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Advantages and limitations of molecular genetic prognostic tests for breast cancer

A systematic literature review

André Berli Delgado

Supervisor: Line Leonore Haugan Moi and Lill-Tove Rasmussen Busund

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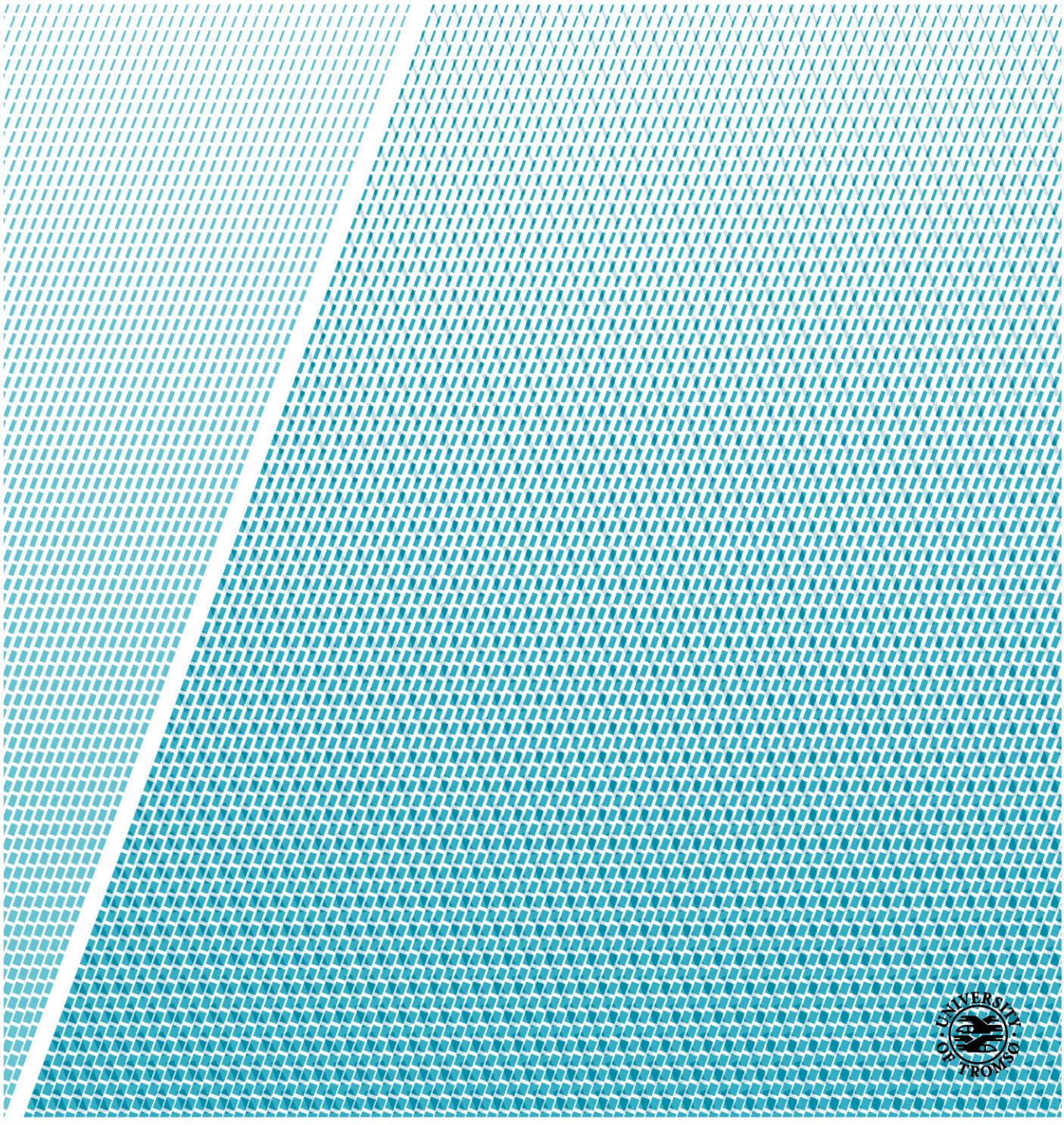

Preface

The purpose of this thesis was to investigate the prognostic and economic aspects of molecular-genetic assays in breast cancer and gather the newest research literature on this topic, and I conducted a systematic literature search to gather an overview on the latest research on this field.

The fields of medical oncology and pathology has been an interest of mine since I started studying medicine, and I have always been interested in scientific research. I therefor applied for the integrated research program for medical students October 2016 and got accepted in May 2017. I joined the Translational Cancer Research Group at the Department of Medical Biology at the University of Tromsø and started my PhD-project on the Norwegian Women and Cancer Study Cohort, researching immunological markers in invasive breast cancer. Through working with my PhD-project I had a growing interest in the genetic aspects of breast cancer.

The project required no extra funding or REK-approval. The literature search and all writing were conducted solely by me. I want to thank my supervisors, postdoc Line Leonore Haugan Moi and professor Lill-Tove Rasmussen Busund, who had the idea for the thesis and have always been very helpful and given excellent counseling and advice.

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André Berli Delgado

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Summary

Background: Breast cancer is the most commonly diagnosed cancer in women internationally, and the most common cause of cancer related death among women. There are many ways to classify breast cancer, and breast cancer can be divided into several subgroups depending on which classification system is used. Pathological reports of breast carcinoma not only depend on one of these systems but include histopathological classification, grade of the tumor, and immunohistochemical (IHC) parameters like estrogen receptor (ER), progesterone receptor (PR), HER2- and Ki67-status. With the development of microarrays, it is now possible to analyze the genes of the cells, and with gene expression profiling (GEP) we have been able to evaluate breast cancer prognosis based on the gene expression of the cancer cells. Different genetic signatures of breast cancer have been obtained through DNA microarray technology, RNA sequencing and bioinformatic models. Some of these signatures have been validated through clinical studies and been translated into commercial prognostic assays. Four such commercial prognostic assays are Oncotype DX, MammaPrint, EndoPredict and PAM5-ROR.

Methods: A literature search were conducted on the databases Medline and Embase. The inclusion criteria of the search were based on the Population, Intervention, Comparison and Outcome (PICO) framework. The search included terms to identify studies assessing the prognostic or economic aspects of Oncotype DX, MammaPrint, EndoPredict or Prosigna. Out of a total of 290 identified studies, 5 were included in this thesis.

Results: Through the systematic literature search only studies focusing on Oncotype DX were included. The litterateur search disclosed that the Oncotype DX recurrence score (RS) is significantly associated with worse prognosis. The Oncotype DX RS were associated with both overall survival, disease free survival and local recurrence. The literature search also disclosed that Oncotype DX may be cost effective, especially in the high-risk RS group, were chemotherapy seemed to be clearly cost-effective because of the gain of additional quality-adjusted life-years (QUALY) at a low cost.

Conclusion: The findings of this thesis suggest that Oncotype DX have an independent prognostic significance and is significantly associated with survival and risk of recurrence and may be helpful to guide treatment. Studies also show that Oncotype DX may be a cost effective alternative when used to guide adjuvant chemotherapy treatment.

Abbreviations

AO	Adjuvant!Online
BCSS	Breast cancer-specific survival
BMI	Body mass index
CI	Confidence interval
DCIS	Ductal carcinoma in situ
DFS	Disease-free survival
DR	Distant recurrence
ER	Estrogen receptor
EUR	Euro
FFPE	Formalin-fixed paraffin-embedded
GBP	Great British Pound
GDP	Gross domestic product
GEP	Gene expression profiling
GRADE	Grades of Recommendation, Assessment, Development and Evaluation
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratio
ICER	Incremental cost-effectiveness ratios
ILC	Invasive lobular carcinoma
LCIS	Lobular carcinoma in citu
LRR	locoregional recurrence
LY	Life years
MeSH	Medical Subject Headings
NGS	Nottingham grading system
NOK	Norwegian kroner
NST	No special type
OR	Odds ratio
OS	Overall survival
PICO	Population, Intervention, Comparison and Outcome
PR	Progesterone receptor
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QUALY	Quality-adjusted-life years
RR	Relative risk

RS	Recurrence score
TNM	Tumor Node Metastasis
USD	United States Dollar
WHO	World Health Organization
YLL	Years of Life Lost

1 Background

1.1 Breast cancer epidemiology

Breast cancer is the most commonly diagnosed cancer in women, both in Norway and internationally, and the most common cause of cancer related death among women (1, 2). It makes up for 22 % of cancer cases among women in Norway, and in 2018 3568 women were diagnosed with breast cancer in Norway, compared to 1235 women in 1970 (3). The prevalence of breast cancer in Norway was 49 314 in 2018, an increase from 34 749 in 2008 (4). Figure 1 demonstrates the trends in breast cancer incidence in Norway from 1980 to 2018 by age groups. In the United States approximately 182 000 women are diagnosed with breast cancer each year, which makes up about 26 % of cancer in women in the US (1). For women in the age group 50 to 74 years the incidence of breast cancer in the UK has increased from 150/100000 to approximately 275/100000 from 1960 to 1990, while in Japan the increase has been from 30/100000 to 60/100000 in the same period of time (5). Since the early 90s the incidence of breast cancer in females has further increased by about 23 % in the UK (6).

Of the 9.6 million registered cancer deaths worldwide in 2018, breast cancer stood for 2.09 million deaths (7). Even though the survival rate of breast cancer is improving with a 5-year survival of 90.7 %, 594 people died from breast cancer in Norway in 2017, of which 586 were women (4). This accounted for 20.0 % of cancer-related deaths in women in Norway in 2017. In stage IV the 5-year survival is decreased to 29.2 % (4). The survival of breast cancer has increased over the last 40 years, from a total survival around 70 to 80 % in the early 80s to almost 91 % today (4). In summary, the mortality of breast cancer has decreased whereas the incidence has increased globally. Figure 2 shows relative survival up to 15 years after diagnosis by age, from 2014 to 2018.

Hormonal factors have been established as key factors in the development of breast cancer through epidemiological studies of the disease. Many of the known risk factors for breast cancer increase the exposure to estrogens in breast tissue, like obesity, early menarche, late menopause, oral contraception, hormonal therapies and alcohol (5, 8, 9).

1.1.1 Possible causes for increasing incidence in breast cancer

The increase in incidence can in part be attributed to breast cancer screening programs introduced in many countries over the last decades (10). The Norwegian Breast Cancer Screening Program started in 1995/96 and included women in the age group 50 to 69 years, and from 1996 to 2007 the screening program detected 67 % of all breast cancers which were diagnosed in this time period (11). An increasing number of women participating in the screening program will most likely lead to an increased incidence, since the screening program can detect tumors that otherwise would go undetected (10, 11). Another possible reason to the increased incidence is the western lifestyle, with high fat consumption, high consumption of alcohol and low physical activity, which are factors associated with increased risk of developing breast cancer. An example of this is the increase in incidence and mortality of breast cancer in Japan, which may be attributed to changes in eating habits to a more western diet with high fat content and low content of fiber (9). Also, in many western countries and Japan, women wait longer to get their first child and also get fewer children than before (9, 12, 13). Low parity is associated with increased risk of breast cancer, probably due to factors such as longer exposure to estrogen and less breastfeeding (5, 6, 8, 9, 14). Another explanation for the increased incidence of breast cancer is younger age at onset of puberty, and thereby earlier menarche and earlier breast development, which in part is caused by increased body mass index (BMI) among children and environmental factors (15-18). As for most types of cancer, one of the most important causes for the increase in breast cancer incidence is increasing age and an older population (10).

1.2 Breast cancer classification

Breast cancer comprises a group of diseases with specific clinical, histopathological and molecular properties. There are many ways to classify breast cancer, and breast cancer can be divided into several subgroups depending on which classification system is used. Pathological reports of breast carcinoma should not only depend on one of these systems, but should include histopathological classification and grade of the tumor, and immunohistochemical (IHC) parameters like estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2)- and Ki67-status (19). Together with clinical variables such as tumor staging, these factors are conventionally used as prognostic and predictive markers (20). More recently, new molecular approaches, named intrinsic subtype classification, have been tested. These approaches focus on the gene expression profiles of the cancer cells. These

molecular approaches are not yet used in routine clinical practice or in treatment guidance, but promising results on the prognostic and predictive importance of molecular subtyping are emerging.

1.2.1 Histopathological classification

The histopathological classification of breast carcinomas is based on the morphological features of the tumors, and is an essential component of the pathological reports of breast cancers (19). Breast carcinomas can broadly be classified into two main groups; invasive carcinoma and carcinoma *in situ*, where carcinoma *in situ* can further be divided into ductal carcinoma *in situ* (DCIS) and lobular carcinoma *in situ* (LCIS). DCIS can be further divided into other groups. Invasive breast carcinomas can also be divided into a number of subclasses, where invasive ductal carcinoma, now referred to as invasive carcinoma of no special type (NST), is by far the most common, accounting for about 70 to 80% of all invasive breast lesions (21). Invasive lobular carcinoma (ILC) is another type of invasive breast cancer, which accounts for 5 to 15 % of invasive breast cancers (22). WHO has made a histopathological classification system for breast tumors, which in its' current version includes about 20 major tumor types and 18 minor subtypes (19).

Since histopathological classification is solely based on the morphological appearance of the cancer cells, this classification system is unable to mirror the heterogeneity of breast cancer because different cells within the same group, and even tumor, has different biological and clinical profiles. Alone, histopathological classification has not sufficient prognostic and predictive implication (19).

Histological tumor grading is a grading system that evaluates the degree of differentiation in the tumor tissue. Today histological grading is one of the best-established prognostic factors for breast cancer, and the Nottingham Grading system (NGS) is the grading system for breast cancer that is recommended by various professional international bodies, like WHO and the EU. NGS is based on the evaluation of the degree of tubular or gland formation, the degree of nuclear pleomorphism (variability in the size and shape of the nuclei), and the mitotic count. Combined with lymph node evaluation and the tumor size, together with the Kalmar Prognostic Index, they constitute the Nottingham Prognostic index (23).

In the NSG, based on the evaluation of the degree of tubular or gland formation, the degree of nuclear pleomorphism, and the mitotic count, each of these features is given a score of either one, two or three points, where 1 point is closest to normal and three points is the least normal. For example, when evaluating the degree of glandular/tubular formation, 1 point is given when >75 % of the tumor area is forming glandular/tubular structures, while 3 points are given when <10 % of the tumor area is forming these structures. Based on the overall score the tumor is graded into grade 1, 2 or 3, where grade 3 has the worst prognosis (24).

1.2.1.1 Carcinoma in situ

Ductal carcinoma *in situ* (DCIS) is a non-invasive intraductal epithelial proliferation that do not infiltrate the basal membrane, hence the term "*in situ*" ("in place"), and is often considered a pre-malignant lesion of the breast (22). The epithelial cells are characterized by cellular and nuclear atypia such as increase in nuclear-cytoplasmic ratio and hyperchromasia (an increase in chromatin content in the nuclei, and thereby increased staining capacity). Women with DCIS have an increased risk of local recurrence of DCIS after resection, and an increased risk of developing invasive breast cancer (25). Lobular carcinoma *in situ* (LCIS) describes an intralobular proliferation of epithelial cells that do not infiltrate the basal membrane. These cells are often small, uniform and loosely cohesive. There are rarely atypical changes like nuclear pleomorphism and/or necrosis like in DCIS (22). The lobular architecture is usually intact, and mitoses are rarely observed. Women with LCIS also have an increased risk of developing invasive breast cancer (26).

1.2.1.2 Invasive carcinoma

No special type (NST) is the most common form of breast cancer and constitutes about 55 % of the incidence of newly diagnosed breast cancer (22), and 80 % of all infiltrative breast cancers (25). NST consists of malignant ductal cells that have broken through the basal membrane and invaded the surrounding tissue and can develop both with and without previous DCIS. The morphology of NST is highly variable, and the tumor can be of varying size. The tumor often shows diffuse sheets of nests of cells and variable degrees of differentiation. Breast cancer that stems from the mammary ducts can also be divided into other different subclasses based on a wide range of criteria (22, 25). Tubular carcinoma is another rare subgroup of breast cancer that is well differentiated. This subgroup is characterized by proliferation of oval elongated tubules with an open lumen. This subgroup

usually has a good prognosis and rarely metastasizes. Invasive cribriform carcinoma is another rare subgroup of ductal cancer that is often associated with a good prognosis and is characterized morphologically by islands of uniform tumor cells. These cells present little morphological atypia and there is no clear invasion of surrounding tissue. Invasive cribriform carcinoma can in some cases be difficult to separate from DCIS, and immunohistochemical staining for myoepithelial cells is necessary. Mucinous carcinomas are tumors that typically consist of small clusters of uniform epithelial cells with mild atypia. These cells secrete mucus. Medullary carcinoma are tumors consisting of poorly differentiated cells with pushing borders. These tumors are associated with prominent lymphoid infiltration, and are associated with good prognosis. In addition to these subgroups there are numerous of very rare subgroups of ductal cancer, like invasive papillary and micropapillary carcinoma, apocrine carcinoma, neuroendocrine carcinoma and metaplastic carcinoma (22, 26).

Invasive lobular carcinoma (ILC) represents 5 to 15 % of all invasive breast tumors. ILC is characterized by small, round, uniform and non-cohesive cells. There are usually only small amounts of cytoplasm. These cells usually infiltrate the stroma in a single-file matter. ILC usually affects older women than NST. ILC can be further divided into subgroups. The most common subgroup of ILC is called “classic type” and is characterized by small uniform cells singly distributed in the stroma, without glandular differentiation. Whether or not the prognosis of ILC is better or worse than NST is not yet determined (22, 26). These carcinomas usually present diffuse infiltration and are often found to be larger than first expected from mammographic imaging and clinical examination and have a tendency to be multifocal.

1.2.2 Immunohistochemical classification

Immunohistochemistry (IHC) is a method used in histopathological diagnostics where antibodies are used to mark and visualize specific molecules/antigens. In the case of breast cancer IHC is routinely used to test for specific receptors. Tissue is harvested and prepared through a process of formalin-fixation, paraffin embedding, sectioning of the tissue blocks, de-paraffinization and blocking of specific and non-specific sites to prevent false positive detection. The stains used can either be chromogenic or fluorescent, and the antigen-detection can be either direct, where the primary antibodies is conjugated to a chromogen or fluorophore, or indirect, where a secondary antibody conjugated to a reporter dye is used (27, 28).

Breast cancer tumor cells are routinely tested for expression of ER, PR and HER2 with IHC in the diagnostic process of breast cancer. Breast tumors are divided into subgroups based on their expression of these receptor proteins; (ER+, PR+) HER2+, which are tumors with either ER or PR positivity and HER2 positivity, (ER+, PR+) HER2-, which are tumors with either ER or PR positivity and HER2 negativity, ER-,PR-, HER2-, which are triple-negative tumors, and ER-, PR-, HER2+, which are tumors with ER and PR negativity and HER2 positivity (29).

The receptor status of the breast cancer has important prognostic and predictive value. The subgroups have different prognosis, as hormone-receptor positive breast cancer tends to grow more slowly, and triple negative breast cancer tend to be more aggressive and spread faster. Further, receptor status also guides targeted treatment, and hormone-receptor positive breast cancer allows for more treatment options. ER-positive breast cancer can be treated with estrogen-receptor blocker or aromatase inhibitors (30, 31), while triple-negative breast cancer does not allow for targeted endocrine treatment.

1.2.3 TNM classification

Tumor Nodal Metastasis (TNM)is the most widely used system for classification of cancer in the world. The TNM-classification is based on the size of the tumor (T), the number of lymph nodes involved (N) and the presence of metastases or not (M). The system used to day is the TNM7-classification (32). Each of these main variables (T, N and M) is further divided into subgroups. T is divided into Tmi, T1, T2, T3 and T4 depending on the size of the main tumor, N is divided into Nx, N1, N2 and N3 depending on the number of lymph nodes that present with metastases, and M is divided into M0 and M1 depending on whether there are distant metastases or not (33). For example, a patient that presents with a primary tumor of 1.5 cm (T1c), has metastasis in 4 lymph nodes in the armpit (N2a), and has no sign of distant metastasis (M0), has a T1cN2aM0 breast cancer, corresponding to a stage IIIA cancer.

1.2.4 Molecular classification

The development of gene technology and microarrays has enabled analyzes of genes of the cells, and gene expression profiling (GEP) has made it possible to evaluate breast cancer prognosis based on gene expression of the cancer cells (20). In 2000 Perou et al. proposed the existence of four molecular-genetic subgroups of breast cancer through high profile molecular

profiling studies of tumor cells; luminal, basal-like, normal breast-like and HER2-enriched breast cancer. These groups were distinguished by differences in their gene expression pattern (34). In a follow-up study in 2001 by Sorlie et al. six intrinsic subgroups of breast cancer were identified (35). Today we classify breast cancer into the 4 intrinsic subgroups luminal A, luminal B, basal-like and HER2-positive. These subgroups have been shown to correspond well with the histopathological classification and receptor-expression measurements of breast cancer, with for example the luminal A usually being ER and PR positive and HER2 negative with a low Ki67, while the HER2-enriched subgroup usually is ER and PR negative and HER2 positive with a high Ki67 (36). Since Perou et al. published their work in 2000 there have been many gene-expression profiling studies on breast cancer, many of them targeted at gaining the ability to better identify patients who will, and will not, benefit from chemotherapy and endocrine therapy (37).

Recent studies have also shown epigenetic modifications, such as DNA methylation in cancer cells, and compared this to the gene expression to investigate the role of the methylation in the prognosis of the patient (38). Gene expression data appears to be superior to e.g. DNA-methylation data for breast cancer subtype classification, but DNA-methylation models may provide addition candidate genes for complementing existing therapy.

1.2.5 Breast cancer classification today

Today, breast cancer patient management still rely on pathology assessment (histologic type, histologic grade and ER/PR/HER2-status) supplemented with a few validated assays testing for biomarkers, even though more clinically relevant intrinsic subtypes are being identified (20, 22). The usefulness of current methods for classification of breast cancer into the intrinsic molecular subtypes (luminal A, luminal B, HER2 and basal-like) were evaluated by a panel of breast cancer and gene expression profiling experts through the 2012 IMPACT task force. The panel concluded that the classification based on ER, PR, HER2 and Ki67 through IHC is not sufficient to modify systemic treatment decisions alone, but the panel still recommends using IHC for ER and HER2 for identification of clinically relevant subgroups of breast cancer as a part of the diagnostic and prognostic evaluation of the disease (39).

There are several limitations with the clinicopathological classification system used today. Nodal status is one of the main deciding factors for using chemotherapy, and women with breast cancer, particularly women with hormone receptor positive, HER2-negative breast

cancer, is at risk of overtreatment. It is important to develop test that objectively stratify patients into risk category. This way treatment can be tailored based on the individual patient prognosis, and not on a standard treatment regimen based on the nodal status of the patient (40).

1.3 Prognostic factors in breast cancer

Prognostic factors estimate clinical outcome and risk of tumor relapse independent of treatment, and help making the decision of which patients are candidates for adjuvant treatment. Predictive factors estimate the likelihood of response to specific treatments, and help making the decision of which type of treatment the patient should be given (41, 42).

There are a lot of different prognostic factors, but the prognostic value of each individual factor varies greatly. Age is observed to be an independent prognostic factor in most types of cancer, and in breast cancer younger age is considered to be unfavorable to the prognosis. Lower age at disease-onset is shown to correlate with higher TNM-stage, high grade tumors, hormone-receptor negativity and HER2-overexpression (43, 44).

Tumor size is another important independent prognostic factor, and larger size of the tumor indicates worse prognosis. Tumors larger than 2 cm are considered intermediate/high-risk cancers, while patients with a tumor <1 cm are reported to have close to 100 % 5-year survival (45). Nodal status is considered to be the most important independent prognostic factor in breast cancer. Studies have found that local lymph node status is a significant predictor for tumor recurrence, distant metastasis and overall survival. Tumor grade is also used to determine the patient's prognosis, and studies have shown that patients with grade 1 tumors have significantly better survival than those with grade 2 and 3 lesions (41, 45).

HER2 status has also been used to determine the patients' prognosis, and studies have generally found that patients with overexpression of HER2 have a worse prognosis. Some studies have shown that patient with HER2 overexpression have twice the mortality rate of women without detectable HER2. ER and PR have also been associated with the patients' prognosis, where high expression of especially ER is associated with a better prognosis (41, 45-47). The fact that some studies have found a better response to hormonal therapy in ER+ patient with the presence of PR indicates a prognostic factor for PR expression as well (42). Measurement of Ki67, a cell proliferation marker, is widely used in many countries to

determine the prognosis of the breast cancer patient, but the use of this marker is controversial, and the results of studies of Ki67 are conflicting. Some studies though conclude that post-chemotherapy Ki67 is a strong predictor for clinical outcome for the patient (47). Some expert panels suggest that Ki67 can be used in combination with other established prognostic factors, while other expert panels are against the use of Ki67 (45).

Today the most widely used prognostic markers are lymph node status, tumor size, tumor grade, and peritumoural lymphovascular invasion (41, 45, 46). These markers also have important predictive value together with hormone receptor status. Lymph node metastases, large tumor size, high tumor grade and infiltrative growth indicates poorer prognosis, and also indicates the need for more aggressive treatment with cytotoxic chemotherapy. For example, in Norway, all patients with lymph node stage 1 to 3 are candidates for systemic treatment with chemotherapeutic drugs. Also, the presence of hotspot Ki67 > 30 % in the tumor tissue provides basis for adjuvant chemotherapeutic treatment. Hormone receptor status also have an important predictive value. Today, endocrine treatment is the main treatment for ER- and PR-positive breast cancer patients. ER-antagonists and aromatase inhibitors are the two main treatment options for ER-positive patients. Also, targeted therapy with the monoclonal antibody against HER2 has greatly increased the prognosis in HER2-positive breast cancer patients, and studies shows up to 50 % reduction in risk of relapse (48).

1.4 Economics of breast cancer

In 2018 approximately 360 billion Norwegian Kroner (NOK) were spent on health in Norway, approximately 10,18 % of the gross domestic product (GDP) (49), a rise from 342 billion NOK in 2017 (50). The total yearly cost of cancer in the Norwegian society is around 40 billion NOK, where 17,5 billion NOK goes to the health care sector and 18 billion comes from lost earnings from the patients. If one includes Years of Life Lost (YLL) the estimated economic burden of cancer in Norway is 100 billion NOK per year (51). The total health costs of breast cancer is estimated to be approximately 1,7 billion NOK, making it the second most expensive form of cancer in Norway, only exceeded by colorectal cancer which has a total health cost of around 2 billion NOK (52).

In 2018 the European average expenditure on health, as well as the expenditure of UK, was 9,6 % of GDP, while Germany used 11,3 % (53) and 17.1 % were used in the USA (49). The estimated total cost of cancer in the USA is estimated to be more than 180 billion United States Dollar (USD) and in 2010 the total cost of treating breast cancer was 16.5 billion USD

(54). The total estimated cost of cancer in the UK is estimated to be 18.3 billion Great British Pounds (GBP) (55).

1.5 Molecular-genetic profiling

As mentioned earlier it is possible to analyze the genes of cancer cells through GEP and been able to evaluate breast cancer prognosis based on their gene expression. Different genetic signatures of breast cancer have been obtained through DNA microarray technology, RNA sequencing and bioinformatic models. Some of these signatures have been validated through clinical studies and been translated into commercial prognostic assays. These tests can stratify patients into different risk categories based on their expression of specific gene signatures and can help guide treatment together with other clinco-pathological factors such as lymph-node status (56). Four such commercial prognostic assays are Oncotype DX, MammaPrint, EndoPredict and PAM50-ROR.

1.5.1 MammaPrint

The Amsterdam 70-gene profile is a prognostic gene signature identified by the Netherlands Cancer Institute. This gene signature was developed through analysis of 78 frozen, node-negative breast cancer tumors in women younger than 55 years. Through comparisons in this cohort, the research group ended up with 70 genes to predict clinical outcome. This prognostic profile has since then been validated in other studies, for example MINDACT (36), and is now available for commercial use as MammaPrint to predict the risk of distant metastasis in T1-2 N0-1, ER-positive/negative and HER2-positive/negative, and to select patients who would benefit from chemotherapy (57, 58). MammaPrint is a microarray prognostic score which measures the mRNA expression of the 70 genes included in the profile in frozen tissue and stratifies the patients' score into a low-risk or a high-risk group (59). Patients with low genomic risk are unlikely to develop distant metastasis and are therefore unlikely to benefit from adjuvant chemotherapy, while high-risk groups most likely will benefit from adjuvant chemotherapy. This test requires either formalin-fixed paraffin-embedded (FFPE) tissue for clinical purpose, or fresh specimens usually for research purpose. All the tests are analyzed in central laboratories in Netherlands and USA (60).

1.5.2 Oncotype DX

The 21-gene recurrence score is a gene-assay test commercially known as Oncotype DX. This test is a RT-PCR-based signature that measures 21 genes. It was developed through the evaluation of 250 genes which correlates to recurrence. Oncotype DX can be used on FFPE tissue samples and gives a score on a scale from 0 to 100, where <18 is defined as low risk, 18-30 as intermediate risk and >30 as high risk of recurrence. The tissue has to be sent to a clinical reference laboratory of Genomic Health, Inc. in Redwood, California in USA, to be analyzed. Oncotype DX can be used to measure the risk of recurrence in women with node-negative, hormone receptor positive breast cancer or with DCIS, and it can be used to identify who would benefit from chemotherapy and/or endocrine therapy. In women with breast cancer with intermediate or high risk of recurrence, the benefits of chemotherapy will likely outweigh the risks of side effects, and in women with high risk of recurrence DCIS the benefits of radiotherapy will likely outweigh the risks of side effects. The test has been validated in many studies, for example TAILORx, and is one of the most used prognostic gene-signature tests in clinical practice (36, 37, 57-59, 61-64).

1.5.3 Prosigna

PAM50-ROR (Prosigna) is a RT-PCR test used on FFPE tissue. It measures the risk of recurrence by using the 50-gene profile from PAM50 classifier, which was originally designed to be a tool to classify the intrinsic subgroups of breast cancer. The score is reported on a scale from 0 to 100, and the patients are divided into low, intermediate or high risk. This test is used to measure the probability of recurrence in hormone receptor positive, early-stage breast cancer patients with 0 to 3 positive nodes. PAM50-ROR has been validated in several studies (36, 37, 59). Prosigna can be used on FFPE-tissue, and is analyzed in specialized molecular pathology laboratories, and is used to guide treatment. These tests may be performed locally, given the laboratories have the right specialized equipment. The test measures the risk of recurrence from 5 to 10 years after being diagnosed with breast cancer in postmenopausal women, after 5 years of therapy. Node-negative cancers are classified as low (risk score 0-40), intermediate (41-60) and high-risk (61-100). Node-positive cancer are classified as low (0-40) or high (41-100) (65, 66). This test has shown to be prognostic for ER-positive, post-menopausal women treated with endocrine therapy alone, and to be both prognostic and predictive of endocrine therapy in pre-menopausal women treated with

adjuvant endocrine therapy. High risk patients have a higher risk of metastasis and may have more benefit for adjuvant treatment (67).

1.5.4 EndoPredict

EndoPredict is a RT-PCR-based assay that measures the RNA of 12 genes, 8 cancer genes and 3 housekeeping genes and 1 control gene. It is used to measure the risk of recurrence in estrogen receptor positive, HER2 receptor negative and node-negative breast cancer patient treated with adjuvant endocrine therapy alone. The prognostic value of EndoPredict has been validated in several studies. EndoPredict stratifies the patient score into two groups; a low risk and a high-risk group. This score is usually combined with other prognostic factors, such as tumor size and node-status, to compute a more comprehensive score, named EPclin (36, 37, 59). This assay can be performed on FFPE-tissue, and can be analyzed in diagnostic molecular pathology laboratories that has established the EndoPredict assay in their routine diagnostics (68). The EPclin score is used to predict the 10-year distant recurrence (DR) rate and is further used to guide treatment decision. High risk patients are more eligible for adjuvant chemotherapy (69). The EPclin Risk Score is given as a number between 1.1 and 6.2, that indicates the risk of recurrence, and the score is separated in two one out of two groups; low-risk score and high-risk score. An EPclin score > 3.3287 indicates high risk of recurrence, > 10%. A score < 3.3287 indicates low risk of recurrence, < 10 %. The EPclin-score is illustrated on a curve (70).

1.6 Aims of the thesis

In this literature review I have looked at the four prognostic gene-signature tests MammaPrint, Oncogene DX, PAM50-ROR and EndoPredict. Through the literature analysis I have looked at the advantages and the limitations of each of these tests, with focus on 2 main aspects, and presented a brief overview:

1. Prognostic information – What are the advantages of molecular-genetic prognostic tests in breast cancer for the individual patient and for the health-care providers? Do they provide any prognostic information outside of the standard classification of breast cancer?

2. Economics – Are there conducted any studies on the cost-effectiveness of the use of these gene-signature tests? Are the tests affordable, and can they be implemented in routine practice, or are they too expensive?

2 Methods

2.1 Data source and search strategy

A systematic literature search was performed to assess the prognostic and economic aspects of molecular-genetic profiling of female breast cancer patients. To perform the literature search I followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). The literature search was conducted May 13. 2020 in Medline and EMbase using the search engine Ovid. The search consisted of a combination of Medical Subject Headings (MeSH) terms and Emtree terms, and highly relevant terms from keywords, title and abstract. The search terms were allocated in three groups: 1. Breast cancer; 2. Molecular-genetic tests; 3. Outcome. The different search terms within each group were combined with "OR", and the categories were combined with "AND". The reference list of the included articles from the literature search were also screened for relevant articles. Figure 3 shows the search terms included in this thesis.

2.2 Selection criteria

The inclusion criteria of this literature review were based on the PICOS-framework: (i) population: Human breast cancer patients; (ii) intervention: either of the molecular genetic tests: MammaPrint, Oncotype DX, Prosigna or EndoPredict; (iii) comparison: not relevant in this thesis; (iv) outcome: direct health care costs of the use, or the prognostic value, of at least one of the molecular genetic tests; (v) study design: costing studies or prognostic studies. Furthermore, the studies had to be human studies, be of English language, full-text and published from year 2016 to present to be included in the search.

I excluded any studies with the following characteristics: studies involving male subjects, studies involving CIS and not invasive carcinoma, non-scientific studies, reviews, studies were there were no full-text article available, and studies with topics irrelevant to this thesis.

2.3 Literature search and data extraction

Through the literature search a total of 290 records were found, 136 in Medline and 154 in Embase. The records were exported to EndNote X9. Here further steps in the selection procedure were carried out. 35 duplicates were identified and removed, and 255 records were

screened in the title and abstract for eligibility. After screening the title and the abstracts, 221 records were excluded due to not matching the inclusion criteria, or due to matching one or more of the exclusion criteria presented in this thesis. The 34 remaining articles were analyzed full text. After reviewing the articles, five articles were chosen as eligible based on the inclusion criteria and included in this thesis. The process of the literature search and data extraction is shown in figure 4.

2.4 Data analysis

Relevant data from the five selected articles included in the thesis were extracted from EndNote X9 to a Microsoft Excel spreadsheet. The excel spreadsheet included author name, journal name, study design, study population, results and conclusion. The results from the articles were divided in prognostic information and/or health costs information. Findings from the prognostic studies were considered significant if the corresponding 95% confidence intervals (CI) for their risk estimates (i.e. odds ratio (OR), hazard ratio (HR) or relative risk (RR)) did not include a point estimate of 1.00, and p-values were less than 0.05.

2.5 GRADE

The GRADE-guidelines (Grades of Recommendation, Assessment, Development and Evaluation) is a method created for appraising controlled studies and make recommendations for systematic reviews and guidelines. The method is used to assess the quality of evidence in controlled studies. This system classifies research articles into four different ranks; Very low, Low, Moderate and High, based on the study design, risk of bias and the size of the effect among other things (71). In this review I have evaluated the five articles from the literature search for quality of evidence, based on the GRADE-method. The GRADE-tables are listed in the end of this thesis.

3 Results

Five articles out of the 290 articles identified in the systematic literature search were included in this thesis. All of the articles were cohort studies, three retrospective cohort studies (72-74), one prospective cohort study (75), and one hypothetical cohort study/modeling study (76). All of the studies were prognostic studies except for the hypothetical cohort study which was an economic study. A complete list of all the studies included in this thesis, including their results, is shown in Table 1. The risk estimate and specific effect size of the prognostic studies is not mentioned in the text but is shown in Table 1. The risk estimates and effect size of the

economic study is discussed in the text. Through the systematic literature search only studies focusing on Oncotype DX were identified. No studies researching MammaPrint, EndoPredict or PAM50-ROR were included in the thesis due to either not fulfilling the inclusion criteria or having one or more exclusion criteria. Many of the studies involving MammaPrint, EndoPredict or PAM50-ROR first identified in the initial search were predictive studies, not prognostic studies. Many were also correlational studies, and not looking at specific endpoints.

3.1 Oncotype DX

All five of the articles selected from the systematic literature search studied Oncotype DX (72-76). All of the prognostic studies found that the Oncotype DX recurrence score (RS) were significantly associated with prognosis, although the studies had different endpoints. All of the studies found that high RS reduced the prognosis. The study by Turashvili et al. (72) found that the risk of locoregional recurrence (LRR) increased more than 4-fold in high RS patients compared to low RS patients. All of the prognostic studies evaluated ER-positive and HER2 negative breast cancer, except for Kizy et al.(73), where they studied the impact of Oncotype DX RS on ILC. Since ILC is mostly HER2-negative they only assume the patients are HER2-negative and did not include HER2 status in the analysis. Kizy et al. is also the only article that studies the impact of Oncotype DX RS on ILC, and found that a high-risk RS is independently associated with increased hazard of death when compared with low-risk RS. Turashvili et al. (72) was the only article that excluded lymph node-positive breast cancer, while the other articles either included pN_{0-1}- or pN_1 breast cancer. None of the articles included breast cancer with distant metastasis.

One article conducted a risk-group specific cost-effectiveness analysis of adjuvant chemotherapy accounting for companion prognostic tests Oncotype DX and Adjuvant!Online (AO) (76). Here they applied a computer simulation model and conducted a hypothetical cohort study of 100000 50-year-old women diagnosed with ER- and/or PR-positive, HER2-negative and lymph node-negative breast cancer. Their outcome of interest included quality-adjusted-life years (QUALY) and number of life years (LY). They also measured incremental cost-effectiveness ratios (ICER; EUR/QUALY). The research group compared their results to a Canadian study conducted by Paulden et al. (77) in a cross-country comparison. They found that in the high-risk RS group, chemotherapy seemed to be clearly cost-effective because of

the gain of additional QUALY at a low cost and found an ICER less than 3500 EUR/QUALY. Chemotherapy was also cost-effective in the RS-group and AO group.

3.2 MammaPrint, Prosigna and EndoPredict

None of the studies included in this systematic literature review studied MammaPrint, Prosigna or EndoPredict. Many of the articles in the initial search focused on these three molecular-genetic profiling tests, but these studies were excluded from the review due to being predictive studies, correlational studies or including male breast cancer. Many of the studies from the initial search investigated the prognostic value of the PAM50 intrinsic subgroups, but not the PAM50-ROR. For this reason, I am not able to evaluate the prognostic or economic aspects of MammaPrint, Prosigna or PAM50 in this thesis.

4 Discussion

In this systematic review, the goal was to evaluate the prognostic and economic impact of the four well known genomic profiles Oncotype DX, MammaPrint, Prosigna and EndoPredict. Only five studies focusing on Oncotype DX fulfilled the inclusion criteria and were included in this thesis. Four of these articles were prognostic studies, and all of them found that Oncotype DX RS is significantly associated with survival outcome in breast cancer, and the RS result provided an independent value in staging of breast cancer. All of the studies showed that a high RS is significantly associated with higher risk of LRR and mortality, while low RS is significantly associated with higher breast cancer-specific survival (BCSS), disease-free survival (DFS) and overall survival (OS).

One article studied the cost effectiveness of the use of Oncotype DX and compared it with AO and found that in intermediate and high-risk RS-groups chemotherapy was effective and potentially cost effective. This is similar to the findings of Blok et al. (78). Blok et al conducted a systematic literature review on the clinical and economical value of gene the expression profiles Oncotype DX, MammaPrint, EndoPredict and Prosigna, and found that most evaluations estimated that genomic testing is cost-effective, with costs that are acceptable in relation to patient outcome.

With regard to MammaPrint, EndoPredict and Prosigna, this systematic review found no studies eligible for inclusion. This is commonly known as “empty review”, and the issues of empty reviews was introduced to the literature by Lang et al. in 2007 (79), and proposed that the authors of empty reviews should note observations from ineligible studies that were

found. This may be problematic, as noted by Green et al. (80), since conclusions based upon studies which do not meet inclusion criteria specified in the review protocol increases the risk of bias and may be misleading. Both Lang et al. and Green et al. outline the benefits and importance of publishing empty reviews as they tell us who is undertaking the reviews and thus who is interested in the topic. Further, they highlight major research gaps, indicate the current state of research evidence and they play an important role in highlighting areas requiring further research.

4.1 Limitations

This thesis has several limitations. First, even though the systematic literature search also included MammaPrint, Prosigna and Endopredict, only studies focusing on Oncotype DX were included in the thesis. There may be several reasons for this. First, the research question we propose may be too specific with overly strict methodological criteria. This secures more relevant articles and higher quality evidence but may result in many articles of interest not being included in the search. Also, MammaPrint, Prosigna and EndoPredict are relatively new tests compared to Oncotype DX, which have already been validated in many studies.

Second, the definition of tumor subtypes varies across the included studies. All of the included studies evaluate the prognostic value of Oncotype DX RS in ER-positive breast cancer, but the TNM-classification and tumor grade vary across the studies. Also, the accepted clinicopathological definition elaborated by the St. Gallen International Breast Cancer Conference Expert Panel recommended implementing Ki67 to the subtype definition (81). Even though this thesis did not aim to evaluate the relation between the molecular-genetic assays and Ki67, one of the included studies did evaluate the impact of RS on DFS within Ki67 subgroups and found a significant correlation.

Third, this thesis only includes one economic study on the cost-effectiveness of Oncotype DX, performed in Austria. This study showed the cost-effectiveness of Oncotype DX in intermediate and high-risk RS groups, and the possible value of implementation of Oncotype DX in the decision making of possible adjuvant chemotherapy. These results are based on Austrian economy and health care system and is not directly transferable to other countries. However, their results were comparable to a Canadian study (77).

4.2 Implications

In this thesis we have disclosed the prognostic and economic value of Oncotype DX in breast cancer. Given the high mortality in late-stage breast cancer and high morbidity associated with adjuvant chemotherapy, it is important to identify which patients are at high risk and which patients are at low risk of advanced disease. Oncotype DX may be able to assist in differentiating between high risk and low risk patients, and who will benefit from adjuvant treatment.

5 Conclusion

The findings of this thesis suggest that Oncotype DX have an independent prognostic significance and is significantly associated with survival and risk of recurrence and may be used to help guide treatment. Studies also show that Oncotype DX may be a cost effective alternative when used to guide adjuvant chemotherapy treatment. This thesis is in agreement with a growing amount of research suggesting that molecular genetic profiling tests have a distinct prognostic value. No information is available from the current systematic literature search regarding the prognostic and economic aspects of MammaPrint, Prosigna and EndoPredict.

6 References

1. Colditz G. Breast Cancer Epidemiology and Risk Factors Medcape2015 [updated Dec 26, 2019; cited 2020 May 14.]. Available from: https://emedicine.medscape.com/article/1697353-overview.

2. Torre L, Islami F, Siegel R, Ward E, Jemal A. Global Cancer in Women: Burden and Trends. Cancer Epidemiol Biomarkes Prev. 2017;26(4):444-57.

3. The Norwegian Directorate of Health. National action program with guidelines for diagnostics, treatment and follow-up of patients with breast cancer, 2019. 2019 [cited 2020 May 14.].

4. Cancer Registry of Norway. Cancer in Norway 2018 - Cancer incidence, mortality, survival, and prevalence in Norway. Oslo; 2019.

5. Kay TJ, Verkasalo PK, Banks E. Epidemiology of breast cancer. The Lancet. 2001;2(3):133-40.

6. Cancer Research UK. Breast cancer statistics. Cancer Research UK [cited 2020 May 14.]. Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer#heading-Zero.

7. WHO. Fact sheet - Cancer: WHO; 2018. [cited 2020 May 14]. Available from: https://www.who.int/news-room/fact-sheets/detail/cancer.

8. Breastcancer.org. Breast Cancer Risk Factors. Breastcancer.org. [cited 2020 May 14]. Available from: https://www.breastcancer.org/risk/factors.

9. Saika K, Sobue T. Epidemiology of breast cancer in Japan and the US. JMAJ. 2009;52(1):39-44.

10. Scowcroft H. Why are breast cancer rates increasing?. Cancer Research UK; 2011 [cited 2020 May 14.]. Available from: https://scienceblog.cancerresearchuk.org/2011/02/04/why-are-breast-cancer-rates-increasing/.

11. The Division of Society and Health. Research-based evaluation of the Norwegian Breast Cancer Screening Program. The Research Council of Norway. Lysaker; 2015.

12. Skaar KM. Overvurderer evne til å få barn i høy alder. Forskning.no: Kjønnsforskning.no; 2014 [cited 2020 May 14.].

13. Matthews TJ, Hammilton BE. Mean Age Mothers is on the Rise: United States, 2000-2014. NCHS data brief. 2016(232).

14. Holford TR, Cronin KA, Mariotto AB, Feuer EJ. Changing Patterns in Breast Cancer Incidence Trends. Journal of the National Cancer Institute Monograms. 2006(36).

15. Colaborative Group on Hormonal Factors in Breast Cancer. Menarche, menopause, and breast cancer risk - individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. Lancet Oncol. 2012;13(11):1141-51.

16. Biro FM, Greenspan LC, Galvez MP. Onset of breast development in a longitudinal cohort. Pediatrics. 2013;132(6):1019-27.
17. Teilmann G, Pedersen CB, Skakkebaek NE, Jensen TK. Increased risk of precocious puberty in internationally adopted children in Denmark. Pediatrics. 2006;118(2):391-9.
18. Aksglaede L, Juul A, Olsen LW, Sørensen TI. Age at puberty and the emerging obesity epidemic. PLoS ONE. 2009;4(12):e8450.
19. Viale G. The current state of breast cancer classification. Annals of Oncology. 2012;23(10):107-210.
20. Dai X, Li T, Bai Z, Yang Y, Liu X, Zhan J, et al. Breast cancer instrinsic subtype classification, clinical use and future trends. Am J Cancer Res. 2015;5(10):2929-43.
21. Malhotra GK, Zhao X, Band H, Band V. Histological, molecular and functional subtypes of breast cancer. Cancer Biol Ther. 2010;10(10):955-60.
22. Makki J. Diversity of Breast Carcinoma: Histological Subtypes and Clinical Relevance. Clinical Medicine Insights: Pathology. 2015;8:23-31.
23. Rakha EA, Reis-Filho JS, Baehner F. Breast cancer prognostic classification in the molecular era: the role of histological grade. Breast cancer research. 2010;12:207.
24. Pathology JH. Overview of histologic grade: Nottingham Histologic Score (”Elston Grade”): Johns Hopkins Medicine; [cited 2020 May 14.]. Available from: https://pathology.jhu.edu/breast/staging-grade/.
25. Bøhler PJ. Histology of Breast Cancer: Oncolex; 2014 [cited 2020 May 14]. Available from: http://oncolex.org/Breast-cancer/Background/Histology.
26. Tse G, Tan PH, Schmitt F. Basic Histopathology of Breast Lesions. Fine Needle Aspiration Cytology of the Breast. Berlin: Springer; 2013.
27. Cell SignalingTechnology. What Is Immunohistochemistry (IHC) Staining? : Cell Signaling Technologies; [cited 2020 May 15.]. Available from: https://www.cellsignal.com/contents/research/what-is-immunohistochemistry-(ihc)-staining/what-is-ihc-staining.
28. ThermoFisher Scientific. Overview of Immunohistochemistry (IHC): ThermoFisher Scientific; [cited 2020 May 15.]. Available from: https://www.thermofisher.com/no/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-immunohistochemistry.html.
29. Dai X, Xiang L, Li T, Bai Z. Cancer Hallmarks, Biomarkers and Breast Cancer Molecular Subtypes. Journal of Cancer. 2016;7(10):1281-94.
30. The American Cancer Society. Breast Cancer Hormone Receptor Status: American Cancer Society; 2019 [cited 2020 august 10.].
31. Bulut N, Altundag K. Does estrogen receptor determination affect prognosis in early stage breast cancers? Int J Clin Exp Med. 2015;8(11):21454-9.

32. Cserni G. The current TNM classification of breast carcinomas: Controversial issues in early breast cancer. Memo. 2011;4:144-8.
33. Cancer Research UK. TNM-staging: Cancer Reaserch UK; [cited 2020 May 14.]. Available from: https://www.cancerresearchuk.org/about-cancer/breast-cancer/stages-types-grades/tnm-staging.
34. Perou C, Sorlie T, Eisen M, Van der Rijn M, Jeffrey S, Rees C. Molecular portraits of human breast tumours. Nature. 2000;406:747-52.
35. Sorlie T, Perou C, Tibshirani R, Aas T, Geisler S, Johnsen H. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implication. Proc Natl Acad Sci. 2001;98(19).
36. Kos Z, Dabbs D. Biomarkers assessment and molecular testing for prognostification in breast cance. Histopathology. 2016;68:70-85.
37. Guler N. Gene Expression Profiling in Breast Cancer and Its Effect on Therapy Selection in Early-Stage Breast Cancer. Eur J Breast Health. 2017;13:168-74.
38. List M, Hauschild A, Tan Q, Kruse TA, Mollenhauer J, Baumbach J, et al. Classificatin of Breast Cancer Subtypes by combining Gene Expression and DNA Methylation Data. Journal of investigative Bioinformatics. 2014;11(2):236.
39. Guiu S, Michiels F, André F, Cortes J, Denkert C, Di Leo A, et al. Molecular subclasses of breast cancer: how we define them? The IMPACT 2012 Working Group Statement. Annals of Oncology. 2012;23:2997-3006.
40. Kittaneh M, Montero A, Glück S. Molecular Profiling for Breast Cancer: A Comprehensive Review. Biomarkers Cancer. 2013;5:61-70.
41. Biganzoli L. Prognostic and Predictive Factors. In: Castiglione M, Piccart M, editors. Adjuvant Therapy for Breast cancer. 151. Boston: Springer; 2009.
42. Weigel MT, Dowsett M. Current and emerging biomarkers in breast cancer: prognosis and prediction. Endocr Relat Cancer. 2010;17:245-62.
43. Assi H, Khoury K, Dbouk H, Khalil L, Mouhieddine T, El-Saghir N. Epidemiology and prognosis of breast cancer in young women. J Thorac Dis. 2013;5(1):2-8.
44. Kataoka A, Iwamoto T, Tokunaga E, Tomotaki A, Kumamaru H, Miyata H, et al. Young adult breast cancer patients have a poor prognosis independent of prognostic clinicopathological factors - a study from the Japanese Breast Cancer Registry. Breast Cancer Res Treat. 2016;160:163-72.
45. Nicolini A, Ferrari P, Duffy MJ. Prognostic and Predictive Biomarkers in Breast Cancer: Past, Present and Future. Semin Cancer Biol. 2018;52(1):56-73.
46. Stickeler E. Prognostic and Predictive Markers for Treatment Decisions in Early Breast Cancer. Breast Care (Basel). 2011;6:193-8.
47. Taneja P, Maglic D, Kai F, Zhu S, Kendig RD, Fry EA, et al. Classical and Novel Prognostic Markers for Breast Cancer and their Clinical Significance. Clin Med Insights Oncol. 2010;4:14-34.

48. Oncolex. Adjuvant behandling ved brystkreft Oncolex: Oncolex; [updated 2014; cited 2020. Available from: http://oncolex.no/Bryst/Prosedyrekatalog/BEHANDLING/Medikamentell-behandling/Adjuvant-behandling-bryst?lg=procedure.
49. Statistics Norway. 68 000 per innbygger til helse: Statistics Norway 2019 [cited 2020 May 15.]. Available from: https://www.ssb.no/nasjonalregnskap-og-konjunkturer/artikler-og-publikasjoner/68-000-per-innbygger-til-helse.
50. Statistics Norway. 65 000 per innbygger til helse: Statistics Norway 2018 [cited 2020 May 15.]. Available from: https://www.ssb.no/nasjonalregnskap-og-konjunkturer/artikler-og-publikasjoner/65-000-per-innbygger-til-helse.
51. Bordvik M. Kreft koster 40 milliarder årlig Dagens Medisin2016 [cited 2020 May 15.]. Available from: https://www.dagensmedisin.no/artikler/2016/10/11/kreft-koster-40-milliarder/.
52. Bordvik M. Dette er de dyreste kreftformene: Dagens Medisin; 2016 [cited 2020 May 15.]. Available from: https://www.dagensmedisin.no/artikler/2016/10/11/dette-er-de-dyreste-kreftformene/.
53. OECD/EU. Health at a Glance: Europe 2018: State of Health in the EU Cycle. Paris; 2018.
54. Ryan S. The Cost of Breast Cancer in the U.S.: Costs of Care; 2015 [cited 2020 May 15.]. Available from: https://costsofcare.org/the-costs-of-breast-cancer-in-the-u-s/.
55. Department of Health and Social Care. 2010 to 2015 government policy: cancer research and treatment. In: Department of Health and Social Care, editor. www.gov.uk: Government of the United Kingdom; 2015.
56. Vieira AF, Schmitt F. An Update on Breast Cancer Multigene Prognostic Tests-Emergent Clinical Biomarkers. Front Med (Lausanne). 2018;5:248.
57. Cheang M, Van der Rijn M, Nielsen T. Gene Expression Profiling of Breast Cancer. Anu Rev Pathol Mech dis. 2008;3:67-97.
58. Bao T, Davidson N. Gene Expression Profiling of Breast Cancer. Advances in Surgery. 2008;42:249-60.
59. Gyorffy B, Hatzis C, Sanft T, Hofstatter E, Aktas B, Pusztai L. Multigene prognostic tests in breast cancer: past, present, future. Breast Cancer Research. 2015;17:11-7.
60. Mittempergher L, Delahaye LJMJ, Witteveen A, Spangler JB, Hassenmahomed F, Mee S, et al. MammaPrint and BluePrint Molecular Diagnostics Using Targeted RNA Next-Generation Sequencing Technology. Journal of Molecular Diagnostics. 2019;21(5):808-23.
61. Reis-Filho J, Pusztai L. Gene expression profiling in breast cancer: classification, prognostication, and prediction. Lancet. 2011;378:1812-23.
62. Breastcancer.org. Oncotype DX Test Breastcancer.org: Breastcancer.org; [updated July 2020; cited 2020 August 2020]. Available from: https://www.breastcancer.org/symptoms/testing/types/oncotype_dx.

63. OncotypeIQ. About the Oncotype DX Breast Recurrence Score® Test OncotypeIQ: OncotypeIQ; [cited 2020 August 2020]. Available from: https://www.oncotypeiq.com/en-US/breast-cancer/healthcare-professionals/oncotype-dx-breast-recurrence-score/about-the-test.
64. McVeigh TP, Kerin MJ. Clinical use of the Oncotype DX genomic test to guide treatment decisions for patients with invasive breast cancer. Breast Cancer - Target and Therapy. 2017;9:393-400.

65. Breastcancer.org. Prosigna Breast Cancer Prognostic Gene Signature Assay Breastcancer.org: Breastcancer.org; [updated July 1, 2020; cited 2020 august 2020]. Available from: https://www.breastcancer.org/symptoms/testing/types/prosigna.
66. NanoString. Overview NanoString: NanoString; [cited 2020 August 2020]. Available from: https://www.nanostring.com/diagnostics/prosigna-uk/overview.
67. Wallden B, Storhoff J, Nielsen T, Dowidar N, Schaper C, Ferree S, et al. Development and verification of the PAM50-based Prosigna breast cancer gene signature assay. BMC Med Genomics. 2015;8:54.
68. Müller BM, Keil E, Lehmann A, Winzer KJ, Richter-Ehrenstein C, Prinzler J, et al. The EndoPredict Gene-Expression Assay in Clinical Practice - Performance and Impact on Clinical Decisions. PLoS ONE. 2013;8(6).

69. Myriad Genetics INC. EndoPredict Executive Summary Myriad Genetics INC: Myriad Genetics INC; [cited 2020 aug 24.]. Available from: https://myriad.com/managed-care/endopredict/.
70. Breastcancer.org. EndoPredict Test Breastcancer.org: Breastcancer.org; [updated July 1, 2020. Available from: https://www.breastcancer.org/symptoms/testing/types/endopredict-test.
71. Goldet G, Howick J. Understanding GRADE - an inroduction. Journal of Evidence-Based Medicine. 2013:50-4.
72. Turashvili G, Chou JF, Brogi E, Morrow M, Dickler M, Norton L, et al. 21-Gene recurrence score and locoregional recurrence in lymph node-negative, estrogen receptor-positive breast cancer. Breast Cancer Res Treat. 2017;166(1):69-76.
73. Kizy S, Huang JL, Marmor S, Tuttle TM, Hui JYC. Impact of the 21-gene recurrence score on outcome in patients with invasive lobular carcinoma of the breast. Breast Cancer Res Treat. 2017;165(3):757-63.
74. Wang M, Wu K, Zhang P, Zhang M, Ding A, Chen H. The Prognostic Significance of the Oncotype DX Recurrence Score in T1-2 N1 M0 Estrogen Receptor-Positive HER2-Negative Breast Cancer Based on the Prognostic Stage in the Updated AJCC 8th Edition. Ann Surg Oncol. 2019;26(5):1227-35.
75. Nitz U, Gluz O, Christgen M, Kates RE, Clemens M, Malter W, et al. Reducing chemotherapy use in clinically high-risk, genomically low-risk pN0 and pN1 early breast cancer patients: five-year

data from the prospective, randomised phase 3 West German Study Group (WSG) PlanB trial. Breast Cancer Res Treat. 2017;165(3):573-83.
76. Jahn B, Rochau U, Kurzthaler C, Hubalek M, Miksad R, Sroczynski G, et al. Personalized treatment of women with early breast cancer: a risk-group specific cost-effectiveness analysis of adjuvant chemotherapy accounting for companion prognostic tests OncotypeDX and Adjuvant!Online. BMC Cancer. 2017;17(1):685.
77. Paulden M, Fanek J, Pham B, Bedard P, Trudeau M, Khran M. Cost-effectiveness of the 21-gene assay for guiding adjuvant chemotherapy decisions in early breast cancer. Value in Health. 2013;16(5):729-39.
78. Blok EJ, Bastiaannet E, van den Hout WB, Liefers GJ, Smit VTHBM, Kroep JR, et al. Systematic review of the clinical and economic value of gene expression profiles for invasive early breast cancer available in Europe. Cancer Treat Rev. 2018;62:74-90.

79. Lang A, Edwards N, A. D. Empty systematic reviews: hidden perils and lessons learned. Journal of Clinical Epidemiology. 2007;60:595-7.
80. Green S, Higgins JPT, Schünemann HJ, Becker L. Response to paper by Lang A, Edwards N, and Fleiszer A. Journal of Clinical Epidemiology. 2007;60:598-9.
81. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ. Strategies for subtypes—dealing with the diversity of breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Annals of Oncology. 2011;22:1736-47.

7 Figures and tables

Figure 1: The incidence of breast cancer in Norway by age, from 1980 to 2018 (4)

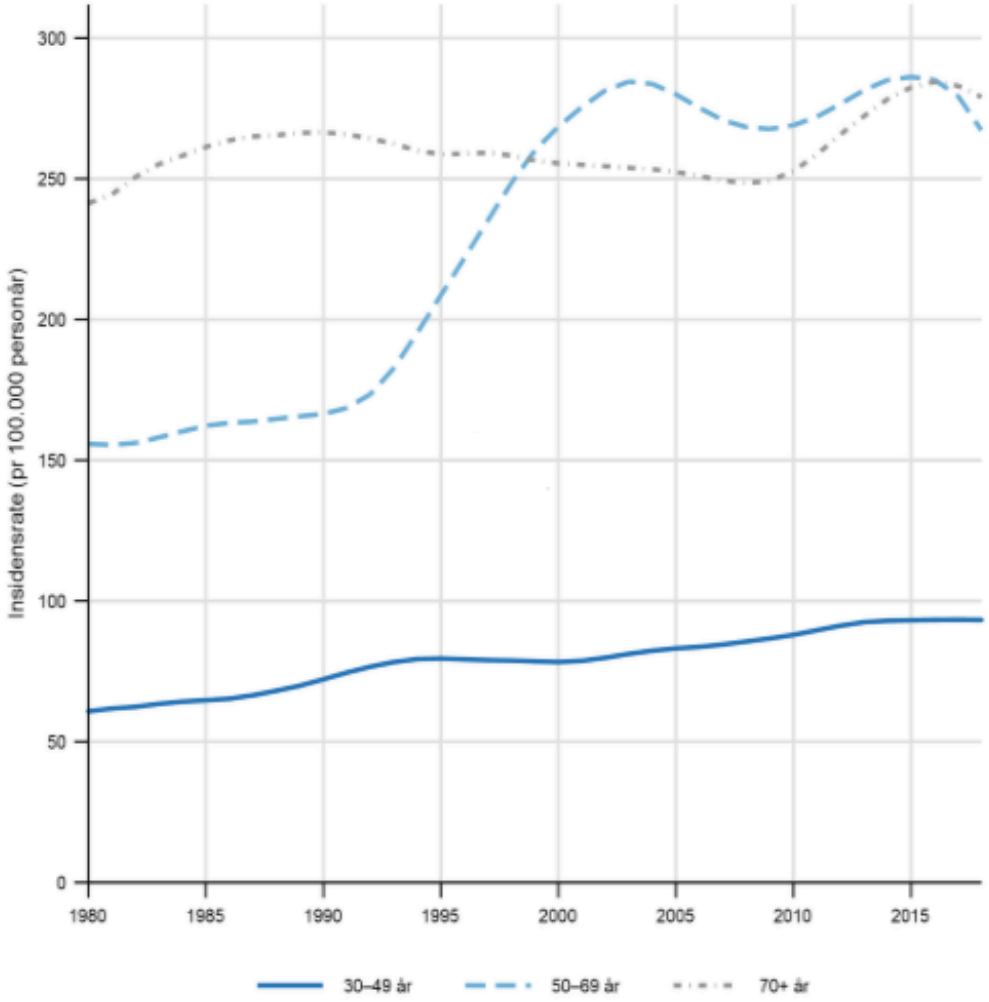

Figure 2: Relative survival of breast cancer in Norway, from 2014 to 2018 (4)

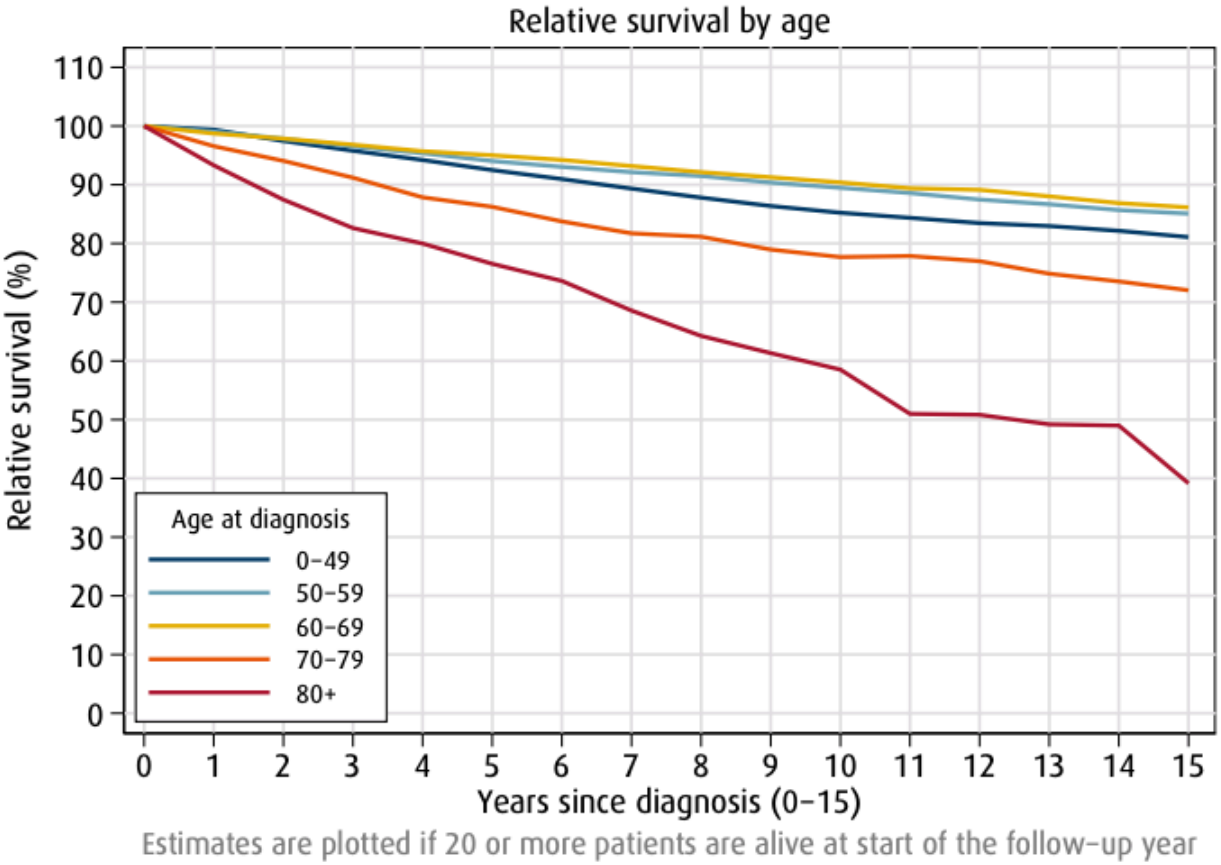

Figure 3: Search terms used in Ovid MEDLINE and EMBASE

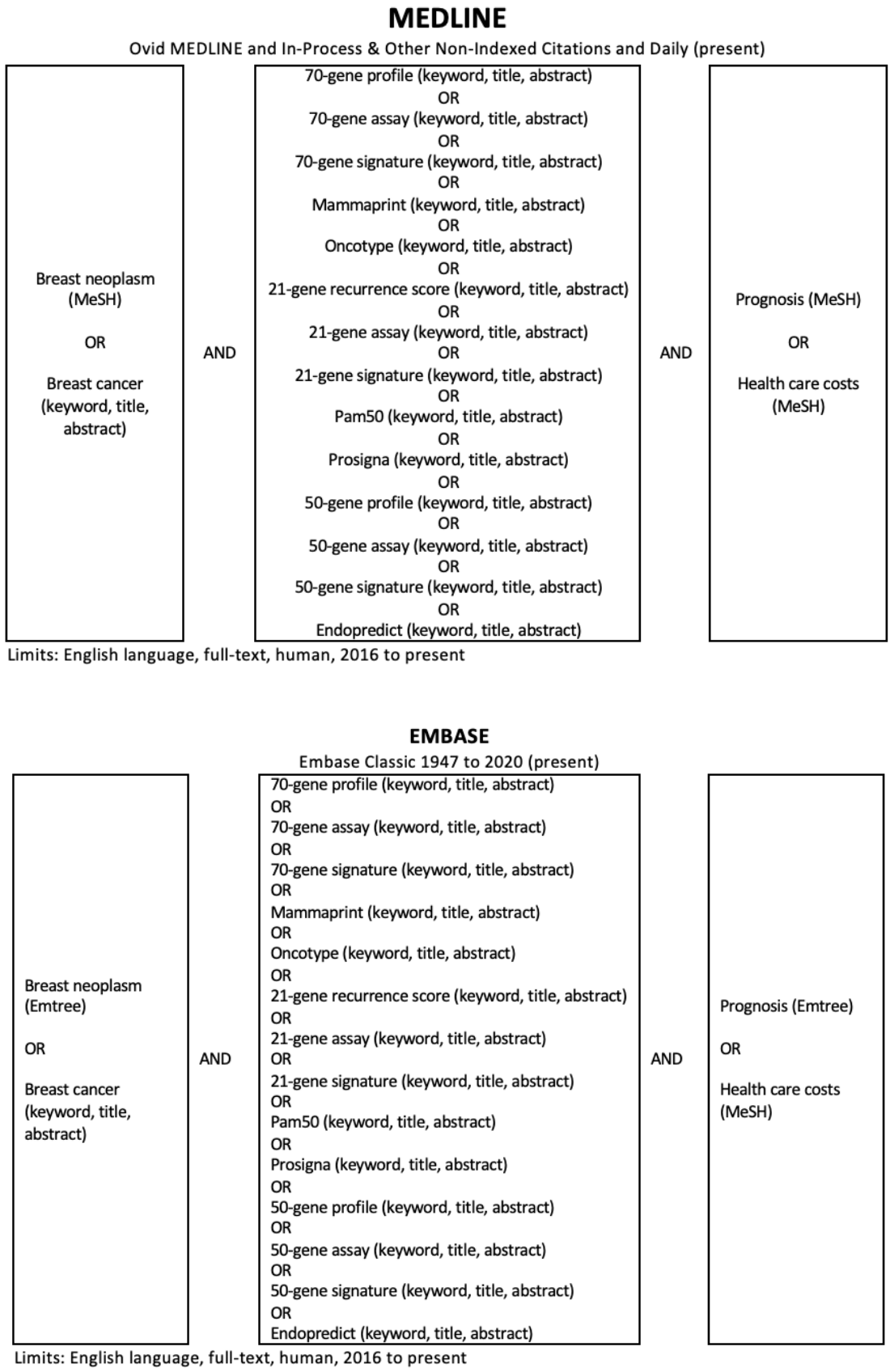

Figure 4: Modified PRISMA flow diagram showing the process of the literature search.

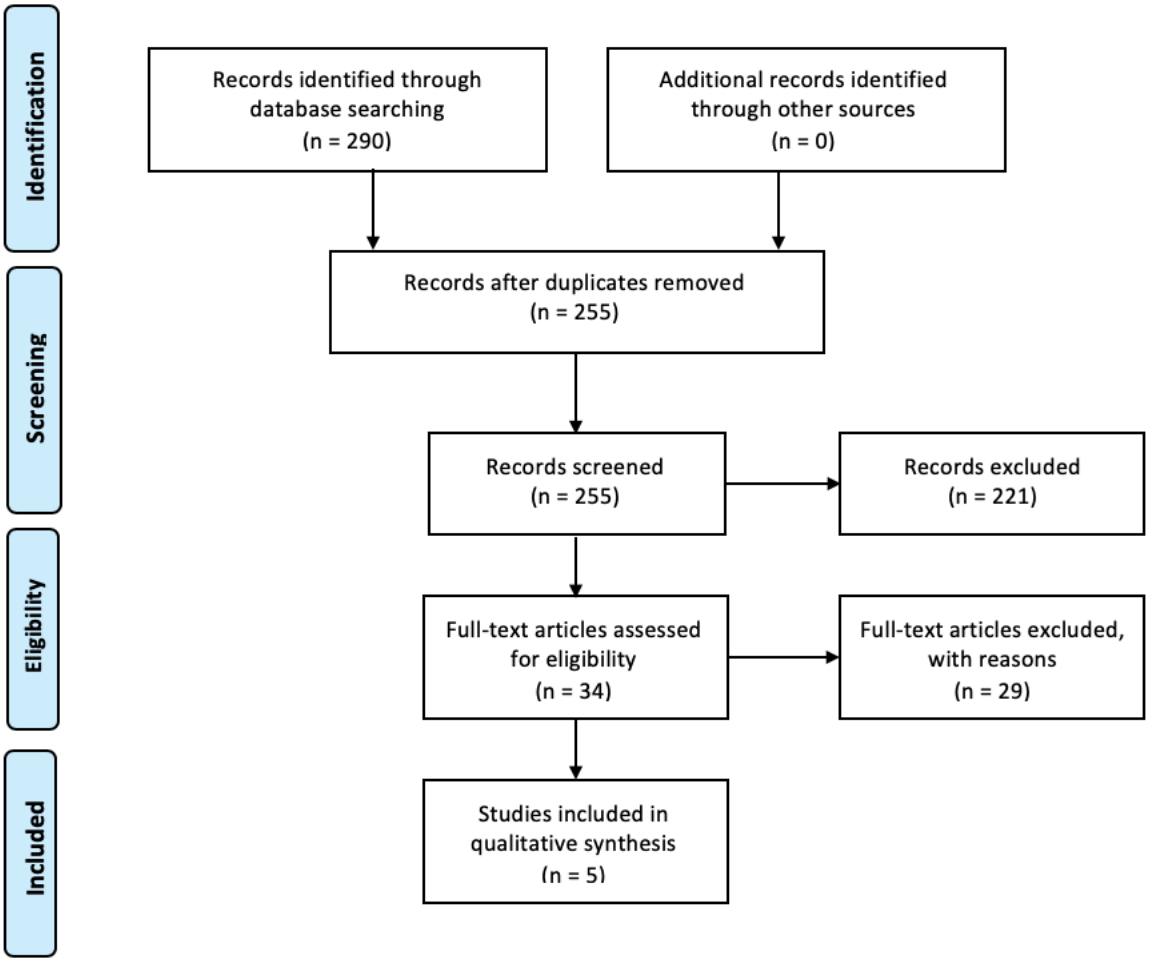

Table 1: Included studies from the literature search

First author	Journal, year	Article name	Study design	Study aims	Population	Results	Conclusion	Molecular test
Turashvili **(72)**	Breast Cancer Res Treat, 2017	21-Gene recurrence score and locoregional recurrence in lymph node-negative, estrogen receptor-positive breast cancer	Retrospective cohort study	To evluate the value of the 21-gene RS assay for predicting the risk of LRR in a cohort of lymph node-negative, ER+/HER2- breast cancer patients treated at a single institution.	2326 women with node-negative, ER-positive, HER2-negative breast cancer, treated from 2008 to 2013.	Univariate analysis showed that the risk of LRR was associated with the RS categories (p<0.01), and RS remained significantly associated with LRR after adjusting for LVI and T stage. Compared to patients with low RS, the risk of LRR was increased more than 4-fold (hazard ratio: 4.61, 95% CI 1.90–11.19, p<0.01), and 3-fold (hazard ratio: 2.81, 95% CI 1.41–5.56, p<0.01) for high and intermediate risk categories, respectively.	The study confirms that RS is significantly associated with the risk of LRR in node-negative, ER+/ HER2- breast cancer patients.	Oncotype DX
Kizy **(73)**	Breast Cancer Res Treat, 2017	Impact of the 21-gene recurrence score on outcome in patients with invasive lobular carcinoma of the breast	Retrospective cohort study	To determine the ability of the RS to predict survival in ILC patients and to predict the benefit of adjuvant chemotherapy in those with high- risk disease.	7316 women (18–74 years old) diagnosed with ER-positive ILC (stage I to III and grade I to III) and who had RS data available.	The 5-year BCSS was 99% in the low-risk, 99% in the intermediate-risk, and 96% in the high-risk groups. A high-risk RS was independently associated with increased mortality (hazard ratio: 2.37, 95% CI 1.14–4.95) when compared to a low-risk RS. In both the high-risk and intermediate-risk groups, adjuvant chemotherapy was not significantly associated with the hazard ratio (high-risk, hazard ratio 1.14, 95% CI 0.55–2.38; interme- diate-risk, hazard ratio 1.08, 95% CI 0.62–1.87).	This study shows that RS is significantly assosiated with survival outcomes in ILC. In the high-risk group, the RS predicted a lower 5-year BCSS. Adjuvant chemotherapy did not seem to confer a survival benefit for either the intermediate- or the high-risk cohorts.	Oncotype DX
Wang **(74)**	Ann Surg Oncol, 2019	The Prognostic Significance of the Oncotype DX Recurrence Score in T1-2N1M0 Estrogen Receptor-Positive HER2-Negative Breast Cancer Based on the Prognostic Stage in the Updated AJCC 8th Edition	Retrospective cohort study	To evaluate the prognostic significance of Oncotype DX in a subgroup of patients stratified into the T1-2N1M0 ER-positive, HER2-negative category based on the pathological prognostic stage in the updated AJCC 8th edition using the Surveil- lance, Epidemiology, and End Results (SEER) 18 database.	4059 female with ER-positive invasive ductal carcinoma cases in T1-2N1M0 stage with Oncotype RS results diagnosed between 2004 and 2012.	The RS risk groups differed significantly in terms of BCSS and OS (P < 0.001). Compared to high RS, the chance of BCSS was increased more than 13-fold (p<0.001 hazard ratio: 13.037, 95% CI 3.846-44.196) and chance of OS more than 3-fold (p<0.001, hazard ratio 3.825, 95% CI 2.2-6.65). Compared with intermediate RS chance of BCSS also increased (p=0.036, hazard ratio 3.516, 95% CI 1.082-11.423) as well as for OS (p=0.044, hazard ratio 1.680, 95% CI 1.015-2.779).	The findings indicate that RS results provide independent prognostic significance to complement the prognostic staging system.	Oncotype DX
Nitz **(75)**	Breast Cancer Res Treat, 2017	Reducing chemotherapy use in clinically high-risk, genomically low-risk pN0 and pN1 early breast cancer patients: five-year data from the prospective, randomised phase 3 West German Study Group (WSG) PlanB trial	Prospective cohort study	To evaluate the prognostic value of RS, Ki-67 and other traditional clinicopathological parameters	3198 female patients, 18–75 years, with histologically confirmed, unilateral primary invasive BC, adequate surgical treatment, without evidence of metastasis.	Five-year DFS in ET-treated RS<11 patients was 94% (in both pN0 and pN1) versus 94% (RS 12–25) and 84% (RS>25) in chemotherapy-treated patients (p \ 0.001). Consistent with DFS, among all locally HR+ patients with available RS, better OS was observed in RS < 11 or RS 12–25 patients than in RS > 25 (p<0.001 for both comparisons). Hazard ratios of 6.46 [2.27–18.42] for RS>25 versus RS<11 and 3.26 [1.87–5.70] for RS>25 versus RS 12–25.	The findings support using RS with standardised pathology for treatment decisions in HR+/HER2- EBC.	Oncotype DX
Jahn **(76)**	BMC Cancer, 2017	Personalized treatment of women with early breast cancer: a risk-group specific cost-effectiveness analysis of adjuvant chemotherapy accounting for companion prognostic tests OncotypeDX and Adjuvant!Online	Modeling study, hypothetical cohort study	To evaluate risk- group specific cost effectiveness of adjuvant chemother-apy for Austrian women with resected ER and/or PR positive, HER-2/neu negative, and lymph node negative early breast cancer.	Hypothetical cohort of 100000 50-year-old women with breast cancer over a lifetime horizon. ER- and/or PR-positive, HER2-negative and lymph node-negative.	Chemotherapy is dominated for patients with 1) low ODX risk independent of AO classification; and 2) low AO risk and intermediate ODX risk. For patients with an intermediate or high AO risk and an intermediate or high ODX risk, the ICER is below 15,000 EUR/QALY.	The findings shows that in the Austrian setting, chemotherapy is usually effective and potentially cost effective for patients classified as intermediate or high risk according to Oncotype DX, independent from their Adjuvant!Online risk classification. Further, the alaysis suggests that risk-group specific cost-effectiveness analyses that include the costs of companion diagnostics, including prognostic tests, are important in PM.	Oncotype DX

8 GRADE tables

Reference: Nitz U, Gluz O, Christgen M, Kates RE, Clemens M, Malter W, et al. Reducing chemotherapy use in clinically high-risk, genomically low-risk pN0 and pN1 early breast cancer patients: five-year data from the prospective, randomised phase 3 West German Study Group (WSG) PlanB trial. Brest Cancer Res Treat. 2017;165:573-583			**Design: Prospective cohort study** Quality of evidence: Moderate Recommendation: None
Aim	**Material and methods**	**Results**	**Discussion/comments**
To evaluate the prognostic value of RS, Ki-67 and other traditional clinicopathological parameters **Conclusion** The excellent five-year outcomes in clinically high-risk, genomically low-risk (RS < 11) pN0-1 patients without adjuvant chemotherapy support using RS with standardised pathology for treatment decisions in HR+ HER2-negative EBC. Ki-67 has the potential to support patient selection for genomic testing. **Country** Germany **Year of data collection** 2009 to 2011	**Data material:** The trial included 3198 female patients, 18–75 years, with histologically confirmed, unilateral primary invasive BC, adequate surgical treatment (free margins, sentinel-node biopsy in node-negative, or axillary dissection in node- positive patients), without evidence of metastasis. **Inclusion criteria:** HER2-negativity; pT1-T4c; pN+ [or pN0 with a risk factor (CpT2, grade 2/3, high uPA/PAI-1, <35 years, or HR-negative)]; ECOG performance status <2 or Karnofsky Index >= 80%; signed informed consent; and (if >=4 positive LN, RS > 11, or HR-negative) willingness to participate in the adjuvant CT PlanB trial. **Exclusion criteria:** Male gender, failure of the assay for any technical reasons and patients receiving neoadjuvant therapy. **Endpoints** The endpoints included prospective evaluation of RS prognostic impact at follow-up target of five years: Clinical outcomes (disease-free survival [DFS], overall survival [OS]) in RS < 11 patients treated with ET alone, and prospective evaluation of the prognostic value of other parameters (Ki-67, IHC4 and histological grade [Elston- Ellis] by local/central assessment) **Statistical methods** For DFS analysis, an event was defined as any invasive cancer event or death (with/without recurrence). Estimates of five-year DFS or OS with approximate 95% confidence intervals [given in brackets] were obtained by the Kaplan– Meier method. Comparisons of DFS or OS among sub- groups used pairwise log-rank tests (reported as significant for $p < 0.05$). Subgroup analyses were performed in RS < 11, RS 12–25, RS>25, and in Ki-67 subgroups. Univariate and multivariate (forward elimination) Cox proportional hazard models for DFS were estimated; RS, Ki-67, ER, PR, and IHC4 were coded as continuous variables using fractional ranks. For a realistic measure of effect sizes, hazard ratios of fractionally ranked variables are reported for 75th versus 25th percentile.	From 2009 to 2011, PlanB enrolled 3198 patients (central tumour bank, n = 3073) with the median age of 56 years, 41.1% pN+, and 32.5% grade 3 EBC. Chemotherapy was omitted in 348/404 (86.1%) eligible RS =< 11 patients. After 55 months of median follow-up, five-year DFS in ET-treated RS =< 11 patients was 94% (in both pN0 and pN1) versus 94% (RS 12–25) and 84% (RS > 25) in chemotherapy-treated patients ($p < 0.001$); five-year overall survival (OS) was 99 versus 97% and 93%, respectively ($p < 0.001$). Nodal status, central/local grade, tumour size, continuous Ki-67, progesterone receptor (PR), IHC4, and RS were univariate prognostic factors for DFS. In a multivariate analysis including all univariate prognostic markers, only pN2-3, central and local grade 3, tumour size > 2 cm, and RS, but not IHC4 or Ki-67 were independent adverse factors. If RS was excluded, IHC4 or both Ki-67 and PR entered the model. The impact of RS was particularly pronounced in patients with intermediate Ki-67 (>10%, < 40%) tumours.	• Is the aim of the study clearly formulated? **Yes** • Were the groups recruited from the same population? **Yes** • Were the groups comparable with respect to underlying factors? **Yes** • Were the exposed individuals representative for a defined population? **Yes** • Were exposures and outcomes measured equally and in a reliable manner in all groups? **Yes** • Were those who evaluated outcomes blinded? **Not mentioned in the study** • Was the study prospective? **Yes** • Were a sufficient amount of participants followed-up? **Yes** • Was the follow-up period sufficient to measure significant results? **Yes** • Were confounding factors adjusted for? **Not mentioned in the study** • Can these results be transferred to the general population? **To some degree, as the participants were recruited from 93 different centers.** • Are these results supported by prior literature? **Yes, the findings are in agreement with results from other studies.** • Do these results have any clinical implication? **Yes, the results indicate that RS may have prognostic and predictive value.** • **Limitations:** - Clinical consequences for CT omission can only be drawn for the relatively small group of RS < 11, pN0-1 patients.

Reference: Wang M, Wu K, Zhang P, Zhang M, Ding A, Chen H. The Prognostic Significance of the Oncotype DX Recurrence Score in T1-2N1M0 Estrogen Receptor-Positive HER2-Negative Breast Cancer Based on the Prognostic Stage in the Updated AJCC 8th Edition. Ann Surg Oncol. 2019;26:1227-35			**Design: Retrospective cohort study** Quality of evidence: Moderate Recommendation: None
Aim	**Material and methods**	**Results**	**Discussion/comments**
We conducted a retrospective study to evaluate the prognostic significance of Oncotype DX in this subgroup of patients stratified into the T1-2N1M0 ER-positive, HER2- negative category based on the pathological prognostic stage in the updated AJCC 8th edition using the Surveil- lance, Epidemiology, and End Results (SEER) 18 database. **Conclusion** Oncotype DX RS provided independent prognostic significance to complement the prognostic staging system. **Country** China, USA (used the SEERS database to collect data) **Year of data collection** 2004 to 2012	**Data material:** This population-based study used data derived from the National Cancer Institute's limited use SEER 18 registry databases released in November 2017. Cases in the SEER database were linked to RS results from assays performed by Genomic Health. The study enrolled 4059 patients who met the inclusion criteria. All cases with RS had negative HER2 per Oncotype DX test via reverse transcription polymerase chain reaction (RT-PCR). We identified female ER-positive invasive ductal carcinoma cases in T1-2N1M0 stage with Oncotype RS results diagnosed between 2004 and 2012. Patients with RS were categorized into low-risk (RS < 11), intermediate-risk (RS 11–25), and high-risk (RS > 25) groups. **Exclusions:** More than one primary cancer, diagnosis at death or autopsy alone, unknown his- tologic grade or PR status, no surgery performed or no record of surgery, or less than 6 months of follow-up evaluation. **Statistical methods** Both BCSS and OS were estimated using the Kaplan- Meier method and compared across RS groups using the log-rank statistic. Adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated using the Cox model to assess the factors independently associated with survival. A two-sided P value lower than 0.05 was con- sidered statistically significant.	The study enrolled 4059 cases categorized into prognostic stages IA to IIB. The RS risk groups were positively correlated with pathological prognostic stages ($P < 0.001$). The RS risk groups differed significantly in terms of BCSS and OS ($P < 0.001$). According to the multivariate analysis, RS risk group was an independent prognostic factor for BCSS and OS together with the pathological prognostic stage. The subgroup analysis showed similar survival rates across pathological prog- nostic stages in the RS low-risk group but significant differences in survival rates among pathological prognostic stages in the RS intermediate-risk group. The survival rates among the RS risk groups also differed significantly in pathological prognostic stage IA.	• Is the aim of the study clearly formulated? **Yes** • Were the groups recruited from the same population? **Yes** • Were the groups comparable with respect to underlying factors? **Yes** • Were the exposed individuals representative for a defined population? **Yes** • Were exposures and outcomes measured equally and in a reliable manner in all groups? **Yes** • Were those who evaluated outcomes blinded? **Yes, as the pathologists who diagnosed the cases were not directly involved in this study.** • Was the study prospective? **No, the study was retrospective** • Were a sufficient amount of participants followed-up? **Yes** • Was the follow-up period sufficient to measure significant results? **Yes** • Were confounding factors adjusted for? **Not mentioned in the study** • Can these results be transferred to the general population? **Yes, to some degree, as the study used the SEER registries, which provide population-based cancer surveillance for 18 geographic areas representing about 28% of the United States.** • Are these results supported by prior literature? **Yes, the findings are in agreement with results from other studies.** • Do these results have any clinical implication? **Yes, the results indicate that RS may have independent prognostic value** • **Limitations:** - The retrospective design had an intrinsic bias despite a large sample size. - The median follow-up period of 57 months might have been relatively too short for the differences in survival outcome to be determined between pathological prognostic stages IA and IB. - Chemotherapy use is known to be underreported in SEER. - The SEER database does not collect information on distant recurrence, although it is the main cause of breast cancer-specific death.

Reference: Turashvili G, Chou JF, Brogi E, Morrow M, Dickler M, Norton L, et al. 21-Gene recurrence score and locoregional recurrence in lymph node-negative, estrogen-receptor positive breast cancer. Breast Cancer Res Treat. 2017;166:69-76			**Design:** Retrospective cohort study Quality of evidence: Moderate Recommendation: None
Aim	**Material and methods**	**Results**	**Discussion/comments**
To evluate the value of the 21-gene RS assay for predicting the risk of LRR in a cohort of lymph node-negative, ER+/HER2- breast cancer patients treated at a single institution. **Conclusion** Our study confirms that RS is significantly associated with the risk of LRR in node-negative, ER?/HER2- breast cancer patients. Our findings suggest that in addition to its value for prognostic stage grouping and decision-making regarding adjuvant systemic therapy, the role of the RS in identifying patients not requiring radio- therapy should be studied. **Country** USA **Year of data collection** September 2008 to august 2013	**Data material:** 2326 consecutive female patients with lymph node-negative (pN0 and pN0[i+]) ER+/HER2- invasive breast carcinoma with known 21-gene RS assay results treated at the researcher medical center between September 2008 and August 2013. The institutional database and electronic medical records were reviewed to record date of last follow-up, date of death, date and type of LRR, and distant recurrence. Clinicopathologic variables included patient age at breast cancer diagnosis, tumor size, histologic type of tumor, LVI, 21-gene RS result, local and systemic treat- ment, and clinical outcome. **Exclusions:** Male gender, failure of the assay for any technical reasons and patients recieving neoadjuvant therapy. **Statistical methods** Multivariable competing risk regression was used to examine the independent effect of RS on LRR, adjusting for other factors that were significantly associated with LRR from the univariate analysis. Univariate associ- ation of RS score on LRR was also examined among the subset of women treated with endocrine therapy and chemotherapy.	Of 2326 patients, 60% (1394) were in the low RS group, 33.4% (777) in the intermediate RS group, and 6.6% (155) in the high RS group. Median follow-up was 53 months. A total of 44 LRRs were observed, with a cumulative incidence of 0.17% at 12 months and 1.6% at 48 months. The cumulative incidence of LRR at 48 months was 0.84%, 2.72% and 2.80% for low, intermediate, and high RS groups, respectively (p \ 0.01). Univariate analysis showed that the risk of LRR was associated with the RS categories (p<0.01), T stage (p<0.01) and lympho- vascular invasion (LVI) (p = 0.009). The RS remained significantly associated with LRR after adjusting for LVI and T stage. Compared to patients with low RS, the risk of LRR was increased more than 4-fold (hazard ratio: 4.61, 95% CI 1.90–11.19, p \ 0.01), and 3-fold (hazard ratio: 2.81, 95% CI 1.41–5.56, p \ 0.01) for high and intermediate risk categories, respectively.	• Is the aim of the study clearly formulated? **Yes** • Were the groups recruited from the same population? **Yes** • Were the groups comparable with respect to underlying factors? **Yes** • Were the exposed individuals representative for a defined population? **Yes** • Were exposures and outcomes measured equally and in a reliable manner in all groups? **Yes** • Were those who evaluated outcomes blinded? **Yes, as the pathologists who diagnosed the cases were not directly involved in this study.** • Was the study prospective? **No, the study was retrospective** • Were a sufficient amount of participants followed-up? **Yes** • Was the follow-up period sufficient to measure significant results? **Yes, in most cases.** • Were confounding factors adjusted for? **Yes, the results were adjusted for LVI and T-stage.** • Can these results be transferred to the general population? **No, not directly, as the population are predominantly screen-detected breast cancer and women from specific regions.** • Are these results supported by prior literature? **Yes, the findings are in agreement with results from other studies.** • Do these results have any clinical implication? **Yes, the results indicate that RS may have prognostic and predictive value.** • **Strengths:** Large, unselected consecutive population whose RS results were prospectively included in the treatment planning, and detailed knowledge of pathologic and treatment variables. • **Limitations:** Retrospective study design, low number of LRR events, follow-up time intervals of less than 5 years in some patients. In addition, the tertiary academic institution predominantly treat patients with screen-detected breast cancer and women from specific geographic regions.

Reference: Kizy S, Huang JL, Marmor S, Tuttle TM, Hui JYC. Impact of the 21-gene recurrence score on outcome in patients with invasive lobular carcinoma of the breast. Breast Cancer Res Treat. 2017;165:757-763			**Design: Retrospective cohort study** Quality of evidence: Moderate Recommendation: None
Aim	**Material and methods**	**Results**	**Discussion/comments**
In this study, our objective was to determine the ability of the RS to predict survival in ILC patients and to predict the benefit of adjuvant chemotherapy in those with high- risk disease. **Conclusion** For patients with ER-positive ILC, 8% were in the high-risk and 72% were in the intermediate-risk groups as per the TAILORx RS cutoffs. In the high-risk group, the RS predicted a lower 5-year BCSS. Adjuvant chemotherapy did not seem to confer a survival benefit for either the intermediate- or the high-risk cohorts. **Country** USA **Year of data collection** 2004 to 2013	**Data material:** To identify women diagnosed with breast cancer during our 10-year study period (2004 through 2013), we used an augmented version of the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) database. The study included 7316 women (18–74 years old) who were diagnosed with ER-positive ILC (stage I to III and grade I to III) and who had RS data available. **Exclusions:** Negative or unknown ER status, unknown PR status, unknown lymph node status, or stage IV disease. **Statistical methods** As per the traditional, as the well as TAILORx, RS cutoffs, we stratified patients into low-, intermediate-, and high-risk groups. To analyze both overall survival (OS) and breast cancer-specific survival (BCSS) rates, we used the Kaplan–Meier method; to analyze factors associated with hazard ratio (HR) of death, we used Cox proportional hazards models. Our Cox proportional hazards models included age at diagnosis, race, tumor size, tumor grade, PR status, lymph node status, use of adjuvant chemotherapy, and RS group. Results were considered statistically significant only for a p value < 0.05 and a 95% confidence interval (CI).	Of the 7316 women included, 21% were in the low-risk; 71%, intermediate-risk; and 8%, high-risk groups as per TAILORx RS cutoffs. The 5-year BCSS was 99% in the low-risk, 99% in the intermediate-risk, and 96% in the high-risk groups. A high-risk RS as per TAILORx cutoff was independently associated with increased mortality (hazard ratio [HR] of death 2.37, 95% confidence interval [CI] 1.14–4.95) when compared to a low-risk RS. In both the high-risk and intermediate-risk groups, adjuvant chemotherapy was not significantly associated with the HR of death (high-risk, HR 1.14, 95% CI 0.55–2.38; intermediate-risk, HR 1.08, 95% CI 0.62–1.87).	• Is the aim of the study clearly formulated? **Yes** • Were the groups recruited from the same population? **Yes** • Were the groups comparable with respect to underlying factors? **Yes** • Were the exposed individuals representative for a defined population? **Yes** • Were exposures and outcomes measured equally and in a reliable manner in all groups? **Yes** • Were those who evaluated outcomes blinded? **Yes, as the pathologists who diagnosed the cases were not directly involved in this study.** • Was the study prospective? **No, the study was retrospective** • Were a sufficient amount of participants followed-up? **Yes** • Was the follow-up period sufficient to measure significant results? **Yes** • Were confounding factors adjusted for? **Yes, the results were adjusted for patient and tumor characteristics** • Can these results be transferred to the general population? **Yes, to some degree, as the study used the SEER registries, which provide population-based cancer surveillance for 18 geographic areas representing about 28% of the United States.** • Are these results supported by prior literature? **Yes, the findings are in agreement with results from other studies.** • Do these results have any clinical implication? **Yes, the results indicate that RS may have prognostic and predictive value.** • **Limitations:** - The SEER database did not record HER2 status until 2010, preventing examination of that parameter in our survival analysis. - The degree of ER positivity, patient comorbidities, use and specific types of endocrine therapy, adjuvant chemotherapy regimens, and duration of systemic treatments is not recorded in the SEER database - Information on use of chemotherapy is occasionally sparse - The SEER database relies on community pathologic examination to diagnose distinct subtypes and does not perform central reviews. - Specific subtypes of ILC that may have affected survival, such as pleomorphic lobular carcinoma, are not reported in the SEER database. - Short follow-up period, and only 5-year survival rates were determined, which may not accurately reflect longer-term survival. - Because of the retrospective nature of this registry review, patients were not randomly assigned to treatment arms.

Reference: Jahn B, Rochau U, Kurtzhaler C, Hubalek M, Miksad R, Sroczynski G, et al. Personalized treatment of women with early breast cancer: a risk-group specific cost-effectiveness analysis of adjuvant chemotherapy accounting for companion prognostic tests OncotypeDX and Adjuvant!Online. BMC Cancer. 2017;17(1):685			**Design: Modeling study, hypothetical cohort study** Quality of evidence: Moderate Recommendation: None
Aim	**Material and methods**	**Results**	**Discussion/comments**
The goal of the current study was to evaluate risk- group specific cost effectiveness of adjuvant chemother- apy for Austrian women with resected ER and/or PR positive, HER-2/neu negative, and lymph node negative early breast cancer. All potential risk groups according to the joint application of AO and ODX are considered. **Conclusion** Our decision analysis shows that in the Austrian setting, chemotherapy is usually effective and potentially cost effective for patients classified as intermediate or high risk according to ODX, independent from their AO risk classification. **Country** Austria **Year of data collection** There were no data collection, the research group used a simulated model with a hypothetical cohort	**Data material:** A previously validated discrete event simulation model was applied to a hypothetical cohort of 100000 50-year-old women with ER- and/or PR-positive, HER2-negative and lymph node-negative breast cancer over a lifetime horizon. We simulated twelve risk groups derived from the joint application of ODX and AO and included respective additional costs. The primary outcomes of interest were life-years gained, quality-adjusted life-years (QALYs), costs and incremental cost-effectiveness (ICER). The robustness of results and decisions derived were tested in sensitivity analyses. A cross-country comparison of results was performed. Model validation is a key modeling step for judging a model's accuracy in making accurate predictions. Following the current ISPOR-SMDM best practice recommendations, the model was validated using face validation, internal validation and cross-model validation	The results for the Austrian setting indicate that chemo- therapy is dominated in the risk groups L-L (low AO, low ODX), L-I (low AO, intermediate ODX), I-L (intermediate AO, low ODX) and H-L (high AO, low ODX). Patients in these risk groups do not on average benefit from chemotherapy with respect to the clinical outcomes (LYs, QALYs). These results are consistent with the results for the Canadian setting with the exception of the L-I risk group (low AO and intermediate ODX). In high risk ODX patients, chemotherapy seems to clearly be cost effective because an additional QALY can be gained at a low additional cost (ICER less than 3500 EUR/QALY). Chemotherapy is also cost effective in patients with an inter- mediate ODX risk and an intermediate or high AO risk chemotherapy with a WTP threshold of 15,000 EUR/QALY. These results are also consistent with the results from the Canadian setting. For patients in our model that are tested only with AO, chemotherapy is mainly cost effective with the exception of those who are AO low risk (L-N). These results differ slightly to the Canadian setting where chemo- therapy for L-N patients is cost effective.	• Is the aim of the study clearly formulated? **Yes** • Were the groups recruited from the same population? **Yes** • Were the groups comparable with respect to underlying factors? **Yes** • Were the exposed individuals' representative for a defined population? **Yes** • Were exposures and outcomes measured equally and in a reliable manner in all groups? **Yes** • Was the study prospective? **No, this is a hypothetical cohort study / modelling study-based om computer simulations** • Were a sufficient amount of participants followed-up? **Yes** • Were confounding factors adjusted for? **Not mentioned in the study** • Can these results be transferred to the general population? **Yes, to some degree, as the study used The Oncotyrol breast cancer model, which is divided into different modules that describe the test-treatment strategies and the respective pathways of patients, their health states and key health events. They also used Drug costs were based on pharmacy hospital prices.** • Are these results supported by prior literature? **Yes, the findings are in agreement with results from other studies.** • Do these results have any clinical implication? **Yes, the results indicate that the use of Oncotype DX may be cost effective in intermediate and high-risk patients** • **Limitations:** - Due to a lack of information about utility parameters and estimates for the risk of distant recurrence, the group applied results from international studies. - The ability to compare these results with other study results is limited due to the different health care settings.