals with its effect on mammographic sensitivity and specificity. It is a well-established fact that higher densities are associated with considerably lower sensitivities and specificities than lower density breasts (135, 136, 197).

In a large study of 329,495 women from 7 registries, Carney et al. (198) described a lower sensitivity of screening mammography in women with extremely dense breasts than in those with fatty breasts with values of 62.2% and 88.2%, respectively (p<0.001). Within the scattered density and heterogeneously dense groups, sensitivity was intermediate.
2.4.5 MBD as a breast cancer risk factor
The clinical importance of mammographic breast density is primarily twofold. In addition to its effect on mammographic sensitivity, nowadays increased MBD is considered an established risk factor for the development of breast cancer (3, 4, 48). A BI-RADS® breast density category increase within 3 years has been associated with an increase in the breast cancer risk, while a decrease in density category is associated with a decrease in risk as compared to those women whose density categories does not change (199). Breast cancer rate was higher when the BI-RADS category changed from 1 to 2 (5.6) and from 1 to 3 (9.9) in comparison to the situation when it remained at 1 (3.0) (199). Moreover, MBD was not associated with any specific breast cancer subtype and was not found to be associated with estrogen exposure (200, 201).

2.4.6 MBD as a prognostic factor for survival
MBD’s role in breast cancer prognosis prediction is controversial and rather poorly studied compared to its role as a risk factor. Studies addressing this issue have produced rather conflicting and inconclusive results, mostly due to the use of different classification systems and different cutpoints for what really constitutes low or high density.

Porter et al. (202) reported that the prognosis of screening or the interval in which invasive breast cancer was detected was unrelated to the mammographic parenchymal pattern. Gierach et al. (203) found that in patients with breast cancer, breast density was not related to the risk of death, except in a certain subset of patients (for patients with BI-RADS1 combined with either obesity or a tumour size greater than 2 cm). Chiu et al. (204) found that high density was suggestively associated with poorer survival, but that the association did not reach statistical significance. Maskarinec et al.’s (205) study of 607 women above the age of 50 reported that in stratified models, percent density was associated with a reduced risk of dying from breast cancer in women who had received radiation, but with an elevated risk in patients who had not received radiation.

Olsen et al. (206) reported that case fatality was lower for patients with dense breasts than for those with non-dense breasts, and speculated that breast cancer was less severe in mixed density breasts than in fatty breasts. However, a potential explanation for their findings is that in their study, the fatty breasts included BI-RADS density 1 and part of BI-RADS density 2, without an adequate explanation of the reason or the actual cut-point used. Olsson et al. (207) also reported that high breast density at the time of diagnosis may associate with decreased survival, with the association being stronger in symptomatic cancers. Sartor et al. (208) reported an association between higher mammographic densities and ER negative and triple negative tumours in clinically detected breast cancers.

In regards to tumour grade and aggressiveness, Sala et al. (209, 210) reported higher tumour grades within patients with dense breasts. In their study, they used the qualitative Wolfe classification to evaluate breast density and reported results for both invasive and in situ cancers combined. In contrast, Ghosh et al. (211) reported an inverse relationship between mammographic density and tumour grade. On the other hand, their study population was assessed using a computer assisted thresholding program.

2.5 HYALURONAN

2.5.1 Hyaluronan and Hyaluronan Synthases 1-3
Hyaluronan (HA) is a ubiquitous high molecular size unbranched polymer. It is a glycosaminoglycan with 2000-25000 repeating N-acetyl-glucosamine (GlcNAc) disaccharides and glucuronic acid (GLcA) units reaching a molecular mass of several million daltons and it possesses many biological functions (212-214). Hyaluronan is widely present in all tissues and body fluids of vertebrates as well as in some bacteria (215). As stated, hyaluronan has many functions in the human body and it acts as a cell surface covering and participates in the maintenance of the extracellular matrix integrity. HA is involved in cell
proliferation, migration, wound healing and the high concentration of hyaluronan in many embryonic tissues correlates with their rates of cell migration and proliferation (5, 216). It has been postulated to provide a pliable matrix for tissue remodeling through its unique physicochemical properties, such as its capacity to bind large amounts of water and form viscous gels at comparatively low concentrations (217). Cells are able to modify their biochemical and biomechanical environment thanks to hyaluronan’s properties, by regulating its turnover during wound repair, inflammation and invasion (215).

Three integral plasma membrane protein isozymes called hyaluronan synthases (HAS1-3) are responsible for the production of hyaluronan in the plasma membrane (6, 218). Prehm et al. (219) was the first to suggest that hyaluronan would be synthesized on the inner surface of the plasma membrane, and that hyaluronan chains were degraded extracellularly by hyaluronidase treatment. In mammals, three genes are mainly responsible for the synthesis of hyaluronan, designated Has1, Has2 and Has3; these are found on chromosomes 19, 8 and 16, respectively (220). Molecular size is a property known to greatly influence the biochemical and biological activity of hyaluronan and this varies according to the different hyaluronan synthases (221). HASs are transmembrane proteins with molecular masses of 60 kDa which have 6-7 putative membrane domains (218).

CD44 is a cell surface protein widely expressed in many cell types including fibroblasts, leukocytes, epithelial cells and keratinocytes and it is considered to be the main receptor for hyaluronan (222, 223). The exact role CD44 plays in cancer is complicated because alternative splicing of its mRNA leads to the production of several variants of CD44 (224, 225). Several isoforms of this receptor have been identified, these variant forms differ primarily from each other in the composition of amino acids within their extracellular domain, whereas the transmembrane and cytoplasmic domains are highly conserved (226). CD44 isoform activation can modulate cell proliferation, migration, aggregation and angiogenesis (225, 227).

The catabolism and breakdown of hyaluronan in tissues occurs due to a group of enzymes called hyaluronidases, five of which have been isolated so far, designated as Hyal1, Hyal2, Hyal3, Hyal4 and PH-20 (228, 229). Of those, the main degrading enzymes are Hyal1 and Hyal2 and these enzyme forms are found in most bodily tissues. Hyal3 is found in testes and bone marrow, while PH-20 is sperm associated and plays a role in the fertilization process (230, 231).

2.5.2 Hyaluronan in human malignancies

Variable amounts of HA are found in tumours that originate from the epithelial, mesenchymal, neural and lymphatic tissues, with the role of HA being more pronounced in adenocarcinomas. This HA has an effect on cellular proliferation, invasion and angiogenesis. Not only HA, but also the catabolic enzymes and the degradation products of this large molecule have a complicated impact on the progression of tumours (5). HA is a molecular marker and effector which has a major influence on breast cancer progression (5).

The link between hyaluronan, its synthetizing enzymes and receptors with cancer is now well established. Tumoral hyaluronan has been linked with the epithelial-mesenchymal transition (217), multidrug resistance (232), host-tumour interactions (233) and angiogenesis (234, 235). It has been hypothesized that this variety of effects is due to hyaluronan’s unique physical properties which adapts the environment so that it is more suitable for cell movement and growth (215).

The progression and development of malignant tumours are multi-stage processes that are accompanied by several cellular, biochemical and genetic alterations that include tumour cell interaction with HA and the other extra-cellular matrix (ECM) components. Thus, hyaluronan plays a major and well established role in malignant tumour transformation, progression and metastasis. Hyaluronan helps the growth of new blood vessels by the virtue of its physicochemical properties that aid in open space formation (236).

HA assists in tumoural cell migration by providing space, and by interacting with its cell
surface receptors, it actively supports tumour cell invasion (237). Many solid malignancies have displayed elevated levels of HA (i.e., breast (238), colon (239), thyroid (240), and lung (241)), and HA has been linked to poor prognosis in several tumours (242-244). The location of hyaluronan accumulation varies with differing types of tumour. For instance, increased hyaluronan content is found within tumour cells in cancers of the stomach, whereas it shows an increase in the stroma right next to the tumours of the thyroid, prostate, lung and breast.

Aggressive tumour behaviours and a high metastasis ratio have been linked with the increase in the hyaluronan content to the extent that elevated hyaluronan expression is considered to be an independent factor for unfavourable prognosis (242, 243). Increased hyaluronan synthesis due to Has2 and CD44 overexpression has been linked to the increased invasiveness of breast cancer cells in in vitro assays (245).

More recently Auvinen et al. (7) reported that HER2 status correlated with intense HA staining in the stroma. Moreover, hyaluronan was associated with large tumour sizes, lymph node positivity, negative hormone receptors, poor differentiation, a high body mass index, increased relapse rate and shortened overall survival. Furthermore, the amount of HA in carcinoma cells correlated with relapse frequency and the association between HER2 positivity and intense stromal HA staining indicates that hyaluronan could be one of the causative agents leading to a poor prognosis for HER2 positive patients.

In another study focusing on breast cancer and HAS enzymes (8), both carcinoma cells and stromal cells were found to be HAS-positive. In carcinoma cells, HAS1 and HA stainings correlated with each other, and furthermore, HAS1 associated with estrogen receptor negativity, HER2 positivity, high relapse rate, and short overall survival. Stromal HAS1 and HAS3 were shown to be independent prognostic factors. The data suggests that increased levels of HAS enzymes contribute to the accumulation of HA in breast cancer, and that HA is synthesized in carcinoma cells and stromal cells.

### 2.5.3 Hyaluronan targeted therapies

There are several preliminary reports claiming that the addition of hyaluronidase to chemotherapeutic regimens could significantly boost efficacy and improve patient outcome (246-248). Unfortunately, the benefits of the bovine hyaluronidase used in those studies were limited therapeutically by immunologic responses mounted against the treatment (246). A newly developed recombinant human hyaluronidase has recently been undergoing clinical trials, and preliminary results indicate that this form helped in overcoming the earlier immunologic issues (249).

### 2.6 BREAST CANCER CLINICOPATHOLOGICAL SCORING SYSTEMS

Prognostic models are widely used for investigating patient outcome in relation to multiple patient and disease characteristics. Since breast cancer is a heterogeneous disease and its outcome remains difficult to predict, it is of great interest to identify patients with favourable prognoses for whom adjuvant therapies would not be beneficial, or groups with poor prognoses that aggressive adjuvant treatments would be truly beneficial and warranted (250). Adjuvant systemic treatments have helped to significantly decrease patient mortality, yet evidence shows that a certain set of patients will suffer from their toxicity without gaining the benefits (251, 252). Strong and accurate prognosis predicting systems are needed to individualize the treatment offered to patients according to their specific needs (253, 254). Several scoring systems have been proposed throughout the years, with only a limited number of them actually being utilized in clinical practice. There is a particular interest in identifying prognostic models for which there has been an evaluation of how successful the models have been when used in a different setting, that is, models which have been validated externally (255).

Wyatt and Altman (255) have observed that “however accurate a model is in statistical terms, doctors will be reluctant to use it to inform their patient management decisions unless
they believe in the model and its predictions.” Moreover, they suggested the following conditions for a scoring system to be considered as clinically credible:

(i) All clinically relevant patient data should have been tested for inclusion in the model
(ii) It should be simple for doctors to obtain all the patient data required, reliably and without expending undue resources, in time to generate the prediction and guide decisions
(iii) Model builders should try to avoid arbitrary thresholds for continuous variables
(iv) The model’s structure should be apparent and its predictions should make sense to the doctors who will rely on them
(v) It should be simple for doctors to calculate the model’s prediction for a patient.

The most commonly used systems are the Nottingham prognostic index (256, 257), Adjuvant! Online (258), and PREDICT (259).

2.6.1 The Nottingham Prognostic Index (NPI)
The NPI is one of the most commonly used clinicopathologic scoring systems and is used to assess patients’ prognosis following breast cancer surgery (256, 257). It was first introduced in 1982 and is based on traditional prognostic factors such as tumour size, lymph node status and histological grade. It also gives clinicians the ability to predict both the clinical outcome of tumours and the need for systemic therapies.
The index is calculated using the formula:

\[ \text{NPI} = [0.2 \times S] + N + G \]

Where:

- \( S \) is the size of the index lesion in centimetres
- \( N \) is the node status: 0 nodes = 1, 1-4 nodes = 2, >4 nodes = 3
- \( G \) is the grade of tumour: Grade I =1, Grade II =2, Grade III =3

The original NPI used lymph node stage, subsequent publications from the same group validated the original model but replaced lymph node stage with the number of lymph nodes involved (260). Prognosis worsens as the NPI numerical value increases and by using cut-off points patients may be stratified into good, moderate and poor prognostic groups (261). The NPI classifies patient into groups with excellent prognoses that do not require adjuvant therapies, after radio- and surgical therapy, and secondly a group for which chemotherapy would be suitable. At first, the NPI was made for relapses in breast cancer patients that were not receiving adjuvant therapies, but it possesses prognostic value in patients receiving currently recommended adjuvant therapies (262).

2.6.2 Validation of the NPI
Since its initial release, the NPI has been confirmed after long-term follow-up (260) and has gone on to be repeatedly validated in several studies based on ethnically diverse patients from around the world (262-268).

In the Nordic countries, a 1994 study by Balslev et al. (265) applied the NPI on a Danish population-based study group comprising 9,149 patients while Sundquist et al. (266) validated the NPI on consecutive Swedish breast cancer patients, advocating its use to increase the comparability of groups of patients receiving different therapies.
2.6.3 Previous attempts to improve the NPI
In a review of prognostic models of breast cancer, Altman et al. (253) concluded that no other prognostic model has emerged that is clearly superior to the NPI despite of all the recent advances and new proposed systems. The authors also note that it’s quite clear that additional factors may enhance the use of NPI. Any improvement in prediction must depend on finding factors which are independent of lymph node stage and pathological grade. The same conclusions were drawn in a report by Williams et al. (269).

One of the most common factors to be trialled with the NPI is patients’ HER2 status. Cooke et al. (270), Suen et al. (271), Van Belle et al. (272) and Rakha et al. (273) all showed that HER2 status was an independent prognostic factor of the NPI, and its incorporation into the NPI could act to substantially improve its prognostic ability. Of those, Van Belle created a new prognostic index by merging both the PR and HER2 status in a system called the iNPI.

Collett et al. (274) trialled both the ER and PR statuses with the NPI, concluding that biological differences exist in subgroups as defined by the index. Hansen et al. (275) reported that a prognostic index including the vascular grade had a clinical impact for 24% of patients, who had a shift in prognostic group, as compared to NPI, and implied a better prognostic dissemination. Malmstrom et al. (276) claimed that “S-phase fraction and urokinase plasminogen activator are better markers for distant recurrences than Nottingham Prognostic Index and histologic grade in a prospective study of premenopausal lymph node-negative breast cancer patients”. Callagy et al. (277) through using tumour tissue microarrays revealed distinct tumour clusters which divided into two main groups correlating with tumour grade and nodal status.
3 Aims of the Study

The general aim of this study was to explore the prognostic effect of mammographic breast density and other mammographic characteristics and to explore their potential associations with Hyaluronan synthesis.

The specific aims were:

1) To examine the prognostic effect of mammographic breast density and other mammographic characteristics in patients with invasive breast cancer.

2) To evaluate whether mammographic breast cancer characteristics are linked to HA metabolism. The relationships between HA, HAS1-3 isoforms, CD44, and HER2 positivity on one side, with MBD and other mammographic features on the other side, were investigated.

3) To examine the possible additional value of very low mammographic breast density (VLD), HER2, ER and PR statuses in a patient group within matched Nottingham Prognostic Index (NPI) categories and whether those variables could be incorporated into the NPI.
8 Conclusions

I. Very low MBD proved to be an independent prognostic feature, associated with higher tumour grade and predicted worse survival even after correcting for possible confounders.

II. A strong reciprocal relationship exists between low MBD and HA expression and synthesis. The expression of both factors simultaneously leads to an especially adverse prognostic effect which might have an impact on treatment decision in the future.

III. Very low breast density and HER2 positivity are prognostic factors for breast cancer independent of the NPI. Moreover, their addition to the NPI helps increase its accuracy and thus offers an improved, but still readily available method for the evaluation of patient prognosis.
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Mammographic breast density (MBD) is a topic of broad and current interest both in the literature and the lay press. While most focus is usually placed on MBD’s effect on screening sensitivity and its role as a breast cancer risk factor, this thesis looks at MBD from a different angle, its role as a breast cancer prognostic factor. This study concluded that very low MBD is indeed an independent prognostic factor for breast cancer, and has a strong reciprocal relationship with high expressions of Hyaluronan and its synthesizing enzymes.