BREAST CANCER SCREENING IN WOMEN WITH HEREDITARY OR FAMILIAL RISK

S. Saadatmand

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Sepideh Saadatmand

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Breast Cancer Screening in Women with Hereditary or Familial Risk

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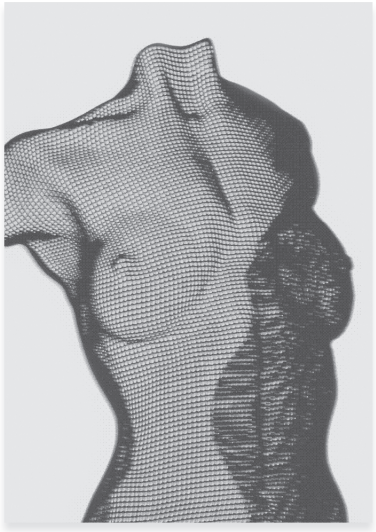
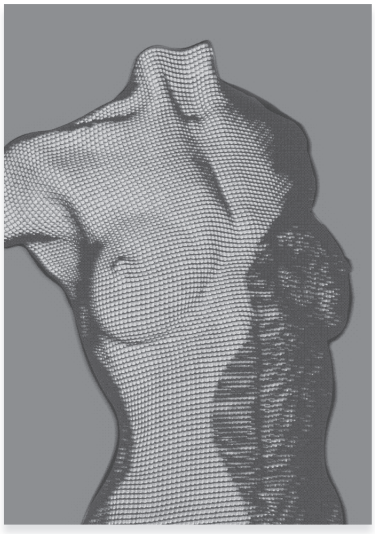
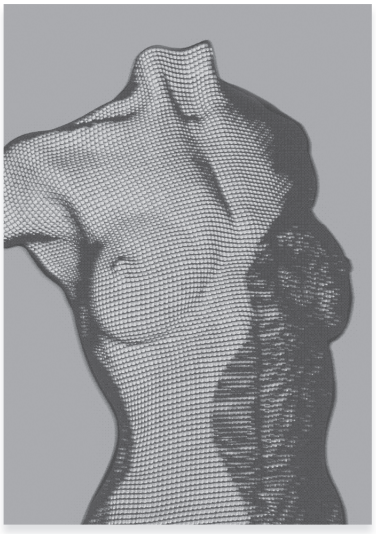
CONTENTS

Chapter 1

Introduction

INTRODUCTION

> "My mother was diagnosed with breast cancer when she was 38 years old. She was operated and received radiation therapy to eliminate the hormonal cycle. It was in the late 50's. At age 54 she developed ovarian cancer. After a long and very painful sickbed she passed away. I was 21 years old, a nurse student, and terrified that this could happen to me as well. However, no one talked about cancer in those days."[1]
>
> *From a BRCA1 mutation carrier.*

Incidence

Breast cancer is the most common female cancer, with almost 1.7 million new cases worldwide,[2] over 464,000 cases in Europe,[3] and approximately 234,000 cases in the United States (US).[4] In other words 1 out of 8 (13%) of the women in the US will

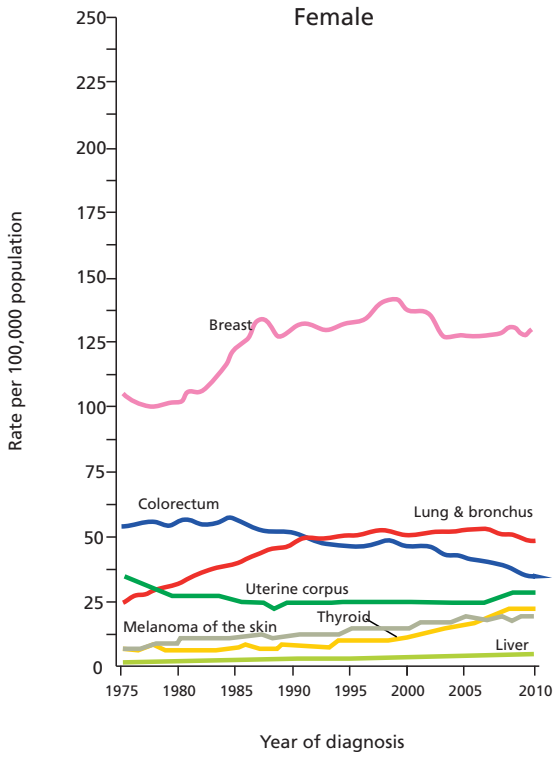


Figure 1 Trends in incidence rates for selected cancers in females, United States, 1975-2011. Rates are age adjusted to the 2000 US standard population and adjusted for delays in reporting. Source: Reproduced with permission from Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA: a cancer journal for clinicians 2015.© 2015 American Cancer Society.

be diagnosed with breast cancer.[4] Breast cancer in men accounts for approximately one per cent of all breast cancer cases.[4] Breast cancer incidence rates in the US have increased steeply from 1980 until 2003, after which they dropped by 7% from 2002 to 2003, most likely due to a reduced use of hormone replacement therapy (HRT).[5,6] Since 2003 breast cancer incidence rates have remained relatively flat with only a slight increase (**Figure 1**).[4] Female breast cancer incidence increases with age. **Figure 2** illustrates this for female citizens of the United Kingdom (UK); age-distribution in Western world is comparable.

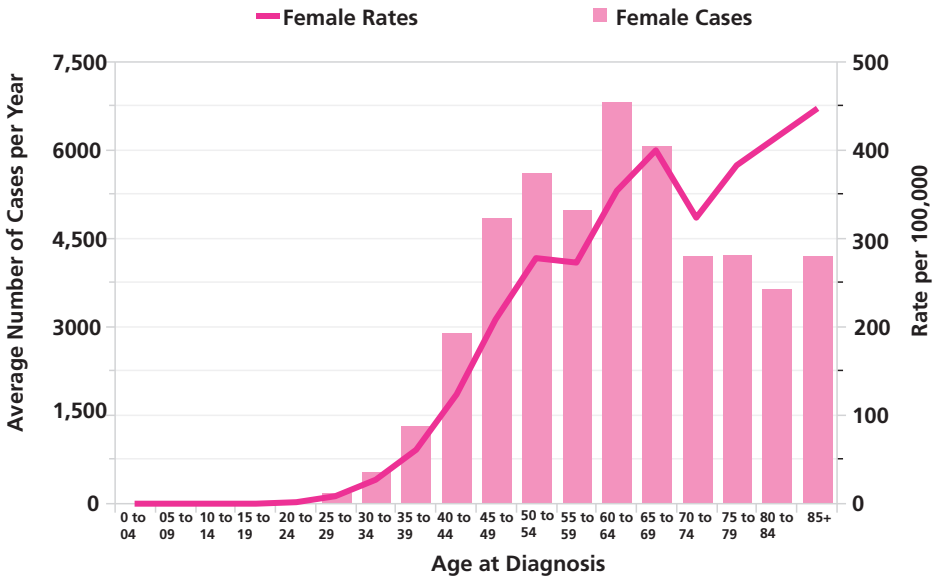


Figure 2 Average number of new female cases per year and age-specific incidence rates of the United Kingdom in the period of 2009-2011. Breast cancer incidence rates increase with age. Source: Reproduced with permission from Cancer Research UK, http://www.cancerresearchuk.org/cancer-info/cancerstats/types/breast/incidence/uk-breast-cancer-incidence-statistics, accessed January 2015.

Risk factors

Besides female gender and increasing age there are several other risk factors associated with breast cancer. One of the most important risk factors is a positive family history, with or without a mutation in one of the high susceptibility breast cancer genes.[7-10] Further risk factors include reproductive risk factors, for which a rule of thumb is the higher the number of uninterrupted menstrual cycles in a woman´s life, the higher the chance of developing breast cancer.[6,7] Examples of reproductive risk factors are lower parity or younger age at menarche.[6,7] Also chest radiotherapy at young age,[11] high body mass index (mainly in postmenopausal women),[12] and high breast density[13] increase breast cancer risk.

Breast density is a measure for the proportion of breast and connective tissue versus fatty tissue.[14] Breast density is high in about 50-74% of women aged between 40-49 years, whereas only 20-44% of women in their 60s have dense breast tissue (**Figure 3**).[15,16] High breast density increases breast cancer risk independent of other factors, but at the same time impairs sensitivity of mammography.[13] An explanation for increased breast cancer risk in high breast density might be that breast and connective tissue stem cells are more susceptible to cancer genesis because they undergo more stem cell divisions, which in turn could lead to DNA mutations.[17] The lower mammography sensitivity in dense breasts is most likely caused by a masking effect, rather than by a higher tumor growth rate.[13] Mammographic density is also an independent breast cancer risk factor in women with a familial risk.[18,19] For *BRCA1/2* mutation carriers results are contradictory.[20-22]

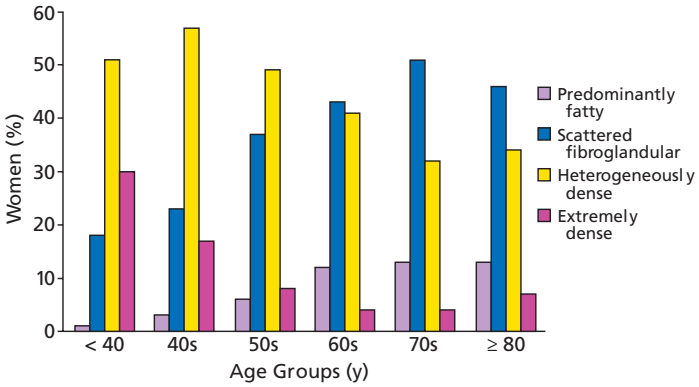


Figure 3 Bar graph showing patient age and breast density classification.
Source: Reproduced with permission from Checka CM, Chun JE, Schnabel FR, Lee J, Toth H. The relationship of mammographic density and age: implications for breast cancer screening. AJR American journal of roentgenology 2012;198:W292-5© 2013, American Roentgen Ray Society.

Familial risk

A strong breast and ovarian cancer family history or a family history of young ages at breast cancer diagnosis of affected family members increase breast cancer risk significantly.[23] Approximately 35% of all breast cancer cases have a positive family history. Less than 10% of all breast cancers are attributable to a mutation in the *BRCA1* or *BRCA2* gene.[24] The 25% without a known gene mutation are probably caused by a combination of multiple gene mutations with low penetrance for breast cancer and environmental factors (**Figure 4**).[23,25,26]

Women with familial breast cancer risk not only have a greater risk of developing breast cancer, their risk also increases at a younger age than in the general population.[27] The risk of developing breast cancer for women with a familial risk can be estimated with several models.[28,29] However, risk estimates for the same woman

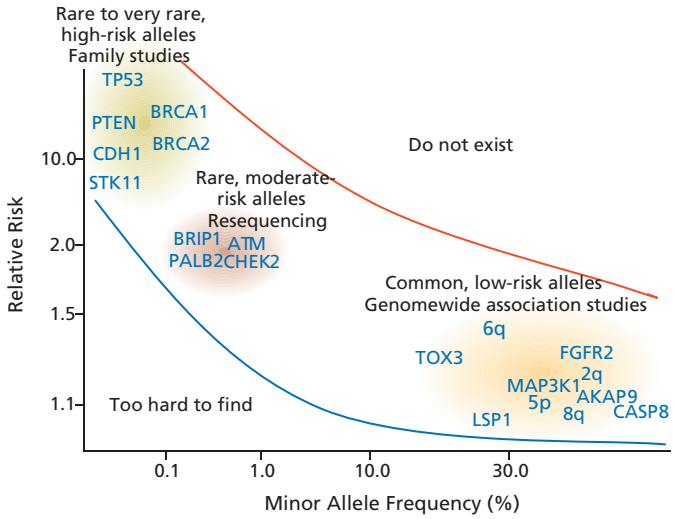


Figure 4 Germline mutations that confer breast cancer susceptibility.
Known breast cancer susceptibility genes are shown between the red and blue lines. No genes are believed to exist above the red line, and no genes have been identified below the blue line. High risk syndrome genes are highlighted in the green area. The moderate penetrance genes highlighted in the red area have an approximately relative risk of 2. In summary, there are a few relatively rare germline gene mutations that lead to high breast cancer risk, and multiple more frequent germline gene mutations that lead to moderate breast cancer risk. Source: Reproduced with permission from Foulkes WD. Inherited susceptibility to common cancers. The New England journal of medicine 2008;359:2143-53, © 2008, Massachusetts Medical Society.

vary greatly between the different models.[30,31] It is therefore difficult to estimate lifetime risk of a particular woman precisely, and even in large cohorts division of women with a familial risk into separate risk categories seems not justified.[28,31]

Except for younger age of onset breast cancers of women with a familial risk do not differ from sporadic ones regarding tumor morphology, grade, steroid receptor status, and growth rate.[32,33] In contrast to *BRCA1/2* mutation carriers, breast cancer patients with a familial risk do not have an increased risk of ipsilateral recurrence or contralateral breast cancer.[32,34] Survival rates of familial risk breast cancer patients are similar to that of women with sporadic breast cancers.[32,34-36]

Genetic predisposition

The cloning of the *BRCA1*[37] and *BRCA2*[38] genes in 1994 made it possible to identify women with a germ-line mutation in these genes, who have a cumulative risk for breast cancer of 43-75% by age 70 (**Figure 5**).[8-10,39] The estimated prevalence at birth in the general population is 0.11% for *BRCA1* carriers and 0.12% for *BRCA2* carriers.[40] Among breast cancer patients, especially when diagnosed young, these percentages are higher.[40]

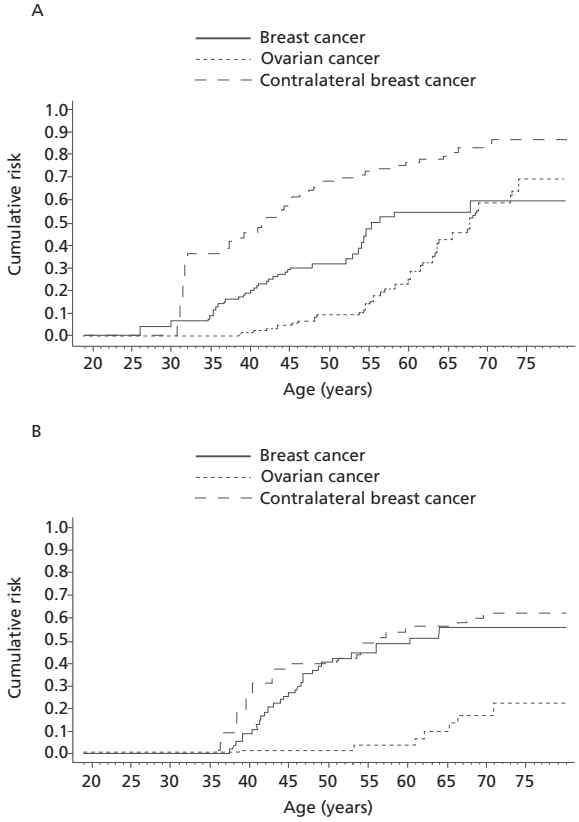


Figure 5 Cumulative risk of breast cancer, ovarian cancer and contralateral breast cancer in: A) *BRCA1*-mutation carriers, and B) *BRCA2*-mutation carriers.
Source: Reproduced with permission from Mavaddat N, Peock S, Frost D, et al. Cancer risks for *BRCA1* and *BRCA2* mutation carriers: results from prospective analysis of EMBRACE. Journal of the National Cancer Institute 2013;105:812-22. © 2015, Oxford University Press.

The median age of diagnosis in mutation carriers is 42 years, more than 20 years earlier than the median age for unselected women in the US and Western Europe.[32,41] *BRCA1* and *BRCA2* are both 'caretaker' genes involved in the maintenance of genomic integrity; DNA repair and recombination, checkpoint control of cell cycle, and transcription.[42] They are, amongst others, involved in double strand DNA breaks repair, like caused by X-rays.[42,43] Because of the role of *BRCA1/2* proteins in DNA repair *BRCA* carriers are more sensitive, especially at younger ages, to the carcinogen effects of ionizing radiation than women in the general population.[44,45] Mutations in these *BRCA* genes not only lead to an autosomal dominant predisposition for breast cancer and ovarian cancer,[9] but also increase risk of other cancers. *BRCA* gene mutations are amongst others associated with higher risk of pancreatic cancer,[41,46] fallopian tube cancer[41], and in men prostate cancer.[41,47,48] Furthermore,

also the risk of second contralateral breast cancer is increased in women with *BRCA* mutation to approximately 40% at 10 years.[49,50] However, this risk is lowered by ovarian estrogen reduction through tamoxifen or oophorectomy.[49,50]

Besides younger age of onset, breast cancers of *BRCA* gene mutation carriers also have other distinguishing characteristics. Breast cancers of *BRCA1* gene mutation carriers in comparison to sporadic cases exhibit higher mitotic counts and are more often high grade,[32,51,52] have more lymphocytic infiltration,[51] and are more likely to be triple negative; i.e. with negative estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status.[53] Breast cancers of *BRCA2* gene mutation carriers in comparison to sporadic cases develop fewer tubules,[51] have lower mitotic count,[51] and are more often estrogen receptor positive.[32]

Furthermore, breast cancers of *BRCA* mutation carriers grow twice as fast as tumors of age-matched non-carriers.[33] The growth rate of a 60 year old *BRCA* mutation carrier is comparable to that of a 37-year old non-carrier. Despite these somewhat unfavorable tumor characteristics *BRCA* gene mutation carriers do not have a worse prognosis than non-carriers. [32,54-56]

Mortality-reducing strategies

Aiming at reduction of breast cancer mortality several strategies have been developed for both *BRCA1/2* mutation carriers, women with familial risk, and women in the general population. Strategies include risk-reducing mastectomy (RRM), risk-reducing salpingo-oophorectomy (RRSO), chemoprevention, and breast cancer screening.

Risk-reducing mastectomy and risk-reducing oophorectomy

Aggressive mortality-reducing strategies are RRM and RRSO, considered for only those women with the highest breast cancer risk, mainly *BRCA1/2* mutation carriers.[57-59] RRM is effective in both decreasing breast cancer incidence as well as increasing survival in *BRCA1/2* mutation carriers.[60-64] However, breast cancer screening with annual magnetic resonance imaging (MRI) and mammography offers comparable survival as RRM in *BRCA1/2* mutation carriers.[61,63,65] Since *BRCA1/2* mutation carriers are also at highly increased risk of developing ovarian or fallopian tube cancer, RRSO is performed to decrease this risk.[61,62,65,66] However, RRSO not only decreases this risk, but also breast cancer incidence, all-cause mortality, breast cancer specific mortality, and ovarian cancer-specific mortality.[61,62,65,66] RRSO reduces breast cancer risk in *BRCA1/2* mutation carriers by approximately 50%.[62]

Chemoprevention

Chemoprevention comprises use of selective estrogen receptor modulators like tamoxifen or raloxifene, that inhibit cell division by binding to the estrogen receptor.[67] Use of these agents to prevent primary breast cancers is still under debate,

and consequently different guidelines over the world offer different recommendations.[57-59]

However, there is increasing evidence of its efficacy in preventing breast cancer.[68-70] Incidence of invasive estrogen-positive breast cancer is reduced by approximately 40% during treatment with tamoxifen or raloxifene, but also for at least 5 years after completion.[70] Downside of these agents is that no effect on breast cancer specific or overall mortality has been demonstrated, and women are reluctant to use these drugs, because of the serious side-effects, including thromboembolic events, endometrial cancer, and cataract.[69-72]

Breast cancer screening: general population

Screening aims to improve survival by early detection of breast cancers. The Dutch, UK, and US guidelines all recommend breast cancer screening with mammography in the general population.[57,73-76] The Dutch, and the UK have national breast cancer screening programs, inviting all women from the age of 50 for mammography screening, biennial and up to 75 years in the Netherlands,[57,76] and every 3 years until 70 years in the UK.[74] The US do not have a national screening program, but the American Cancer Society guideline advises annual mammography from the age of 40 years, without an upper age limit,[75] and the US Preventive Services Task Force advises biennial mammography screening from the ages of 50 through 74 years.[73] There has been extensive debate about potential harms and benefits of mammography screening focusing on overdiagnosis and mortality reduction.[77-79] At this time the balance seems to be in favor of screening, since national screening programs are continued, and the UK national breast cancer screening program currently is even being extended to women aged 47-49 as well as those aged 71-73.[74,77]

Breast cancer screening: BRCA1/2 mutation carriers

For *BRCA1/2* mutation carriers screening with annual clinical breast examination, mammography and MRI is recommended from the age of 30 years in most screening guidelines.[57-59,75] The Dutch guideline recommends starting clinical breast examination and MRI screening from the age of 25 years.[57] Considering the sensitivity of *BRCA1/2* mutation carriers for radiation induced breast cancers mammography should be postponed until after the age of 30.[45] Recently the additional value of even digital mammography screening was shown to be very small (2%) beside MRI screening for *BRCA1* mutation carriers below 40 years.[80] Further prospective studies are needed to confirm these results. Continuation of breast cancer screening is not advised after risk-reducing mastectomy since there is hardly any residual risk of breast cancer.[60,62,64]

Screening with yearly MRI in addition to mammography is considered cost-effective for female *BRCA1* and *BRCA2* gene mutation carriers aged 30–60 years or women who have a 50% chance of carrying such a mutation.[81-83] Guidelines differ in the upper age limit of MRI screening. The British NICE guideline recom-

mends MRI screening at least until 50, and considering continuing MRI screening for women with dense breasts.[59] Dutch guidelines advise MRI screening until 60 years, and screening with biennial mammography in the national breast cancer screening program afterwards.[57] American guidelines advice annual screening with MRI and mammography without an upper age limit.[75] There are several reasons why a less intensive screening protocol may not be adequate for *BRCA1/2* mutation carriers above 60 years. First of all, breast cancer incidence remains high in mutation carriers above 60 years.[8,10,84] Secondly, there is no evidence that screening with mammography alone is effective for *BRCA1/2* mutation carriers above 60.[80] In the Dutch prospective MRI Screening Study (MRISC) *BRCA1/2* mutation carriers and women with familial risk for breast cancer aged 25 to 70 years were screened with annual mammography and MRI.[85] Sensitivity of mammography was just 25% for *BRCA1* mutation carriers and 62% for *BRCA2* mutation carriers, whilst MRI sensitivity was approximately 68%.[85] Other studies showed similar numbers.[85-88] In a recent meta-analysis from Phi et al. MRI above the age of 50 years improved breast cancer sensitivity by a magnitude similar to that of younger *BRCA1/2*.[89] Finally, it is questionable if reducing screening frequency is optimal for *BRCA1/2* mutation carriers above 60 years as their tumors grow twice as fast as tumors of age-matched non-carriers.[33]

Breast cancer screening: familial risk

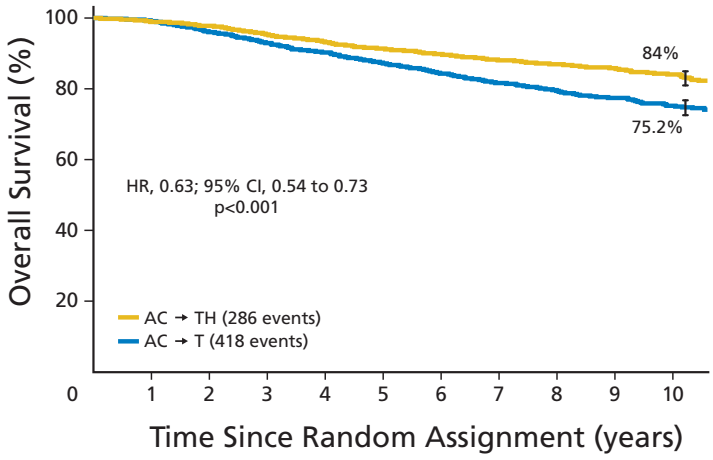
There is a lot of debate on what breast cancer screening modalities should be used for women with familial risk. In the last decade several screening trials comparing MRI and mammography in high-risk women have been completed.[85,86,88] The Dutch MRISC was one of the first prospective studies to conclude that adding MRI to mammography screening improves sensitivity for breast cancer detection in women with a familial or genetic predisposition.[85] As a result of this, and subsequent published studies that confirmed these findings,[86-88] guidelines for breast cancer screening were modified globally.[90] Currently, annual mammography, clinical breast examination, and MRI screening is advised for *BRCA1/2* gene mutation carriers of 30 years and older,[57-59] and in the American guidelines also for women with a cumulative lifetime risk (CLTR) of at least 20% due to a positive family history.[75] Dutch and British screening guidelines omit MRI screening for women with a familial risk, and start screening for women with a moderate familial risk from 40 years.[57,59]

How is it possible that the same screening studies led to different guidelines for women with a familial risk? There are several reasons; although the MRISC and successors demonstrated that MRI detects invasive breast cancers in an earlier stage than mammography, long term metastasis-free survival and mortality reduction of MRI screening remains unknown. Furthermore, MRI screening is far more expensive than mammography screening, and is associated with more false-positive results, leading to additional costs and anxiety of patients.[85-88] Cost-effectiveness studies for

this specific risk group have not been performed. Finally, mammography screening might have a better sensitivity for pre-invasive breast cancer: ductal carcinoma in situ (DCIS).[85] Although, both increasing experience with MRI and recent advances in MRI technology and methods may have improved results.[91,92]

Survival and prognostic factors

In the last decades breast cancer survival rates have increased significantly all over the world.[4,93,94] In the US the overall 5-year relative survival rate for female breast cancer patients has improved from approximately 75% in 1975 through 1977 to 90.3% in 2003 through 2009.[95] The relative survival rates are 83% after 10 years and 78% after 15 years.[95] This survival improvement can mainly be explained by an equal effect of better treatment options and earlier diagnosis as a result of breast cancer screening, though this effect may differ for ER-positive and ER-negative breast cancers.[96-98] However, the balance may be different now, since patients included in these studies were diagnosed with breast cancer in 2004 latest, and trastuzumab (herceptin) was not prescribed regularly at that time. Trastuzumab is a humanized monoclonal antibody that targets the extracellular domain of the HER2 protein[99] that is overexpressed in about 25% of invasive breast cancers.[100]



No. at risk

AC → TH	2,028	1,995	1,959	1,897	1,843	1,785	1,709	1,506	1,085	735	439
AC → T	2,018	1,962	1,883	1,806	1,730	1,640	1,534	1,336	944	604	353

Figure 6 Overall survival of female HER2 positive breast cancer patients treated with chemotherapy (AC: doxorubicin and cyclophosphamide followed by T: paclitaxel) with or without trastuzumab (H).

Source: Reproduced with permission from Perez EA, Romond EH, Suman VJ, et al. Trastuzumab plus adjuvant chemotherapy for human epidermal growth factor receptor 2 positive breast cancer: planned joint analysis of overall survival from NSABP B-31 and NCCTG N9831. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2014;32:3744-52. © 2014, American Society of Clinical Oncology.

Overexpression of the HER2 protein, or HER2 positive cancer, leads to a worse prognosis than HER2 negative disease,[100] but after adding trastuzumab to chemotherapy both short-term prognosis[101] and long-term prognosis have increased significantly (**Figure 6**).[102]

In addition to HER2 status, cancer-related factors that influence survival include tumor grade, tumor size, hormone receptor status, and the number of axillary lymph nodes involved.[103-106] However, recent publications have not included women diagnosed after 2007, and breast cancer treatment has changed considerably. The use of trastuzumab is only one example. It is questionable if the above mentioned prognostic factors are still relevant in the current era. Recently, guidelines concerning axillary lymph node therapy have been modified,[107] following a study of Giuliano et al. in which patients with limited positive sentinel lymph nodes treated with breast conserving therapy and systemic therapy had comparable survival outcomes as those treated additionally with lymph node dissection.[108] Furthermore, the same group published data indicating that in patients with small breast cancers lymph node micro metastases are not of any prognostic value.[106] These data are pointing to the possibility that known prognostic factors possibly are no longer applicable in the current era of new therapy.

Aims and outlines of this thesis

In this thesis we aim to investigate remaining questions of breast cancer screening in women with hereditary or familial risk for breast cancer. In **chapter 2**, we illustrate risk-reducing options for women with a familial or genetic predisposition using three patient cases. This chapter focuses on screening guidelines in the Netherlands.

There is a lot of debate on MRI screening for women with familial risk. Guidelines are equivocal, partly because of MRI screening costs are high due to MRI prices and false-positive results leading to additional examinations. We analyzed survival data of both *BRCA1/2* mutation carriers and women with a familial risk in the largest prospective MRI screening study; MRISC in **chapter 3**. Furthermore, in **chapter 4,** we estimate cost-effectiveness of additional MRI screening for women with a familial risk in the MRISC.

Since all studies that investigated sensitivity of additional MRI screening had a non-randomized design it is difficult to gain certainty regarding what stage tumors would have been detected by either test. Furthermore, breast density, which increases breast cancer risk, and decreases mammography sensitivity,[13,18] has not been taken into account in previous studies. Breast density may be a key discriminator for selecting the optimal screening strategy for women with familial risk: mammography or MRI. For this reason the Familial MRI Screening Study (FaMRIsc) has been designed. In **chapter 5**, we describe the study design of FaMRIsc.

Recently efficacy of mammography screening for *BRCA1* mutation carriers below 40 years was questioned in a small retrospective study.[80] Substantial early breast

cancer detection by mammography is needed to outweigh the possible breast cancer induction by x-rays in *BRCA1/2* mutation carriers.[45] In **chapter 6**, we analyze individual patient data from 6 prospective MRI screening studies to estimate the additional value of mammography to MRI screening in *BRCA1* and *BRCA2* mutation carriers for different age categories.

In **chapter 7**, we continue on the subject of optimal breast cancer screening ages in *BRCA1/2* mutation carriers. Less intensive screening is advised for *BRCA1/2* mutation carriers above the age of 60 in both the British NICE guideline and the Dutch guideline.[57,59] It is questionable if this is justified. To address the clinical relevance and extent of this issue, we first assess the proportion of *BRCA1/2* mutation carriers with remaining breast tissue at risk at 60 years in an ongoing nationwide cohort study and a family cancer clinic cohort. Secondly, to determine the optimal breast cancer screening strategy we compared tumor stage at detection per screening strategy in *BRCA1/2* mutation carriers diagnosed with breast cancer when 60 years or older.

Current prognostic factors for breast cancer survival, like tumor size and lymph node status, do not differ in effect between women with a familial risk and sporadic breast cancer cases.[34] It is reasonable to assume that this is also the case for other prognostic factors. Furthermore, large datasets with long follow-up time are needed to identify prognostic factors for breast cancer survival. Therefore, it is more feasible to primarily discover these prognostic factors in datasets of unselected breast cancer cases, and validate them secondarily in smaller familial risk patient datasets. In **chapter 8**, we evaluate the prognostic value of tumor expression of several cell adhesion molecules; N-cadherin, E-cadherin, carcinoembyonic antigen (CEA) and epithelial CAM (Ep-CAM), in a large unselected dataset with extensive follow-up. Cell adhesion molecules play an important role in the process of metastasis.

In **chapter 9**, we assess the relevance of established prognostic factors in the current era of new therapies in a prospective nationwide population-based study using data of the Dutch national cancer registry. Results of this study are of particular interest to screening related studies in smaller subgroups like women with familial risk. Screening studies in women with familial risk generally use prognostic factors, like breast cancer stage at diagnosis, as primary outcome, reasoning that in general, and also in hereditary and familial breast cancer, these prognostic factors are related to both risk of metastases and overall survival.[32,34,103-106]

Finally a general discussion and summary of results reported in this thesis is given in **chapter 10**. In **chapter 11** a summary in Dutch is given and in **chapter 12** a list of publications, Curriculum Vitae, PhD portfolio, and acknowledgements are provided.

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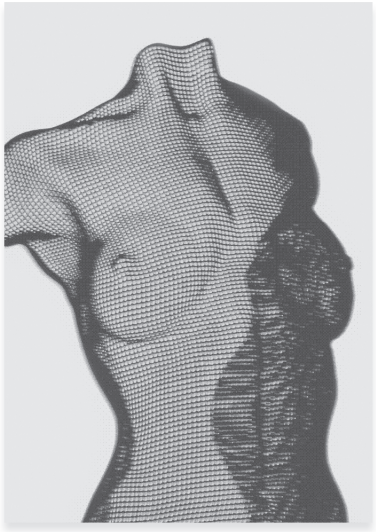
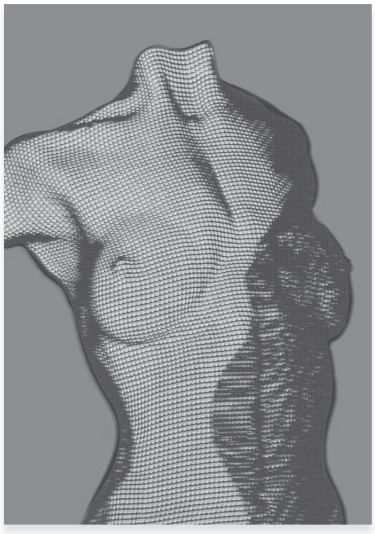
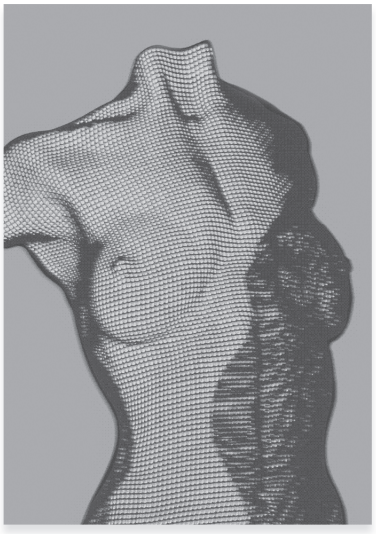

Chapter 2

Choices for women with hereditary breast cancer risk

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ABSTRACT

Background

BRCA1/2 mutation carriers and women with a family history have an increased risk of developing breast cancer. Mortality-reducing strategies for these women include prophylactic mastectomy, breast cancer screening with mammography, MRI or a combination.

Methods

In this clinical lesson we discuss relevant literature exemplified by the choices of three women diagnosed with breast cancer; a 32 year old *BRCA1* mutation carrier, a 63 year old *BRCA2* mutation carrier, and a 52 year old woman with familial breast cancer risk.

Results

For *BRCA1/2* mutation carriers bilateral preventive oophorectomy, combined with either MRI screening or bilateral preventive mastectomy can contribute to a considerably better life expectancy. Both options are nearly equally effective. Breast conserving treatment can be a safe choice for young *BRCA1/2* mutation carriers despite a 40% risk of contralateral cancer within 10 years and possibly a higher risk of a second ipsilateral cancer. The contribution of mammography to early breast cancer detection seems small for MRI screened *BRCA1* carriers below 40 years; less than 10% according to recent literature. High tumor growth rate and high breast cancer incidence in *BRCA1/2* carriers above the age of 60 are arguments to screen more frequently than the current mammogram every 2 years. For women with a familial risk the optimal screening strategy; annual MRI versus mammogram, is being investigated in a randomized controlled trial (www.famrisc.nl). Age of onset of breast cancer is determined by both family history and risk group. If known, the ages of family members at diagnosis may assist in determining at what age preventive measures should be started for high-risk women.

Conclusions

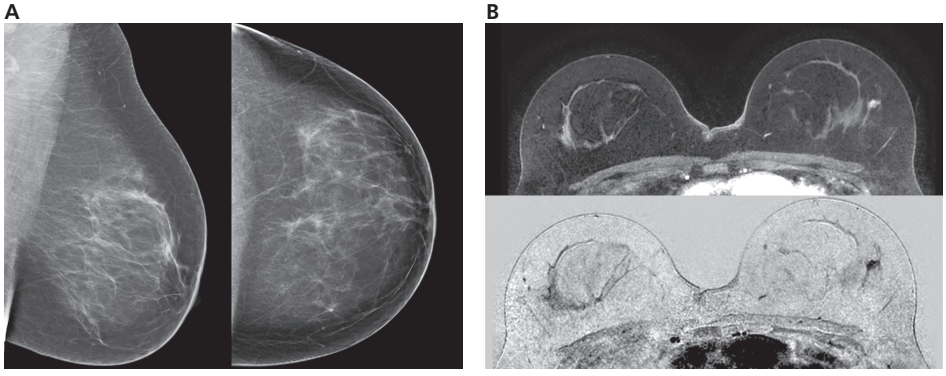
Bilateral preventive mastectomy and MRI screening for *BRCA1/2* mutation carriers are equally safe mortality-reducing strategies. The optimal screening method for women with familial risk is being investigated in the FaMRIsc trial.

Dames en heren,

Een jonge steractrice die bekend maakt dat ze heeft gekozen voor preventieve bilaterale mastectomie, omdat ze draagster is van een borstkanker genmutatie, trekt aandacht. Maar vrouwen kunnen ook kiezen voor jaarlijkse screening met MRI en mammografie. Bij het maken van deze afweging spelen leeftijd en familiegeschiedenis een belangrijke rol.

Van de 13.000 vrouwen bij wie in Nederland jaarlijks borstkanker wordt gediagnosticeerd, hebben minstens 1300 een duidelijke familiaire belasting. Bij ongeveer 300 zal een *BRCA1*- of *BRCA2*-mutatie bekend zijn. De vrouwen in deze laatste groep hebben een geschat risico van 45-70% om voor ze 70 jaar oud zijn borstkanker te krijgen.[1] Is het mogelijk om in te schatten welke draagster tot de 20-30% hoort die voor haar 50e borstkanker krijgt, wie pas op hogere leeftijd en wie helemaal niet? Wat is de beste keuze als een vrouw pas op hogere leeftijd hoort dat ze draagster is van een mutatie, zoals vaak het geval is? Als bij een jonge draagster borstkanker blijkt, wat zijn dan haar opties? En ligt dat anders voor een vrouw met een sterk belaste familie zonder genafwijking? Aan de hand van 3 vrouwen laten we in deze klinische les zien welke overwegingen meespelen als een vrouw hoort dat ze erfelijk belast is met borstkanker.

Patiënt A is een vrouw die zich vanaf haar 30e liet screenen op borstkanker met een jaarlijkse mammografie, MRI en lichamelijk onderzoek volgens de landelijke richtlijn.[2] Zij erfde de *BRCA1*-genmutatie via haar vader. Bij haar nichtje was op 29-jarige leeftijd borstkanker geconstateerd en op 42-jarige leeftijd in de contralaterale borst. De moeder van haar vader had beiderzijds borstkanker op 50 en 52 jaar, de zus van vader op 42 jaar. Mevrouw had geen kinderen, maar wel een kinderwens. De mammografie was goed te beoordelen: er was weinig dicht klierweefsel en er waren geen afwijkingen. Na 2 jaar screening liet de MRI links een afwijking



Figuur 1 A) Goed te beoordelen mammografie van patiënt A die ten tijde van de diagnose geen verandering laat zien ten opzichte van de vorige mammografie; B) MRI van patiënt A gemaakt rond hetzelfde moment als de mammografie, waarop een 12 mm groot mammacarcinoom zichtbaar is links lateraal.

zien van 12 mm (BI-RADS 3, ofwel een kleine kans op maligniteit; BI-RADS staat voor 'Breast Imaging-Reporting Data System') (**Figuur 1**). Echografisch was een afwijking herkenbaar van 9 mm die gering verdacht was. Cytologisch onderzoek van het punctaat wees op een adenocarcinoom. Gezien haar leeftijd van 32 jaar, de ingeschatte grootte en het feit dat *BRCA1*-tumoren meestal hooggradig zijn, was adjuvante chemotherapie geïndiceerd bij de behandeling. Patiënte sprak een gynaecoloog die gespecialiseerd is in fertiliteitsbehoud, maar zag af van de geboden mogelijkheid van cryopreservatie voor latere IVF. Besloten werd tot lumpectomie met schildwachtklierprocedure, zodat patiënte tijdens de daarop volgende chemotherapie rustig de mogelijkheid van ablatio met reconstructie kon overwegen en ook kon nadenken over eventuele preventieve contralaterale ablatio. De tumor bleek 7 mm, N0, graad 3 en oestrogeenreceptor (ER) negatief, progesteronreceptor (PR) negatief en humane epidermale groeifactorreceptor (HER2) negatief. De adjuvante chemotherapie – 3 kuren FEC (fluorouracil, epirubicine, cyclofosfamide) en 3 kuren docetaxel – viel zwaar. Patiënte maakte de borstsparende behandeling af door radiotherapie van de borst en continueerde de follow-up met MRI. Nu, 2 jaar later, is er geen recidief.

Patiënt B hoort op haar 56e dat ze draagster is van een *BRCA2*-mutatie. Een nicht met die mutatie kreeg mammacarcinoom toen ze 41 jaar oud was, een tante toen ze 51 was en 2 andere familieleden toen ze 65 en 70 jaar waren. Mevrouw menstrueert nog onregelmatig en laat preventieve bilaterale ovariëctomie verrichten, zonder hormonale substitutie daarna. Na 7 jaar jaarlijkse screening toont de MRI een 4 mm grote afwijking boven de linker tepel (BI-RADS 3). Bij lichamelijk onderzoek, mammografie en echografie worden geen afwijkingen geconstateerd. Op de herhalings-MRI na 6 maanden blijkt de afwijking enigszins te zijn toegenomen: van 4 naar 5 mm, ook is er verdachte contrastopname. De afwijking wordt nu wel herkend op een echo. Histologisch onderzoek van een biopt toont een invasief carcinoom. Bij lumpectomie met schildwachtklierprocedure blijkt het ductaal carcinoom 4 mm, N0, graad 2, ER positief, PR negatief en HER2 negatief. Na radiotherapie van de borst wordt de follow-up van de 63-jarige patiënte gecontinueerd met om en om MRI en mammografie, met geleidelijk toenemende tussenpozen.

Patiënt C is een 52-jarige nullipara die de anticonceptiepil nog gebruikt. Haar moeder kreeg beiderzijds mammacarcinoom toen ze 50 was. De overige familieanamnese is negatief en het geschatte 'lifetime'-risico op borstkanker is 24%, zodat patiënte volgens de landelijke richtlijn kon deelnemen aan het landelijk bevolkingsonderzoek. Mevrouw besloot echter tot deelname aan de Familiare MRI screeningsstudie (FaMRIsc-studie) naar de waarde van MRI–screening bij familiair belaste vrouwen bij wie geen sprake is van een *BRCA1/2*-genmutatie. Ze randomiseerde voor de MRI-groep, waarbij vrouwen jaarlijks met MRI en om het jaar met mammografie worden gescreend. Op de 1e mammografie in de studie, die goed beoordeelbaar was, werden links retromamillair over een lengte van 2 cm nieuwe calcificaties gezien verdacht voor maligniteit. Op de 1e MRI werd zowel mediaal

boven, als caudaal van de linker tepel contrastopname gezien passend bij ductaal carcinoma in situ (DCIS). Twee biopten op enkele centimeters van elkaar toonden DCIS graad 3. Vanwege de ligging en de grootte van de afwijking in verhouding tot de grootte van de borst werd in overleg met patiënte tot ablatio met schildwachtklierprocedure besloten. Er werd 3 cm DCIS gevonden.

Hierna zet patiënte erfelijkheidsonderzoek in gang. Als blijkt dat zij draagster is van een *BRCA1/2*-mutatie wil zij contralateraal preventieve ablatio met 'deep inferior epigastric perforator'(DIEP)-reconstructie beiderzijds. Als ze geen draagster is kiest ze voor eenzijdige reconstructie. Op dit moment is de uitslag van het erfelijkheidsonderzoek nog niet bekend.

BESCHOUWING

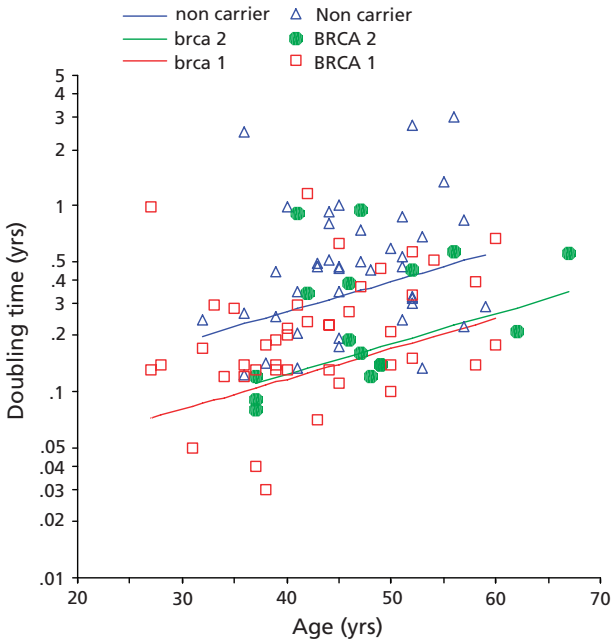
Screening bij *BRCA1/2*-mutaties

In de 3 hierboven beschreven casussen kozen alle patiënten voor screening, zoals ongeveer 60% van de gezonde draagsters van een *BRCA1/2*-mutatie doet. Bij alle 3 werd de tumor gedetecteerd toen hij <1 cm was en in stadium N0. Het tumorstadium heeft ook bij *BRCA1/2*-mutatiedraagsters significante invloed op de prognose.[3]

De Nederlandse richtlijn adviseert om draagsters van een *BRCA1/2*-mutatie en vrouwen met 50% kans daarop jaarlijks te screenen met MRI vanaf 25 tot 60 jaar en jaarlijks een mammografie te maken van 30 tot 60 jaar.[2] Hoe effectief is MRI-screening vergeleken met preventieve mastectomie? Naar schatting neemt de kans om 70 jaar te worden voor een *BRCA1*-mutatiedraagster toe van 53% zonder screening of preventieve chirurgie naar 79% door preventieve salpingo-ovariëctomie op 40-jarige leeftijd en preventieve mastectomie op 25-jarige leeftijd.[4] Als in plaats van preventieve mastectomie wordt gekozen voor MRI-screening tussen het 25ste en 70e levensjaar zou die kans 74% zijn. Bij *BRCA2*-mutatiedraagsters stijgt de kans om 70 jaar te worden in het eerste scenario van 71% naar 83% en in het tweede naar 80%.[4] Een prospectieve studie volgde in de periode 1994-2011 570 gezonde *BRCA1/2*-draagsters, waarvan 212 op enig moment voor preventieve mastectomie kozen.[5] De 10-jaarsoverleving was 99% in de groep die preventieve mastectomie onderging versus 96% in de screeningsgroep; dit verschil was niet significant.

De eerlijkheid gebiedt te zeggen, dat mevrouw B frequenter werd gescreend dan de Nederlandse richtlijn aangeeft. Volgens de richtlijn kunnen *BRCA1/2*-mutatiedraagsters na het 60e levensjaar volstaan met eens per 2 jaar screenen met mammografie, door deelname aan het landelijk bevolkingsonderzoek of door screening in het ziekenhuis. De vraag is of dit echt zo is. De incidentie neemt namelijk boven de leeftijd van 60 jaar niet af. Wel neemt de groeisnelheid van de borsttumor af met toenemende leeftijd, even sterk als bij niet-genmutatiedraagsters.[6] Maar er is wél aangetoond, dat de groeisnelheid van borstkanker bij *BRCA1/2*-draagsters op

iedere leeftijd 2 maal zo snel is als bij niet-draagsters, zoals weergegeven in **Figuur 2**. Gemiddeld groeit de borstkanker bij een *BRCA1/2*-draagster van 60 even snel als bij een niet-draagster van 40, voor wie wel jaarlijkse screening is geïndiceerd.[6] Recent is in het Erasmus MC Kankerinstituut uitgezocht, dat 72% van de *BRCA1/2*-draagsters van 60 jaar of ouder nog borstklierweefsel heeft en dus risico heeft op borstkanker. Data over optimale screening bij *BRCA1/2*-mutatiedraagsters boven de 60 jaar ontbreken, maar als bovenstaande wordt bevestigd met onderzoek is het te verwachten dat de richtlijn wordt aangepast en het advies wordt om vaker dan eens per 2 jaar een mammografie te maken.



Figuur 2 Tumorverdubbelingstijd en leeftijd bij diagnose voor 100 invasieve carcinomen in draagsters van *BRCA1*, *BRCA2* en vrouwen met een familiair risico zonder bekende genmutatie. Gemiddeld is de groeisnelheid bij *BRCA1/2* op ieder gegeven leeftijd 2x zo hoog (ofwel de verdubbelingstijd 2x zo kort) als bij een vrouw met familiair risico. Bron: Clinical Cancer Research. 2007; 13:7357-62.

In de Nederlandse MRISC-studie was bij *BRCA1*-mutatiedraagsters <40 jaar die jaarlijks gescreend werden met MRI het tumorstadium slechter en het percentage intervalcarcinomen hoger dan bij oudere *BRCA1*-mutatiedraagsters, bij gescreende *BRCA2*-mutatiedraagsters en bij vrouwen met familiair risico. Dit suggereert dat het screeningsinterval te lang is. De bijdrage aan borstkankerdetectie van mammografie was <10%.[6,7] Of 2 keer per jaar MRI-screening en het achterwege laten

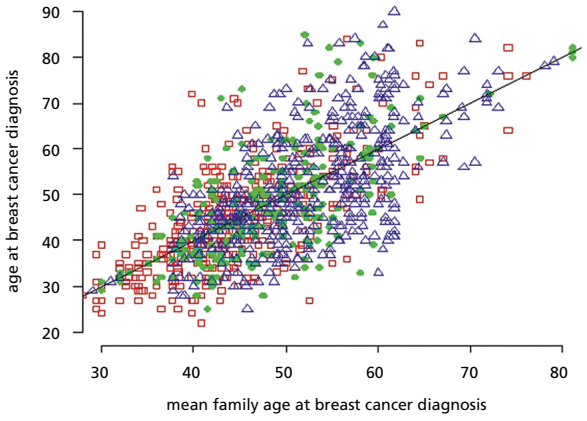
van mammografie bij jonge *BRCA1*-mutatiedraagsters kosteneffectief is als borstkanker jong in de familie voorkomt, is nog niet onderzocht.

Screening bij familiair risico

Bij patiënt C werd het hooggradige DCIS zowel met mammografie als met MRI gedetecteerd. Patiënte deed mee met de FaMRIsc-studie, die onderzoekt of een deel van de vrouwen met een screeningsindicatie vanwege familiair risico zonder *BRCA1/2*-mutatie baat heeft bij MRI-screening.[8,9] Mogelijk zijn dat vrouwen met dicht borstklierweefsel, want de incidentie van borstkanker is bij hen het hoogst. Daarnaast is de sensitiviteit van mammografie, zelfs die van digitale mammografie, bij hen minder hoog. De FaMRIsc-studie (www.famrisc.nl) loopt in het Antoni van Leeuwenhoekziekenhuis, 6 academische centra en enkele grote Nederlandse ziekenhuizen.

Timing van preventie

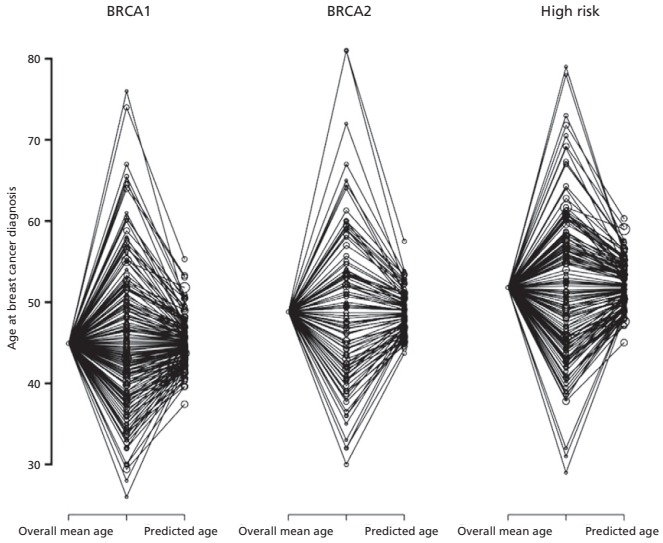
Als we wisten welke vrouw met familiair risico of een *BRCA1/2*-genmutatie de ziekte jong zou krijgen en wie pas postmenopauzaal of zelfs helemaal niet, dan zouden veel *BRCA1/2*-mutatiedraagsters screening of preventieve operaties later kunnen plannen. Patiënt A had een nichtje dat met 29 jaar borstkanker kreeg; zijzelf was 32. Bij patiënt B was de laagste leeftijd waarop een familielid borstkanker kreeg 41 jaar; zijzelf was 63. Bij beide families valt op dat er een grote spreiding is in de leeftijd waarop de diagnose 'borstkanker' wordt gesteld: 29-52 jaar in familie A en 41-70 jaar in familie B.



Figuur 3 Leeftijd ten tijde van de diagnose borstkanker per gemiddelde diagnoseleeftijd in de familie.
Weergegeven zijn de leeftijden van 1304 vrouwen uit de MRISC-studie. Vrouwen uit dezelfde familie staan op 1 verticale lijn: *BRCA1*-draagster (□), *BRCA2*-draagster (●), vrouw met een familiair risico (△). Bron: International Journal of Cancer 2013. 133:156-63.

Uit een recente Nederlands-Engelse studie bleek dat als van veel familieleden bekend is op welke leeftijd borstkanker optrad, dit een indicatie geeft op welke leeftijd de ziekte is te verwachten.[10] Er blijven echter ruime marges bestaan (**Figuur 3**). Naarmate meer diagnoseleeftijden bekend zijn in de familie is beter te voorspellen op welke leeftijd een draagster borstkanker kan ontwikkelen (**Figuur 4**). Die kennis zou, naast gegevens over andere risicofactoren, gebruikt kunnen worden bij de timing van bijvoorbeeld preventieve operaties. Als er slechts 1 of 2 leeftijden bekend zijn, zoals bij patiënt C, kunnen hierop geen aannames worden gebaseerd.

De Nederlandse richtlijn adviseert screening met jaarlijks mammografie van 40-50 jaar bij een geschat lifetimerisico van 20-30% en van 35-60 jaar bij een lifetimerisico ≥30% zonder aangetoonde *BRCA1*- of *BRCA2*-mutatie of 50% risico daarop. Het familiaire risico wordt hoger geschat als de borstkankerdiagnoses in de familie op jongere leeftijd waren. Het is dus verstandig om bij een hoger risico op een



Figuur 4 Leeftijd ten tijde van de diagnose 'borstkanker' voor 203 *BRCA1*-families (links), 105 *BRCA2*-families (middelste figuur) en 106 familie met een erfelijk risico zonder bekende mutatie (rechts).

In alle 3 de figuren is de punt links de gemiddelde diagnoseleeftijd in de hele groep patiënten. In het midden staat de gemiddelde leeftijd per familie. Rechts in iedere figuur is per familie de voorspelde leeftijd weergegeven waarop een gezond familielid mogelijk borstkanker krijgt. Als er maar 1 of 2 familieleden met borstkanker bekend waren, ligt de voorspelde leeftijd dicht bij de gemiddelde leeftijd in de hele groep. Als er veel diagnoseleeftijden van familieleden bekend zijn komt de voorspelde leeftijd dicht bij het familiegemiddelde. Screening wordt aangeraden vanaf 15 jaar vóór de voorspelde leeftijd. Bron: Int J Cancer. 2013;133:156-63.

jongere leeftijd te starten met screenen. Er zijn overigens verschillende methodes om tot een schatting van het risico te komen. Bij dezelfde vrouw kunnen deze tot aanzienlijk verschillende uitkomsten leiden.

Behandelopties

Therapeutische ablatio en contralaterale preventieve ablatio verbeteren de overleving van *BRCA1/2*-draagsters niet meer dan mammasparende therapie van borstkanker.[3] Maar de kans om nogmaals de diagnose 'borstkanker' te krijgen is hoog. Voor *BRCA1*-draagsters die hun eerste mammacarcinoom voor hun 50e krijgen, zoals patiënte A, is het risico op een contralateraal carcinoom 40% na 10 jaar. Als het 1e carcinoom op een leeftijd >50 jaar wordt vastgesteld is die kans 12%, niet hoger dan bij het sporadische mammacarcinoom.[3] Ook een 2e ipsilateraal carcinoom lijkt vaker voor te komen, hoewel de incidentie in de literatuur wisselt.[3] Veel vrouwen willen niet nog eens de diagnose horen en de chemotherapie ondergaan en kiezen daarom voor preventieve contralaterale mastectomie met directe reconstructie, zoals patiënt C aangeeft te zullen doen als ze *BRCA1/2*-mutatiedraagster blijkt te zijn. Kiezen voor mammasparende behandeling en screening met MRI continueren is ook voor *BRCA1/2*-draagsters verantwoord.[3] Het kan verstandig zijn denktijd te winnen door zonder uitstel een lumpectomie uit te voeren met schildwachtklierprocedure gevolgd door eerst adjuvante chemotherapie in plaats van eerst bestraling. Dan is het tumorstadium en de totale noodzakelijke behandeling duidelijk en kunnen alle behandelopties rustig worden overwogen, zoals bij patiënt A werd gedaan.

Voor familiair belaste vrouwen zonder *BRCA1/2*-mutatie is het risico op contralateraal carcinoom en ipsilateraal recidief meestal niet verhoogd.[3]

Dames en Heren, *BRCA1/2*-draagsters kunnen preventief hun borsten laten verwijderen, maar het is net zo verantwoord om te screenen met MRI en mammografie. Ook een mammasparende behandeling gevolgd door MRI-screening kan een acceptabele optie voor een mutatiedraagster zijn, als ze de risico's op recidief kent. Het geeft veel inzicht in de gevoelens en ervaringen van een erfelijk belaste vrouw als de familiegeschiedenis uitgebreid met haar wordt besproken. Dit helpt haar bij het maken van de moeilijke keuzes.

LEERPUNTEN

- Vrouwen met een *BRCA1*- of *BRCA2*-mutatie kunnen kiezen voor ofwel MRI-screening conform de richtlijn ofwel preventieve ablatio. Het verschil in effectiviteit tussen beide opties is klein.
- Bij borstkanker kan borstsparende behandeling ook voor jonge *BRCA1/2*-mutatiedraagsters een verantwoorde keuze zijn.

- Bij vrouwen jonger dan 40 met een *BRCA1*-mutatie die jaarlijks een MRI krijgen, draagt mammografie nauwelijks bij aan vroege detectie van borstkanker.
- Het is nog onduidelijk of screening via het landelijk bevolkingsonderzoek wel voldoende frequent is voor *BRCA1/2*-mutatiedraagsters ouder dan 60 jaar.
- Een deel van de vrouwen met een familiair risico op borstkanker, maar zonder *BRCA1/2*-mutatie heeft mogelijk baat bij MRI-screening; om welke vrouwen dat gaat wordt momenteel onderzocht in de FaMRIsc-studie.

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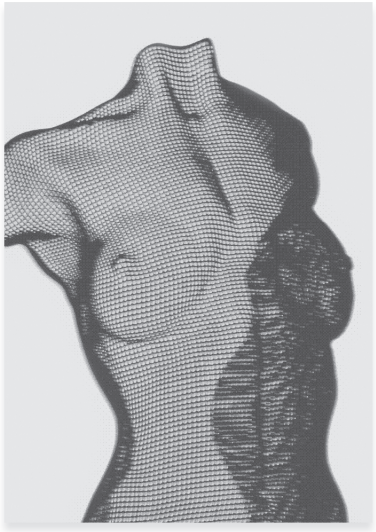
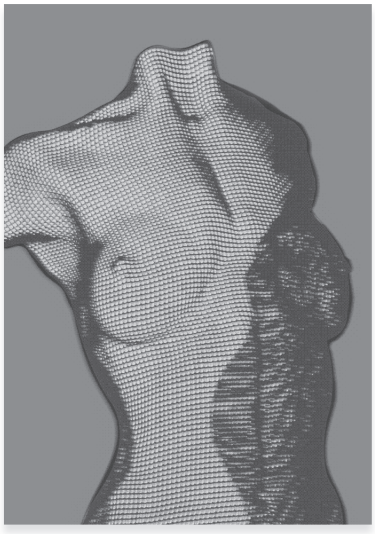
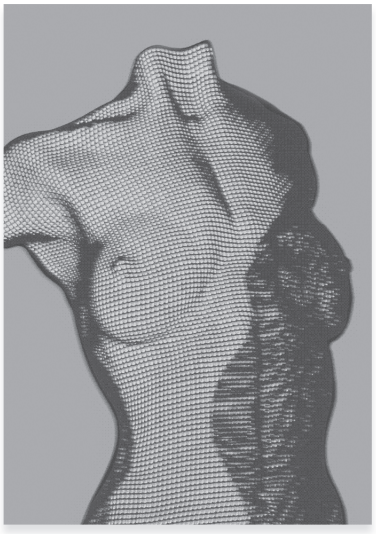

Chapter 3

Survival benefit in women with *BRCA1* mutation or familial risk in the MRI Screening Study (MRISC)

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ABSTRACT

Background

Adding MRI to annual mammography screening improves sensitivity for breast cancer detection in women with familial risk or *BRCA1/2* mutation. Metastasis free survival remains unknown. We describe long-term survival of the largest prospective MRI Screening Study: MRISC.

Methods

Breast cancer specific metastasis free survival (MFS) was compared between MRISC patients and 1:1 matched controls. Controls, unscreened if<50 years, and screened with biennial mammography if≥50 years, were matched on risk category (*BRCA1*, *BRCA2*, familial risk), year and age of diagnosis.

Results

Of 2308 MRISC participants, breast cancer was detected in 93 (97 breast cancers), who received MRI <2 years before breast cancer diagnosis; 33 *BRCA1* mutation carriers, 18 *BRCA2* mutation carriers, and 42 with familial risk. MRISC patients had smaller (87% versus 52% <T2, p<0.001), more often node negative (69% versus 44%, p=0.001) tumors, and received less chemotherapy (39% versus 77%, p<0.001) and hormonal therapy (14% versus 47%, p<0.001) than controls. Median follow-up time was 9 years (range 0-14). Breast cancer metastasized in 9% (8/93) of MRISC patients and in 23% (21/93) of controls (p=0.009). MFS was better in MRISC patients overall (log-rank p=0.008, HR 0.36, 95% CI 0.16-0.80), in women with familial risk (log-rank p=0.024, HR: 0.21, 95% CI 0.04-0.95), and in *BRCA1* mutation carriers, though borderline significant (log-rank p=0.055, HR 0.30, 95% CI 0.08-1.13). MFS remained better in MRISC patients after lead time correction (log-rank p=0.020, HR 0.40, 95% CI 0.18-0.90). Overall survival was non-significantly better in MRISC patients (log-rank p=0.064, HR 0.51, CI 0.24-1.06).

Conclusions

Annual screening with MRI and mammography improves metastasis free survival in women with *BRCA1* mutation or familial predisposition.

INTRODUCTION

Aiming at mortality reduction through early cancer detection, breast cancer screening is used as a less invasive alternative to prophylactic bilateral mastectomy for women with a familial or genetic predisposition for breast cancer.[1-3] The Dutch MRISC (MRI Screening Study) was one of the first prospective studies to conclude that adding MRI to mammography screening improves sensitivity for breast cancer detection in women with a familial or genetic predisposition for breast cancer.[4-8] As a result of this, and subsequent published studies that confirmed these findings, guidelines for breast cancer screening were modified globally.[9] Additional MRI screening was advised, at least for *BRCA1* and *BRCA2* gene mutation carriers,[1,3] and in the American guidelines for all women with a cumulative lifetime risk (CLTR) of ≥20% due to familial risk.[2,10] The MRISC and successors demonstrated that MRI detects breast cancers in an earlier stage than mammography, however long term metastasis free survival and mortality reduction of MRI screening remains unknown.

All studies that investigated sensitivity of additional MRI screening had a non-randomized design; therefore it is difficult to gain certainty regarding the survival benefit of MRI screening. For ethical reasons it is very unlikely that a randomized controlled trial, with a non-screened control group is ever going to be performed. The best available alternative is comparison of a prospective cohort with matched controls. We describe long-term survival of the MRISC breast cancer patients, the largest prospective MRI screening study to date. To address the non-randomization issue, we compared MRISC survival data with those of controls matched for risk group, year of diagnosis, and age at diagnosis.

METHODS

Study Population

The Dutch MRISC was a non-randomized multicentre prospective cohort study comparing efficacy of mammography with MRI for surveillance of women with a familial or hereditary predisposition for breast cancer. Enrolment started November 1, 1999 and ended August 1, 2007.[11] Methods and results have been described previously.[11] The study was approved by the institutional review boards of all 6 participating university hospitals. Included were women aged 25-70 years with a genetic or familial predisposition for breast cancer, whose CLTR according to the modified tables of Claus et al.[12] was ≥15% and who gave written informed consent. Women with symptoms or a medical history of breast cancer were excluded.[4,11]

We subdivided participants into the following risk categories on the basis of their estimated CLTR: *BRCA1* gene mutation carriers, *BRCA2* gene mutation carriers, and patients with a familial risk (estimated CLTR 15-50%). The one patient with *PTEN* mutation was not assigned to a risk group and only included in analyses concern-

ing the whole patient set. Current risk models, and also the modified tables of Claus used in MRISC,[12] have wide confidence intervals for women with a familial risk.[13] Furthermore, the breast cancer incidence rate was comparable in the two MRISC risk groups and no other statistical difference was found regarding patient or tumor characteristics.[14] Therefore, the division into a moderate and high risk group in the MRISC does not seem justified and was not performed.

All patients underwent clinical breast examination (CBE) every six months, and annual mammography and MRI. True cancer status was ascertained by histological examination. Patients were subsequently treated according to national guidelines for local and systemic therapy. An interval cancer was defined as a carcinoma symptomatically detected in between two consecutive screening rounds, after initially negative findings on screening. All MRISC patients with breast cancer detected before August 1, 2007 and who received MRI screening in accordance with the study protocol within two years before breast cancer diagnosis were included. Patients with only breast cancers detected at preventive mastectomy were excluded from comparative analyses. In case of bilateral or metachronous breast cancer data of the first cancer detected were used.

Control group

To determine whether breast cancer specific distant metastasis free survival (MFS) was more favorable in patients screened with annual MRI and mammography, we compared breast cancer patients from the MRISC with matched controls with breast cancer who received no screening if younger than 50 years of age, or were screened with biennial mammography in the Dutch national breast cancer screening program if 50 years or older. Controls with a *BRCA1/2* gene mutation were not screened, according to the protocol for gene mutation carriers, because they were not aware of their mutation status, since testing was done after breast cancer diagnosis (except for two patients who presented with a palpable breast lesion within one month after their DNA result). Controls were diagnosed with breast cancer or treated for breast cancer in the ErasmusMC - Cancer Institute and had given informed consent for enrolment in a prospective institutional registry. Patients were matched 1:1, because of lack of availability of multiple controls. Matching was performed on; risk group (*BRCA1*, *BRCA2*, or familial risk with CLTR >15% according to the modified tables of Claus et al),[12] year of diagnosis (+/- 2 years), and age at diagnosis (+/- 2 years). If incidentally for one MRISC patient there were multiple controls eligible as a match, the one with the smallest differences in both match criteria was used. Matching was performed first, blinded for pathology, and without knowledge of outcomes.[15]

The following data were registered: date of DNA test in case of a *BRCA1* or *BRCA2* gene mutation, age at diagnosis of breast cancer, tumor characteristics, local and systemic therapy, loco regional and distant tumor recurrence, and survival. For tumor size pathological tumor category was used (pT), unless the patient was

given neo-adjuvant therapy, then stage was determined based on imaging studies and clinical examination (cT). In the Netherlands human epidermal growth factor receptor 2 (HER2) status was routinely determined from 2005 and onward.[16] If the patient was no longer under surveillance in the hospital of inclusion, information regarding relapse and/or death was obtained from the medical files of the general practitioner. Last date of follow-up was August, 2013.

Statistical Analysis

Differences in stage distributions, lymph node status, and tumor characteristics between the MRISC patients and controls or the different risk groups in the MRISC were calculated using Pearson's χ^2 tests or Fisher's exact tests, as appropriate, differences in median age at diagnosis and median year of diagnosis of breast cancer were assessed with the Mann-Whitney *U* test. MFS was defined as time from diagnosis of breast cancer until breast cancer specific distant metastasis. Median follow-up time was determined by reversed censoring.[17] Kaplan-Meier curves for breast cancer specific distant metastasis were plotted and compared with log-rank tests. Women were censored when developing distant metastases of other cancers than breast cancer, in case of death or at date of last follow-up. Kaplan-Meier curves for overall survival were also plotted and compared with log-rank tests. Women were censored at date of last follow-up. Median potential follow-up was defined as the median follow-up if none of the patients were censored. Cox proportional hazard models were developed to estimate unadjusted hazard ratios with 95% confidence intervals (CI) for distant metastases and also for overall survival. The assumption of proportional hazards was found to be valid by graphically plotting the log-log survival curves. Data-analyses were also performed stratified for risk group. A two-sided P value $\leq$0.05 was considered statistically significant. Missing values were excluded from analyses. Statistical analyses were performed by S.S. using SPSS Statistics for Windows, version 20.0 (IBM Corp, Armonk, NY, US).

Lead time bias and survival bias correction

Lead time is the amount of time by which the diagnosis has been advanced by screening.[18] To correct for potential lead time bias, we subtracted estimated lead times from MFS of patients with screen-detected breast cancers. For patients with interval cancers no correction was performed, since per definition there is no lead time. If MFS was shorter than the lead time a MFS of 0 was used. Lead time was estimated with MISCAN (micro simulation screening analysis), a well-validated micro simulation model,[19] calibrated earlier for the MRISC *BRCA1* and *BRCA2* cohort,[20] the MRISC familial risk cohort,[14] and the Dutch national breast cancer screening program.[21] Since survival bias may be present for controls with a *BRCA1/2* mutation; they were tested after their breast cancer diagnosis and must have lived long enough to undergo a DNA test, this was also taken into account when correcting for lead time. Mean lead time correction in our cohort was estimated at: 1 year for

BRCA1 carriers, 3 years for *BRCA2* carriers, 4 years for women with a familial risk, and 3 years for controls aged ≥50 years screened in the Dutch national breast cancer screening program. Furthermore, we performed sensitivity analyses subtracting lead times of 1, 2, and 3 years from MFS of all MRISC patients with screen-detected breast cancers. In these sensitivity analyses no distinction was made between lead time adjustment for the different risk categories and no lead time correction was applied to controls with screen-detected breast cancers.

RESULTS

MRISC patients and matched controls

A total of 2308 women participated in the MRISC; 711 gene mutation carriers (706 *BRCA1/2*, 2 *PTEN*, and 3 *P53*), and 1597 women with a familial risk. In 110 MRISC patients, 115 breast cancers were detected, 10 of them by chance at prophylactic mastectomy. In total 93 breast cancer patients had received MRI screening in accordance with the study protocol within two years before their breast cancer diagnosis. In these 93 patients, 97 breast cancers were detected; 80 (83%) screen-detected, 14 (14%) interval carcinomas and 3 (3%) breast cancers detected at contralateral prophylactic mastectomy after therapeutic mastectomy. These 93 patients consisted of 33 *BRCA1* mutation carriers, 18 *BRCA2* mutation carriers, 41 patients with a familial risk, and 1 patient with a *PTEN* mutation. Women were matched 1:1 on risk group (*BRCA1*, *BRCA2*, or familial risk with CLTR >15%). The patient with *PTEN* mutation was matched with a control with familial risk. For 97% (90/93) of patients year of diagnosis and age at diagnosis was matched within a range of 2 years. For the remaining 3% of patients year of diagnosis and age at diagnosis was matched within 3 years. In the control group, median time between breast cancer diagnosis and date of DNA test was 1 year (range 0-7 years). There were 23 controls that received biennial mammography screening in the national breast cancer screening program because they were aged above 50 years, of these 7 were diagnosed with interval cancers, and 16 with screen-detected breast cancers. Six MRISC patients, 5 *BRCA1* mutation carriers, and 1 *BRCA2* mutation carrier, underwent prophylactic bilateral salpingo-oophorectomy (PBSO) before breast cancer diagnosis. None of the controls underwent PBSO before breast cancer detection.

Tumor Stage and Metastasis Free Survival

MRISC patients had smaller breast cancers at detection, <T2; 87% in comparison to 52% in controls (p<0.001), in *BRCA1* 79% versus 51% (p=0.020), in *BRCA2* 94% versus 50% (p=0.003), and in women with familial risk 90% versus 51% (p<0.001). MRISC breast cancers were more often node negative; 69% versus 44% in controls (p=0.001), and MRISC patients received less chemotherapy; 39% versus 77% (p<0.001), and less hormonal therapy; 14% versus 47% (p<0.001) than controls.

Complete information on receptor status (e.g. ER, PR and HER2 status known) was available from 45 MRISC patients and 50 controls. Triple negative breast cancer was diagnosed in 40% (18) of MRISC patients and also in 40% (20) of controls (p=1.00). Patient and tumor characteristics of MRISC patients and matched controls are shown in **Table 1**.

Median potential follow-up was 10 years (range 4-14). Median follow-up time for MFS was 9 years (range 0-14) for all patients, 9 years (range 1-12) for the MRISC cohort and 8 years (range 0-14) for controls. Local recurrence was found in 8% (7/93) of MRISC patients, compared to 10% (9/93) of controls (p=0.601). Nine per cent (8/93) of MRISC patients had breast cancer specific distant metastases, compared to 23% (21/93) of patients in the matched control group (p=0.009). Median age at breast cancer diagnosis was not significantly different between MRISC patients

Table 1 Patient and tumor characteristics of MRISC patients and matched controls*

Patient and tumor characteristics	MRISC		Control		P value
Number of breast cancer patients	93		93		-
Age at diagnosis in years, median (range)	44	(26-67)	44	(26-68)	1.00
Year of diagnosis, median (range)	2003	(2000-2007)	2003	(1999-2009)	0.85
Pathological tumor category overall, no (%)†					**<0.001**
Ductal carcinoma in situ	15	(16%)	6	(6%)	
T1a/T1b	37	(40%)	8	(9%)	
T1c	29	(31%)	34	(37%)	
T2+	12	(13%)	45	(48%)	
Total	93	(100%)	93	(100%)	
Pathological tumor category *BRCA1*, no (%)†					**0.02**
Ductal carcinoma in situ/T1	26	(79%)	17	(51%)	
T2+	7	(21%)	16	(49%)	
Total	33	(100%)	33	(100%)	
Pathological tumor category *BRCA2*, no (%)†					**0.003**
Ductal carcinoma in situ/T1	17	(94%)	9	(50%)	
T2+	1	(6%)	9	(50%)	
Total	18	(100%)	18	(100%)	
Pathological tumor category familial risk, no (%)†					**<0.001**
Ductal carcinoma in situ/T1	37	(90%)	21	(51%)	
T2+	4	(10%)	20	(49%)	
Total	41	(100%)	41	(100%)	
Pathological node category overall, no (%)†‡§					**0.001**
Node positive / micro metastasis, 0.2-2.0 mm	24	(31%)	48	(56%)	
Node negative / isolated tumor cells	54	(69%)	38	(44%)	
Total	78	(100%)	86	(100%)	

Table 1 Patient and tumor characteristics of MRISC patients and matched controls* (continued)

Patient and tumor characteristics	MRISC		Control		P value
Pathological node category *BRCA1*, no (%)†‡					0.79
Node positive / micro metastasis, 0.2-2.0 mm	11	(37%)	10	(32%)	
Node negative / isolated tumor cells	19	(63%)	21	(67%)	
Total	30	(100%)	31	(100%)	
Pathological node category *BRCA2*, no (%)†‡§					**0.007**
Node positive / micro metastasis, 0.2-2.0 mm	5	(33%)	13	(81%)	
Node negative / isolated tumor cells	10	(67%)	3	(19%)	
Total	15	(100%)	16	(100%)	
Pathological node category familial risk, no (%)†‡					**<0.001**
Node positive / micro metastasis, 0.2-2.0 mm	8	(24%)	25	(66%)	
Node negative / isolated tumor cells	25	(76%)	13	(34%)	
Total	33	(100%)	38	(100%)	
Histological subtype, no (%)‡					0.51
Ductal cancer	65	(83%)	69	(79%)	
Other	13	(17%)	18	(21%)	
Total	78	(100%)	87	(100%)	
Bloom & Richardson grade, no (%)‡‖					0.08
Grade 1	20	(26%)	13	(16%)	
Grade 2	25	(33%)	22	(26%)	
Grade 3	32	(42%)	49	(58%)	
Total	77	(100%)	84	(100%)	
Receptor status, no (%)‡‖					
Estrogen positive	42	(57%)	54	(63%)	0.44
Estrogen negative	32	(43%)	32	(37%)	
Total	74	(100%)	86	(100%)	
Progesterone positive	39	(52%)	47	(56%)	0.62
Progesterone negative	36	(48%)	37	(44%)	
Total	75	(100%)	84	(100%)	
HER2 over expression	2	(5%)	8	(16%)	0.10
HER2 no over expression	41	(95%)	43	(84%)	
Total	43	(100%)	51	(100%)	
Breast cancer treatment§					
Breast conserving therapy	27	(29%)	43	(47%)	**0.01**
Mastectomy	66	(71%)	49	(53%)	
Total	93	(100%)	92	(100%)	
Chemotherapy, adjuvant	34	(37%)	63	(67%)	**<0.001**
Chemotherapy, neo-adjuvant	2	(2%)	9	(10%)	
No chemotherapy	57	(61%)	21	(23%)	
Total	93	(100%)	93	(100%)	
Hormonal therapy	13	(14%)	44	(47%)	**<0.001**
No hormonal therapy	80	(86%)	49	(53%)	
Total	93	(100%)	93	(100%)	

*MRISC patients were screened with annual mammography and MRI. Controls received no screening below age 50, or were screened with biennial mammography in the Dutch national breast cancer screening program from age 50 onwards. Controls were matched on risk category, age of diagnosis and year of diagnosis. All percentages were calculated vertically. Two-sided P value for difference between MRISC patients and controls, differences in median age at diagnosis and median follow-up were calculated from the Mann-Whitney *U* test. All other differences were obtained from $\chi 2$ or Fisher exact tests, as appropriate. All statistical tests were two-sided. Missing data were excluded from analyses. MRISC= MRI Screening Study, no=number, HER2= human epidermal growth factor receptor 2.
†In case of neo-adjuvant chemotherapy the clinical Tumor category (cT) and clinical Node category (cN) was used, determined by clinical examination and imaging.
‡Except ductal carcinoma in situ (DCIS).
§One of the controls did not receive breast conserving therapy or mastectomy, nor sentinel node procedure or axillary lymph dissection, since she presented with symptoms of breast cancer metastases. Not included in this analysis.
‖In the pathology reports of invasive cancers grade was not described in 2% (4/165) of patients, estrogen receptor status was not described in 3% (5/165) of patients, and progesterone receptor status was not described in 4% (6/165) of patients. HER2 status was not routinely determined during the whole period of the MRISC, and was missing in 43% (71/165) of patients.

and controls with breast cancer metastases (p=0.26). Of the MRISC *BRCA1* mutation carriers also 9% (3/33) developed metastases versus 27% (9/33) of the controls (p=0.056). Two of the MRISC *BRCA1* mutation carriers developing metastases had undergone PBSO before breast cancer diagnosis. The difference in the *BRCA2* group was non-significant: 11% (2/18) metastases in the MRISC versus 17% (3/18) of controls (p=1.000). Five per cent (2/41) of patients in the MRISC familial risk group developed metastases versus 22% (9/41) in the familial control group (p=0.023). Detailed patient and tumor information regarding metastasized breast cancers can be found in **Table 2**. Kaplan-Meier curves for breast cancer specific distant metastasis for all risk groups separately were plotted, and differences between the MRISC patients and controls were compared using log-rank tests (**Figure 1**).

Ten-years MFS was: 90% for the overall MRISC group versus 77% for controls (log-rank p=0.008, HR 0.36, 95% CI 0.16-0.80): for the *BRCA1* group 88% for MRISC versus 72% for controls (log-rank p=0.055, HR 0.30, 95% CI 0.08-1.13), for the *BRCA2* group 88% for MRISC versus 83% for controls (log-rank p=0.739, HR 0.74 95% CI 0.12-4.45), and for the familial risk group 95% for MRISC versus 78% for controls (log-rank p=0.024, HR 0.21, 95% CI 0.04-0.95). MFS remained better in all MRISC patients after risk-category specific correction for lead time and survival bias as shown in **Figure 2** (log-rank p=0.020, HR 0.40, 95% CI 0.18-0.90). MFS also remained better in MRISC patients compared to controls when a variable lead time bias correction was applied (**Supplementary table 1**).

Median follow-up time for overall survival was 9 years (range 1-14) for all patients. Twelve per cent (11/93) of MRISC patients died, compared to 22% (20/93) of patients in the matched control group (p=0.114). Sixty-four per cent (7/11) of

Table 2 Patient, tumor, and treatment characteristics of patients with breast cancer specific distant metastasis; MRISC patients and matched controls*

ID	Age at diagnosis (years)	Risk group	MFS (months)	pT†	Grade	pN†	Therapy primary tumor		Contralateral breast cancer	Local recurrence prior to distant metastasis	Site of distant metastasis	Death‡
							Chemo	HT				
MRISC												
1	37	*PTEN*	98	Tis	2	N-	No	No	No	Yes, invasive (T2N+)	Bone	No
2	37	*BRCA1*	20	T1b	3	N-	No	No	No	Yes, simultaneously	Bone, liver	Yes
3	57	*BRCA1*	103	T1c	2	N+	No	Yes	No	No	Lung, bone	Yes
4§	58	*BRCA1*	20	T2	3	N-	Yes	No	No	No	Brain	Yes
5	36	*BRCA2*	57	T1c	3	N+	Yes	Yes	No	No	Bone, liver	Yes
6	44	*BRCA2*	56	T1a	2	N-	No	No	Yes, T1bN-	No	Bone	Yes
7	52	Familial	53	T1c	2	N+	Yes	Yes	No	No	Lung, pericardium	Yes
8	55	Familial	35	T1b	2	N-	No	No	No	No	Lung	Yes
Controls												
1	27	*BRCA1*	23	T1c	3	N+	Yes	No	No	No	Lung, liver, bone	Yes
2	29	*BRCA1*	16	T2	3	N+	Yes	No	Yes, T1cN+	No	Lung, liver	Yes
3	36	*BRCA1*	1	T4	3	N+	Yes, Neo	Yes	No	No	Bone	Yes
4	37	*BRCA1*	31	T2	3	N+	Yes	No	No	Yes	Lung, kidney	Yes
5	40	*BRCA1*	52	T2	3	N-	No	No	No	No	Lung	Yes
6	40	*BRCA1*	20	T2	3	N+	Yes	Yes	No	No	Lung	Yes
7	44	*BRCA1*	41	T2	2	N+	Yes	Yes	No	No	Bone	Yes
8	45	*BRCA1*	42	T1c	2	N-	No	No	Yes, Tis	Yes	Bone, liver	Yes
9	48	*BRCA1*	78	T1c	3	N-	Yes	No	Yes, T1bN-	Yes	Lung, distant lymph node	Yes
10‖	36	*BRCA2*	0	T1c	Unknown	-	Yes	Yes	No	No	Bone, liver	Yes
11	38	*BRCA2*	70	T3	2	N+	Yes	Yes	No	No	Lung, liver	Yes

Table 2 Patient, tumor, and treatment characteristics of patients with breast cancer specific distant metastasis; MRISC patients and matched controls* (continued)

ID	Age at diagnosis (years)	Risk group	MFS (months)	pT†	Grade	pN†	Therapy primary tumor		Contralateral breast cancer	Local recurrence prior to distant metastasis	Site of distant metastasis	Death‡
							Chemo	HT				
12	42	*BRCA2*	101	T2	3	N+	Yes, Neo	Yes	Yes, T1cN+	No	Lung, liver	Yes
13¶	31	Familial	45	Tis	3	N+	Yes	Yes	No	No	Liver	Yes
14	39	Familial	60	T2	2	N-	No	No	No	No	Lung, liver, bone	Yes
15	40	Familial	79	T1c	1	N+	Yes	No	No	No	Soft tissue	No
16	44	Familial	15	T4	3	N+	Yes, Neo	Yes	No	No	Bone, liver	Yes
17	44	Familial	1	T1c	2	N+	Yes	Yes	No	No	Bone	Yes
18	54	Familial	13	T2	3	N+	Yes	No	No	No	Lung, liver, bone	Yes
19	54	Familial	23	T2	3	N+	Yes	Yes	No	Yes	Lung	Yes
20	55	Familial	0	T3	1	N+	Yes, Neo	Yes	No	No	Bone	No
21	55	Familial	10	T2	3	N+	Yes	No	No	Yes	Lung	Yes

*MRISC patients were screened with annual mammography and MRI. Controls received no screening below age 50, or were screened with biennial mammography in the Dutch national breast cancer screening program from age 50 onwards. Controls were matched on risk category, age of diagnosis and year of diagnosis. ID= identification number, HT=hormonal therapy, MFS= breast cancer specific distant metastasis, MRISC=MRI Screening Study, Neo= neo-adjuvant chemotherapy N-=node negative, N+=node positive and/or micro metastasis, pN=pathology nodal category, pT=pathology primary tumor size, Tis=Ductal carcinoma in situ, T1a≤5 mm, T1ub=6-10 mm, T1c=11-20 mm, T2=20-50 mm, T3 >50 mm, T4=tumor with extension to chest wall, or the skin.

†In case of neo-adjuvant chemotherapy the clinical tumor and clinical node category was used.

‡Breast cancer was cause of death for all deceased patients.

§Breast cancer was not screen-detected, but interval cancer.

||Did not receive breast conserving therapy or mastectomy, nor sentinel node procedure or axillary lymph dissection, since she presented with symptoms of breast cancer metastases.

¶Ductal carcinoma in situ pathologically confirmed throughout the whole breast, and micro metastasis in the lymph node, however invasive component not demonstrated.

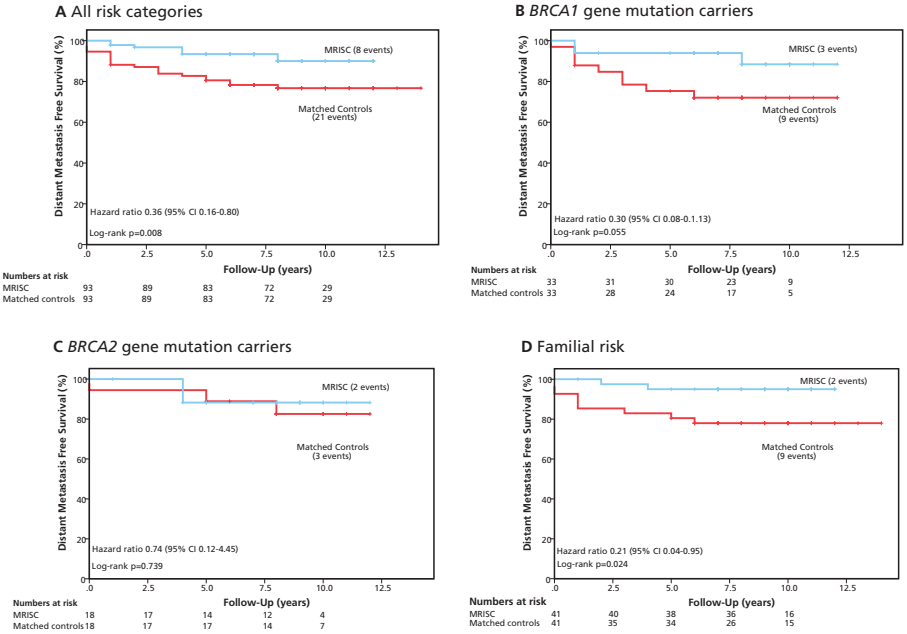

Figure 1 Distant metastasis free survival of breast cancer patients in the MRISC compared to matched controls per risk category.
MRISC patients were screened with annual mammography and MRI. Controls received no screening if younger than 50 years, or were screened with biennial mammography in the Dutch national breast cancer screening program if 50 years or older. Controls were matched on risk category, age of diagnosis and year of diagnosis. Distant metastasis free survival was defined as time from histological diagnosis until breast cancer specific distant metastasis. Differences in breast cancer specific distant metastasis free survival were compared by means of a log-rank test. The unadjusted hazard ratio for breast cancer specific distant metastasis of the MRISC patients in comparison to the controls is shown. CI= Confidence Interval, MRISC= MRI Screening Study.

deceased MRISC patients died of breast cancer, in comparison to 95% (19/20) of controls (p=0.023). Overall survival was better in MRISC patients, though not statistically significant (log-rank p=0.064, HR 0.51, 95% CI 0.24-1.06) (**Figure 3**).

DISCUSSION

This study reports survival data, with a potential follow-up of 10 years, and a median follow-up of nine years, of the largest prospective MRI screening study to date: MRISC.[11] By matching breast cancer patients that were screened with annual MRI and mammography in the MRISC with controls from the same risk group

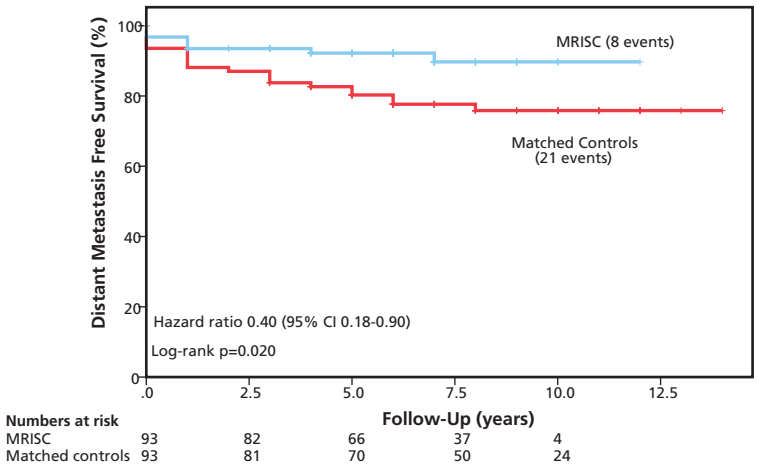

Figure 2 Distant metastasis free survival of breast cancer patients in the MRISC compared to matched controls per risk category, corrected for lead time bias.
MRISC patients were screened with annual mammography and MRI. Controls received no screening if younger than 50 years, or were screened with biennial mammography in the Dutch national breast cancer screening program if 50 years or older. Controls were matched on risk category, age of diagnosis and year of diagnosis. Distant metastasis free survival was defined as time from histological diagnosis until breast cancer specific distant metastasis. Lead time bias was corrected, by subtracting lead time as estimated by a micro simulation analysis model (MISCAN) from the metastasis free survival time of patients with screen-detected breast cancers (*BRCA1*: 1 year, *BRCA2*: 3 years, familial risk: 4 years, national breast cancer screening program: 3 years). Differences in breast cancer specific distant metastasis free survival were compared by means of a log-rank test. The unadjusted hazard ratio for breast cancer specific distant metastasis of the MRISC patients in comparison to the controls is shown. CI= Confidence Interval, MRISC= MRI Screening Study.

(unscreened if <50 years, screened with biennial mammography if ≥50 years), we showed that screening with annual mammography and MRI improves breast cancer metastasis free survival for women with a genetic or familial predisposition. MRISC patients were almost three times less likely to develop metastases compared to controls. This difference in hazard ratio was even more pronounced for women with familial risk (HR 0.21), and was also seen in *BRCA1* gene mutation carriers when analyzed separately, but borderline significant (log-rank p=0.055). The difference in MFS for *BRCA2* gene mutation carriers was non-significant, but their numbers were small and therefore conclusions for this group separately cannot be drawn. Overall survival was better in MRISC patients, though due to small numbers of events not statistically significant. However, there was a clear trend in favor of MRISC patients, and cause of death was significantly more often breast cancer in controls (p=0.023).

Supplementary Table 1 Distant metastasis free survival of breast cancer patients in the MRISC compared to matched controls per risk category, sensitivity analyses of lead time bias correction only in screen-detected MRISC breast cancer patients*

Mean lead time assumed	Log-rank P value	Hazard Ratio	95% Confidence Interval
1 year	0.011	0.37	0.16-0.83
2 years	0.017	0.39	0.17-0.88
3 years	0.025	0.41	0.18-0.93

*MRISC patients were screened with annual mammography and MRI. Controls received no screening if younger than 50 years, or were screened with biennial mammography in the Dutch national breast cancer screening program if 50 years or older. Controls were matched on risk category, age of diagnosis and year of diagnosis. Distant metastasis free survival was defined as time from histological diagnosis until breast cancer specific distant metastasis. To correct for lead time bias a sensitivity analysis was performed by subtracting different lead times (1, 2, or 3 years) from the metastasis free survival time of MRISC patients with screen-detected breast cancers. Differences in breast cancer specific distant metastasis free survival were compared by means of a log-rank test. The unadjusted hazard ratio for breast cancer specific distant metastasis of the MRISC patients in comparison to the controls is shown. CI= Confidence Interval, MRISC= MRI Screening Study.

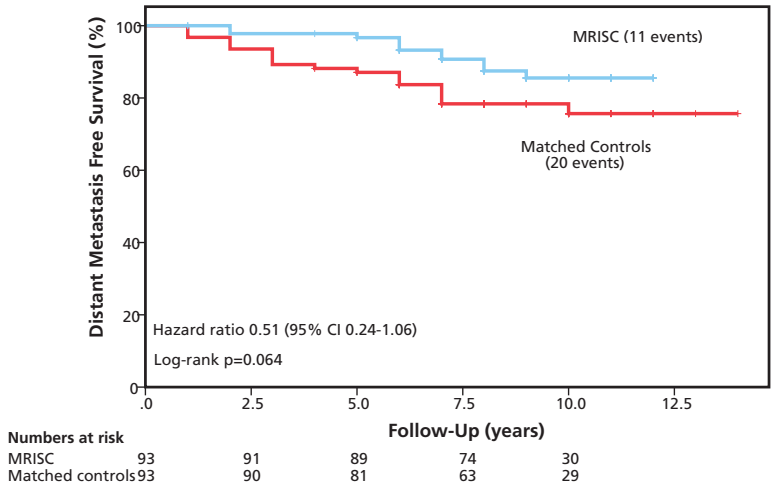

Figure 3 Overall survival of breast cancer patients in the MRISC compared to matched controls. MRISC patients were screened with annual mammography and MRI. Controls received no screening if younger than 50 years, or were screened with biennial mammography in the Dutch national breast cancer screening program if 50 years or older. Controls were matched on risk category, age of diagnosis and year of diagnosis. Overall survival was defined as time from histological diagnosis until death. Differences in overall survival were compared by means of a log-rank test. The unadjusted hazard ratio for overall survival of the MRISC patients in comparison to the controls is shown. CI= Confidence Interval, MRISC= MRI Screening Study.

There was a significant difference in breast cancer treatment between MRISC patients and controls. MRISC patients had undergone mastectomy more often. Most likely because *BRCA1* and *BRCA2* gene mutation carriers with breast cancer regularly opt for mastectomy and simultaneous preventive contralateral mastectomy.[22] In our control group *BRCA1* and *BRCA2* gene mutation carriers were not aware of their mutation status at the time of diagnosis. However, breast conserving therapy is equally safe as mastectomy, also in *BRCA1/2* mutation carriers.[23]

Importantly, since Dutch guidelines do not advise systemic therapy for very favorable tumor stages at detection,[1] there were three times more controls who received hormonal therapy ($p<0.001$) and twice as many who received chemotherapy ($p<0.001$) than MRISC patients. Systemic therapy improves breast cancer survival,[24] also in *BRCA1/2* mutation carriers,[25] but the fact that controls received more systemic therapy could not compensate sufficiently for their poorer tumor stage. PBSO, the oldest form of hormonal therapy, improves breast cancer survival in *BRCA1/2* mutation carriers.[23] Coincidentally, in our study 2 out of 6 *BRCA1/2* mutation carriers with prior PBSO developed distant breast cancer metastases. Influence of PBSO cannot be shown in our MRISC patients.

Two smaller studies presented survival data of their high-risk MRI screening trial; the Canadian study presented survival data of 28 *BRCA1/2* mutation carriers, without a familial risk group or controls. They describe a single metastasis (4%) in a median follow-up time of 8 years,[26] slightly lower than our distant breast cancer metastases rate of 9% in *BRCA1* mutation carriers and 11% in *BRCA2* mutation carriers, though in range considering the small numbers in that study.

Very recently the multicentre UK high-risk MRI-screening study MARIBS published survival results combined with a more recent single-centre MRI study.[27] Their conclusion is also that early detection by screening improves survival. However patients were not matched, and groups were not comparable regarding age at diagnosis, year of diagnosis, and median follow-up time.[27] There was no lead time bias correction, nor information regarding (adjuvant) therapy.[27] Combining the UK and Dutch results suggests that intensive screening improves survival in all 3 risk-groups.

Recent results with more MRI experience and newer MRI techniques, show higher MRI sensitivity compared to the earlier results of the MRISC, therefore these and future survival results may be better.[28,29] Furthermore, some MRI sensitivity results published in early prospective studies were slightly higher than those of MRISC.[4,6] The favorable results of our study are also supported by a smaller study that demonstrated comparable survival in a cohort *BRCA1/2* mutation carriers screened with annual MRI and mammography compared to a cohort choosing prophylactic bilateral mastectomy.[28] By modeling Kurian et al. predicted a comparable result as our study.[30] Our numbers of MRISC patients detected $\geq$50 years are unfortunately too small for a valid separate analysis of this group and the controls screened with biennial mammography.

Our study does have limitations. Due to the non-randomized design some effects of bias could be present. The most important screening related biases are: lead time bias, length bias, overdiagnosis, and selection bias.[18] The exact lead time in high risk patients is unknown; with the well validated MISCAN model adjusted to this group we sought an optimal correction. Also after correction for this bias MFS remained significantly better in MRISC patients compared to controls. Furthermore, the majority of breast cancer metastases are detected within the first 5 years of diagnosis,[31,32] with a peak in the second year after diagnosis,[33] and our median follow-up is twice as long. Even if there would be lead time bias, after such a long follow-up, the amount of breast cancer metastases in the MRISC patients should be comparable to the matched controls, though the detection peak would be later. However, after this long follow-up period there were 8 (9%) metastases detected in MRISC patients versus 21 (23%) in controls (p=0.009).

As for length bias, which involves screening detecting tumors with a more favorable prognosis e.g. slower growing,[18] we countered this bias by also including all interval cancers in our analyses.

Overdiagnosis is an often mentioned disadvantage of screening. It involves screening detecting breast cancers that would never become symptomatic, and are unlikely to cause the patient's death.[18] Though this is seen in population screening, which usually involves postmenopausal older women, it is unlikely to be a problem in our population of mainly young women, and *BRCA1/2* mutation carriers with very fast growing tumors.[34]

Finally selection bias, which implies that screening attracts healthier women with a higher socio-economic status, who might have a better prognosis.[18] Our control group was not aware of their mutation; they were possibly from families with few women or a milder familial penetrance and therefore did not attend a screening program. Moreover, controls aged above 50 at diagnosis all did participate in the Dutch national breast cancer screening program, making selection bias in this specific group unlikely.

In conclusion, our study is the first to show with actual survival data that screening with annual MRI and mammography improves breast cancer specific metastasis free survival substantially for women with a *BRCA1* mutation or familial risk.

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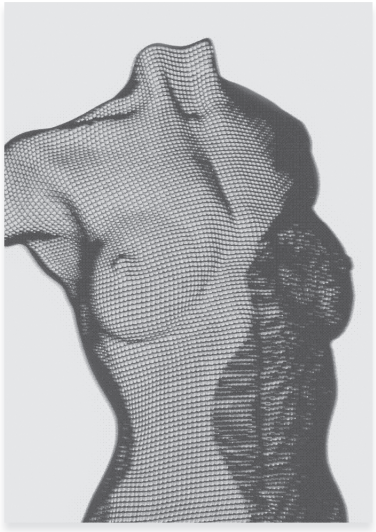
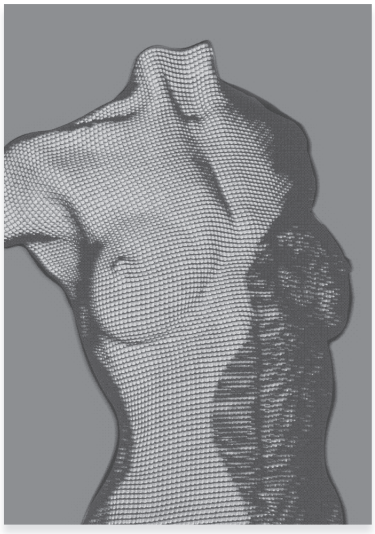
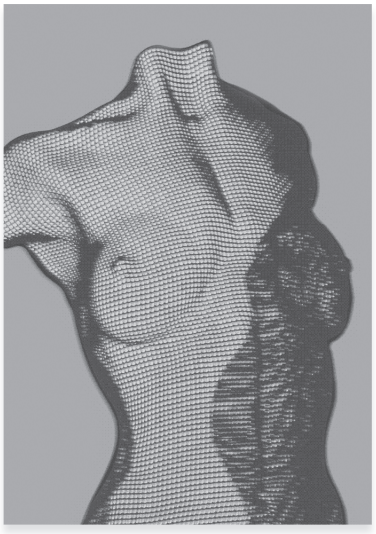

Chapter 4

Cost-effectiveness of screening women with familial risk for breast cancer with Magnetic Resonance Imaging

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ABSTRACT

Background

To reduce mortality, women with a family history of breast cancer are often screened with mammography before age 50 years. Additional magnetic resonance imaging (MRI) improves sensitivity and is cost-effective for *BRCA1/2* mutation carriers. However, for women with a family history without a proven mutation, cost-effectiveness is unclear.

Methods

We evaluated data of the largest prospective MRI screening study (MRISC). Between 1999 and 2007, 1597 women (8370 woman years at risk) aged 25 to 70 years with an estimated cumulative lifetime risk of 15% to 50% for breast cancer were screened with clinical breast examination every 6 months and with annual mammography and MRI. We calculated the cost per detected and treated breast cancer. After incorporating MRISC data into a micro simulation screening analysis model (MISCAN), different schemes were evaluated, and cost per life-year gained (LYG) was estimated in comparison with the Dutch nationwide breast cancer screening program (biennial mammography from age 50 to 75 years). All statistical tests were two-sided.

Results

Forty-seven breast cancers (9 ductal carcinoma in situ) were detected. Screening with additional MRI costs $123,672 (€93,639) per detected breast cancer. In increasing age-cohorts, costs per detected and treated breast cancer decreased, but, unexpectedly, the percentage of MRI-only detected cancers increased. Screening under the MRISC-scheme from age 35 to 50 years was estimated to reduce breast cancer mortality by 25% at $134,932 (€102,164) per LYG (3.5% discounting) compared with 17% mortality reduction at $54,665 (€41,390) per LYG with mammography only.

Conclusions

Screening with MRI may improve survival for women with familial risk for breast cancer but is expensive, especially in the youngest age categories.

INTRODUCTION

Breast cancer is the leading cause of cancer death in women worldwide, accounting for 14% of cancer deaths in 2008, mainly from metastatic disease, rather than the primary tumor.[1] The risk of metastases in sporadic and familial breast-cancer is related to both the size of the breast cancer at detection and the number of axillary lymph nodes involved.[2] It is therefore crucial to diagnose breast cancer in an early stage of disease. With that aim population breast cancer screening programs for women aged 50-69 years have been incorporated in several countries and they have reduced the death rate from breast cancer.[3,4]

Approximately 15-20% of all female breast cancers occur in women with a family history of breast cancer, in whom no causative hereditary gene mutation has been found.[5] These women have a greater risk of developing breast cancer and at a younger age than in the general population.[6] Therefore, to reduce mortality, these women are often offered annual screening with mammography before they are 50 years old.[7,8] However, mammography may not be the ideal screening method for all young women. Breast density is high in about 50% of women between 40 and 49 years, whereas only 20-44% of women in their 60s have dense breast tissue.[9,10] Increased breast density lowers sensitivity of mammography; moreover, it increases the risk of breast cancer, independent of other factors.[11,12]

Adding annual Magnetic Resonance Imaging (MRI) to mammography screening strongly increases sensitivity of detection.[13-15] Yearly screening with MRI is cost-effective for women aged 30-60 years with a *BRCA1* or *BRCA2* gene mutation or women who have a 50% chance of being a carrier.[16-18] For these women, MRI screening is advised by the American Cancer Society (ACS), the American College of Radiologists (ACR), the United Kingdom's National Institute for Health and Care Excellence (NICE) guideline and the European guideline of the European Society of breast imaging (EUSOBI).[19-22] For women with a family history without a proven genetic predisposition, guidelines are equivocal.[19,22,23]

To the best of our knowledge, no study has been published assessing cost-effectiveness of MRI specifically for these women. We evaluate cost-effectiveness of additional MRI for women with a familial risk in the largest prospective study: the MRI Screening Study (MRISC)[15] to date.

METHODS

Patient characteristics

The MRISC was a Dutch multi-centered trial comparing efficacy of mammography with MRI for surveillance of women with a hereditary predisposition for breast cancer from November 1, 1999 to August 1, 2007.[15] Methods and results have been described previously.[15] Included were women aged 25-70 years with a familial or

genetic predisposition for breast cancer, whose cumulative lifetime risk (CLTR) according to the modified tables of Claus et al.[24,25] was ≥15% and who gave written informed consent. Women with evident symptoms or a medical history of breast cancer were excluded.[15]

Participants were subdivided into the following risk categories on the basis of their estimated CLTR: carriers of the *BRCA1*, *BRCA2*, *P53* or *PTEN* mutation or a 50% likelihood of such a mutation (CLTR ≥50%), a high risk group (estimated CLTR 30-50%) and a moderate risk group (estimated CLTR 15-30%). All patients underwent clinical breast examination (CBE) every six months, and annual mammography and MRI. True cancer status was ascertained by pathology. Of the 1597 participants in the moderate and high risk group, the following data were used: number of diagnosed breast cancers, number of interval breast cancer cases, age distribution of the study population, and age at cancer diagnosis, tumor characteristics and attendance rates at successive rounds of the MRISC study. Median follow-up was 6.4 years.

Statistical Methods

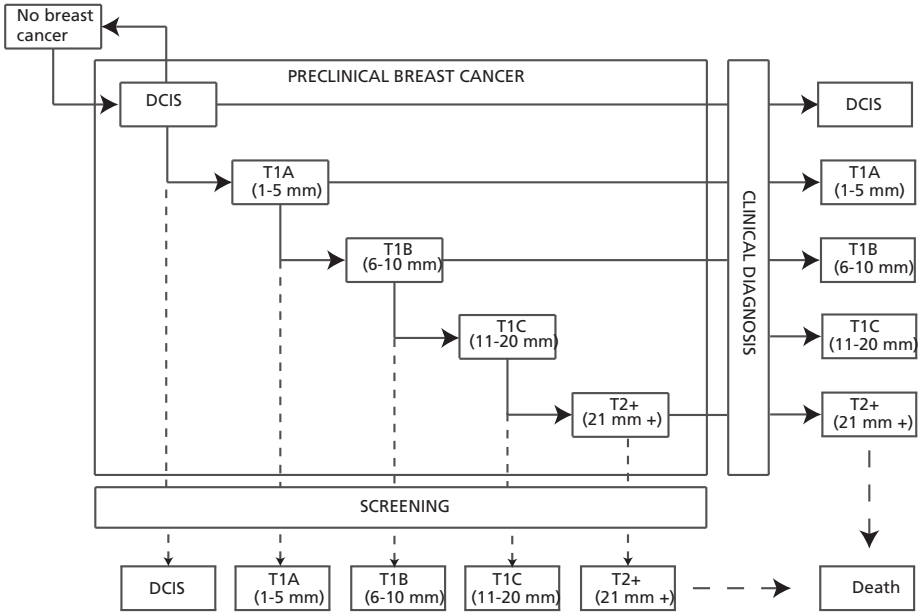
Breast cancer incidence rates were calculated as the total number of breast cancers detected (including DCIS) per 1000 woman years at risk. Differences between the high risk group and the moderate risk group in incidence rates, stage distribution, lymph node status and tumor characteristics were calculated using χ^2 tests or Fisher's exact tests, as appropriate, differences in median age with the Mann-Whitney *U* test. A two-sided P value ≤0.05 was considered statistically significant. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS), version 17.0.2 (Chicago, US). All statistical tests were two-sided.

Actual cost calculation

We calculated the cost per detected breast cancer by dividing the total costs of the MRISC screening program by the number of cancers detected. We also calculated the cost per detected and treated breast cancer by dividing the total costs of screening, diagnosis and treatment of all carcinomas found in the program by the number of cancers detected. Additionally, screening, diagnosis and treatment costs per detected breast cancer by age category were calculated.

Micro simulation screening analysis (MISCAN)

MISCAN was used to simulate the MRISC trial and evaluate the cost-effectiveness of different screening strategies for women with a family history of breast cancer. MISCAN is a well-validated micro simulation model, originally developed to estimate the cost-effectiveness of the population-based screening program in the Netherlands.[3,4,26] In MISCAN the natural history of breast cancer is modeled as a progression through 5 pre-clinical and invasive disease stages (**Supplementary figure 1**). At each pre-clinical stage, a tumor may either be clinically diagnosed or grow into the next pre-clinical stage. Screening may detect the tumor in a preclini-



Supplementary figure 1 The structure of the MISCAN model.
The natural history of breast cancer is modelled as a progression through the successive disease stages DCIS, T1A, T1B, T1C and T2+. A certain percentage of the modelled population develops pre-clinical disease. In each pre-clinical stage, a tumour may become clinically diagnosed or grow into the next pre-clinical stage. When a screening programme is applied, the pre-clinical tumour may also be detected by screening. Transition probabilities, durations of the various stages and survival after diagnosis, are governed by a series of age and stage dependent parameters.

cal stage. Survival after clinical diagnosis or screen detection is based on data of the Dutch nation-wide screening program.[3,27] The improvement of prognosis after detection by screening is based on the long-term effects of Swedish trials.[28-30] A detailed description of the model has been published.[31]

Calibration and application of MISCAN

We developed the MISCAN family history risk model by using the number of women with a family history in the MRISC, age distribution at entry of the study, duration of follow-up and screening protocol, attendance and sensitivity of different screening methods as inputs. Average screening attendance in the MRISC, 90%, was used in the model. Test sensitivities, dependent on age, stage and screening method were also estimated from the study.[15] Stage-specific sensitivities of clinical breast examination (CBE) in women 55 years or older were based on the Canadian National Breast Screening study.[26] For women under 50 years old, CBE sensitivity was assumed to be 50% of the sensitivity for women >55 years. For women aged

50-55 years, all test sensitivities were linearly interpolated. Estimated values for sensitivities are shown in **Supplementary table 1**.

The model was calibrated using the MRISC number of screens, number of screen detected cancers and interval cancers, cancer stage distribution and age at diagnosis. Likelihood ratio tests were used to compare the goodness of fit. Parameters for the lifetime incidence, onset of disease, duration of the stages and stage transition probabilities were estimated by minimizing the difference between observed and predicted counts, measured as a sum of the χ^2 quantities using the simplex method of Nelder and Mead.[32] Model parameters are shown in **Supplementary table 2**.

Using the calibrated model, predictions of number of screens, number of screen

Supplementary table 1 Estimated sensitivity of CBE, MAM and MRI by stage and age

	Age < 50 years			Age > 55 years*		
Stage	**CBE**	**Mx**	**MRI**	**CBE**	**Mx**	**MRI**
DCIS	0.027	0.360	0.360	0.053	0.720	0.360
T1A (≤ 5 mm)	0.004	0.101	0.778	0.008	0.470	0.975
T1B (6-10 mm)	0.079	0.140	0.778	0.158	0.620	0.975
T1C (11 - 20 mm)	0.239	0.450	0.810	0.478	0.900	0.975
T2+ (> 20 mm)	0.325	0.515	0.810	0.649	0.980	0.975

*For women aged 50-55 years, sensitivities were linearly interpolated. CBE= clinical breast examination, Mx= mammography, MRI= magnetic resonance imaging.

detected and interval cancers, stage distribution, mortality reduction and life years gained were made for the following screening protocols:

(1) Yearly mammography combined with CBE (**Figure 1B**)
(2) CBE every six months, and yearly mammography and MRI combined (MRISC) (**Figure 1E**)
(3) Yearly MRI, 6 months later mammography combined with CBE (**Figure 1D**)
(4) Yearly CBE combined with alternating mammography or MRI (**Figure 1C**)

All screening protocols were run in the model starting screening at age 35 years. Separate runs were done ending screening at 50 years and 60 years. Additional runs were done for the most cost-effective MRI screening schedule starting screening at 40 years and ending screening at 50 years. All runs included screening by biennial mammography in the national breast cancer screening program until age 75 years, after the end of the screening protocol.[23]

A cohort of 5 million women, born in 1975 was simulated. All costs and effects were predicted for a life-time follow-up. Results are presented per 1000 women aged 35 years in 2010. Cost-effectiveness ratios are expressed as cost per life year gained (LYG). Mortality reduction, life years gained and costs per life year gained are estimated in comparison to the Dutch national breast cancer screening program, which consists of biennial mammography from the age 50 to 75 years

Supplementary table 2 Model parameters on natural history of breast cancer and survival after screening in MISCAN*

Onset probability*				**0.462**					
Cumulative onset									
Age									
20	0.0007								
40	0.006								
60	0.030								
80	0.16								
Mean duration (years) of screen-detectable preclinical phases by age									
Age	DCIS	T1AN-	T1AN+	T1BN-	T1BN+	T1CN-	T1CN+	T2+N-	T2+N+
40	3.1	0.0	0.0	1.6	1.6	4.7	4.7	5.0	5.0
50	3.1	0.0	0.0	2.2	2.2	6.4	6.4	6.7	6.7
60	3.1	0.0	0.0	3.1	3.1	9.2	9.2	9.7	9.7
Long term relative survival by clinical stage and age									
Age	DCIS	T1AN-	T1AN+	T1BN-	T1BN+	T1CN-	T1CN+	T2+N-	T2+N+
40	1.00	0.876	0.721	0.838	0.648	0.753	0.499	0.568	0.254
50	1.00	0.887	0.743	0.852	0.674	0.773	0.530	0.598	0.283
60	1.00	0.874	0.717	0.836	0.643	0.749	0.490	0.561	0.240
Reduction in risk of dying from breast cancer by age and stage after screen detection at age 50									
	N-	N+							
T1A	0.887	0.743							
T1B	0.852	0.674							
T1C	0.773	0.530							
T2+	0.598	0.283							

*The onset probability is the probability that a woman develops breast cancer in a situation without screening. In the model, the decision whether a woman will eventually have the onset of breast cancer is made at birth. It is possible that before onset of breast cancer the woman dies of other causes and therefore the life-time risk is lower.

(**Figure 1A**).[23] Incremental cost-effectiveness ratios that compared two alternative screening programs were calculated by dividing the difference in total net costs and the difference in life years gained between two alternative screening policies. Policies that were estimated to be both more expensive and less effective were referred to as dominated, and no incremental cost-effectiveness ratios were calculated. Incremental cost-effectiveness ratios were expressed as additional cost per additional LYG. In order to weigh costs and health gains in relation to the time at which they occur, costs and effects were discounted at an annual rate of 3.5%.[33]

A CLTR of 16% was used as an age specific incidence function; sensitivity analyses of the most MRI cost-effective strategy were performed increasing CLTR to 26%. Further sensitivity analyses were performed by varying key model parameters, i.e., test sensitivity and costs of MRI and mammography.

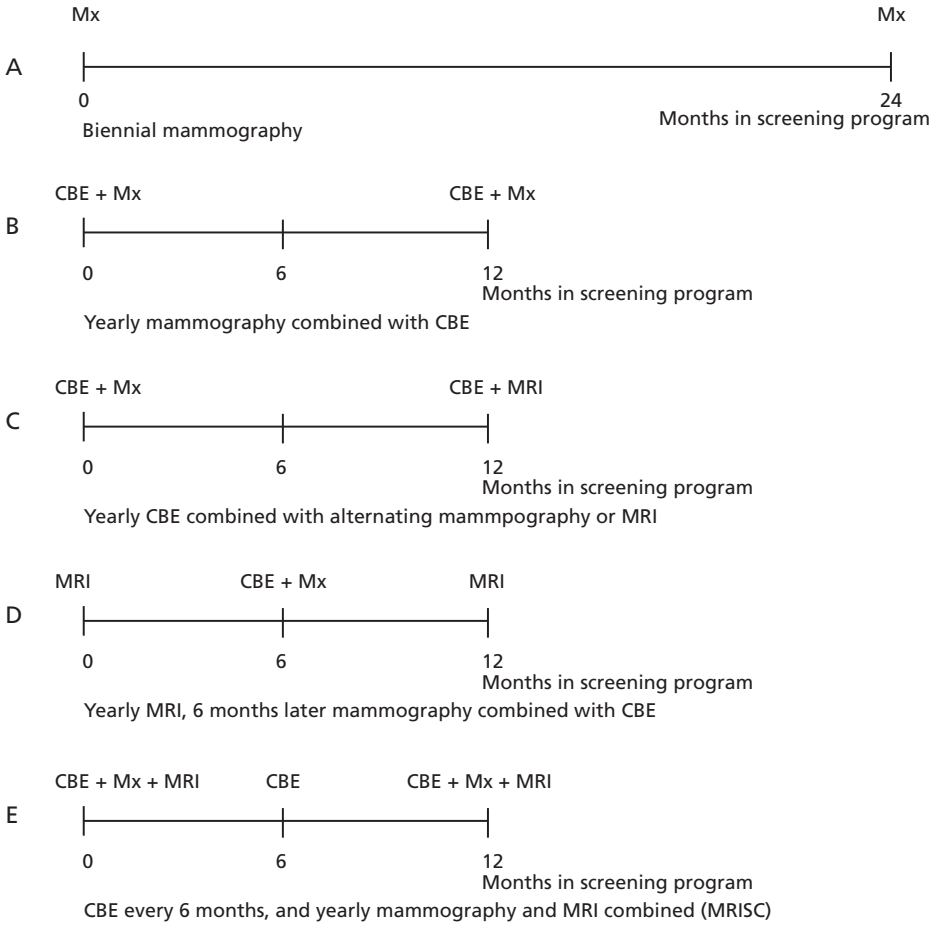


Figure 1 Screening protocols modeled in the micro simulation screening analysis.
A) Mammography (Mx) is performed every 2 years. B) Mammography and clinical breast examination (CBE) is performed every year. C) The first year mammography and clinical breast examination are performed; the second year magnetic resonance imaging (MRI) and clinical breast examination are performed. D) MRI is performed, followed by mammography and clinical breast examination 6 months later. E) MRI, mammography, and clinical breast examination are performed; after 6 months clinical breast examination is performed.

Costs

Costs of screening and additional investigations of each participant were taken into account using the information in the MRISC. Additional investigations were defined as all diagnostic tests conducted due to an 'uncertain' or 'suspicious' screening test and included; ultrasonography with or without fine-needle aspiration, histological biopsy, and repeating mammography or MRI.[15] Costs were calculated in Euros (€) (converted to US dollars ($) for publication). The number of

Supplementary table 3 Cost of treatment per cancer stage in USD, treatment according to current standards and with 30% HER2 amplification assumed*

	Number of tumors	Surgery	Localization	Radiotherapy	Systemic treatment	Total	Mean cost/tumor
DCIS	9	10,001 (7,572)	1,042 (789)	43,263 (32,757)	0	54,306 (41,117)	6,034 (4,569)
T1a, N-	5	6,295 (4,766)	520 (394)	17,306 (13,103)	4,491 (3,400) (1 patient <35 years)	28,611 (21,663)	5,722 (4,333)
T1b, N-	11	1,1681 (8,844)	1,215 (920)	60,568 (45,859)	0	73,463 (55,623)	6,678 (5,057)
T1c, N-	10	10,639 (8,055)	1,042 (789)	51,916 (39,308)	83,616 (63,310)	147,212 (111,462)	14,721 (11,146)
T2, N-	1	967 (732)	173 (131)	8,652 (6,551)	4,491 (3,400)	14,282 (10,815)	14,282 (10,815)
T1a, N+	0	0	0	0	0	0	21,267 (16,103)‡
T1b, N+	1	1,181 (894)	0	0	7,726 (5,850)	8,907 (6,744)	8,907 (6,744)
T1c, N+	5	7,964 (6,030)	347 (263)	34,610 (26,205)	94,578 (71,610)	137,499 (104,108)	27,500 (20,822)
T2, N+	4	6,626 (5,017)	0	17,306 (13,103)	55,643 (42,130)	79,574 (60,250)	19,894 (15,063)
Total†	47	56,244 (42,585)	4,514 (3,417)	24,2272 (183,437)	255,034 (193,100)	558,064 (422,540)	117,947 (89,304)

*All costs mentioned are in 2013 USD with Euros in parentheses. DCIS= ductal carcinoma in situ, HER2= human epidermal growth Factor 2, N= nodal status.

†Miss size of 1 tumor, but treatment data were available.

‡No T1a, N+ in MRISC: breast conserving therapy/mastectomy rate assumed comparable to T1a, N-, 80% assumed hormone positive.

investigations performed in the study was used for calculation of the screening and diagnostic costs (**Supplementary table 3**).

Costs of systemic treatment were based on current national guidelines.[23] HER2 status was not routinely determined during the whole period of the MRISC study. For calibration of MISCAN, we assumed that 30% of patients receiving systemic therapy would be HER2 positive and according to current guidelines, should receive trastuzumab.[34,35] Equipment, staff, and treatment costs were based on current prices provided by the Erasmus Medical Center-Daniel Den Hoed cancer center, Rotterdam, the Netherlands. At this center an MRI costs $484.71 (€367). For mammography, costs were averaged with costs of two other Dutch centers: the Academic Medical Center, Amsterdam, and the University Medical Center Sint Radboud, Nijmegen. A standard mammographic study, two views (oblique and craniocaudal) of each breast, costs on average $136.04 (€103). The unit cost of a mammography performed in the national breast cancer screening program is $78.86 (€59.71). Further costs are presented in **Supplementary table 4**.

Supplementary table 4 Unit costs of screening, diagnosis and treatment procedures

Procedure	Cost, USD (€)*
Screening & Diagnosis	
Consult including clinical breast examination	91.20 (69.05)
Mammography	136.34 (103.23)
Mammography (national breast cancer screening program)	78.86 (59.71)
Magnetic Resonance Imaging (MRI)	485.49 (367.59)
Ultrasound	101.80 (77.08)
Fine Needle Aspiration	187.19 (141.73)
Biopsy	232.26 (175.86)
Localization & Surgery	
Wire-guided localization of malignant lesion	173.62 (131.46)
Lumpectomy	891.70 (675.15)
Lumpectomy including sentinel node biopsy	966.65 (731.90)
Mastectomy	1106.41 (837.72)
Mastectomy including sentinel node biopsy	1181.36 (894.47)
Lymph node dissection	1112.17 (842.08)
Modified radical mastectomy	1444.35 (1093.59)
Radiotherapy & Chemotherapy	
Radiotherapy	8652.56 (6551.31)
Chemotherapy	4490.51 (3400.00)
Chemotherapy followed by 1 year trastuzumab	32463.72 (24580.00)
5 years of hormonal therapy	3235.81 (2450.00)

*All costs mentioned are in 2013 USD with Euros in parentheses.

RESULTS

Tumor characteristics

Forty-seven breast cancer cases, of which 9 were DCIS (19%), were diagnosed in 8370 woman years screened. Two (4%) of the breast cancers were interval carcinomas. **Table 1** lists incidence rates per age category and risk category, and tumor characteristics of breast cancer cases according to risk category. Incidence rates increase with age. No breast cancer was diagnosed in women aged <30 years. The incidence rate in the moderate risk group (estimated CLTR 15-30%) was not lower than in the high risk group (estimated CLTR 30-50%). Nor was there a statistically significant difference between the tumor stage and characteristics in the high compared to moderate risk group; therefore cost-effectiveness calculations were assumed similar in both groups and a subdivision was no longer made.

Actual costs

The cost per detected cancer by screening with CBE, mammography and MRI was $123,672 (€93,639). **Table 2** describes costs per detected cancer by age category, separately for screening, diagnosis, treatment and overall costs. Since there were no tumors detected in the group of women aged <30 years, total overall costs instead of costs per detected cancer are depicted.

After adding treatment costs, the cost per detected and treated cancer was $133,760 (€101,277). The costs per detected and treated cancer decreased in increasing age categories, most likely due to the higher cancer incidence rate in the older age categories (**Figure 2**). However, the percentage MRI-only detected cancers increased in older age-cohorts.

Cost-effectiveness of different screening strategies

After calibration the model predicted distribution of cancers detected per age category (**Supplementary figure 2**) and stage distribution quite well (**Supplementary figure 3**). Using the model, we estimated the effects and costs of screening women with a CLTR for breast cancer between 15-50%, with biennial mammography screening from ages 50-75 years, the protocol of the current Dutch nation-wide breast cancer screening program, compared to no screening. This would lead to an estimated mortality reduction of 37% at a cost per LYG of $14,922 (€11,298) (costs per LYG are 3.5% discounted). An additional mortality reduction of 17% at a cost per LYG of $54,665 (€41,390) can be gained if women are also screened with annual mammography and CBE from the age 35 to 50 years, according to guidelines for women with high familial risk.

Screening under the MRISC scheme (CBE every 6 months and annual mammography and MRI from age 35 to 50 years followed by biennial mammography until 75 years) would lead to a mortality reduction of 25%, at a cost per LYG of $134,932

Table 1 Observed breast cancer cases, overall cumulative incidence rates and tumor characteristics in the MRISC study for CLTR categories: 30-50% and 15-30%

Patient & tumor characteristics	CLTR of 30-50%		CLTR of 15-30%		Overall		P value
Number of women at entry	1089		508		1597		
Woman years at risk	5608		2762		8370		
Diagnosed breast cancers	28		19		47		
Screen detected	27	(96%)	18	(95%)	45	(96%)	
Interval	1	(4%)	1	(5%)	2	(4%)	1.000
Age at diagnosis in years, median (range)	50	(34-65)	46	(31-61)	48	(31-65)	0.193
Incidence Rates (per 1000 woman years) [CI]	5.0	[3.36-7.21]	6.9	[4.1-10.7]	5.6	[4.1-7.5]	0.278
<30 years	0.0	[0.0-13.2]	0.0	[0.0-21.0]	0.0	[0.0-8.1]	
30-39years	2.9	[0.9-6.7]	2.2	[0.3-7.8]	2.6	[1.1-5.4]	
40-49 years	4.2	[1.9-7.9]	11.9	[6.1-20.7]	6.6	[4.1-10.1]	
50-59 years	9.2	[4.6-16.4]	7.0	[1.9-17.9]	8.5	[4.7-14.0]	
≥60 years	12.1	[2.5-35.4]	13.7	[0.4-76.1]	12.5	[3.4-32.0]	
Cancer cases by tumor size†							0.211
DCIS	4	(15%)	5	(26%)	9	(19%)	
T1a/T1b (tumor ≤ 10 mm)	8	(30%)	9	(47%)	17	(37%)	
T1c (tumor 11-20 mm)	12	(44%)	3	(16%)	15	(33%)	
T2+ (tumor >20 mm)	3	(11%)	2	(11%)	5	(11%)	
Nodal status†‡							0.710
N- / isolated cells	16	(70%)	11	(79%)	27	(73%)	
N+ / micro metastasis (0.2-2.0 mm)	7	(30%)	3	(21%)	10	(27%)	
Tumor Characteristics†							
ER+	21	(87%)	11	(79%)	32	(84%)	0.153
ER-	3	(13%)	1	(7%)	4	(11%)	
Unknown	0	(0%)	2	(14%)	2	(5%)	
PR+	19	(79%)	11	(79%)	30	(79%)	0.107
PR-	5	(21%)	1	(7%)	6	(16%)	
Unknown	0	(0%)	2	(14%)	2	(5%)	
HER2 over expression	2	(8%)	0	(0%)	2	(5%)	0.340
HER2 no over expression	9	(38%)	8	(57%)	17	(45%)	
Unknown	13	(54%)	6	(43%)	19	(50%)	
Bloom & Richardson Grade§							0.727
Grade 1	11	(46%)	5	(42%)	16	(44%)	
Grade 2	12	(50%)	7	(58%)	19	(53%)	
Grade 3	1	(4%)	0	(0%)	1	(3%)	

*All percentages are calculated vertically. Two-sided P value for difference between two risk groups, differences in stage distribution, lymph node status and tumor characteristics were obtained from $\chi 2$ or Fisher's exact tests, as appropriate. Differences in median age were calculated from the Mann-Whitney *U* test. All statistical tests were two-sided. CI= confidence interval, CLTR= cumulative lifetime risk, DCIS= ductal carcinoma in situ, ER= estrogen receptor, HER2= human epidermal growth factor 2, MRISC= MRI Screening Study, N= nodal status, PR= progesterone receptor.

†Missing size of 1 tumor.

‡Except DCIS.

§Missing data of two patients.

Table 2 Carcinomas detected and costs per detected cancer by age category*

Age in years	Number of carcinomas	MRI-only† detected, number (%)		Woman years at risk	Screening costs, USD (€)	Additional investigation costs, USD (€)	Treatment costs, USD (€)	Total costs, USD (€)
<30	0	-		455	320,791 (242,888)‡	13,139 (9,948) ‡	0‡	333,930 (252,836)‡
30-39	7	1/5	(20)	2661	243,891 (184,663)	15,825 (11,982)	10,960 (8,298)	270,677 (204,944)
40-49	21	7/18	(39)	3167	99,772 (75,543)	8,018 (6,071)	8,613 (6,521)	116,403 (88,135)
50-59	15	6/14	(43)	1772	74,989 (56,778)	4,044 (3,062)	12,457 (9,432)	91,491 (69,273)
≥60	4	0/2	(0)	321	50,024 (37,876)	2,862 (2,167)	7,428 (5,624)	60,315 (45,668)
Total	47	14/39	(36)	8376	115,918 (87,768)	7,754 (5,871)	10,088 (7,638)	133,760 (101,277)

*All costs mentioned are in 2013 USD with Euros in parentheses. MRI= magnetic resonance imaging.
†Percentage of carcinomas or ductal carcinomas in situ detected by MRI, but not by mammography.
‡Since there were no tumors detected in this group, costs mentioned are total costs for the women screened in this group.

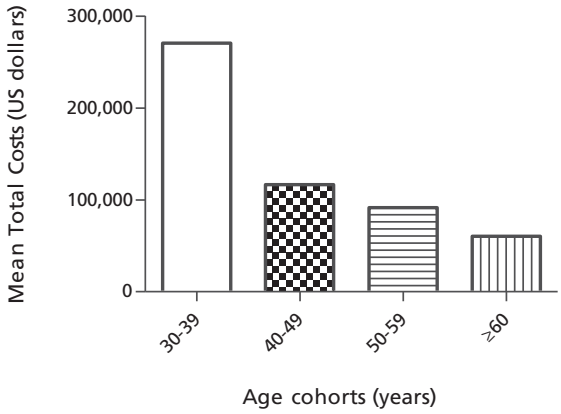
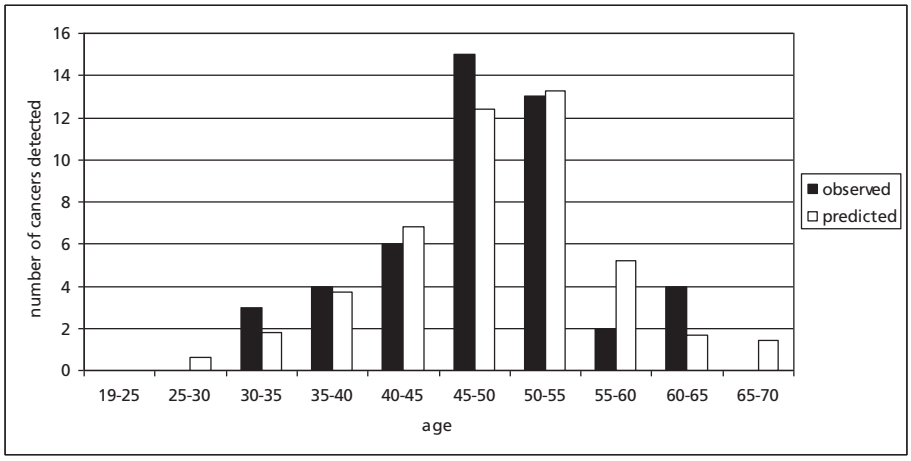
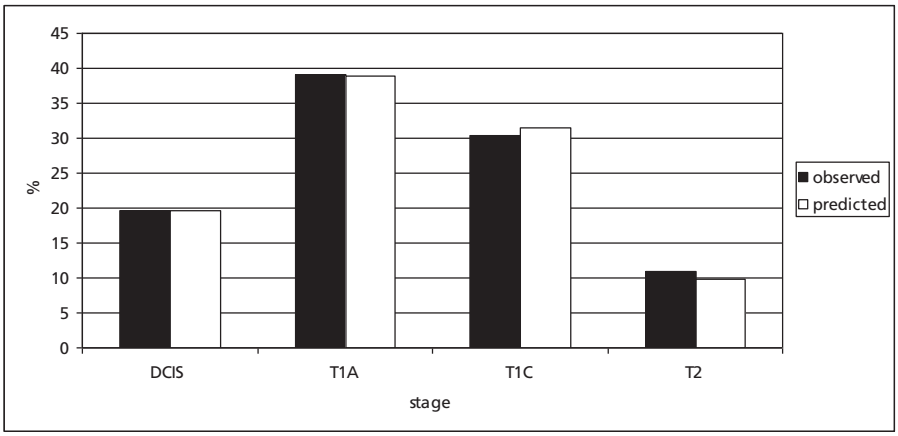


Figure 2 Costs of diagnosis and treatment per detected cancer by age.
Costs decreased in increasing age categories, most likely because of the lower cancer incidence rate in the younger age categories.



Supplementary figure 2 Observed (MRISC) and predicted (MISCAN) number of cancers detected by age category.
The number of cancers observed in the MRISC compared with the number of diagnoses predicted by the calibrated MISCAN model.



Supplementary figure 3 Observed (MRISC) and predicted (MISCAN) stage distribution.
The stage distribution of the total number of cancers diagnosed observed in the MRISC in percentages, compared with the percentages predicted by the calibrated MISCAN model, divided in DCIS, T1A/T1B (≤ 10 mm), T1C (10 - 20 mm), T2+ (> 20 mm).

(€102,164) compared to screening with biennial mammography from age 50 to 75 years.

Screening annually with MRI followed by mammography and CBE 6 months later is more efficient than screening under the MRISC protocol, as it also leads to an estimated mortality reduction of 25%, but decreases costs to $118,936 (€90,053) per LYG.

The most cost-effective MRI screening scheme consisted of alternating screening with mammography in year 1 and MRI in year 2. This gave an estimated mortality reduction of 21% at a cost per LYG of $79,654 (€60,310). When performing sensitivity analyses of this screening scheme, costs per LYG vary between $47,854 (€36,233) and $90,139 (€68,249) (**Supplementary table 5**). If the start of this scheme was postponed until 40 years of age, mortality reduction decreased to 18% at a cost per life year gained of $60,267 (€45,631). Further results, depicted per 1000 women screened, are presented in **Tables 3** and **4**.

DISCUSSION

We evaluated cost-effectiveness of adding yearly MRI to the screening schedule for women with a familial risk in the MRISC prospective study. The costs of diagnosis and treatment per detected cancer in our study were $133,760 (€101,277) over all age categories. Costs strongly and continuously decreased with increasing age, most likely because of the higher incidence rate in the older age categories. Although cost per detected and treated breast cancer was most expensive in the

Supplementary table 5 Sensitivity analyses of cost per life year gained of yearly screening with CBE combined with alternating MRI or Mx (ages 35-50 years), after which biennial Mx in the national breast cancer screening program*

Assumption	Cost per life year gained, USD (€)
a. Base model	79,654 (60,310)
Costs	
b. MRI €250	62,967 (47,676)
c. Mammography €59.71	73,399 (55,574)
Cumulative Lifetime Breast Cancer Risk	
d. 26%	47,854 (36,233)
e. 14%	90,139 (68,249)
Sensitivity of screening tools	
f. MRI: <50; DCIS: 0.720, T1A/B: 0.810; >55† DCIS: 0.720, T1A/B: 0.975	78,540 (59,467)
g. Mammography: <50; DCIS: 0.400, T1A/B: 0.400; >55† DCIS: 0.720, T1A/B: 0.620	77,766 (58,881)
h. Both MRI & mammography as in f. & g.	76,712 (58,083)

* In comparison with biennial mammography from the ages 50-75 years. All costs mentioned are in 2013 USD with Euros in parentheses. Both costs and effects 3.5% discounted. CBE= clinical breast examination, Mx= mammography, MRI= magnetic resonance imaging.
†For women aged 50-55 years, sensitivities are linearly interpolated.

youngest age categories, one would expect additional MRI also to be most useful, as breast density is more often high in premenopausal women,[9,10] which decreases sensitivity of mammography, but not of MRI.[11] Unexpectedly, the percentage of MRI-only detected cancers increased with rising age. MRI sensitivity was also higher in older women in a recent study,[36] but numbers are small and this needs confirmation in an independent series.

We show MRI screening to be expensive, especially in young women. Our results might seem difficult to translate to other practices because costs of mammography and MRI differ greatly between institutions and countries. However, by comparing the ratio of the MRI/mammography costs (approximately 3.5 in our study) a reasonable estimate can be made.

Estimated with MISCAN, the cost per LYG is approximately 2.5 times higher when MRI is added to annual screening with mammography and CBE, but estimated mortality reduction rises from 17% to 25%, caused by a shift in stage at detection of breast cancer. A less costly, quite effective alternative -alternating screening with mammography in year 1 and MRI in year 2- needs confirmation in clinical practice. Also, screening closer to the expected age at onset may reduce costs.[37]

We have used the largest database currently available to assess the cost-effectiveness of MRI screening in women with a familial risk for breast cancer without a proven genetic predisposition. Although studies have been published that analyze cost-effectiveness of MRI screening for women with hereditary risk, our study is

Table 3 Costs and effects of screening protocols for ages 35 to 50 years, after which biennial mammography until 75 years, compared with biennial mammography from 50-75 years*

Cancers and costs	Biennial Mx (50-75 years)	Annual Mx + CBE	Alternating: year 1: CBE+MRI/year 2: CBE + Mx	Screening twice a year: MRI /Mx+CBE	Screening twice a year: MX+MRI+CBE/CBE (MRISC)
Carcinomas	235	240	240	243	243
Invasive	206	203	203	203	203
DCIS	29	37	37	40	40
BC deaths	31	25	24	23	23
Mortality reduction†‡	-	17%	21%	25%	25%
Extra life years gained‡	-	139	166	196	195
Cost per LYG, USD (€)*‡	-	20,534 (15,547)	30,236 (22,893)	46,098 (34,903)	52,491 (39,744)
3.5% discounted					
Total costs, **USD (€)***	2,378,880 (1,801,176)	4,669,372 (3,535,429)	6,345,618 (4,804,604)	9,360,429 (7,087,277)	10,258,897 (7,767,555)
Cost per LYG, **USD (€)*‡**	-	54,665 (41,390)	79,654 (60,310)	118,936 (90,053)	134,932 (102,164)
Inc cost per LYG, **USD (€)*‡§**	-	54,665 (41,390)	212,183 (160,655)	338,743 (256,480)	Dominated‖

*Results are per 1000 women screened. Screening programs are in order of increasing total net costs. All costs mentioned are in 2013 USD with Euros in parentheses. BC= breast Cancer, CBE= clinical breast examination, Inc= incremental, LYG= life year gained, MRI= Magnetic Resonance Imaging, MRISC= MRI Screening Study, Mx= mammography.

†Percentages are computed using unrounded numbers.

‡In comparison with biennial mammography from the ages 50-75 years.

§Incremental cost per life year gained represents the marginal cost per life year gained when a more expensive screening program (higher net costs) is compared with a less expensive program.

‖A program is dominated if another program is more effective at the same or lower costs.

Table 4 Costs and effects of screening protocols ages 35 to 60 years, after which biennial mammography until 75 years, compared with biennial mammography from 50-75 years*

Cancers and costs	Biennial Mx (50-75 years)	Annual Mx+CBE	Alternating: year 1: CBE+MRI/year 2: CBE + Mx	Screening twice a year: MRI /Mx+CBE	Screening twice a year: MX+MRI+CBE/CBE (MRISC)
Carcinomas	235	243	242	246	246
Invasive	206	203	203	203	203
DCIS	29	40	39	43	43
BC deaths	31	24	23	21	21
Mortality reduction†,‡	-	21%	25%	31%	30%
Extra life years gained‡	-	162	192	233	229
Cost per LYG, USD (€)*,‡	-	26,526 (20,084)	40,646 (30,775)	61,211 (46,346)	70,695 (53,527)
3.5% discounted					
Total costs, **USD (€)***	2,378,880 (1,801,176)	5,392,744 (4,083,132)	7,727,812 (5,851,136)	11,906,426 (9,014,986)	13,160,745 (9,964,698)
Cost per LYG, **USD (€)*,‡**	-	63,316 (47,940)	95,007 (71,935)	140,524 (106,398)	161,405(122,208)
Inc cost per LYG, **USD (€)*,‡,§**	-	63,316 (47,940)	268,399 (203,219)	363,357 (275,117)	DominatedII

*Results are per 1000 women screened. Screening programs are in order of increasing total net costs. All costs mentioned are in 2013 USD with Euros in parentheses. BC= breast Cancer, CBE= clinical breast examination, Inc= incremental, LYG= life year gained, MRI= Magnetic Resonance Imaging, MRISC= MRI Screening Study, Mx= mammography.

†Percentages are computed using unrounded numbers.

‡In comparison with biennial mammography from the ages 50-75 years.

§Incremental cost per life year gained represents the marginal cost per life year gained when a more expensive screening program (higher net costs) is compared with a less expensive program.

IIA program is dominated if another program is more effective at the same or lower costs.

the first to assess this specifically for non-*BRCA1/2* women.[16-18,38-40]. Furthermore, no studies taking costs of treatment into account have been published and none of the published studies assess costs per age category.

Our study differs from others performed in various aspects. Three studies[17,18,39] published data solely on *BRCA1/2* mutation carriers. The cost-effectiveness article of the MARIBS-study included, but did not analyze separately, women without a proven *BRCA1/2* mutation.[16] Their non-*BRCA1/2* group was small, with 12 invasive carcinomas detected in 419 women.[14] Furthermore, no treatment-related costs were taken into account and no cost per LYG was estimated. In two recent studies cost-effectiveness analyses were done on estimates based on published data of several prospective MRI screening cohort studies. However, because limited data were published on tumor sizes and probability of nodal involvement for interval tumors, essential data were missing in their analyses.[38,40]

Our study has some limitations. The estimated CLTR is based on modified tables of Claus,[24] which only take familial risk into account. Other models incorporate additional risk factors. However, risk estimates for the same woman vary greatly with different models.[41] Current risk models have wide confidence intervals, when estimating risk at the personal level and even in large groups as shown in the MRISC study. Therefore, the division into a moderate and high risk group in the MRISC does not seem justified.[41,42] This most likely explains our comparable incidence rate in the two risk groups. Second, survival data of patients with screen-detected tumors that are treated with new targeted therapies like trastuzumab are not yet available.[28-30] Furthermore, both increasing experience with MRI screening[43] and recent advances in MRI technology and methods may improve results. Still, since cost-effectiveness is largely determined by cancer incidence and basic screening costs, these effects are limited.

For future research women with a familial risk, who have high breast density, might be an interesting group. The results of a randomized controlled trial currently in progress may clarify cost-effectiveness for this group [44].

In conclusion screening with additional MRI is expensive, but it can improve survival for women with familial risk for breast cancer. Still, it may be more cost-effective in select groups.

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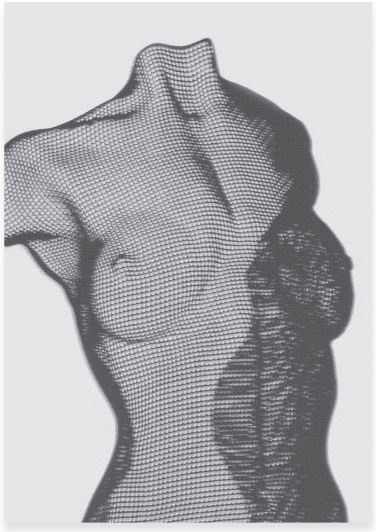
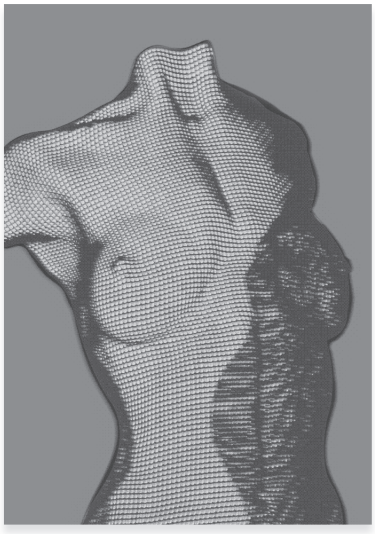
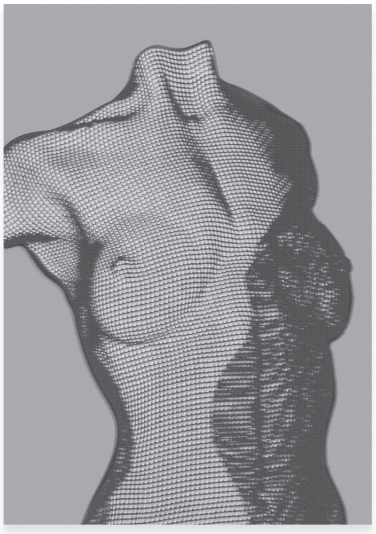

Chapter 5

Breast density as indicator for the use of mammography or MRI to screen women with familial risk for breast cancer (FaMRIsc): a multicenter randomized controlled trial

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ABSTRACT

Background

To reduce mortality, women with a family history of breast cancer often start mammography screening at a younger age than the general population. Breast density is high in over 50% of women younger than 50 years. With high breast density, breast cancer incidence increases, but sensitivity of mammography decreases. Therefore, mammography might not be the optimal method for breast cancer screening in young women. Adding MRI increases sensitivity, but also the risk of false-positive results. The limitation of all previous MRI screening studies is that they do not contain a comparison group; all participants received both MRI and mammography. Therefore, we cannot empirically assess in which stage tumors would have been detected by either test. The aim of the Familial MRI Screening Study (FaMRIsc) is to compare the efficacy of MRI screening to mammography for women with a familial risk. Furthermore, we will assess the influence of breast density.

Methods

This Dutch multicentre, randomized controlled trial, with balanced randomization (1:1) has a parallel grouped design. Women with a cumulative lifetime risk for breast cancer due to their family history of ≥20%, aged 30-55 years are eligible. Identified *BRCA1/2* mutation carriers or women with 50% risk of carrying a mutation are excluded. Group 1 receives yearly mammography and clinical breast examination (n=1000), and group 2 yearly MRI and clinical breast examination, and mammography biennially (n=1000). Primary endpoints are the number and stage of the detected breast cancers in each arm. Secondary endpoints are the number of false-positive results in both screening arms. Furthermore, sensitivity and positive predictive value of both screening strategies will be assessed. Cost-effectiveness of both strategies will be assessed. Analyses will also be performed with mammographic density as stratification factor.

Discussion

Personalized breast cancer screening might optimize mortality reduction with less over diagnosis. Breast density may be a key discriminator for selecting the optimal screening strategy for women <55 years with familial breast cancer risk; mammography or MRI. These issues are addressed in the FaMRIsc study including high risk women due to a familial predisposition.

Trial Registration: Netherland Trial Register NTR2789

INTRODUCTION

A positive family history is one of the most important risk factors for breast cancer.[1] Women with a family history of breast cancer are not only at greater risk of developing breast cancer, but their risk also increases at a younger age than in the general population.[2] In over 75% of the families that display clear clustering of breast cancer no causative gene mutation like *BRCA1* or *BRCA2* can be detected.[3] Tumor stage at detection is of key influence on survival.[4] Aiming at early detection and ultimately to reduce mortality risk, these women, with a positive family history for breast cancer, are often offered annual screening with mammography before age 50.[5-7] However, screening also causes false-positive test results.

In the last decade several screening trials in high-risk women have been completed and Magnetic Resonance Imaging (MRI) had a significantly higher sensitivity for invasive breast cancer than mammography in all studies.[8-12] However, MRI was expensive and was associated with significantly more false-positive results in most studies. Furthermore, mammography had better sensitivity for the pre-invasive stage of breast cancer: ductal carcinoma in situ (DCIS).[13] Therefore, mammography should perhaps not be omitted completely when MRI screening is offered.

Despite the higher costs of MRI and the false-positive results, screening with yearly MRI in addition to mammography is considered cost-effective for female *BRCA1* and *BRCA2* gene mutation carriers aged 30-60 years or women who have a 50% chance of carrying such a mutation.[14-16] For women with a familial risk, from families without a proven genetic predisposition, results are inconclusive.[17,18] Since previous screening studies have performed MRI and mammography simultaneously the difference in stage of the tumors when detected by mammography alone is not known. A randomized controlled trial is therefore needed.

Apart from a positive family history and age, high breast tissue density is a well documented risk factor for breast cancer. Breast density increases breast cancer incidence significantly.[19,20] At the same time, high mammographic density greatly impairs the sensitivity of mammography,[19-22] but far less the sensitivity of MRI.[23] The lower sensitivity of mammography in dense breasts is most likely caused by a masking effect, rather than by a higher tumor growth rate in denser tissue.[21,24] Breast density is high or very-high in about 50-74% of women between 40 to 49 years of age, whereas only 20-44% of women in their 60s have dense or extremely dense breast tissue.[25,26] This dual effect of breast density on cancer incidence and sensitivity of mammography results in women with the highest risk being screened with a tool with limited effectiveness: mammography.

To the best of our knowledge, no study has been published assessing the cost-effectiveness of MRI specifically in women with a familial risk for breast cancer, without a known genetic predisposition. Therefore, guidelines for breast cancer screening for women with a familial risk vary widely internationally and are weakly underpinned. The 2008 American Cancer Society and 2010 American College of

Radiologists guidelines advise MRI screening for women with a familial cumulative lifetime risk (CLTR) > 20%,[18] while the Dutch guidelines advise screening with mammography only.[17]

Robust cost-effectiveness analyses cannot be based on the published studies, as all had a paired design (i.e. all participants received both mammography and MRI). These studies cannot examine the improvement in tumor stage at diagnosis, as one cannot know in what stage the tumor would have been diagnosed by either test alone. A randomized controlled trial is needed for a valid answer to these questions.

Furthermore, cost-effectiveness of either imaging technique may vary across categories of mammographic density. Breast density has not yet been evaluated as a parameter to identify sub-groups of women with a familial risk, for whom MRI is cost-effective. A prospective randomized trial in women with increased breast cancer risk, taking breast density into account, will give robust evidence on which screening tool, MRI or mammography, is best suited for a particular woman. These issues are addressed in the Familial MRI Screening study (FaMRIsc).

METHODS

Trial design

The FaMRIsc study is a multicentre, randomized controlled trial (RCT), with balanced randomization (1:1), and a parallel group design conducted in the Netherlands. The study is in compliance with the Helsinki declaration and ethical approval has been granted on 8 November 2010 by the Institutional Review Board of the Erasmus University Medical Centre, Rotterdam, the Netherlands (reference-number: MEC-2010-292).

Participants

Eligible participants are women aged 30-55 years with a cumulative lifetime risk (CLTR) of >20% because of a familial predisposition according to the modified tables of Claus[1,27] or as assessed at a Clinical Genetics Centre. *BRCA1* and/or *BRCA2* mutation carriers or women with a 50% likelihood of such a mutation are excluded, since MRI screening is already advised for these women by the American Cancer Society (ACS), the American College of Radiologists (ACR), the United Kingdom's NICE guideline and the European guideline of the European Society of breast imaging (EUSOBI).[18,28-30] Exclusion criteria are previous invasive cancer (potentially of influence on survival data), and a contraindication for contrast-enhanced MRI (decreased creatinin clearance, metal implants or claustrophobia).

Study settings

Participants are recruited from outpatient breast or family cancer clinics at all eight academic medical centers in the Netherlands and the Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital (**Supplementary table 1**). Women who are already in a screening program because of an increased familial risk and meeting inclusion criteria are sent study information 2 weeks before a scheduled visit. Women who meet all criteria and visit the outpatient clinic for an initial screening are given information on site.

Supplementary table 1 Academic Medical Centers participating in the FaMRIsc in the Netherlands

University Medical Centre, Utrecht
Academic Medical Hospital, Maastricht
Radboud University, Nijmegen
University Medical Centre, Groningen
VU University Medical Centre, Amsterdam

Interventions

After informed consent is obtained participants are randomized through a computer-generated randomization sequence with stratification for centre, in one of the two groups. Group 1 receives screening according to the 2012 Dutch guidelines[17] with yearly mammography and clinical breast examination (CBE). Group 2 is screened with yearly MRI and CBE, and mammography biennially (**Figure 1**). Additional investigations are performed if deemed necessary due to findings at clinical examination, on mammography or MRI. Mammography still has a place in both arms, since DCIS is generally easier to detect with mammography,[8,10,31-33] although in one study MRI was found to detect more aggressive grade III DCIS than mammography.[34] In the intervention arm however, the frequency of mammography is reduced from annually to biennially. DCIS not detected by MRI will most likely be low-grade, progress slowly, and be detected by the next mammographic examination. Leaving out mammography every other year seems safe in the MRI arm and may prevent over diagnosis of low-grade DCIS. Mammographic examination is done using full field digital mammography (FFDM). All examinations are scored in a standardized way, according to the Breast Imaging Reporting and Data System (BI-RADS) mammography classification of the American College of Radiology.[30] To determine mammographic density an automated breast density measurement is done on raw data of the first FFDM of all participants.[35,36] Dynamic breast MRI with gadolinium-containing contrast medium is performed according to standard protocol. In premenopausal women, the MRI is performed between day 5 and 20 of the menstrual cycle.[37]

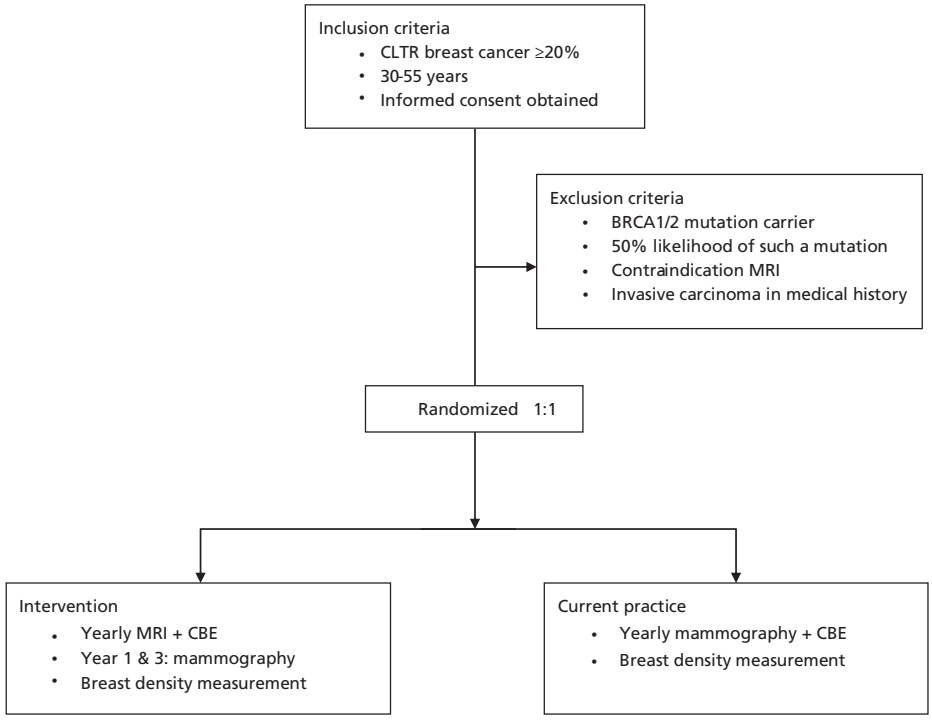

Figure 1 Flow diagram Familial MRI Screening Study (FaMRIsc).
CLTR: Cumulative Lifetime Risk, MRI: Magnetic Resonance Imaging, CBE: Clinical Breast Examination.

Outcomes

Primary endpoints are the number and stage of detected breast cancers, both DCIS and invasive, in each arm. Secondary endpoints are the false-positive results in the screening arms, and the sensitivity and positive predictive value of both screening strategies. Furthermore, cost-effectiveness and breast cancer mortality reduction of both strategies will be assessed. All analyzes will also be performed stratified for mammographic density.

A positive screening test is defined as a mammographic or MRI examination with a BI-RADS score of 0, 3, 4, or 5 and/or a clinical breast examination classified as 'suspicious'. Interval cancers are defined as tumors diagnosed after a negative screening examination but before the next scheduled screening examination. Sensitivity is calculated as the number of screen-detected tumors divided by the total of screen-detected and interval tumors. The positive predictive value of a screening strategy is calculated as the proportion of women with a positive screening test, which after pathology indeed proved to be breast cancer.

Prospective assessment of mortality reduction requires a very lengthy follow-up and a large study population, which may not be feasible. To study this issue we will start with a less costly and time-consuming approach and estimate mortality reduction through a micro simulation model: MISCAN, a well-validated micro simulation model, originally developed to estimate the cost-effectiveness of the population-based screening program in the Netherlands.[38-40] In the model, the natural history of breast cancer is modeled as a progression through 5 pre-clinical and invasive disease stages. At each pre-clinical stage, a tumor may either be clinically diagnosed or grow into the next pre-clinical stage. Screening may detect the tumor in a preclinical stage. Transition probabilities, stage durations and survival after clinical diagnosis or screen detection are based on data from the Dutch nation-wide screening program.[41,42] The improvement of prognosis after detection by screening is based on the long-term effects of Swedish trials.[43-45] A detailed description of the model has been published previously.[39]

We will develop a family history risk model by using the number of women enrolled in the study, the age distribution at entry of the study, the duration of follow-up and the screening protocol, attendance and sensitivity of different screenings methods as inputs. The model will be calibrated using the number of screens, the number of screen detected cancers and interval cancers, the stage distribution and the age at diagnosis. Likelihood ratio tests will be used to compare the goodness of fit. Using the calibrated model, predictions of the number of screens, number of screen detected and interval cancers, the stage distribution, the mortality reduction and the life years gained will be made for the different screening arms in the study.

A cohort of 5 million women will be simulated. All costs and effects will be predicted for a life-time follow-up. The costs will be presented in European currency (€). Cost-effectiveness ratios will be expressed as cost per life year gained (LYG). Costs and effects will be discounted at an annual rate of 3.5%.

Sample size: power calculation

Our primary aim is to detect a difference in tumor stage between the intervention and the current practice group. In the Dutch MRI Screening Study (MRISC) study, conducted from 1999 to 2007, over 1500 women with familial risk were included in the 6 participating centers.[11] The incidence rate in this risk group was 7/1000 women years screened. Since the FaMRIsc study will have three more participating centers we intend to include 2000 women. We expect to detect about 50 breast cancers (both DCIS and invasive) in 4 years. With this number we are able to detect a difference in tumor size of 8 mm (SD tumor size: 9 mm) as statistically significant (two sided alpha =0.05) with a power of 80%. Eight mm is considered to be a clinically relevant difference.

Stopping guidelines

The accrual will be evaluated after two years. If adequate inclusion numbers cannot be achieved, appropriate measures will be taken in the remaining two years, consisting of expansion of the number of participating centers or longer continuation of the study.

Statistical Methods

Primary outcome will be incidence and the difference in mean tumor size at diagnosis between the two arms. If normally distributed, this will be tested by means of the independent samples (unpaired) t-test. If not normally distributed, medians will be estimated and differences between distributions will be tested with the non-parametric Mann-Whitney *U* test. Breast cancer incidence rates will be calculated as the total number of breast cancers detected per 1000 woman years at risk. This will be calculated both including and excluding DCIS. Differences between these proportions will be compared using a χ^2 test or Fisher's exact test as appropriate. All tests will also be performed stratified by mammographic density to examine the influence of density on the efficacy of MRI screening versus usual care. The influence of breast density on detection rates, tumor stage and false positive results in both arms will be analyzed by means of analysis of variance (ANOVA).

DISCUSSION

Twenty-five percent of all breast cancers occur before age 50 and especially familial breast cancer is seen at younger ages.[2] A randomized controlled trial can provide the best evidence for any breast cancer mortality reduction attributable to digital mammography or MRI screening in this population.

Studies that offered MRI and mammography screening simultaneously have several shortcomings due to their paired design. Sensitivity of neither test without the other can be assessed. Nor the stage in which either test separately would have detected tumors.

With the results of our study we will be able to estimate the mortality reduction for screening women with familial risk with either digital mammography or additional MRI. Furthermore, we will be able to assess whether mortality reduction by earlier detection differs with increasing breast density between screening with digital mammography or with additional MRI.

Breast density may be a key discriminator for choosing the optimal screening strategy below age 50 years for women with familial risk. If we can assess this, personalized cancer screening can be offered, based on a woman's age, risk and breast density. This may optimize mortality reduction, whilst possibly decreasing

over diagnosis. Compliance to screening will be best if there is convincing evidence that the most effective tool with the lowest side-effects is offered.

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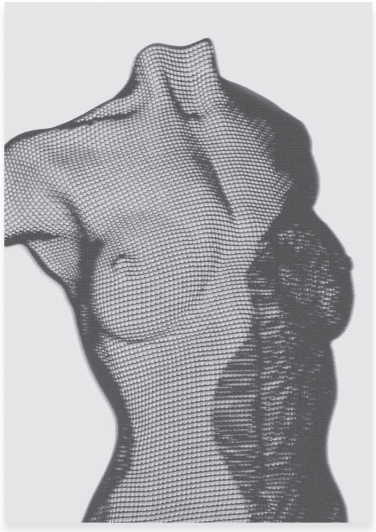
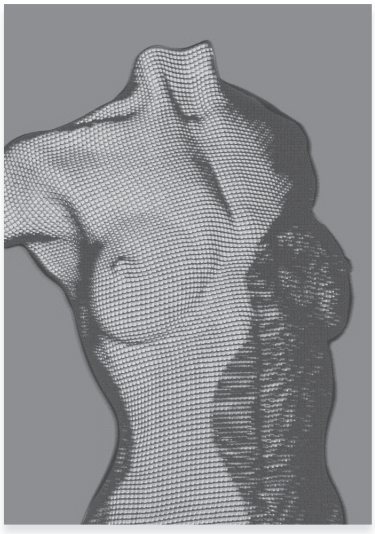
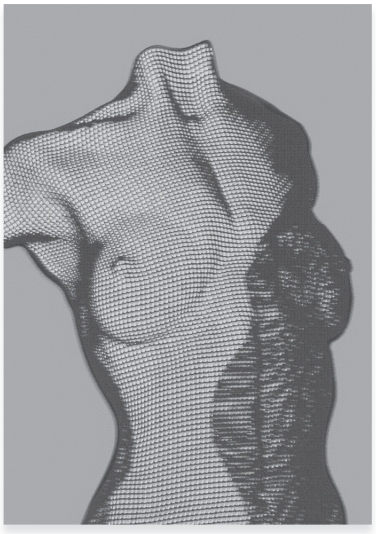

Chapter 6

Quantifying the additional value of mammography to MRI screening in *BRCA1* and *BRCA2* mutation carriers by *BRCA* status and age; an individual patient data meta-analysis

Phi XA, Saadatmand S, de Bock GH, Warner E, Sardanelli F, Leach MO, Riedl CC, Trop I, Hooning MJ, Mandel R, Santoro F, Kwan-Lim G, Helbich TH, Tilanus-Linthorst MM, van den Heuvel ER, Houssami N

Submitted

ABSTRACT

Background

Breast screening sensitivity in *BRCA1/2* mutation carriers is higher for MRI than for mammography, and mammography has the disadvantage of potential radiation harm. This study investigated the additional contribution of mammography to screening accuracy in *BRCA1/2* mutation carriers screened with MRI at different ages using individual patient data (IPD) meta-analysis.

Methods

IPD were assembled and pooled from six high-risk screening studies. Characteristics of *BRCA1/2* mutation carriers and their breast cancers were analyzed. Sensitivity and specificity of MRI, mammography and the combination of these tests were compared stratified for *BRCA* mutation and age using generalized linear mixed models with random effect for studies. Number of screens needed for additional mammography-only detected cancer was estimated.

Results

In *BRCA1/2* mutation carriers of all ages, adding mammography to MRI did not statistically significantly increase screening sensitivity (increased by 3.9% in *BRCA1* and 12.6% in *BRCA2* mutation carriers, $p>0.05$). Mammography in *BRCA1* mutation carriers detected three DCIS and two invasive tumors (not detected by MRI) from a total of 112 breast cancers (4.5%). Mammography in *BRCA2* mutation carriers detected four DCIS and seven invasive tumors (not detected by MRI) from 72 cancers (15.3%). However in women with *BRCA2* mutation younger than 40 years around one-third of breast cancers were detected by mammography only. Number of screens needed for mammography to detect one breast cancer not detected by MRI was much higher for *BRCA1* than *BRCA2* mutation carriers at initial and repeat screening.

Conclusion

The additional screening sensitivity from mammography above that from MRI is limited in *BRCA1* mutation carriers, whereas mammography contributes to screening sensitivity in *BRCA2* mutation carriers especially those $\leq$40 years. The evidence from our work highlights that a differential screening schedule by *BRCA* status is worth considering.

INTRODUCTION

Women with a *BRCA1* or *BRCA2* mutation have limited choices to prevent mortality resulting from their 40-80% lifetime risk for breast cancer.[1] Screening with yearly MRI from age 25 years on, and additional mammography from age 30 years is recommended in international guidelines,[2-5] and is estimated to be slightly less effective than preventive mastectomy.[6,7] Several prospective high-risk screening studies have included both MRI and mammography,[8,9] given that randomized controlled trials have shown that mammography reduces breast cancer mortality in population screening[10] whereas there have been no randomized controlled trials for MRI.

The addition of mammography to MRI screening of *BRCA1/2* carriers in most guidelines, from the age of 30 or 40 years,[2-5] is based on several arguments. First, mammography is expected to be sensitive in fatty breasts (generally in older women) but is less sensitive in young women who are most likely to have denser breasts. Nonetheless a meta-analysis, using Individual Patient Data (IPD) from six large MRI screening studies in *BRCA1/2* mutation carriers, showed sensitivity of MRI was superior to that of mammography both under and above 50 years.[11] Second, screening with mammography could lead to the induction of breast cancer by x-rays at younger ages.[12] Proper repair of DNA double-strand breaks that are caused by low dose x-rays is impaired at any age in both *BRCA1* and *BRCA2* mutations carriers.[13] This makes *BRCA1* and *BRCA2* mutation carriers more susceptible than non-carriers, possibly also at older ages, to the cumulative effect of yearly mammograms. Given these potential disadvantages of mammography, it is important to balance the advantages and disadvantages of mammography screening in *BRCA1/2* mutation carriers. Substantial early breast cancer detection by mammography screening is needed to outweigh the disadvantages of possible breast cancer induction[12] in *BRCA1/2* mutation carriers of all ages.

We meta-analyzed IPD from six prospective MRI screening studies to determine if mammography screening in *BRCA1/2* mutation carriers in addition to MRI improves screening accuracy, and whether this effect differs between *BRCA1* and *BRCA2* gene mutation carriers or in different age groups.

METHODS

An IPD meta-analysis was conducted by pooling individual data from relevant prospective MRI screening studies.[11] Studies were eligible if mammography and MRI breast cancer sensitivity and specificity were compared in women with a *BRCA1/2* mutation. After searching PubMed, twelve studies met eligibility requirements and were sought to contribute data.[11] Six of these provided IPD data,[14-19] and were included in this meta-analysis (the reasons for non-inclusion of some studies have

been reported in our earlier work).[11] All the included studies were assessed in terms of reporting quality, and were qualified as high quality.[11] The data were assembled and cross-checked with the original publications. Inclusion criteria for analyses were women with a *BRCA1/2* mutation, screened annually with both mammography and MRI. Breast cancer diagnosis was confirmed by pathology and absence of breast cancer at one year follow-up.[11]

Primary outcome and definition

Primary outcome was sensitivity and specificity of mammography and MRI separately, as well as combined. Analyses were stratified for mutation type (*BRCA1* or *BRCA2*) and age in years at screening (40 and younger, between 41 and 50, over 50). Sensitivity was defined as the number of breast cancers detected by a screening modality (MRI or mammography or the combination) out of the total number of breast cancers diagnosed during the study course. Specificity of a screening modality was defined as the number of true negative out of the total number of true negative plus false positive results. A true positive was defined as a positive screening result (BI-RADS 0,3,4,5) followed by a pathology-proven breast cancer. A false positive was defined as a positive screening result (BI-RADS 0, 3, 4, 5) not followed by a pathology-proven breast cancer within 1 year of follow-up. A true negative was defined as a negative screening result (BI-RADS 1, 2) not followed by pathology-proven breast cancer within 1 year of follow-up. A false negative case was defined as a negative screening result (BI-RADS 1, 2) followed by a pathology-proven breast cancer within one year of follow-up.

Statistical analysis

Descriptive statistics of women and breast cancer characteristics were performed. Breast cancer incidence was calculated per 1,000 woman years. The related 95% confidence intervals were computed based on a Poisson distribution. Related 95% confidence intervals were presented between square brackets. Differences in breast cancer incidence and interval cancer incidence were compared using z-tests. Differences in proportion of ductal carcinoma in situ (DCIS), invasive tumor size and grade were compared between two groups using χ^2 tests or Fishers' exact tests. All analyses were also performed stratified for *BRCA* mutation and age groups. The numbers of screens needed (NSN) for mammography to detect one breast cancer missed by MRI were calculated, and stratified according to *BRCA* mutation, age-group, and screening round (first or subsequent rounds) strata.

Sensitivity and specificity of MRI alone, mammography alone and the combination of mammography and MRI were estimated using generalized linear mixed models. For the three age groups separate models were used to estimate sensitivity and specificity stratified by type of *BRCA* mutation. Screening modality, *BRCA* status, and their interaction were introduced as a fixed effect in the model. The repeated screening results were summarized to form binomial counts for each

woman: the number of true positive, the number of true negative, the number of total screening visits with or without breast cancer detected. We modeled the counts with a Binomial distribution conditionally on the random effects for studies using two separate models for sensitivity and specificity. All the analyses were performed using SAS 9.4. P values <0.05 were considered statistically significant.

RESULTS

Study population and breast cancer characteristics

The analyses were based on 1951 *BRCA1/2* mutation carriers with 6085 woman years of follow-up (**Table 1**). There was no significant difference in cancer incidence between *BRCA1* mutation carriers and *BRCA2* mutation carriers. Five breast cancers were diagnosed before the age of 30 in *BRCA1* mutation carriers, and none in *BRCA2* mutation carriers. The proportion of DCIS differed between *BRCA* groups in all age groups (**Table 1**) as follows: ≤40 years 19.6% for *BRCA1* versus 16.7% for *BRCA2* (p=1.00), 41-50 years 8.1% for *BRCA1* versus 35.1% in *BRCA2* (p=0.010), and >50 years 10.3% for *BRCA1* versus 35.3% for *BRCA2* (p=0.058).

Sensitivity and specificity in *BRCA1* mutation carriers

In *BRCA1* mutation carriers, there were no statistically significant differences in sensitivity and specificity between mammography and MRI combined compared to MRI alone. Sensitivity of the combination was higher than that of MRI alone in all age groups (age ≤40: 86.8% [63.1-96.2] versus 77.5% [57-90], p=0.441; age 41-50: 94.1% [74.5-98.9] versus 93.1% [70.8-98.7], p=0.895; age >50: 89.3% [71.3-96.6] versus 89.1% [54.8-98.2], p=0.986). Combining mammography and MRI decreased specificity compared to MRI screening alone in all age groups (age ≤40: 81% [73.9-86.5] versus 84.3% [78.7-88.7], p=0.409; age 41-50: 77.2% [70.5-82.8] versus 82.9% [77.9-87], p=0.135; age >50: 87.4% [79.3-92.6] versus 89.9% [82.6-94.3], p=0.566). Further results are shown in **Table 2**.

Sensitivity and specificity in *BRCA2* mutation carriers

In *BRCA2* mutation carriers, there were no significant differences in sensitivity or specificity between combined mammography and MRI and MRI alone in all the age groups. Sensitivity of the combination was higher than that of MRI alone in all age groups (age ≤40: 87.2% [56.1-97.3] versus 52.7% [27.2-76.8], p=0.075; age 41-50: 91.2% [70.4-97.9] versus 86.4% [58.2-96.7], p=0.646; age >50: 94.1% [67.5-99.2] versus 85% [43.7-97.7], p=0.474). Combining mammography and MRI decreased specificity compared to MRI screening alone in all age groups (age ≤40: 75.3% [66.6-82.4] versus 80.2% [72.9-85.8], p=0.351; age 41-50: 80% [73.3-85.3] versus 86% [81.1-89.8], p=0.105; age >50: 88.6% [80.7-93.6] versus 91% [84-95.2], p=0.565).

Table 1 Overview of women and their breast cancers: stratified by BRCA status and age at screening*

	All ages		Age ≤40		Age 41-50		Age >50	
	BRCA1	BRCA2	BRCA1	BRCA2	BRCA1	BRCA2	BRCA1	BRCA2
Women, number	1219	732	605	301	482	308	310	228
Follow-up time (woman years)	3840	2245	1691	749	1216	812	895	673
BC, number	112	72	46	18	37	37	29	17
BC incidence [95% CI]	29.2 [24-35.1]	32.1 [25.1-.40.4]	27.2 [19.9-36.3]	24 [14.2-38]	30.4 [21.4-41.9]	45.6 [32.1-62.8]	45.6 [32.1-46.5]	25.3 [14.7-40.4]
DCIS, number (%)†	15 (13.4%)	22 (30.6%)	9 (19.6%)	3 (16.7%)	3 (8.1%)	13 (35.1%)	3 (10.3%)	6 (35.3%)
Number of invasive cancers (%)	97 (86.6%)	50 (69.4%)	37 (80.4%)	15 (83.3%)	34 (91.9%)	24 (64.9%)	26 (89.7%)	11 (64.7%)
Invasive <1cm (% of invasive)	26 (26.8%)	17 (34.0%)	8 (21.6%)	2 (13.3%)	9 (26.4 %)	11 (45.8%)	9 (34.6%)	4 (36.4%)
Invasive 1-2 cm (% of invasive)	34 (35.1%)	12 (24.0%)	13 (35.1%)	5 (33.3%)	9 (26.4%)	3 (12.5%)	13 (50.0%)	4 (36.4%)
Grade 1 (% of invasive)	8 (8.2%)	7 (14.0%)	1 (2.7%)	1 (6.7%)	4 (11.8%)	2 (8.3%)	3 (11.5%)	4 (36.4%)
Early stage tumor (DCIS or invasive <1cm), number (% of invasive)	41 (36.6 %)	39 (54.2%)	17 (37.0%)	5 (27.8%)	11 (29.7%)	24 (64,9%)	12 (41.4%)	10 (58.8%)

*Incidence was expressed per 1000 woman years of follow-up. Age group was defined based on age at screening, therefore, women can belong to two or more age groups. BC= breast cancer, DCIS= ductal carcinoma in situ, 95% CI= 95% confidence interval.

†P value for difference in proportion of DCIS between BRCA1 and BRCA2 mutation carriers was calculated with $\chi 2$ tests or Fishers' exact tests as appropriate, and was for all ages p=0.008, ≤40 p= 1.00, 41-50 p=0.010, >50 p=0.058.

Table 2 Sensitivity and specificity of screening modalities in women with *BRCA1* mutation stratified by age*

	Mammography			MRI			Combination		
	No of BC detected	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	No of BC detected	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	No of BC detected	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]
All ages (n=112)	39	35.7	93.4	92	88.6	84.4	98	92.5	80.4
		[25.9-46.9]	[89.3-96.5]		[73.4-95.6]	[78.7-88.3]		[80.1-97.4]	[72.8-86.2]
Age ≤40 (n=46)	18	39.1	94.9	34	77.5	84.3	38	86.8	81
		[26.2-53.9]	[91.2-97.1]		[57-90]	[78.7-88.7]		[63.1-96.2]	[73.9-86.5]
Age 41-50 (n=37)	13	34.2	91.5	33	93.1	82.9	34	94.1	77.2
		[21-50.5]	[86.7-94.6]		[70.8-98.7]	[77.9-87]		[74.5-98.9]	[70.5-82.8]
Age >50 (n=29)	8	29.4	96.8	25	89.1	89.9	26	89.3	87.4
		[12.8-54.2]	[91.9-98.8]		[54.8-98.2]	[82.6-94.3]		[71.3-96.6]	[79.3-92.6]

* BC= breast cancer, No= number, 95% CI= 95% confidence interval.

Table 3 Sensitivity and specificity of screening modalities in women with *BRCA2* mutation stratified by age*

	Mammography			MRI			Combination		
	No of BC detected	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	No of BC detected	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	No of BC detected	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]
All ages (n=72)	31	44.6	93.4	53	80.1	82.3	64	92.7	80.5
		[31.9-58]	[88.4-96.3]		[58.9-91.9]	[79.6-89.6]		[79.3-97.7]	[72.8-86.4]
Age ≤40 (n=18)	10	55.6	92.3	9	52.7	80.2	15	87.2	75.3
		[32.9-76.1]	[86.6-95.7]		[27.2-76.8]	[72.9-85.8]		[56.1-97.3]	[66.6-82.4]
Age 41-50 (n=37)	14	37.8	92	30	86.4	86	33	91.2	80
		[23.8-54.3]	[87-95.2]		[58.2-96.7]	[81.1-89.8]		[70.4-97.9]	[73.3-85.3]
Age >50 (n=17)	7	45.5	97.4	14	85	91	16	94.1	88.6
		[19.3-74.4]	[92.8-99.1]		[43.7-97.7]	[84-95.2]		[67.5-99.2]	[80.7-93.6]

* BC= breast cancer, No= number, 95% CI= 95% confidence interval.

Mammography contribution to sensitivity in *BRCA1* mutation carriers

In *BRCA1* mutation carriers: overall adding mammography to MRI screening increased sensitivity by roughly 4% to 92.5% (**Table 2**) (p=0.553). In the ≤40 years group, the addition of mammography increased sensitivity by 9.3% (**Table 2**). Without mammography, 3 of 46 (6.5%) breast cancers, including 2 DCIS, would not have been detected (**Table 4**) in this subgroup. In the 41-50 years group, additional mammography increased sensitivity by only 1% (**Table 2**), detecting one DCIS (2.7%) (**Table 4**). Similarly, in the >50 group, mammography detected one additional breast cancer (3.4% of cancers) (**Table 4**).

Mammography contribution to sensitivity in *BRCA2* mutation carriers

In *BRCA2* mutation carriers: overall adding mammography to MRI screening increased sensitivity by 12.6 % to 92.7% (**Table 3**) (p=0.154). In the ≤40 group additional mammography increased sensitivity by 34.5% (**Table 3**). Without mammography six of 18 cancers (33.3%), including 2 DCIS, would not have been detected in this young age group (**Table 4**). In women aged 41-50 years, adding mammography insignificantly increased sensitivity by nearly 5% (**Table 3**) and detected 3 cancers, including 1 DCIS, which were not detected by MRI (8.1% of cancers). In the >50 year age-group screening sensitivity increased insignificantly by approximately

Table 4 Mammography-only detected breast cancers per BRCA mutation status*

No	BRCA status	Age at diagnosis	Tumor type	Invasive tumor size	Invasive tumor grade	Screening round
1	*BRCA1*	31	DCIS	-	-	2
2	*BRCA1*	33	DCIS	-	-	2
3	*BRCA1*	40	IDC	1-2cm	Grade 3	1
4	*BRCA1*	42	DCIS	-	-	3
5	*BRCA1*	56	IDC	<1cm	Grade 3	4
1	*BRCA2*	36	DCIS	-	-	1
2	*BRCA2*	37	DCIS	-	-	1
3	*BRCA2*	35	IDC	<1cm	Grade 2	4
4	*BRCA2*	36	IDC	1-2cm	Grade 3	1
5	*BRCA2*	37	ILC	2-5cm	Grade 2	1
6	*BRCA2*	39	Other	NA	NA	3
7	*BRCA2*	42	DCIS	-	-	4
8	*BRCA2*	53	DCIS	-	-	2
9	*BRCA2*	44	ILC	>5cm	3	1
10	*BRCA2*	47	ILC	>5cm	2	5
11	*BRCA2*	51	NA	NA	NA	1

*NA: information was not available in the database.

9% (**Table 4**), and mammography detected two cancers (11.8%) which were not detected by MRI, including one DCIS.

Number of screens needed (NSN)

For the first screening round, the NSN for mammography to detect one breast cancer not detected by MRI was 527 for women with *BRCA1* mutation and 94 for women with *BRCA2* mutation for all ages (**Table 5**). For subsequent screening rounds, the NSN for mammography to detect an additional breast cancer for women with *BRCA1* mutation (717 screens) was roughly three times that for women with *BRCA2* mutation (231 screens).

DISCUSSION

Adding mammography to MRI screening in *BRCA1* mutation carriers overall leads to a very modest increase in sensitivity of 3.9% of 112 breast cancers (p=0.553), and a decrease in specificity (by 4%, p=0.154). One invasive cancer and two DCIS (6.5%) of the 46 *BRCA1* breast cancers detected before the age of 40, and only one DCIS

Table 5 Number of screens needed for one additional mammography-only detected cancer for first and subsequent screening rounds*

BRCA	Age-group	Number of BC in study subjects	Number of screens	BC only detected by mammography	NSN for mammography to detect one BC missed by MRI
First screening round					
BRCA1	All ages	45	1053	2	527
	Age ≤40	19	555	2	278
	41-50	14	304	0	NA
	Age >50	12	194	0	NA
BRCA2	All ages	18	564	6	94
	Age ≤40	10	221	4	55
	41-50	10	204	1	204
	Age >50	8	139	1	139
Subsequent (repeat) screening rounds					
BRCA1	All ages	67	2150	3	717
	Age ≤40	27	775	1	775
	41-50	23	797	1	797
	Age >50	17	578	1	578
BRCA2	All ages	54	1155	5	231
	Age ≤40	8	281	2	141
	41-50	27	444	2	222
	Age >50	9	430	1	430

* BC= breast cancer, NA= not applicable, NSN= number of screens needed.

and one invasive cancer <1 cm (3%) in a total of 66 *BRCA1* breast cancers would not have been detected at that screen after the age of 40. The percentage of early-stage (DCIS or <1cm invasive) cancers detected with both MRI and mammography screening of 37% (41/112) could decrease by 4% (37/112) if mammography was not performed. With MRI and mammography, 63% of the cancers were more advanced breast cancer (invasive >1cm), 0.9% was only detected by mammography. In order to detect one breast cancer missed by MRI, we estimated that 641 screens with mammography would be needed.

The contribution of mammography above MRI to screening sensitivity in the 72 *BRCA2* mutation carriers was 12.6% (p>0.05). Additional mammography in *BRCA2* mutation carriers also decreased specificity. Without additional mammography one third of breast cancers would not have been detected in *BRCA2* mutation carriers aged 40 years and younger, but this proportion was 9.3% in those older than 40 years. The percentage of *BRCA2* cancers detected at very early stage, as DCIS or <1cm, with both MRI and mammography screening of 54% (39/72) might decrease to 47% (34/72) without mammography, and only 156 mammography screens are needed to detect a breast cancer missed with MRI. Without mammography 4 advanced BRCA2 breast cancers (5.6% of 72 BRCA2 breast cancers total) would have been missed. The advantage of mammography over MRI has been the ability to detect DCIS through visualizing micro calcifications. The proportion of DCIS is larger for women with *BRCA2* mutation than for women with *BRCA1* mutation, explained by the difference in nature of *BRCA*-associated breast cancers.[20] This partly explains the higher sensitivity of mammography for women with *BRCA2* compared to that for women with *BRCA1* mutations.[14,16]

The modest additional value of digital-only mammography to current MRI screening of *BRCA1* mutation carriers was recently shown in a retrospective study by Obdeijn et al.[21] Only two (2%) DCIS of 94 breast cancers were detected by mammography alone, none in women aged below 40 and no invasive cancers. Importantly, in this retrospective study with more recent data MRI screening detected 67% of the breast cancers detected as DCIS or <1 cm, considerably more than the 41-44% published for the Dutch, UK and Canadian studies of our IPD meta-analyses.[15,16,22]

At the time of the studies included in our current analyses, radiologists may not have had extensive experience with breast MRI screening. Most likely both a learning curve, as expected for any new screening modality, and improved techniques explain the relatively improved MRI sensitivities in more recent studies. A learning curve for the diagnostic performance of MRI screening in high-risk women was evident for the Canadian study, in particular for DCIS detection.[23] Although in a previous report in this study population,[11] the sensitivity of MRI and mammography fluctuated over the years, and heterogeneity across different studies may have hindered observation of any potential effect of timeframe. In balance, learning curve effects are also expected for modern mammography. A cohort study from the Netherlands showed that digital mammography had higher sensitivity

compared to studies reporting film mammography (and a transition to digital).[21] However, in the Italian HIBCRIT-1 study, transition from film-screen to digital mammography (resulting in screening with roughly equal mix of film-screen and digital) did not increase mammography sensitivity in high-risk women.[17] Newer mammography technologies such as tomosynthesis (or 3D-mammography) which has better screening sensitivity than standard mammography[24] have not been compared to modern MRI screening of *BRCA* carriers. This lacking evidence in high-risk screening is worthy of research effort but would still imply increased ionizing radiation from tomosynthesis.[25]

In contrast to benefits of possible earlier breast cancer detection, there are also possible harmful effects of additional mammography. The twofold increase in breast cancers in *BRCA1/2* mutation carriers after exposure to 4 or more radiographs, compared to non-exposure, described by Pijpe et al,[26] was significant below age 30 years, but not at 30-39 years, possibly due to small numbers. Two other studies could not demonstrate tumor induction in *BRCA1/2* mutation carriers by screening mammography or low dose contralateral irradiation from breast conserving treatment.[27,28] However this may have been due to the short follow-up time in these studies, with a mean follow-up time of 7.5 and 8.5 years, while the latency time for radiation induced breast cancer is 10-15 years.[12,29]

In this meta-analysis, we combined IPD from six large prospective studies, making this the largest study in the world with prospectively collected screening data on *BRCA1/2* mutation carriers, although numbers are still modest in subgroups and in *BRCA2* mutation carriers. Based on our findings, the contribution of mammography to MRI screening was different for women with *BRCA1* and women with *BRCA2* mutation. It is questionable whether additional detection from mammography in *BRCA1* mutation carriers who receive MRI screening outweighs the disadvantages, such as potential breast cancer induction or more false-positive results. In MRI screening for *BRCA2* mutation carriers, the contribution of additional mammography seems more relevant. Different screening routines for these two groups of women defined by *BRCA* mutation status should be considered on the basis of balancing the contribution of mammography and its potential harms.

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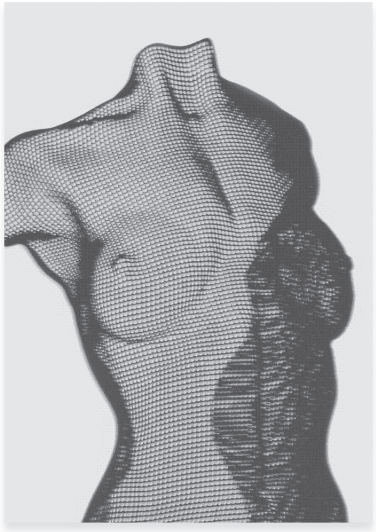
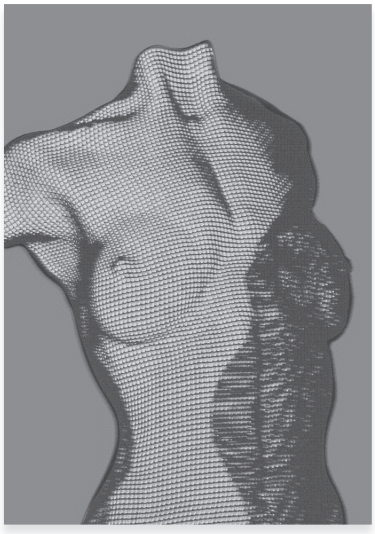
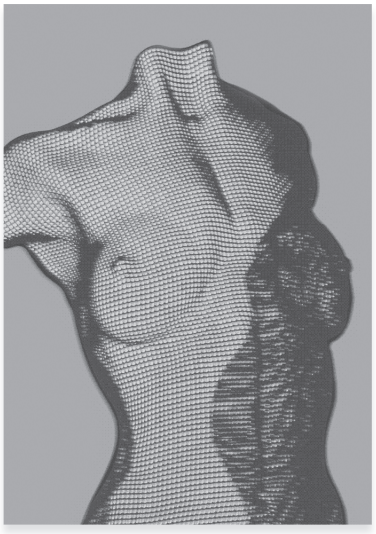

Chapter 7

Relevance and efficacy of breast cancer screening in *BRCA1* and *BRCA2* mutation carriers above 60 years; a national cohort study

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ABSTRACT

Background

Annual MRI and mammography is recommended for *BRCA1/2* mutation carriers to reduce breast cancer mortality. Less intensive screening is advised ≥60 years, although effectiveness is unknown.

Methods

We identified *BRCA1/2* mutation carriers without bilateral mastectomy before age 60 to determine for whom screening ≥60 is relevant, in the Rotterdam Family Cancer Clinic and HEBON: a nationwide prospective cohort study. Furthermore, we compared tumour stage at breast cancer diagnosis between different screening strategies in *BRCA1/2* mutation carriers ≥60. Tumours>2 cm, positive lymph nodes, or distant metastases at detection were defined as 'unfavourable'.

Results

Of 548 *BRCA1/2* mutation carriers ≥60 years in 2012, 395 (72%) did not have bilateral mastectomy before the age of 60. Of these 395, 224 (57%) had a history of breast or other invasive carcinoma. In 136 *BRCA1/2* mutation carriers, we compared 148 breast cancers (including interval cancers) detected ≥60, of which 84 (57%) were first breast cancers. With biennial mammography 53% (30/57) of carcinomas were detected in unfavourable stage, compared to 21% (12/56) with annual mammography (adjusted odds ratio: 4.07, 95% confidence interval [1.79-9.28], p=0.001). With biennial screening 40% of breast cancers were interval cancers, compared to 20% with annual screening (p=0.016). Results remained significant for *BRCA1* and *BRCA2* mutation carriers, and first breast cancers separately.

Conclusions

Over 70% of 60-year old *BRCA1/2* mutation carriers remain at risk for breast cancer, of which half has prior cancers. When life expectancy is good, continuation of annual breast cancer screening of *BRCA1/2* mutation carriers ≥60 is worthwhile.

INTRODUCTION

The cloning of the *BRCA1*[1] and *BRCA2*[2] genes in 1994 made it possible to identify women with a germ-line mutation in these genes, who have a cumulative risk for breast cancer of 43-75% by age 70.[3-5]

Without preventive intervention, survival probability by age 80 for *BRCA1* and *BRCA2* mutation carriers is estimated 33-52%, in comparison to 66% in the United States (U.S.) general female population.[6] The two main strategies to reduce breast cancer mortality are prevention of breast cancer through risk-reducing mastectomy or optimizing survival chances by early detection through breast screening with annual magnetic resonance imaging (MRI) and mammography.[7-9] Both strategies offer comparable improved survival in modelling studies,[6] and in most countries the majority of mutation carriers opt for screening.[10,11]

In most international guidelines screening with annual clinical breast examination, MRI, and mammography is advised for *BRCA1/2* mutation carriers until the age of 50.[7-9,12,13] Above the age of 50 guidelines differ. The recently updated British NICE guideline recommends extending the period of annual mammography screening until 69 years, and considering continuing MRI screening for women older than 50 with dense breasts.[12] From the age of 60 (≥60) Dutch guidelines advise screening with only biennial mammography.[13,14] American guidelines advice annual screening with MRI and mammography without an upper age limit.[7,9]

There are several reasons why a less intensive screening protocol may not be adequate for *BRCA1/2* mutation carriers ≥60 years. First of all, breast cancer incidence remains high in mutation carriers ≥60.[4,5,15] Secondly, there is no evidence that screening with mammography alone is effective for *BRCA1/2* mutation carriers ≥60.[16] In the Dutch prospective MRISC cohort screening study *BRCA1/2* mutation carriers and women with familial risk for breast cancer aged 25-70 years were screened with annual mammography and MRI. Sensitivity of mammography was just 25% for *BRCA1* mutation carriers and 62% for *BRCA2* mutation carriers, whilst MRI sensitivity was approximately 68%.[17] Since only few *BRCA1/2* mutation carriers ≥60 have participated in the large prospective screening trials no sensitivity analyses have been performed for this subgroup specifically.[17-19] Finally, it is questionable if reducing screening frequency is optimal for *BRCA1/2* mutation carriers ≥60 as their tumours grow twice as fast as tumours of age-matched non-carriers.[20] The growth rate of a carcinoma of a 60 year old *BRCA1/2* mutation carrier is comparable to that of a 37 year old non-carrier.[20]

To address the clinical relevance and extent of this issue, we first assess the proportion of *BRCA1/2* mutation carriers with remaining breast tissue at risk at age 60, in an ongoing nationwide cohort study and a family cancer clinic cohort. Secondly, to determine the optimal breast cancer screening strategy for *BRCA1/2* mutation carriers ≥60, we compared tumour stage at detection per screening strategy.

METHODS

BRCA1/2 mutation carriers with breast tissue at risk at age 60

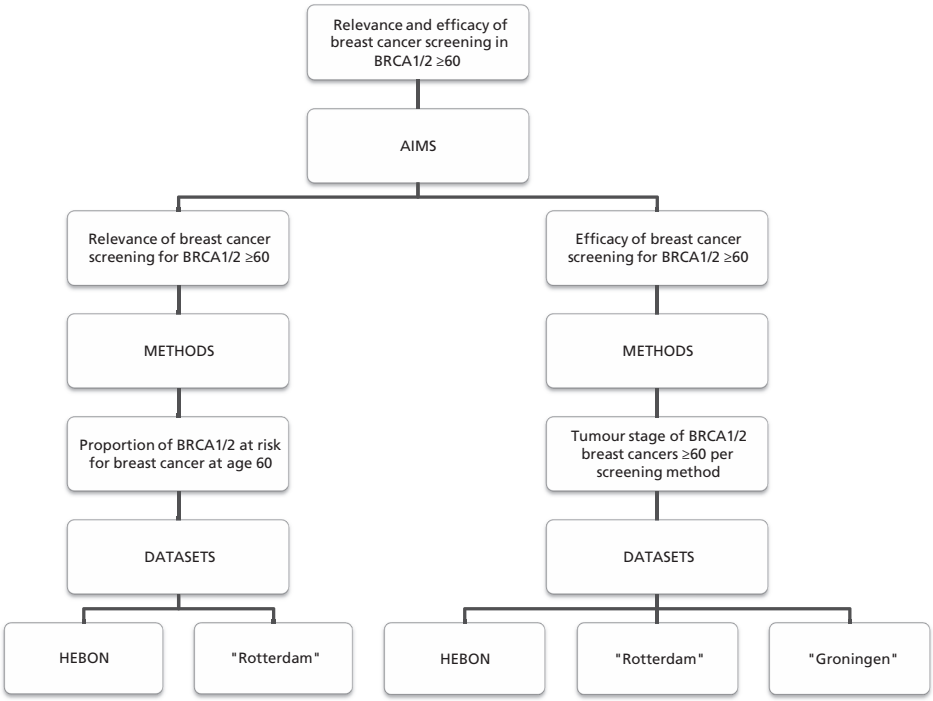
We assessed the proportion of *BRCA1/2* mutation carriers who were at risk of breast cancer at age 60, i.e. did not have bilateral therapeutic or risk-reducing mastectomy performed before age 60. In addition, in the women with remaining breast tissue present at age 60, we evaluated the proportion of women with a history of invasive carcinoma, other than non-melanoma skin cancer, since relevance of screening also depends on a woman's life expectancy. We selected all female *BRCA1/2* mutation carriers aged 60 years or older in 2012, i.e. born before 1-1-1953, from the prospective cohorts with follow-up of the Family Cancer Clinic of the Erasmus University Medical Centre, Cancer Institute, Rotterdam ("Rotterdam") and the Netherlands Collaborative Group on Hereditary Breast Cancer (HEBON)[21] a nationwide cohort study of women tested for *BRCA1/2* mutations in the Netherlands. Patients included in both cohorts were identified, and registered in the "Rotterdam" cohort only. Informed consent was obtained from all patients, prior to inclusion in these databases. Women were selected irrespective of their prior cancer status. Women who died before reaching the age of 60 were excluded.

Ethics committee

The research protocol of the "Rotterdam" cohort was approved by the institutional board of the Erasmus Medical Centre, Rotterdam and the HEBON study was approved by the Institutional Review Board of all participating centres. All participants of "Rotterdam" and HEBON provided written informed consent.

Assessment of the optimal screening strategy

To evaluate the optimal screening strategy for *BRCA1/2* mutation carriers ≥60, we conducted a case-case study, comparing tumour stage at breast cancer detection (including ductal carcinoma in situ; DCIS) in women ≥60 between screening strategies (biennial mammography, annual mammography). Breast cancer cases were selected from the "Rotterdam" and HEBON cohorts and from the department of Clinical Genetics of the University Medical Centre Groningen ("Groningen"), updated until September 2011[5] and entered into an anonymised database (**Supplementary figure 1**). All first breast cancers, contralateral breast cancers and second primary ipsilateral breast cancers detected above 60 years were included. Second primary ipsilateral breast cancer was distinguished from breast cancer recurrence by the multidisciplinary team of the treating hospital based on differences in tumour characteristics and/or localization between the first and second breast cancer. Tumours >2 cm, with positive lymph nodes, or with distant metastases at detection were defined as 'unfavourable'. Women with missing data on screening method or tumour stage were excluded from analysis.



Supplementary figure 1 Relevance and efficacy of breast cancer screening in *BRCA1/2* ≥60; aims, methods and datasets used.
BRCA1/2= *BRCA1* and *BRCA2* gene mutation carriers, HEBON= the Netherlands Collaborative Group on Hereditary Breast Cancer, "Rotterdam"= Erasmus University Medical Centre, Cancer Institute, Rotterdam, the Netherlands, "Groningen"= the department of Clinical Genetics of the University Medical Centre Groningen, the Netherland.

Data collection

To answer the two research questions breast cancer characteristics were extracted from the three databases or from additional medical files in case of missing data. Medical files from the hospitals and the national breast cancer screening program were searched. The following data were collected: age at diagnosis, mode of detection, lateralization, tumour size, nodal status, histological type and grade, oestrogen receptor status, progesterone receptor status and human epidermal growth factor receptor 2 (HER2) status. Furthermore, data on prior cancer status, therapeutic or prophylactic breast surgery, and of method and frequency of breast cancer screening were gathered.

Statistical analysis

Continuous data were presented as median (range). To evaluate the optimal screening strategy for *BRCA1/2* mutation carriers ≥60 differences between different screening strategies were analysed. Differences in discrete outcomes were

analysed using Pearson χ^2 tests or Fisher's exact tests, as appropriate, differences in median age at diagnosis of breast cancer were assessed with the Mann-Whitney *U* test. Data analyses were stratified for *BRCA1* mutation carriers and *BRCA2* mutation carriers, and for first breast cancer (yes or no). The association of frequency of mammography screening with tumour stage (favourable/unfavourable) was estimated by multivariable backwards logistic regression models with adjustment for all possible clinical relevant factors known in our dataset: period of diagnosis (<2005 or >2005; after 2005 generally digital mammography was used in the Netherlands), mutation status (*BRCA1* or *BRCA2*), first breast cancer (yes or no), and age at detection of breast cancer. Odds ratios (OR) for unfavourable tumour stage were computed and presented with 95% confidence intervals (CI). We started with the most extensive logistic regression model, including all possible confounders, using a backward stepwise approach to remove all variables that were shown to have a non-significant contribution to the model by the likelihood-ratio test. A two-sided P value of 0.05 or less was considered statistically significant. Missing values were excluded from analyses. Statistical analyses were performed using SPSS Statistics for Windows, version 20.0 (IBM Corp, Armonk, NY).

RESULTS

BRCA1/2 ≥60 with breast tissue at risk

We identified 588 *BRCA1/2* mutation carriers born before 1953. Forty (7%) patients died before reaching the age of 60 and were excluded from analyses. Of the remaining 548 *BRCA1/2* mutation carriers, 264 patients were from "Rotterdam", and an additional 284 patients from HEBON.

There were 413 patients with a *BRCA1* mutation, 133 with a *BRCA2* mutation, and two with both a *BRCA1* and a *BRCA2* mutation. **Table 1** gives the distribution of *BRCA1* and *BRCA2* mutation carriers and information on breast tissue present in the "Rotterdam" and HEBON cohorts. In our total dataset 395/548 (72%) had one or both breasts present at age 60. Among these 395 women with breast tissue present, 171 (43%) women had no history of breast cancer or other invasive carcinoma (non-melanoma skin cancer excluded), 144 (37%) had a history of breast cancer (including DCIS), 47 (12%) had a history of another type of cancer, and 33 (8%) women had a history of breast cancer as well as another type of cancer (**Figure 1**).

Differences in tumour stage per screening modality

The HEBON, "Rotterdam" and "Groningen" series of *BRCA1/2* mutation carriers included 154 breast cancers detected in patients aged 60 years or older. Six were excluded due to missing screening data. The 148 remaining breast cancers were detected in 86 *BRCA1* mutation carriers, 50 *BRCA2* mutation carriers and one *BRCA1*

Table 1 *BRCA1* and *BRCA2* gene mutation carriers; information on breast tissue present at age 60 and history of cancer*

Breast tissue present (%)		"Rotterdam"		HEBON only		Total	
BRCA1	Bilateral	100	(52%)	120	(55%)	220	(53%)
	Unilateral	39	(20%)	36	(16%)	75	(18%)
	None	54	(28%)	64	(29%)	118	(29%)
	Total	193	(100%)	220	(100%)	413	(100%)
BRCA2	Bilateral	44	(62%)	31	(50%)	75	(56%)
	Unilateral	12	(17%)	12	(19%)	24	(18%)
	None	15	(21%)	19	(31%)	34	(26%)
	Total	71	(100%)	62	(100%)	133	(100%)
Total	Bilateral	144	(55%)	151	(53%)	295	(54%)
	Unilateral	51	(19%)	49	(17%)	100	(18%)
	None	69	(26%)	84	(30%)	153	(28%)
	Total	264	(100%)	284	(100%)	548	(100%)

*Two HEBON patients included with gene mutations in both *BRCA1* and *BRCA2*. *BRCA1*= *BRCA1* gene mutation carrier, *BRCA2*= *BRCA2* gene mutation carrier, HEBON= prospective cohort of the Netherlands Collaborative Group on Hereditary Breast Cancer, "Rotterdam"= prospective cohort of Erasmus University Medical Centre, Rotterdam, %= per cent.

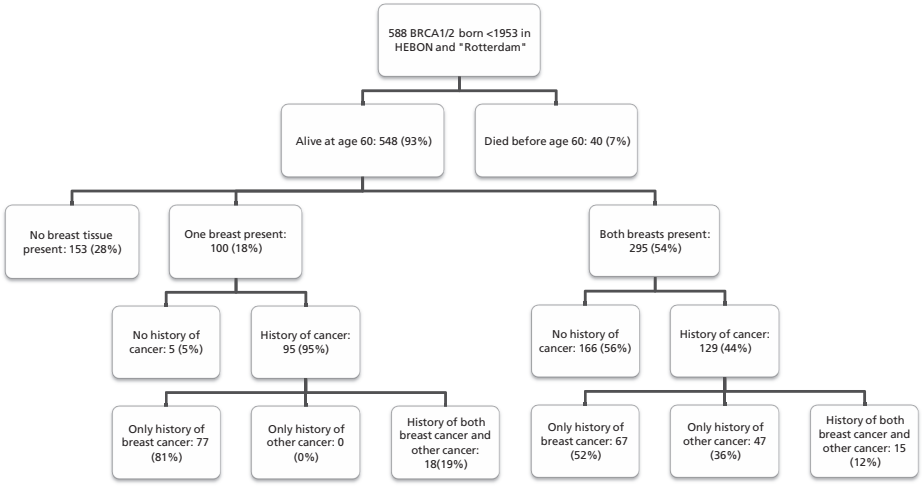


Figure1 *BRCA1* and *BRCA2* gene mutation carriers; proportion with breast tissue present at age 60 and history of cancer.
BRCA1/2= *BRCA1* and *BRCA2* gene mutation carriers, %= per cent.

& *BRCA2* mutation carrier. Of these tumours 15 (10%) were a second primary ipsilateral carcinoma, 49 (33%) were a second primary contralateral breast cancer, and

Table 2 Tumour characteristics and mode of screening of *BRCA1/2*-associated breast cancers diagnosed≥60*

	BRCA1		BRCA2		Overall†		P value
Number of patients	86		50		136		
Number of diagnosed breast cancers	92		55		147		
First breast cancer	52	(57%)	31	(56%)	83	(57%)	0.985
Second breast cancer (ipsilateral and contralateral)	40	(44%)	24	(44%)	64	(44%)	
Median age at diagnosis in years (range)	64 (60-81)		65 (60-79)		64 (60-81)		0.487
Year of diagnosis							0.055
Detected < 2005	47	(51%)	37	(67%)	84	(57%)	
Detected ≥ 2005	45	(49%)	18	(33%)	63	(43%)	
Screening at time of detection‡							**0.008**
No screening	9	(10%)	13	(24%)	22	(15%)	
Biennial mammography	30	(33%)	26	(47%)	56	(39%)	
Annual mammography	42	(47%)	14	(25%)	56	(39%)	
Annual mammography and MRI	9	(10%)	2	(4%)	11	(7%)	
Detection							0.069
Interval carcinoma	21	(23%)	12	(22%)	33	(22%)	
Screen-detected	62	(67%)	30	(54%)	92	(63%)	
No screening	9	(10%)	13	(24%)	22	(15%)	
Stage							0.423
Favourable (T1 & N- & M-)	58	(63%)	31	(56%)	89	(60%)	
Unfavourable	34	(37%)	24	(44%)	58	(40%)	
Tumour size§							0.225
Tis/T1a/T1b (tumour ≤ 10 mm)II	32	(36%)	12	(22%)	44	(31%)	
T1c (tumour 11-20 mm)	33	(37%)	24	(44%)	57	(40%)	
T2+ (tumour >20 mm)	24	(27%)	18	(33%)	42	(29%)	
Tumour characteristics of invasive cancers¶							
Nodal status							0.359
N- / isolated cells	68	(77%)	38	(70%)	106	(75%)	
N+ / micro metastasis (0.2-2.0 mm)	20	(23%)	16	(30%)	36	(25%)	
Histological subtype							0.234
Ductal cancer	78	(89%)	44	(82%)	122	(86%)	
Other	10	(11%)	10	(18%)	20	(14%)	
Bloom & Richardson Grade							0.092
Grade 1/2	22	(29%)	20	(44%)	42	(34%)	
Grade 3	55	(71%)	26	(57%)	81	(66%)	
Oestrogen status							**<0.001**
Positive	28	(36%)	35	(76%)	63	(51%)	
Negative	50	(64%)	11	(24%)	61	(49%)	
Progesterone status							0.052

Table 2 Tumour characteristics and mode of screening of *BRCA1/2*-associated breast cancers diagnosed≥60* (continued)

	BRCA1		BRCA2		Overall†		P value
Positive	20	(27%)	19	(44%)	39	(33%)	
Negative	55	(73%)	24	(56%)	79	(67%)	
HER2 status							0.978
Overexpression	4	(9%)	2	(9%)	6	(9%)	
No overexpression	41	(91%)	20	(91%)	61	(91%)	

*****Two-sided P value for difference between two risk groups; all differences were obtained from χ^2 test or Fisher's exact tests, as appropriate, except for differences in median age which were computed using the Mann-Whitney *U* test. All statistical tests were two-sided. *BRCA1*= *BRCA1* gene mutation carrier, *BRCA2*= *BRCA2* gene mutation carrier**,** HER2= human epidermal growth factor 2, N-= node negative, N+= Node positive, %= per cent.
†Data of the one breast cancer in a patient with both *BRCA1* and *BRCA2* gene mutation are not shown in this table.
‡Two patients were screened according to a different protocol, not included in analyses.
§Size of four breast cancers unknown (three axillary cancers, primary breast cancer undetected).
IIFive Tis, four in the *BRCA1* gene mutation group, one in the *BRCA2* gene mutation group.
¶Missing data not shown.

84 (57%) were first breast cancers. Further tumour characteristics and method of screening per mutation type (*BRCA1* or *BRCA2*) are depicted in **Table 2**.

In **Table 3** the characteristics of *BRCA1/2*-associated breast cancers diagnosed ≥60 with either biennial or annual mammography screening are compared. Of the 113 breast cancers detected ≥60 while being screened with mammography, 64 (57%) were first breast cancers. For *BRCA1/2* mutation carriers screening with biennial mammography detected 53% (30/57) of tumours in an unfavourable stage versus 21% (12/56) with annual mammography (OR: 4.07, 95% CI [1.79-9.3], p=0.001). Also when analysing first breast cancers separately this percentage was 53% (24/45) versus 21% (4/19) respectively (OR: 4.29, 95% CI [1.23-14.94], p=0.017). This statistically significant difference was also seen for *BRCA1* mutation carriers separately; biennial screening detected 57% of all breast cancers in unfavourable stage versus 24% with annual mammography (OR: 4.19, 95% CI [1.5-11.5], p=0.005), and for *BRCA2* mutation carriers separately; biennial screening detected 50% of tumours in an unfavourable stage compared to annual screening 14% (OR: 6.00, 95% CI [1.11-32.3], p=0.026). In the overall biennial mammography group 40% (23/57) of tumours detected were interval cancers, compared to 20% (11/56) in the annual mammography group (p=0.016). In the overall biennial mammography group 74% (17/23) of interval cancers was detected in unfavourable stage, versus 36% (4/11) in the annual mammography group (p=0.060).

In univariable logistic regression models mammographic screening frequency (annual or biennial) was the only significant variable influencing tumour stage at

Table 3 Characteristics of *BRCA1/2*–associated breast cancers diagnosed≥60 per screening strategy*

	Biennial Mx		Annual Mx		Overall		P value
Number of patients	56		49		105		
Number of diagnosed breast cancers	57		56		113		
First breast cancer	45	(79%)	19	(34%)	64	(57%)	**<0.001**
Second breast cancer	12	(21%)	37	(66%)	49	(43%)	
Median age at diagnosis in years (range)	63	(60-75)	64	(60-77)	64	(60-77)	0.428
Year of diagnosis							0.220
Detected < 2005	37	(65%)	30	(54%)	67	(60%)	
Detected ≥ 2005	20	(35%)	26	(46%)	46	(40%)	
Detection							**0.016**
Interval	23	(40%)	11	(20%)	34	(30%)	
Screen-detected	34	(60%)	45	(80%)	79	(70%)	
***BRCA1* / *BRCA2*†**							
Stage							**0.001**
Favourable (≤T1 & N- & M-)	27	(47%)	44	(79%)	71	(63%)	
Unfavourable	30	(53%)	12	(21%)	42	(37%)	
Tumour size‡							**0.001**
Tis/T1a/T1b (tumour ≤ 10 mm)§	9	(16%)	21	(39%)	30	(27%)	
T1c (tumour 11-20 mm)	24	(43%)	26	(48%)	50	(46%)	
T2+ (tumour >20 mm)	23	(41%)	7	(13%)	30	(27%)	
Nodal statusII							**0.005**
N- / isolated cells	36	(64%)	48	(87%)	84	(76%)	
N+ / micro metastasis (0.2-2.0 mm)	20	(36%)	7	(13%)	27	(24%)	
BRCA1							
Detection							0.087
Interval	12	(40%)	9	(21%)	21	(29%)	
Screen-detected	18	(60%)	33	(79%)	51	(71%)	
Stage							**0.005**
Favourable (T1 & N- & M-)	13	(43%)	32	(76%)	45	(63%)	
Unfavourable	17	(57%)	10	(24%)	27	(38%)	
Tumour size‡							**0.004**
Tis/T1a/T1b (tumour ≤ 10 mm)	6	(20%)	16	(40%)	22	(32%)	
T1c (tumour 11-20 mm)	9	(31%)	19	(48%)	28	(40%)	
T2+ (tumour >20 mm)	14	(48%)	5	(13%)	19	(28%)	
Nodal status§							**0.045**
N- / isolated cells	19	(66%)	36	(86%)	55	(76%)	
N+ / micro metastasis (0.2-2.0 mm)	10	(35%)	6	(14%)	16	(23%)	

Table 3 Characteristics of *BRCA1/2*–associated breast cancers diagnosed≥60 per screening strategy* (continued)

	Biennial Mx		Annual Mx		Overall		P value
BRCA2							
Detection							0.157
Interval	10	(39%)	2	(14%)	12	(30%)	
Screen-detected	16	(62%)	12	(86%)	28	(70%)	
Stage							**0.026**
Favourable (T1 & N- & M-)	13	(50%)	12	(86%)	25	(63%)	
Unfavourable	13	(50%)	2	(14%)	15	(38%)	
Tumour size¶							
Tis/T1a/T1b (tumour ≤ 10 mm)	3	(12%)	5	(36%)	8	(20%)	
T1c (tumour 11-20 mm)	14	(54%)	7	(50%)	21	(53%)	
T2+ (tumour >20 mm)	9	(35%)	2	(14%)	11	(28%)	
Nodal status							0.063
N- / isolated cells	16	(62%)	12	(92%)	28	(72%)	
N+ / micro metastasis (0.2-2.0 mm)	10	(39%)	1	(8%)	11	(28%)	
First Breast Cancers							
Stage							**0.017**
Favourable (T1 & N- & M-)	21	(47%)	15	(79%)	36	(56%)	
Unfavourable	24	(53%)	4	(21%)	28	(44%)	
Second Breast Cancers							
Stage							0.059
Favourable (T1 & N- & M-)	6	(50%)	29	(78%)	35	(71%)	
Unfavourable	6	(50%)	8	(22%)	14	(29%)	

*Two-sided P value for difference between two risk groups; all differences were obtained from χ^2 test or Fisher's exact tests, as appropriate, except for differences in median age which were computed using the Mann-Whitney *U* test. All statistical tests were two-sided. *BRCA1*= *BRCA1* gene mutation carrier, *BRCA2*= *BRCA2* gene mutation carrier, HER2= human epidermal growth factor 2, N-= node negative, N+= Node positive, %= per cent.
†Including one breast cancer found in a patient with a *BRCA1* and *BRCA2* gene mutation.
‡Size unknown for three breast cancers (two axillary cancers, primary breast cancer undetected).
§Only one Tis per screening group.
IIOnly invasive cancers included in analysis (one DCIS excluded).
¶Numbers too small for χ^2 test, more than 20% of expected frequencies less than 5.

detection (favourable/unfavourable) (**Table 4**). In multivariable backwards logistic regression, after adjustment for period of diagnosis (before 2005 or after 2005), mutation status (*BRCA1* or *BRCA2*), first breast cancer (yes or no), and age at detection of breast cancer, mammography screening frequency remained the only significant variable (OR: 4.07, 95% CI [1.79-9.3], p=0.001) influencing tumour stage at detection. Therefore, results of univariable and multivariable logistic regres-

sion with backward stepwise approach were equal for mammography screening frequency.

Screening with annual MRI and mammography detected two of 11 (18%) breast cancers in an unfavourable stage. Fourteen of 22 (64%) breast cancers were detected in an unfavourable stage, when women were not screened at all. Since so few

Table 4 Odds ratios for unfavourable tumour stage with factors in *BRCA1/2*-associated breast cancers diagnosed above 60 years*†

Variable investigated	Univariable OR (95% CI)	P value	Multivariable OR (95% CI)‡	P value‡
Screening frequency		**0.001**		**0.001**
Annual	1.00		1.00	
Biennial	4.07 (1.79-9.28)		4.07 (1.79-9.28)	
First breast cancer		0.100		
Yes	1.00			
No	0.51 (0.23-1.14)			
Period of diagnosis		0.407		
After 2005	1.00			
Before 2005	1.40 (0.64-3.06)			
Type of mutation*				
BRCA2	1.00	1.000		
BRCA1	1.00 (0.45-2.22)			
Age at diagnosis	0.98 (0.90-1.07)	0.614		

*Univariable regression analysis and multivariable logistic regression analyses with backward stepwise approach for unfavourable tumour stage. *BRCA1*= *BRCA1* gene mutation carrier, *BRCA2*= *BRCA2* gene mutation carrier, OR= odds ratio, 95% CI= 95 per cent confidence interval.
†Excluding one breast cancer found in a patient with a *BRCA1* and *BRCA2* gene mutation.
‡Multivariable OR only shown for factors with a significant contribution to the model.

BRCA1/2 mutation carriers ≥60 were screened with annual MRI and mammography, or not screened at all, no comparative analyses were performed for these groups.

DISCUSSION

This is the first study assessing relevance and optimal strategy of breast cancer screening in *BRCA1/2* mutation carriers ≥60. Our results, based on data derived from a prospective nationwide cohort study and a large family cancer clinic suggest that more than 70% of *BRCA1/2* mutation carriers still have breast tissue (one or both breasts) present at age 60 and are therefore at risk for breast cancer. An unacceptably high percentage of breast cancers (53%) were detected in unfavourable stage with biennial mammography. Continuing screening annually is therefore advisable.

Some explanations for the large proportion of *BRCA1/2* mutation carriers at risk for breast at age 60 in our study are: approximately 60% of *BRCA1/2* mutation carriers opt for screening over risk-reducing bilateral mastectomy in the Netherlands,[22] and in case of malignancy, breast conserving therapy is considered equally safe as mastectomy.[23,24] Furthermore, most *BRCA1/2* mutation carriers with breast tissue present at age 60 in our study did not have a history of breast cancer. Older age at onset, also in *BRCA1/2* mutation carriers, can be a family trait.[25] Because in *BRCA1/2* mutation carriers ≥60, breast cancer incidence remains high,[4,5,15] screening effectiveness in this group is a highly relevant question. Also for *BRCA1/2* mutation carriers ≥60 with a history of breast cancer, screening is important, since contralateral breast cancer risk is high; up to 56% after 25 years,[26] and survival with timely diagnosis is good.[27]

While screening biennially is beneficial in the general population,[28,29] our results suggest that continuation of annual screening is the advisable strategy for *BRCA1/2* mutation carriers ≥60. Biennial screening compared to annual screening resulted in twice as many *BRCA1/2*-associated cancers detected as interval cancers (40%) and twice as many breast cancers detected in an unfavourable stage (53%). Differences in tumour stage remained significant when breast cancers of *BRCA1* and *BRCA2* mutation carriers were analysed separately.

Since no prior studies have been published that assess the most effective screening frequency in *BRCA1/2* mutation carriers ≥60 specifically, it is not possible to compare our results with those of others. However, our results are supported by knowledge of the biology of *BRCA1/2* tumours. *BRCA1/2* breast cancers grow twice as fast as sporadic breast cancers[20,30] and are more often detected as interval cancers.[17] There is no evidence for a sudden change in tumour biology of *BRCA1/2* mutation carriers at age 60. This is supported by our findings and those of others, that like *BRCA1/2* breast cancers at younger age, *BRCA1* breast cancers ≥60 are significantly more often oestrogen-negative than *BRCA2* tumours ≥60.[27,31] Compared with the results of the Dutch population-based national breast cancer screening program, in which women in the ages of 50-75 are screened with biennial mammography, the results of biennial screening in *BRCA1/2* mutation carriers are disappointing. In the general population less than 30% of tumours (DCIS included) are diagnosed in an unfavourable stage; this percentage is almost doubled with biennial screening of *BRCA1/2* mutation carriers.[32] Annual screening is therefore more appropriate.

Although carefully designed and executed, our study does have limitations. We have reported extensive information on mastectomies and prior cancers in *BRCA1/2* mutation carriers from a prospectively collected national hereditary breast cancer cohort and a large family cancer clinic cohort to describe our population at risk. However, there is a considerable variability between countries in the uptake of risk reducing strategies by *BRCA1/2* mutation carriers.[10] In the Netherlands and the United States of America relatively many women opt for bilateral risk-reducing mastectomy.[11] Therefore our results may be an underestimation of the proportion

of women at risk for breast cancer aged ≥60 in countries, like France and Italy, where more carriers opt for screening.[11] In these countries there might be an even larger proportion of *BRCA1/2* mutation carriers for whom continuation of annual screening is relevant.

Secondly, our study does not have a randomized design, and there was a significant difference in the screening of *BRCA1* mutation carriers and *BRCA2* mutation carriers ≥60. *BRCA2* mutation carriers were less intensively screened and more often not screened at all. Moreover, patients with a second breast cancer were more often screened with annual screening as opposed to patients with a first breast cancer, introducing selection bias. An explanation might be that, although Dutch guidelines do not advise annual screening for *BRCA1* and *BRCA2* mutation carriers above the age of 60,[13] doctors often do not feel safe, sending these mutation carriers with a history of breast cancer, to the national breast cancer screening program where they are screened biennially. Furthermore, Dutch guidelines advise screening with annual mammography for the first 5 years after breast cancer detection, irrespective of age.[13] However, in stratified analyses the percentage breast cancers found in unfavourable tumour stage with biennial mammography was comparable for first and second breast cancers. Furthermore, adjusting for first breast cancer (yes or no) in multivariable analysis did not influence the significant difference in tumour stage at detection found between annual and biennial screening in *BRCA1* and *BRCA2* breast cancers.

Furthermore, we have chosen not to analyse survival data, as interpretation will be extremely difficult, due to a large number of patients with a history of a previous breast cancer (39%) and/or other invasive cancer (additional 18%). In general, and also in *BRCA1/2*-associated breast cancers, the risk of metastases is related to both tumour size and the number of axillary lymph nodes involved.[27,33] Consequently, 'unfavourable tumour stage', provides a good alternative outcome for prognosis.

Also, our study does not report on the number of false-positives, the number of additional examinations, or the extra costs of a more frequent screening scheme with mammography. However, the differences in tumour stage between annual and biennial mammography screening were so large, that it is likely that the advantages of annual screening outweigh the disadvantages. Finally, since so few *BRCA1/2* mutation carriers were screened with MRI, no comparative analyses were performed for this group to guarantee statistical accurateness. Screening with additional MRI in younger *BRCA1* and *BRCA2* mutation carriers is generally considered both effective, sensitivity of at least 70%,[17,34] as well as cost-effective.[35] The preferred method of breast cancer screening for *BRCA1/2* mutation carriers ≥60; MRI and/or mammography, remains to be assessed. The results of this comparison might also be influenced by breast density, which is associated with increased breast cancer incidence, also in *BRCA1/2* mutation carriers,[36] and decreased sensitivity of mammography.[37]

In conclusion, the majority of *BRCA1/2* mutation carriers are still at risk for breast cancer after the age of 60. If life expectancy is good, annual mammography screening of *BRCA1/2* mutation carriers ≥60 should be considered over biennial screening.

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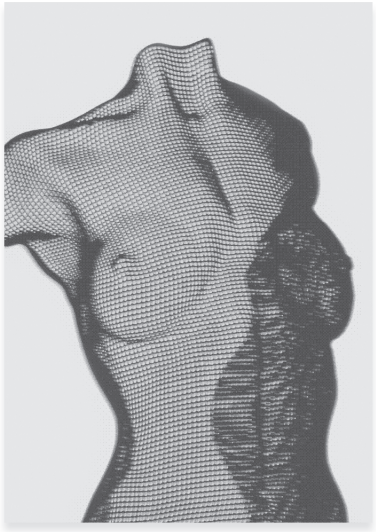
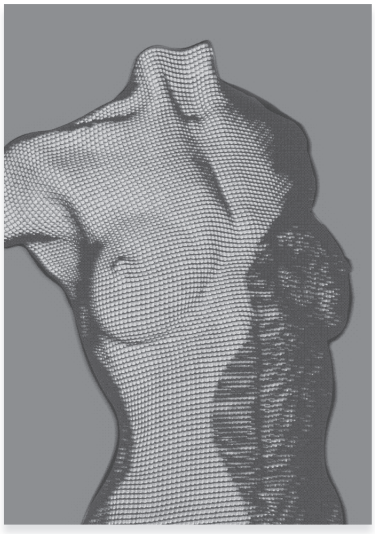
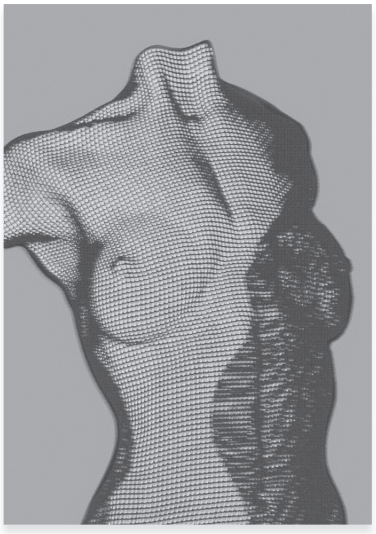

Chapter 8

Expression of cell adhesion molecules and prognosis in breast cancer

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ABSTRACT

Background

Cell adhesion molecules (CAMs) play an important role in the process of metastasis. The prognostic value of tumour expression of N-cadherin, E-cadherin, carcinoembryonic antigen (CEA) and epithelial CAM (Ep-CAM) was evaluated in patients with breast cancer.

Methods

A tissue microarray of the patient cohort was stained immunohistochemically for all markers and analysed by microscopy. Expression was classified into two categories, with the median score as cut-off level. For CEA, the above median category was further subdivided in two subgroups based on staining intensity (low or high intensity).

Results

The cohort consisted of 574 patients with breast cancer with a median follow-up of 19 years. Below median expression of E-cadherin ($p=0.015$), and above median expression of N-cadherin ($p=0.004$), Ep-CAM ($p=0.046$) and CEA ($p=0.001$) all resulted in a shorter relapse-free period. Multivariable analysis revealed E-cadherin and CEA to be independent prognostic variables. Combined analysis of CEA and E-cadherin expression showed a 3.6 times higher risk of relapse for patients with high-intensity expression of CEA, regardless of E-cadherin expression, compared with patients with below median CEA and above median E-cadherin tumour expression (hazard ratio 3.60, 95 per cent confidence interval 2.12 to 6.11; $p<0.001$). An interaction was found between expression of these two CAMs ($p<0.001$), suggesting a biological association.

Conclusions

Combining E-cadherin and CEA tumour expression provides a prognostic parameter with high discriminative power that is a candidate tool for prediction of prognosis in breast cancer.

INTRODUCTION

Breast cancer is the leading cause of cancer mortality in women worldwide.[1] Metastatic disease is responsible for most cancer deaths.[2] For a tumour to metastasize a sequential series of steps has to be completed: detachment from the primary tumour, intravasation into the circulation, extravasation into distant new organs and initiation of growth of secondary lesions with simultaneous neoangiogenesis.[3] It has been hypothesized that the capacity of tumour cells to complete these steps is gained through epithelial–mesenchymal transition (EMT).[4] This is a process whereby epithelial cells switch to a mesenchymal progenitor-cell type enabling them to lose their polarity and adhesive contacts to become invasive.

One of the features of EMT is loss of intercellular adhesions. Cell adhesion molecules (CAMs) play an important role in this process. CAMs are of importance in the first step of tumour metastasis: detachment from the primary tumour. To detach, intercellular adhesion, accomplished by CAMs, needs to be reduced. CAMs are cell-surface proteins that conduct cell–cell or cell–extracellular matrix interactions. CAMs are divided into four groups: cadherins, integrins, selectins and immunoglobulins such as carcinoembryonic antigen (CEA). CAMs that might play an important role in EMT are CEA, E-cadherin, N-cadherin and epithelial CAM (Ep-CAM).

CEA, described by Gold and Freedman in 1965, mediates, among others, cell–cell adhesion.[5] Although the association between CEA expression and prognosis for breast cancer has been evaluated in the past, the results have not been conclusive.[6-12]

The hallmark of EMT is the cadherin switch: the transposition of E-cadherin to N-cadherin expression on tumour cells.[13-15] E-cadherin and N-cadherin are CAMs that influence cell–cell adhesion by Ca^{2+}-dependent homotypic epithelial cell–cell interactions. E-cadherin prevents cells from detaching and invading the surrounding tissue, promotes cell differentiation and suppresses proliferation.[13,16,17] N-cadherin is normally found in fibroblasts and neural cells. The presence of N-cadherin increases cell motility and migration, thereby promoting dissemination.[18,19]

There are also CAMs that cannot be classified into any of the four groups mentioned above. Amongst these is Ep-CAM,[20-22] which is believed to be one of the regulators of cadherins.[23,24] Expression of this CAM is associated with poor prognosis in breast cancer.[25,26] This might be because Ep-CAM, by inducing cytoskeletal rearrangements, weakens the intercellular adhesions mediated by classical cadherins such as E-cadherin.[23,24]

To investigate the association between CAMs and prognosis in breast cancer, the tumour expression of CAM was correlated with survival in patients with breast cancer.

METHODS

The study group comprised a cohort of patients with non-metastasized breast cancer primarily treated surgically in the Leiden University Medical Centre (LUMC) between 1985 and 1994. Patients with bilateral tumours or a previous history of cancer other than basal cell carcinoma or cervical carcinoma in situ were excluded from the analysis. The following data were known: age, tumour differentiation grade and morphology, tumour node metastasis (TNM) stage, local and systemic therapy, locoregional and distant tumour recurrence, survival, and expression of oestrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 (HER2).[27]

Antibodies

Antibodies used for immunohistochemical staining were: mouse monoclonal anti-Ep-CAM antibody (323A3; provided by the Department of Pathology, LUMC), rabbit polyclonal anti-CEA antibodies (A0115; Dako, Glostrup, Denmark), anti-E-cadherin (AB53033; AbCAM, Cambridge, UK) and anti-N-cadherin (AB12221; AbCAM).

Immunohistochemistry

Slices of 4 μm were cut from a previously constructed tissue microarray (TMA) and stained immunohistochemically according to procedures described previously.[27] For each staining, a TMA slide with various tissue types served as positive control. A TMA slide stained without primary antibodies served as negative control.

Evaluation of immunostaining

The percentage of tumour cells showing membranous staining was analysed microscopically by two observers blinded to the clinical data to ensure consistency. The interclass agreement was calculated using Cohen's κ coefficient.

Statistical analysis

Continuous data are presented as median (range). The χ^2 test was used to evaluate associations between various clinicopathological variables and the expression of CEA, Ep-CAM, E-cadherin and N-cadherin. The relapse-free period (RFP) was defined as the time from date of surgery until locoregional and/or distant recurrence. RFP is reported as a cumulative incidence function, after accounting for death as a competing risk.[28] The Kaplan–Meier method was used for survival plotting and the log-rank test for comparison of survival curves. To examine whether CAMs were associated with RFP, univariable Cox proportional hazard analyses were performed. Multivariable analyses were carried out using the Cox proportional hazards model with stepwise regression, with inclusion of CAMs and clinicopathological variables shown to have an influence on outcome in univariable analysis (defined as $p<0.100$). For all regression analyses, hazard ratio estimates were calculated with 95 per cent

confidence intervals. All statistical testing was two-tailed with 0.05 as the level of significance. Missing data were not accounted for in the analyses. Statistical analyses were done using the statistical package SPSS version 16.0 for Windows (IBM, Armonk, New York, USA).

RESULTS

A total of 667 patients with non-metastatic breast cancer were treated with a primary surgical resection during the study period. Tumour material was obtained from pathology archives and incorporated in the TMA for 574 patients (86.1 per cent).[29] Ductal breast cancer was diagnosed in 513 patients in (89.4 per cent). Tumour category was T1 in 211 patients (36.8 per cent), T2 in 272 (47.4 per cent) and T3/T4 in 72 patients (12.5 per cent). Mastectomy was performed in 331 patients (57.7 per cent). Further clinicopathological and treatment characteristics are shown in **Supplementary table 1**. The median age of the women was 57 (23–96) years. Median follow-up was 19 (14–23) years.

Expression of cell adhesion molecules

Microscopic quantification of expression in tumours was successful in 530 (92.3 per cent) of 574 patients for CEA, in 537 (93.6 per cent) for Ep-CAM, in 502 (87.5 per cent) for E-Cadherin and in 486 (84.7 per cent) for N-cadherin. Cohen's κ coefficient values for the percentages of positive tumour cells were 0.675 for E-cadherin, 0.668 for N-cadherin, 0.939 for CEA and 0.999 for Ep-CAM. As the data were not distributed normally, all scores were classified into two categories, with the median expression of each CAM as cut-off point. There was a wide variety of intensity of staining of CEA in the above median group. Therefore, the above median category was further subdivided in subgroups based on intensity (low or high). No further subdivision based on intensity was made for the other CAMs.

The median percentage of positive tumour cells was 55 per cent for CEA, 0 per cent for Ep-CAM, 53 per cent for E-cadherin and 67 per cent for N-cadherin, and ranged from 0 to100 per cent for all stainings. Above median expression of Ep-CAM was seen in 147 (27.4 per cent) of 537 tumours, of E-cadherin in 245 (48.8 per cent) of 502, and of N-cadherin in 226 (46.5 per cent) of 486 (**Supplementary figure 1 A-F**). For CEA, below median expression was found in 252 (47.5 per cent) of 530 tumours; the remaining tumours showed above median expression, with low-intensity staining in 236 (44.5 per cent) and high-intensity staining in 42 (7.9 per cent) (**Supplementary figure 1 G-I**).

Above median Ep-CAM expression correlated with higher tumour grade ($p<0.001$) and lower progesterone receptor expression ($p=0.009$) (**Supplementary table 1**). Above median CEA expression levels correlated with HER2 overexpression ($p=0.008$). Above median E-cadherin expression correlated with ductal cancer

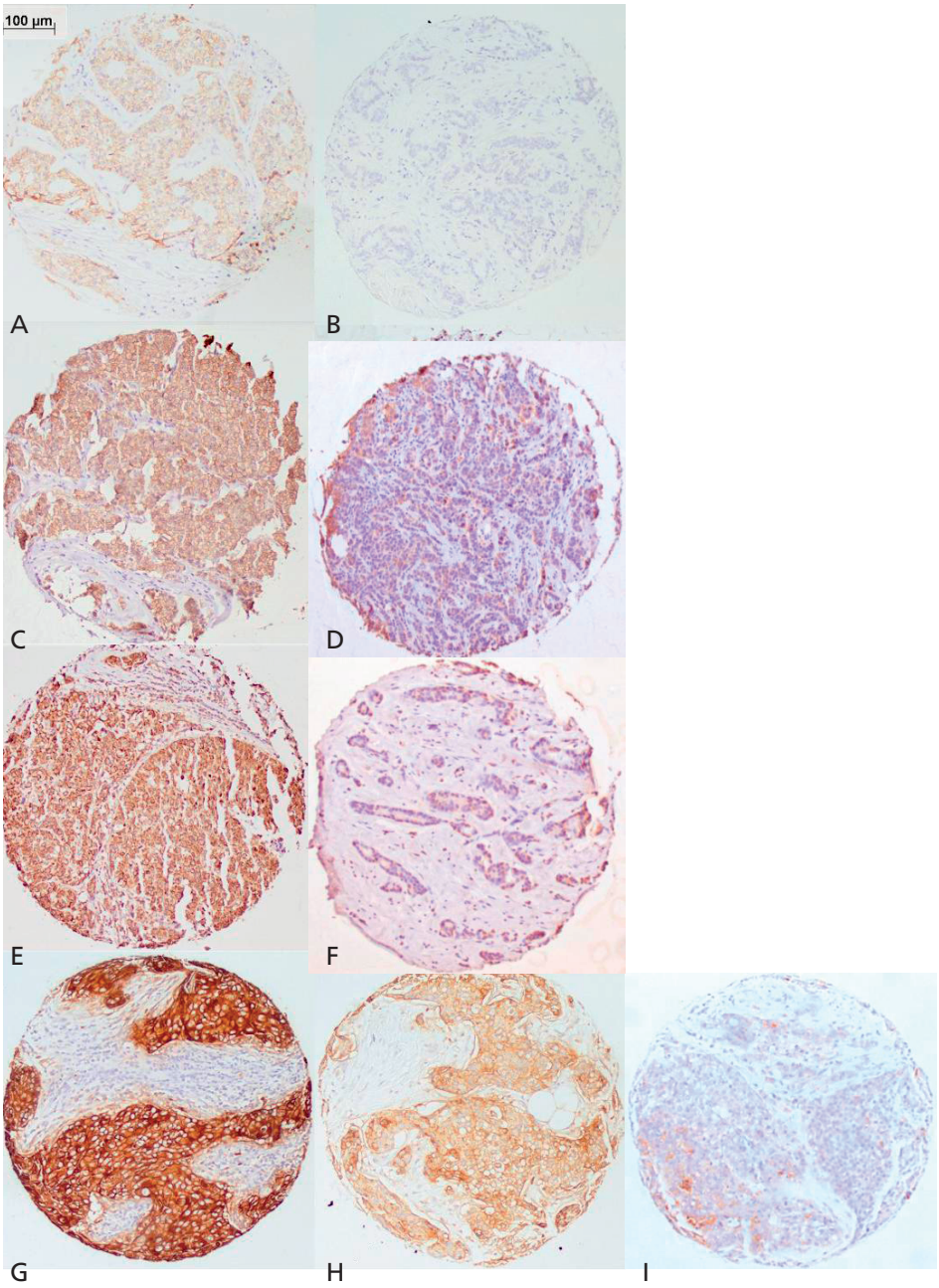
Supplementary table 1 Relationships between epithelial cell adhesion molecule, carcinoembryonic antigen, E-cadherin and N-cadherin tumour expression and prognostic factors in patients with breast cancer*

		Ep-CAM			CEA				E-cadherin			N-cadherin		
	All patients	Below median	Above median	P value	Below median	Above median, low intensity	Above median, high intensity	P value	Below median	Above median	P value	Below median	Above median	P value
Age (years)				0.243				0.313			0.637			0.221
<40	48 (8)	33 (9)	10 (7)		25 (10)	16 (7)	2 (5)		21 (8)	19 (8)		23 (9)	18 (8)	
40–50	145 (25)	103 (26)	32 (22)		69 (27)	53 (23)	9 (21)		59 (23)	66 (27)		72 (28)	49 (22)	
50–60	132 (23)	81 (21)	42 (29)		58 (23)	50 (21)	12 (29)		59 (23)	60 (25)		52 (20)	61 (27)	
>60	249 (43)	173 (44)	63 (43)		100 (40)	117 (50)	19 (45)		118 (46)	100 (41)		113 (44)	98 (43)	
Tumour grade				<0.001				0.546			0.911			0.028
I	80 (14)	61 (16)	11 (8)		36 (15)	32 (14)	2 (5)		32 (13)	33 (14)		44 (17)	18 (8)	
II	282 (50)	201 (53)	63 (43)		125 (51)	117 (51)	23 (55)		129 (51)	118 (49)		118 (46)	119 (53)	
III	203 (36)	119 (31)	72 (49)		86 (35)	82 (36)	17 (40)		92 (36)	89 (37)		93 (37)	88 (39)	
Histological subtype				0.379				0.813			0.008			0.156
Ductal cancer	513 (91)	341(89)	136 (93)		223 (90)	209 (91)	39 (93)		222 (87)	227 (95)		237 (93)	198 (88)	
Lobular	53 (9)	41 (11)	10 (7)		25 (10)	22 (10)	3 (7)		33 (13)	13 (5)		18 (7)	27 (12)	
Pathological tumour category				0.117				0.393			0.474			0.002
pT1	211 (38)	147 (40)	46 (32)		96 (40)	84 (37)	11 (27)		96 (39)	80 (34)		108 (43)	61 (28)	
pT2	272 (49)	174 (47)	83 (57)		120 (50)	110 (48)	23 (56)		118 (47)	125 (53)		119 (47)	119 (55)	
pT3/4	72 (13)	51 (14)	17 (12)		26 (11)	34 (15)	7 (17)		35 (14)	32 (14)		27 (11)	38 (17)	
Pathological node category				0.258				0.105			0.369			0.028
pN0	307 (55)	212 (57)	74 (51)		145 (59)	113 (50)	22 (52)		141 (56)	123 (52)		148 (58)	105 (48)	
pN+	250 (45)	163 (44)	71 (49)		99 (41)	114 (50)	20 (48)		110 (44)	113 (48)		107 (42)	114 (52)	
Oestrogen receptor				0.169				0.272			0.690			0.638
Negative	203 (38)	139 (37)	63 (44)		97 (41)	79 (34)	18 (43)		90 (37)	92 (39)		92 (37)	87 (39)	

Supplementary table 1 Relationships between epithelial cell adhesion molecule, carcinoembryonic antigen, E-cadherin and N-cadherin tumour expression and prognostic factors in patients with breast cancer* (continued)

		Ep-CAM			CEA				E-cadherin			N-cadherin		
	All patients	Below median	Above median	P value	Below median	Above median, low intensity	Above median, high intensity	P value	Below median	Above median	P value	Below median	Above median	P value
Positive	337 (62)	235 (63)	81 (56)		141 (59)	152 (66)	24 (57)		155 (63)	147 (62)		155 (63)	134 (61)	
Progesterone receptor				0.009				0.698			0.009			0.284
Negative	223 (42)	144 (39)	75 (51)		100 (42)	93 (41)	20 (48)		83 (34)	110 (46)		108 (43)	82 (38)	
Positive	313 (58)	227 (61)	71 (49)		139 (58)	136 (59)	22 (52)		159 (66)	129 (54)		143 (57)	133 (62)	
HER2				0.442				0.008			0.879			0.072
No overexpression	435 (81)	264 (90)	109 (88)		167 (92)	170 (90)	31 (76)		171 (90)	179 (89)		188 (92)	153 (86)	
Overexpression	103 (19)	28 (10)	15 (12)		14 (8)	20 (11)	10 (24)		20 (11)	22 (11)		17 (8)	25 (14)	
Local therapy				0.495				0.037			0.140			0.002
MAST	223 (39)	149 (38)	59 (40)		95 (38)	90 (38)	24 (57)		99 (39)	94 (38)		94 (36)	95 (42)	
MAST + RT	108 (19)	72 (19)	32 (22)		42 (17)	53 (23)	8 (19)		42 (16)	56 (23)		42 (16)	57 (25)	
BCS	243 (42)	169 (43)	56 (38)		115 (46)	93 (39)	10 (24)		116 (45)	95 (39)		124 (48)	74 (33)	
Systemic therapy				0.167				0.810			0.310			0.071
CT alone	112 (20)	81 (21)	26 (18)		47 (19)	49 (21)	9 (21)		49 (19)	55 (22)		57 (22)	47 (21)	
ET alone	75 (13)	57 (15)	13 (9)		34 (14)	32 (14)	4 (10)		30 (12)	36 (15)		30 (12)	35 (16)	
CT and ET	18 (3)	14 (4)	4 (3)		7 (3)	8 (3)	3 (7)		5 (2)	8 (3)		3 (1)	10 (4)	
None	369 (64)	238 (61)	104 (71)		164 (65)	147 (62)	26 (61)		173 (67)	146 (60)		170 (65)	134 (59)	
Total	574	390	147		252	236	42		257	245		260	226	

*Two-sided P value for difference between two risk groups; all differences were obtained from χ^2 test or Fisher's exact tests, as appropriate. Values in parentheses are percentages. Missing values or unknown status are not shown in table. Ep-CAM= epithelial cell adhesion molecule, CEA= carcinoembryonic antigen, HER2= human epidermal growth factor receptor 2, MAST= mastectomy, MAST + RT= mastectomy followed by radiotherapy of the chest, BCS= breast-conserving surgery, CT= chemotherapy, ET= endocrine therapy.



Supplementary figure 1 Representative examples of immunohistochemical staining for A,B epithelial cell adhesion molecule (EpCAM), C,D E-cadherin, E,F N-cadherin and G–I carcinoembryonic antigen (CEA) expression in breast cancer.

(p=0.008) and lack of progesterone receptor expression (p=0.009). Above median N-cadherin expression correlated with higher tumour grade (p=0.028), higher pathological tumour status (p=0.002) and more frequent positive lymph nodes (p=0.028). Testing for associations between expression levels of CEA, Ep-CAM, E-cadherin and N-cadherin revealed a positive correlation between CEA and Ep-CAM expression (p<0.001). There was no correlation between E-cadherin and N-cadherin (p=0.635), CEA (p=0.717) or Ep-CAM (p=0.453). Nor was there any correlation between N-cadherin and Ep-CAM (p=0.273) or CEA (p=0.411).

Prognostic value of cell adhesion molecules

Associations with clinical outcome were tested for all CAMs separately. Above median expression levels of N-cadherin (p=0.004), Ep-CAM (p=0.046) and CEA (p=0.001), and below median expression of E-cadherin (p=0.015), all resulted in a shorter RFP (**Figure 1**).

In Cox univariable regression analyses all four CAMs showed a statistically significant association with RFP (**Table 1**). In multivariable analyses, the only independent factors for RFP were expression of E-cadherin (p=0.002) and CEA (p=0.041) (**Table 1**).

When E-cadherin and CEA expression were combined as a single variable, above median expression levels of CEA combined with below median expression of E-cadherin resulted in a worse RFP (p<0.001) (**Figure 2**). Most notably, above median expression of E-cadherin combined with below median expression of CEA resulted in a better RFP, whereas above median expression and high-intensity staining of CEA independent of E-cadherin expression resulted in a worse RFP (p<0.001) (**Figure 2**). All other combinations of expressions of E-cadherin and CEA showed similar RFP. Therefore, the combination variable of CEA and E-cadherin expression was classified into three groups: group 1, below median expression of CEA combined with above median expression of E-cadherin (104 of 469 tumours, 22.2 per cent); group 2, above median expression, low-intensity staining of CEA independent of expression of E-cadherin, or below median expression of both E-cadherin and CEA (325 of 469 tumours, 69.3 per cent); and group 3, above median expression, high-intensity staining of CEA independent of E-cadherin expression (40 of 469 tumours, 8.5 per cent). The combined variable of CEA and E-cadherin expression was a prognostic variable for RFP (p<0.001) (**Figure 2**), predicting a 1.9 times greater risk of relapse for patients in group 2 compared with those in group 1, and a 3.6 times greater risk of relapse for group 3 compared with group 1 (**Table 2**).

The interaction between E-cadherin and CEA was tested in Cox regression analysis. The results showed a significant interaction of both E-cadherin (p<0.001) and CEA expression (p<0.001) in prognostic effect on RFP.

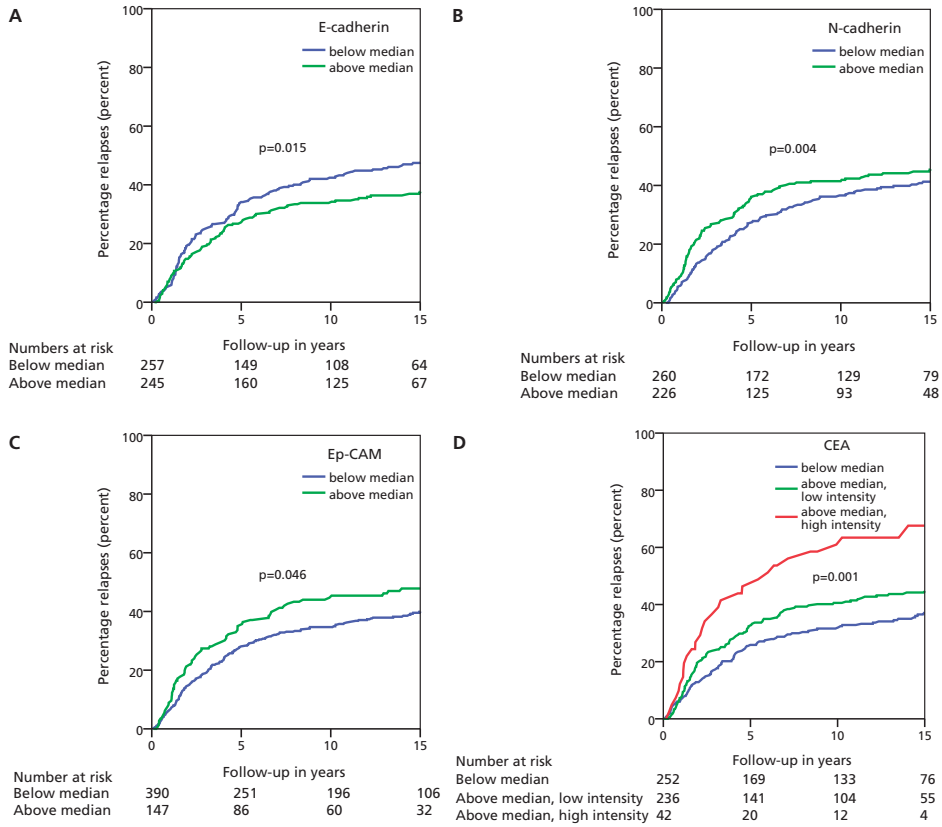


Figure 1 Relapse-free period in relation to levels of A) E-cadherin, B) N-cadherin, C) epithelial cell adhesion molecule (Ep-CAM) and D) carcinoembryonic antigen (CEA) in patients with breast cancer. Staining was classified with the median score as cut-off point (Ep-CAM, E-cadherin and N-cadherin). For CEA, the above median category was further subdivided into low-intensity and high-intensity subgroups. A) p= 0.015, B) p= 0.004, C) p= 0.046 and D) p= 0.001 (log-rank test).

Table 1 Cox univariable and multivariable analysis of clinicopathological variables and expression of cell adhesion molecules in relation to relapse-free period in breast cancer patients*

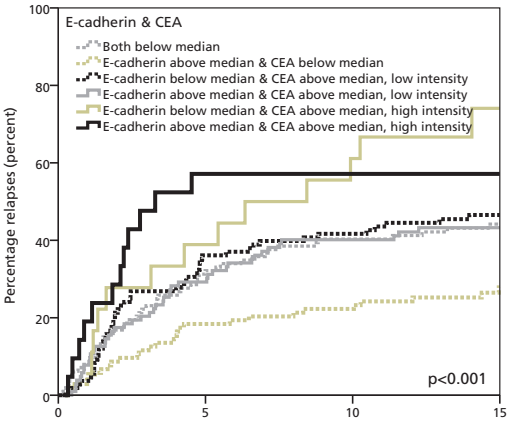
	No. of patients	Univariable analysis		Multivariable analysis	
		Hazard ratio	P value	Hazard ratio	P value
Age (years)			0.422		
<40	48	1.00			
40–50	145	0.97 (0.61, 1.54)			
50–60	132	1.17 (0.73, 1.85)			
>60	249	0.90 (0.57, 1.41)			
Tumour grade			0.001		0.065
I/II	362	1.00		1.00	
III	203	1.52 (1.19, 1.94)		1.31 (0.98, 1.75)	

Table 1 Cox univariable and multivariable analysis of clinicopathological variables and expression of cell adhesion molecules in relation to relapse-free period in breast cancer patients* (continued)

	No. of patients	Univariable analysis		Multivariable analysis	
		Hazard ratio	P value	Hazard ratio	P value
Histological subtype			0.291		
Ductal cancer	513	1.00			
Other	53	1.24 (0.83, 1.85)			
Pathological tumour category			<0.001		0.080
pT1/2	483	1.00		1.00	
pT3/4	72	1.90 (1.36, 2.66)		1.41 (0.96, 2.08)	
Pathological node category			<0.001		<0.001
pN0	307	1.00		1.00	
pN+	250	3.06 (2.38, 3.95)		2.85 (2.10, 3.88)	
Oestrogen receptor			0.725		
Negative	203	1.00			
Positive	337	1.05 (0.81, 1.36)			
Progesterone receptor			0.744		
Negative	223	1.00			
Positive	313	0.96 (0.74, 1.24)			
HER2			0.401		
No overexpression	378	1.00			
Overexpression	44	1.21 (0.78, 1.88)			
Endocrine therapy			0.197		
No	481	1.00			
Yes	93	1.24 (0.90, 1.71)			
Chemotherapy			0.839		
No	444	1.00			
Yes	130	0.97 (0.73, 1.29)			
E-cadherin			0.004		0.002
Above median	245	1.00		1.00	
Below median	257	1.38 (1.06, 1.79)		1.56 (1.17, 2.08)	
N-cadherin			0.004		0.455
Below median	260	1.00		1.00	
Above median	226	1.46 (1.13, 1.90)		1.12 (0.83, 1.50)	
Ep-CAM			0.047		0.284
Below median	390	1.00		1.00	
Above median	147	1.32 (1.00, 1.73)		1.18 (0.87, 1.61)	
CEA			<0.001		0.041
Below median	252	1.00		1.00	
Above median, low intensity	236	1.42 (1.09, 1.86)		1.38 (1.01, 1.88)	
Above median, high intensity	42	2.41 (1.59, 3.65)		2.27 (1.42, 2.64)	

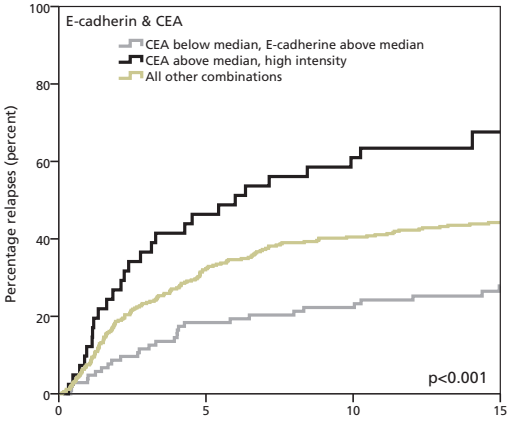
Values in parentheses are 95 per cent confidence intervals. CEA= carcinoembryonic antigen, Ep-CAM= epithelial cell adhesion molecule, HER2= human epidermal growth factor receptor 2, No.=number.

A



Number at risk				
Both below median	113	68	53	35
E-cadherin above median & CEA below median	104	77	63	35
E-cadherin below median & CEA above median, low intensity	109	61	42	22
E-cadherin above median & CEA above median, low intensity	103	66	50	26
E-cadherin below median & CEA above median, high intensity	18	10	6	3
E-cadherin above median & CEA above median, high intensity	22	8	2	1

B



Number at risk				
CEA below median, E-cadherin above median	104	77	63	35
CEA above median, high intensity	40	18	12	4
All other combinations	325	195	145	83

Figure 2 Relapse-free period in relation to E-cadherin and carcinoembryonic antigen (CEA) expression in patients with breast cancer: A) separate combinations and B) combined groups: group 1, below median expression of CEA combined with above median expression of E-cadherin; group 2, above median expression, low-intensity staining of CEA independent of expression of E-cadherin, or below median expression of both E-cadherin and CEA; and group 3, above median expression, high-intensity staining of CEA independent of E-cadherin expression. A) p<0.001, B) p<0.001 (log-rank test).

Table 2 Cox univariable and multivariable analysis of clinicopathological variables and combined expression of E-cadherin and carcinoembryonic antigen in relation to relapse-free period in patients with breast cancer*

	No. of patients	Univariable analysis		Multivariable analysis	
		Hazard ratio	P value	Hazard ratio	P value
Age (years)			0.422		
< 40	48	1.00			
40–50	145	0.97 (0.61, 1.54)			
50–60	132	1.17 (0.73, 1.85)			
> 60	249	0.90 (0.57, 1.41)			
Tumour grade			0.001		0.170
I/II	362	1.00		1.00	
III	203	1.52 (1.19, 1.94)		1.22 (0.92, 1.61)	
Histological subtype			0.291		
Ductal cancer	513	1.00			
Other	53	1.24 (0.83, 1.85)			
Pathological tumour category			< 0.001		0.112
pT1/2	483	1.00		1.00	
pT3/4	72	1.90 (1.36, 2.66)		1.36 (0.93, 1.97)	
Pathological node category			<0.001		<0.001
pN0	307	1.00		1.00	
pN+	250	3.06 (2.38, 3.95)		2.85 (2.12, 3.83)	
Oestrogen receptor			0.725		
Negative	203	1.00			
Positive	337	1.05 (0.81, 1.36)			
Progesterone receptor			0.744		
Negative	223	1.00			
Positive	313	0.96 (0.74, 1.24)			
HER2			0.401		
No overexpression	378	1.00			
Overexpression	44	1.21 (0.78, 1.88)			
Endocrine therapy			0.197		
No	481	1.00			
Yes	93	1.24 (0.90, 1.71)			
Chemotherapy			0.839		
No	444	1.00			
Yes	130	0.97 (0.73, 1.29)			
E-cadherin–CEA			<0.001		<0.001
Group 1	104	1.00		1.00	
Group 2	325	1.99 (1.35, 2.94)		1.94 (1.30, 2.90)	
Group 3	40	3.39 (2.01, 5.72)		3.60 (2.12, 6.11)	

*Values in parentheses are 95 per cent confidence intervals. CEA= carcinoembryonic antigen, HER2= human epidermal growth factor receptor 2, Group 1= below median expression of CEA combined with above median expression of E-cadherin, Group 2= above median expression, low-intensity staining of CEA independent of expression of E-cadherin, or below median expression of both E-cadherin and CEA, Group 3= above median expression, high-intensity staining of CEA independent of E-cadherin expression.

DISCUSSION

In the present study of the prognostic value of CEA, E-cadherin, N-cadherin and Ep-CAM in breast cancer, all four CAMs were associated with RFP. E-cadherin and CEA expression were identified as independent prognostic factors for RFP in multivariable analysis. By combining the independent prognostic markers E-cadherin and CEA, a strong prognostic predictor was created. RFP was almost four times shorter for patients with CEA tumour expression above median and with high-intensity staining compared with that of patients with above median E-cadherin and below median CEA tumour expression. Cox regression analysis revealed that E-cadherin and CEA expression interacted and together determined the prognostic effect on RFP.

The findings in the present study are consistent with previous investigations in which E-cadherin was shown to be a tumour dissemination suppressor.[13,16,17] Studies assessing CEA expression on breast cancer tissue have not been conclusive.[7-12] Results of these studies are difficult to compare for various reasons. Tumour expression of CEA has been assessed in different patient populations using different antibodies and different immunohistochemical cut-offs for defining high CEA tumour expression.[7-12] The number of patients studied, and the follow-up times also differed.[7-12] The present results have confirmed that there is a difference in RFP for patients with high versus low CEA tumour expression.[9-11]

The conventional prognostic factors for breast cancer survival are tumour size, lymph node status and tumour grade. However, the value of these prognostic factors decreases with follow-up time.[30] Survival associated with these prognostic factors usually shows a pronounced decrease in the first 5 years, then stabilizes. Thus, the influence of these traditional prognostic variables appears to attenuate over time. However, survival for patients with breast cancer differs from that of the general population even 10 years after diagnosis and so there is a need for prognostic factors that reflect long-term survival.

Several prognostic factors have been found for outcome in patients with breast cancer. Microarray-based gene expression analysis, as well as measurement of urokinase plasminogen activator (uPA) and plasminogen activator inhibitor (PAI) 1 are recommended for routine clinical use by the American Society of Clinical Oncology (ASCO).[31] Microarray-based multigene tests, such as MammaPrint® (Agendia, Irvine, California, USA), a 70-gene expression profile, and Oncotype DX® (Genomic Health, Inc, Redwood City, CA, USA), a 21-gene-based recurrence score, are currently being tested as prognostic tools in large prospective cohorts.[32] Evidence is accumulating that these complex gene profiles quantify tumour characteristics such as tumour grade, oestrogen receptor expression, HER2, cell cycle and cell proliferation.[33,34] However, gene expression arrays cannot always be used in daily clinical practice as most tests require fresh breast tissue for mRNA extraction,[35] and are expensive and difficult to perform. Gene profiles of both Oncotype DX® and MammaPrint®

cannot be determined in local hospitals, but have to be analysed in larger centres. By contrast, immunohistochemistry is a cheap and simple method, and can be performed in all diagnostic centres. Although UPA and PAI-1 are recommended for testing by ASCO, this is a subject of debate and they are currently being evaluated in the prospective Node-Negative Breast Cancer III study. Patient recruitment was stopped in 2009, but the analysis has yet to be performed.[36] In a recent study, different tumour markers, including carbohydrate antigen15-3, were correlated with different breast cancer subtypes.[37] It might therefore be interesting to investigate the association of CAMs with these tumour markers and different subtypes of breast cancer.

The results of the present study suggest that CAMs are of importance for tumour metastasis, possibly via mechanisms of EMT.[4,21] Although the exact roles of CEA and E-cadherin still remain unclear, E-cadherin and CEA expression interact, and together have a prognostic effect on RFP. This suggests a biological association, in which loss of E-cadherin and gain of CEA expression seem essential steps in tumour cell migration. The combination of E-cadherin and CEA expression distinguishes between patients of different prognosis with high discriminative power.

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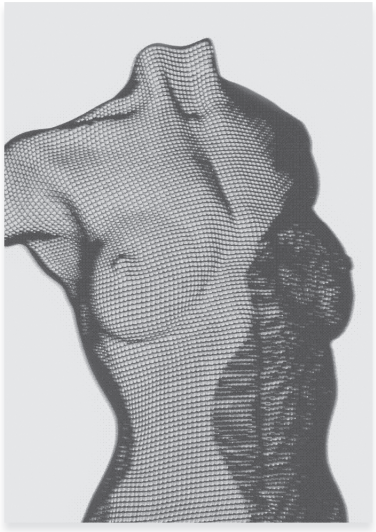
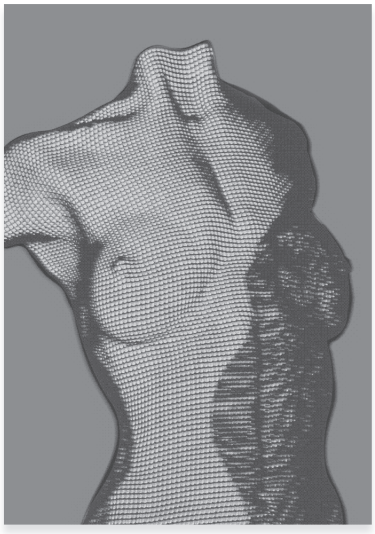
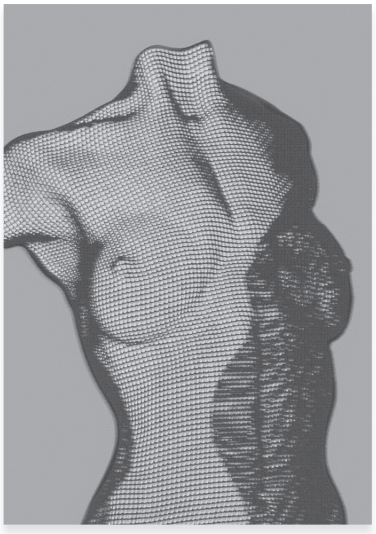

Chapter 9

Influence of tumor stage at breast cancer detection on survival in modern times; a population-based study in 173,797 patients

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Submitted

ABSTRACT

Background

Breast cancer survival has increased significantly, partly due to more effective systemic therapy. To what extent stage still determines survival in contemporary times of better systemic therapy is unknown.

Methods

Female primary breast cancer patients diagnosed between 1999-2005 and 2006-2012 were selected from the nationwide Netherlands Cancer Registry. Clinicopathological characteristics were compared with χ^2-tests. Relative survival was compared between both cohorts. Influence of traditional prognostic factors on overall survival was analyzed for both cohorts separately with Cox regression.

Results

Compared to 1999-2005 (n=80,228) patients from 2006-2012 (n=93,569) had smaller ($\leq$T1 65% versus 60%, p<0.001), more often lymph node negative (N0 68% versus 65%, p<0.001) tumors, but they received more chemotherapy, hormonal therapy, and targeted therapy (neo-adjuvant/adjuvant systemic therapy 60% versus 53%, p<0.001). Median follow-up was 9.8 years for 1999-2005 and 3.9 years for 2006-2012. Relative 5-years survival rates were higher in 2006-2012 for all tumor and nodal stages, and 100% in tumors $\leq$1 cm. Adjusted, for tumor type, surgery type, radiotherapy and systemic therapies, survival decreased with increasing tumor size in both cohorts (2006-2012 T1c versus T1a HR 1.54, 95% CI 1.33-1.78), but without significant difference in invasive breast cancers until 1 cm (2006-2012 T1b versus T1a HR 1.04, 95% CI 0.88-1.22). Survival decreased independently with progressing number of positive lymph nodes (2006-2012 N1 versus N0 HR 1.25, 95% CI 1.17-1.32).

Conclusion

Tumor stage at breast cancer diagnosis influences overall survival significantly also in the current era of effective systemic therapy. Early breast cancer detection remains vital.

INTRODUCTION

In the last decades breast cancer survival rates have increased significantly all over the world.[1-3] In the US the 5-year relative survival rates for female breast cancer patients have improved from approximately 75% in 1975 thru 1977 to 90.3% in 2003 thru 2009.[4] This survival improvement can mainly be explained by an effect of both earlier diagnosis as a result of breast cancer screening and awareness, and better treatment options.[5,6]

The risk of metastases and death increases with both breast cancer size at detection and number of axillary lymph nodes involved.[7-10] Screening aims to improve survival, by decreasing risk of metastases through early breast cancer detection. In the Netherlands the national breast cancer screening program with biennial mammography was implemented for all women aged 50-69 years in 1989, and in 1998 the program was extended to the ages 71-74 year.[11]

Next to tumor size and lymph node involvement cancer-related factors that influence survival are; tumor grade, hormone receptor status, and human epidermal growth factor receptor 2 (HER2).[8-10,12] Surgery, the cornerstone of breast cancer treatment, changed in this period: to assess lymph node positivity in 1999 sentinel lymph node biopsy (SLNB) was first described in Dutch guidelines,[13] although regional implementation had already started. The proportion of early stage breast cancer patients who underwent SLNB increased from approximately 9% in 1998 to over 70% in 2003.[14] Recently, Mittendorf et al. published data indicating that in patients with small breast cancers lymph node micro metastases are not of any prognostic value.[12] An explanation might be the increasing effectiveness of systemic therapy.

In more recent years (neo-)adjuvant systemic breast cancer treatment has improved considerably and is applied more often. Improvements include the use of trastuzumab that increases both short-term[15] and long-term prognosis significantly in HER2 positive breast cancer patients.[16] Trastuzumab was implemented in the Netherlands between 2005 and 2006.[17,18] Moreover, there has been a switch to more effective chemotherapy regimens. CMF (Cyclofosfamide, Methotrexaat, 5-Fluorouracil) was prescribed to 90% of breast cancer patients receiving chemotherapy in 2000 and to almost none in 2005.[19] It was gradually replaced by the more effective anthracyclines (4% use in 2000 to 96% in 2005) at first, which in turn were partly replaced by taxane-containing regimens later.[19]

The effect of screening and better treatment options on survival may be distributed differently nowadays, since data published were based on patient cohorts with breast cancer diagnosis in 2004 latest, and changes to more recent systemic therapy had not yet occurred. Possibly traditional prognostic factors, like tumor size and number of positive lymph nodes, no longer predict survival in the current era of new systemic therapy. And if these factors do affect survival, the size of this effect is unknown. To quantify the effect of traditional prognostic factors, both

long-term and in the current era, we describe overall survival of female breast cancer patients of two time cohorts (1999-2005 and 2006-2012) in a nationwide population-based study using data of the Netherlands Cancer Registry.

METHODS

Patient population

Female breast cancer patients diagnosed with primary breast cancer between January 1, 1999 and December 31, 2012 were selected from the Netherlands Cancer Registry (NCR). Excluded were patients with; a prior history of invasive cancer, or lack of information on both clinical and pathological tumor size.

The NCR is a nationwide prospective population-based cancer registry in which all newly pathologically confirmed malignancies in the Netherlands are recorded. New malignancies are detected through the national Pathology Archive (PALGA), in which all pathological reports of Dutch hospitals are collected. Trained registrars from the NCR collect patient and tumor characteristics, and primary treatment directly from the patient's medical records. By linkage to the municipal administration vital status and date of death, if applicable, is verified. Last date of linkage was December 31, 2013. Follow-up was complete for all, except women who emigrated out of the Netherlands before that time. This study was conducted in accordance with the Helsinki declaration. The study was approved by the privacy committee of the Netherlands Cancer Registry.

We subdivided patients into two time cohorts; 1999-2005 and 2006-2012 on the basis of their breast cancer diagnosis. These cohorts were chosen, because in 2005 and onward chemotherapy schemes used were changed, trastuzumab was implemented,[17] and Dutch guidelines were more liberal on who should receive adjuvant treatment.[18] Analyses of the 1999-2005 cohort were performed to confirm long-term effects of traditional prognostic factors on survival in earlier times in our Dutch population-wide cohort.

The following data were registered: date and age at breast cancer diagnosis, tumor characteristics, local and systemic therapy, vital status, second primary breast cancer, date of follow-up and date of death. Local recurrence and occurrence of distant metastases were not registered by the NCR. Second primary breast cancer was defined as contralateral ductal carcinoma in situ or invasive epithelial breast cancer.[20] For local breast therapy the most extensive surgery performed within 1 year of diagnosis was used. Data on whether patients underwent axillary lymph node dissection was registered all years, but data on sentinel lymph node biopsy procedure was only registered from 2011 on. Staging of primary tumors was based on the pathological AJCC Cancer Staging classification 7th edition.[21] If pathological tumor size was missing, clinical stage based on imaging studies and clinical examination was used. Tumor stage (T-stage) was defined on the greatest dimension of

the largest tumor size; Tis= ductal carcinoma in situ, T1a= ≤0.5 cm (including micro invasion), T1b= >0.5 cm and ≤ 1 cm, T1c= >1 cm and ≤ 2 cm, T2= >2 cm and ≤5 cm, T3= >5 cm, T4= any size with direct extension to chest wall and/or to skin. Lymph node status (N-stage) was described depending on the number of regional lymph nodes with pathologically proven metastasis, e.g. positive. Lymph node positivity was determined including results of sentinel lymph node biopsy. Lymph nodes with only isolated tumor cells were defined lymph node negative. N0= no pathologically proven positive lymph nodes, N1= 1-3 positive, N2= 4-9 positive, N3= ≥10 positive. Grading of tumors was based on the modified Bloom & Richardson grading system.[22] Patients were considered estrogen (ER) and progesterone (PR) positive in case of more than 10% nuclear staining. Hormone receptor status was registered from 2005 and onward, and HER2 status was registered in the NCR from 2006 onward.

Statistical Analysis

Differences in stage distributions, lymph node status, and tumor characteristics between the two time cohorts were calculated using Pearson's χ^2 tests, differences in age distribution at breast cancer diagnosis were assessed with the Mann-Whitney *U* test.

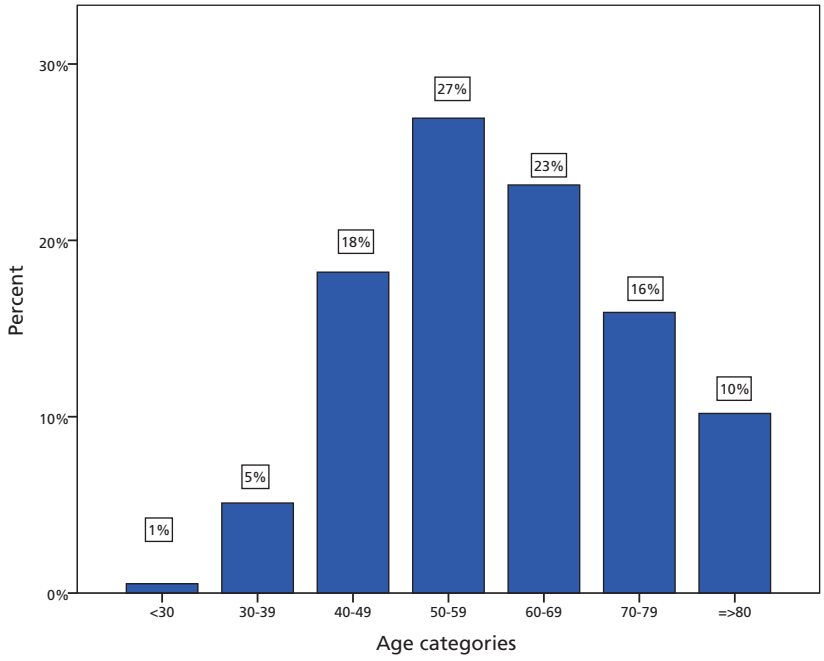
Overall survival was defined as time from breast cancer diagnosis to death resulting from any cause.[23] Relative survival was defined as the relative excess risk of death, or the observed survival of our study population divided by the expected survival of the corresponding general population by sex, age, and year of diagnosis.[24] Relative survival was calculated using the Ederer II method. Dutch national life-tables of the central bureau of statistics (CBS) were used to estimate expected survival in the general population. Relative 5-year survival rates were estimated. Relative survival curves stratified for tumor stage and nodal stage were plotted. Women were censored at date of last follow-up. Since the follow-up of women in the two time cohorts differed considerably no statistical comparisons were performed between the two time periods, and all analyses were performed stratified for time period of breast cancer detection.

Cox univariable and multivariable proportional hazard models for overall survival were developed for invasive breast cancers to estimate hazard ratios with 95% confidence intervals (CI). Since DCIS is expected to be 100% no influence of prognostic factors on overall survival of DCIS was expected and Cox regression univariable and multivariable analyses were performed only for invasive breast cancers. In multivariable analyses all clinicopathological relevant variables and variables with a P value <0.05 in univariable analyses were included. The assumption of proportional hazards was found to be valid by graphically plotting the log-log survival curves. A two-sided P value ≤0.05 was considered statistically significant. Missing values were analyzed as separate unknown group within the same variable. Statistical analyses were performed using SPSS Statistics for Windows, version 20.0 (IBM Corp, Armonk, NY, US) and relative survival in SAS (version 9.4, SAS Institute Inc, Cary, NC,US).

RESULTS

Patient & tumor characteristics

In the Netherlands 173,797 female patients were diagnosed with primary breast cancer between 1999-2012; 80,228 (46%) diagnosed from 1999 through 2005 and 93,569 (54%) from 2006 through 2012. Median age at diagnosis was 59 years for both time periods, and age distribution was comparable (p=0.168). **Supplementary figure 1** shows distribution in different age categories at breast cancer diagnosis. Compared to 1999-2005 tumors diagnosed from 2006-2012 were smaller (≤T1 65% versus 60%, p<0.001), more often lymph node negative (N0 68% versus 65%, p<0.001), and low grade (invasive cancers grade 1 21% versus 16%, p<0.001). Recently diagnosed breast cancer patients underwent breast conserving therapy more often (54% versus 48%, p<0.001), while less often axillary node dissection was performed (p<0.001). Uptake of radiotherapy and systemic therapy was increased (p<0.001); hormonal therapy (+10%), chemotherapy (+7%), targeted therapy (mainly trastuzumab +7%) and the combination (+7%). Hormone receptor status



Supplementary figure 1 Age distribution at breast cancer diagnosis in the Netherlands Cancer Registry from 1999-2012.

The Netherlands Cancer Registry is an ongoing nationwide prospective population-based cancer registry in which all newly pathologically confirmed malignancies in the Netherlands are recorded. The age distribution in years of 173,797 females diagnosed with breast cancer between 1999-2012 is shown. There is a peak around menopause, age category 50-59 years.

was available for invasive breast cancers of the most recent cohort of 2006-2012 only and is described in **table 1**.

Relative and overall survival

Median follow-up was 9.8 (0-15) years for the first cohort 1999-2005 and 3.9 (0-8) years for the second cohort. During follow-up there were 27,924 events in the first cohort (1999-2005) and 11,177 events in the second cohort (2006-2012). Relative survival curves for both tumor stage (**Figure 1**) and nodal stage (**Figure 2**) were plotted. Compared to 1999-2005 5-year relative survival rates and overall survival rates were higher for the 2006-2012 cohort for all tumor and nodal stages (**Table 2**). Relative survival of DCIS was 100% after 15 years for the 1999-2005 cohort and 101% after 8 years for the 2006-2012 cohort. Relative survival decreased with increasing tumor and nodal stages, except for T1b versus T1a (1999-2005 100% versus 99%, 2006-2012 101% versus 100%). Relative survival in the 1999-2005 cohort did not decrease after 9 years for all tumor sizes ≤T1c and after 13 years for tumors ≥T2. In the 2006-2012 cohort no relative survival decrease was seen in tumor sizes ≤T2 after 6 years and for tumor sizes >T2 after 7 years.

Prognostic factors: cohort 1999-2005

Since relative survival of DCIS was ≥100% no influence of prognostic factors on overall survival of DCIS was expected and Cox regression univariable and multivariable analyses were performed only for invasive breast cancers. Patients with breast surgery classified as 'other' were excluded from analyses, due to the small numbers (n=93 in all invasive breast cancer patients of both time cohorts), and heterogeneity of this group. Of 73,245 invasive breast cancer patients diagnosed between 1999-2005 26,717 (37%) deceased during follow-up. With univariable and multivariable Cox regression analyses we assessed influence on overall survival of; age, second primary breast cancer, tumor and nodal stage, grade, morphology, breast surgery, axillary lymph node dissection, chemotherapy, hormonal therapy, targeted therapy, and radiotherapy. Cox regression univariable and multivariable analyses showed that corrected for above mentioned factors higher tumor stage and lymph node positivity decreased overall survival (**Table 3**).

Prognostic factors: cohort 2006-2012

In the 2006-2012 cohort 10,778 (13%) of 83,191 invasive breast cancer patients deceased during follow-up. In Cox regression univariable analyses all clinicopathological variables were significantly associated with overall survival in the 2006-2012 cohort (**Table 4**). In multivariable analysis we adjusted for age, tumor and nodal stage, grade, morphology, hormone receptor and HER2 status, breast surgery, axillary lymph node dissection, chemotherapy, hormonal therapy, targeted therapy, radiotherapy, and second primary breast cancer. Tumor stage and nodal stage were both significantly associated with overall survival, although tumor size was only of

Table 1 Patient and tumor characteristics and treatment by time period in breast cancer patients in the Netherlands Cancer Registry from 1999-2012*

Patient and tumor characteristics	1999-2005		2006-2012		Total		P value
Number of breast cancer patients (%)	80,228	(46)	93,569	(54)	173,797	(100)	-
Age at diagnosis in years, median (range)	59	(17-100)	59	(18-103)	59	(17-103)	0.168
Second primary breast cancer							**<0.001**
No	74,689	(93)	89,836	(96)	164,525	(95)	
Yes	5,539	(7)	3,733	(4)	9,272	(5)	
Pathological tumor category, no (%)							**<0.001**
Ductal carcinoma in situ	6,920	(9)	10,348	(11)	17,268	(10)	
T1a	2,398	(3)	3,846	(4)	6,244	(4)	
T1b	9,599	(12)	12,213	(13)	21,812	(13)	
T1c	29,114	(36)	34,163	(37)	63,277	(36)	
T2	26,624	(33)	27,946	(30)	54,570	(31)	
T3	2,711	(3)	3,213	(3)	5,924	(3)	
T4	2,862	(4)	1,840	(2)	4,702	(3)	
Pathological node category, no (%)							**<0.001**
N0	52,238	(65)	63,544	(68)	115,782	(67)	
N1	19,012	(24)	21,901	(23)	40,913	(24)	
N2	5,985	(8)	5,400	(6)	11,385	(7)	
N3	2,993	(4)	2,724	(3)	5,717	(3)	
B&R grade, ductal carcinoma in situ only, no (%)							**<0.001**
Grade 1	986	(14)	1,667	(16)	2,653	(15)	
Grade 2	1,798	(26)	3,162	(31)	4,960	(29)	
Grade 3 (including anaplastic)	2,863	(41)	4,842	(47)	7,705	(45)	
Unknown	1,273	(18)	677	(7)	1,950	(11)	
B&R grade, invasive cancers only, no (%)							**<0.001**
Grade 1	11,939	(16)	17,334	(21)	29,273	(19)	
Grade 2	26,923	(37)	32,672	(39)	59,595	(38)	
Grade 3 (including anaplastic)	21,119	(29)	22,269	(27)	43,388	(28)	
Unknown	13,327	(18)	10,946	(13)	24,273	(16)	
Morphology, invasive cancers only, no (%)							**<0.001**
Ductal carcinoma or ductal mixed type	56,144	(77)	66,124	(80)	122,268	(78)	
Lobular carcinoma	8,133	(11)	9,133	(11)	17,003	(11)	
Other	9,031	(12)	7,964	(10)	17,266	(11)	
Estrogen receptor status, no (%)†							-
Negative	-	-	13,876	(17)	-	-	
Positive	-	-	67,993	(82)	-	-	
Unknown	-	-	1352	(2)	-	-	
Progesterone receptor status, no (%)†							-
Negative	-	-	26,268	(32)	-	-	
Positive	-	-	53557	(64)	-	-	
Unknown	-	-	3,396	(4)	-	-	

Table 1 Patient and tumor characteristics and treatment by time period in breast cancer patients in the Netherlands Cancer Registry from 1999-2012* (continued)

Patient and tumor characteristics	1999-2005		2006-2012		Total		P value
HER2 status, no (%)†							-
Negative	-	-	67,418	(81)	-	-	
Positive	-	-	10,899	(13)	-	-	
Unknown/inconclusive	-	-	4,904	(6)	-	-	
Breast surgery, no (%)							**<0.001**
No surgery	3,319	(4)	4,877	(5)	8,196	(5)	
Breast conserving therapy	38,638	(48)	50,313	(54)	88,951	(51)	
Mastectomy	38,040	(47)	38,307	(41)	76,347	(44)	
Other	231	(0)	72	(0)	303	(0)	
Axillary lymph node dissection, no (%)							**<0.001**
No	34,790	(43)	62,548	(67)	97,338	(56)	
Yes	45,438	(57)	31,021	(33)	76,459	(44)	
Systemic therapy, no (%)‡							**<0.001**
No	38,043	(47)	37,167	(40)	75,210	(43)	
Yes	42,185	(53)	56,402	(60)	98,587	(57)	
Chemotherapy, no (%)							**<0.001**
No	56,199	(70)	58,750	(63)	114,949	(66)	
Yes	24,029	(30)	34,819	(37)	58,848	(34)	
Hormonal therapy, no (%)							**<0.001**
No	48,908	(60)	48,212	(52)	97,120	(56)	
Yes	31,320	(39)	45,357	(49)	76,677	(44)	
Targeted therapy, no (%)							**<0.001**
No	79,503	(99)	86,158	(92)	165,661	(95)	
Yes	725	(1)	7,411	(8)	8,136	(5)	
Radiotherapy, no (%)							**<0.001**
No	33,303	(42)	34,469	(37)	67,772	(39)	
Yes	46,925	(59)	59,100	(63)	106,025	(61)	

*All percentages were calculated vertically. Total of percentages may not equal 100% due to rounding. Two-sided P value for difference between the two time cohorts, differences in age distribution at diagnosis were calculated from the Mann-Whitney *U* test. All other differences were obtained from χ^2. Missing values were analyzed as separate unknown group within the same variable. B&R= Bloom & Richardson, HER2= human epidermal growth factor receptor 2, N0= no pathologically assessed regional lymph nodes with metastasis/isolated tumor cells, N1= metastasis in 1-3 regional lymph nodes, N2= metastasis in 4-9 ipsilateral regional lymph nodes, N3= metastasis in ≥10 regional lymph nodes, T1a= ≤0.5 cm (including micro invasion), T1b= >0.5 cm and ≤1 cm, T1c= >1 cm and ≤ 2 cm, T2= >2 cm and ≤5 cm, T3= >5 cm, T4= any size with direct extension to chest wall and/or to skin.

†Hormone and HER2 status was only available for the 2006-2012 cohort.

‡Systemic therapy includes chemotherapy, hormonal therapy, and all targeted therapy (mainly trastuzumab).

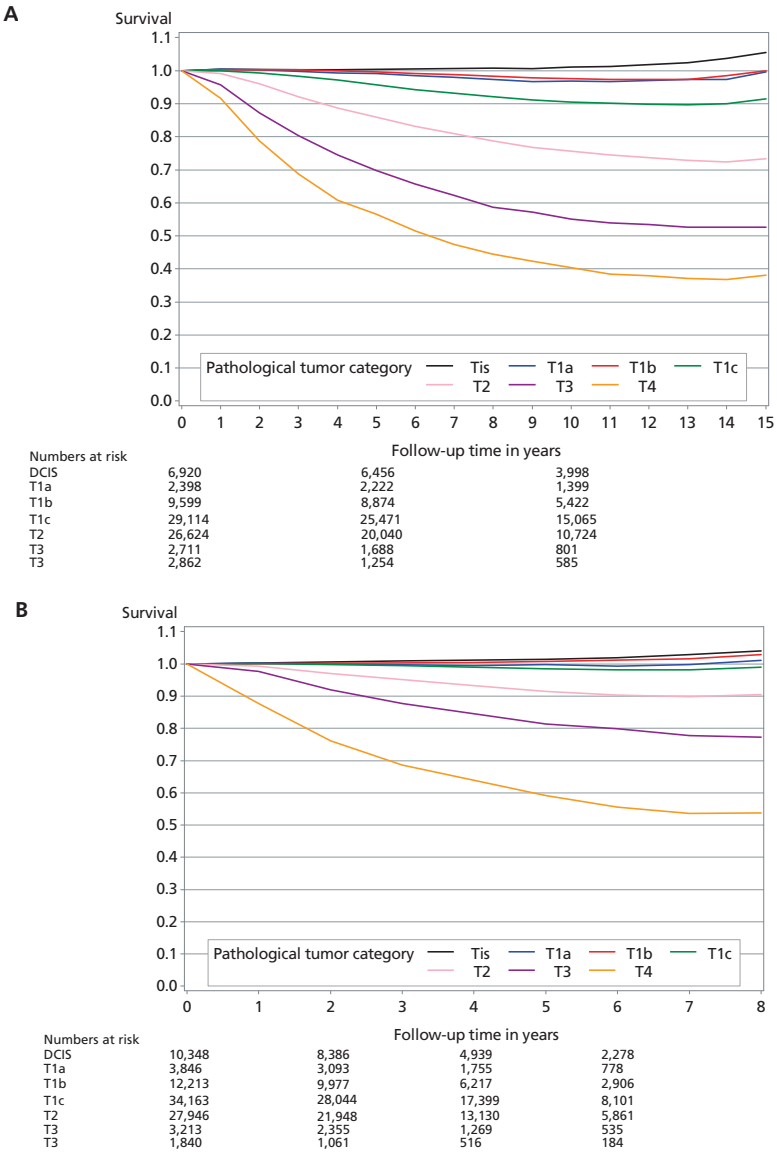
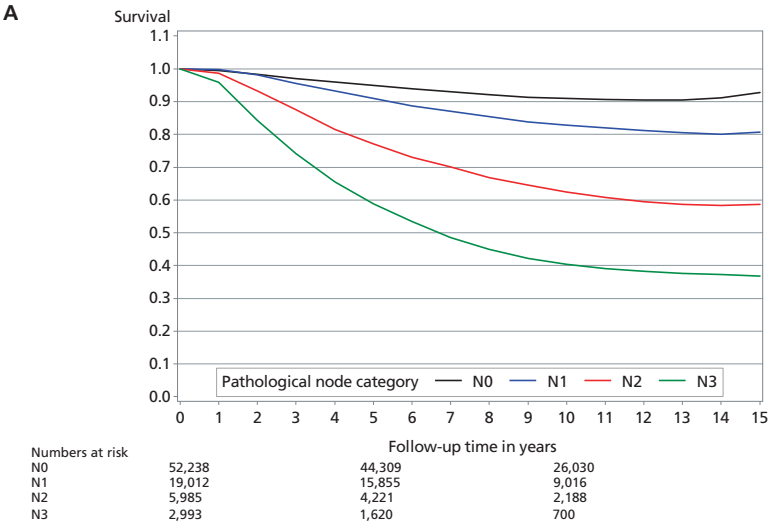


Figure 1 Tumor stage specific relative survival of breast cancer patients in the Netherlands Cancer Registry diagnosed with breast cancer in A) 1999-2005 and B) 2006-2012.
The Netherlands Cancer Registry is an ongoing nationwide prospective population-based cancer registry in which all newly pathologically confirmed malignancies in the Netherlands are recorded. Relative survival per tumor stage of female breast cancer patients is depicted. Relative survival was defined as observed survival divided by expected survival of the corresponding general population, matched by sex, age, and year of diagnosis. Relative survival was calculated using the Ederer II method. Women were censored at date of last follow-up. Tis= ductal carcinoma in situ. T1a= ≤0.5 cm (including micro invasion), T1b= >0.5 cm and ≤1 cm, T1c= >1 cm and ≤ 2 cm, T2= >2 cm and ≤5 cm, T3= >5 cm, T4= any size with direct extension to chest wall and/or to skin.



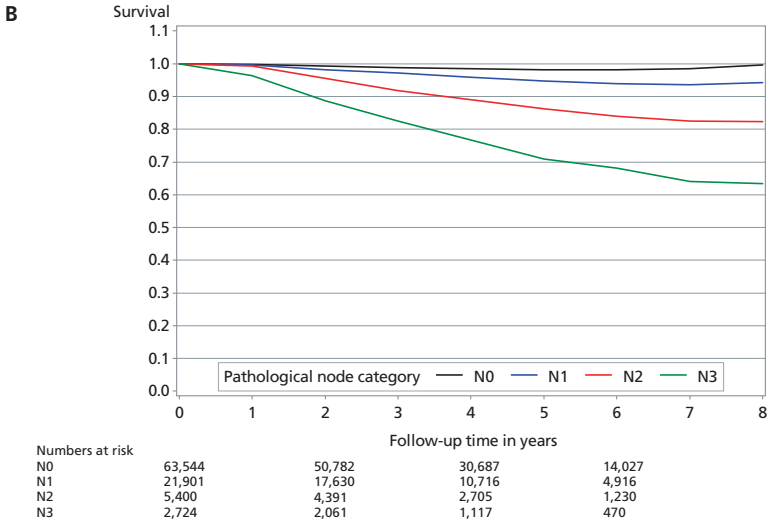


Figure 2 Nodal stage specific overall survival of breast cancer patients in the Netherlands Cancer Registry diagnosed with breast cancer in A) 1999-2005 and B) 2006-2012.
The Netherlands Cancer Registry is an ongoing nationwide prospective population-based cancer registry in which all newly pathologically confirmed malignancies in the Netherlands are recorded. Relative survival per tumor stage of female breast cancer patients is depicted. Relative survival was defined as observed survival divided by expected survival of the corresponding general population, matched by sex, age, and year of diagnosis. Relative survival was calculated using the Ederer II method. Women were censored at date of last follow-up. N0= no pathologically assessed regional lymph nodes with metastasis/isolated tumor cells, N1= metastasis in 1-3 regional lymph nodes, N2= metastasis in 4-9 regional lymph nodes, N3= metastasis in ≥10 regional lymph nodes.

Table 2 Estimated 5-year overall survival and relative survival rates per tumor stage and nodal stage by time period in breast cancer patients in the Netherlands Cancer Registry from 1999-2012*

	Overall survival (%)		Relative survival (%)	
	1999-2005	2006-2012	1999-2005	2006-2012
All patients	83	88	91	96
DCIS	94	96	100	101
T1a	93	95	99	100
T1b	93	95	100	101
T1c	88	91	96	98
T2	76	82	86	92
T3	63	73	70	81
T4	44	45	57	59
N0	85	90	95	98
N1	84	88	91	95
N2	71	81	77	86
N3	55	66	59	71

*Overall survival was defined as time from breast cancer diagnosis to death from any cause. Relative survival was defined as observed survival divided by expected survival of the corresponding general population, matched by sex, age, and year of diagnosis. Relative survival was calculated using the Ederer II method. Women were censored at date of last follow-up.

significant influence when larger than 1 cm (T1b versus T1a HR 1.04, 95% CI 0.88-1.22, p=0.677). Positive hormone receptors lowered hazard of death with 20-30% (p<0.001), in contrast to positive HER2 status which lost significance in multivariable analyses (HR 1.00, 95% CI 0.93-1.08, p=0.933). Axillary lymph node dissection increased hazard rate (HR 1.29, 95% CI 1.21-1.38, p<0.001) and breast conserving therapy decreased hazard rate (HR 0.87 95% CI 0.81-0.93, p<0.001). Treatment with chemotherapy, hormonal therapy, targeted therapy and radiotherapy all led to lower hazard rates. (p<0.001). Contrary to univariable analysis second primary breast cancer had no effect on overall survival in multivariable analysis (HR: 1.01, 95% CI 0.93-1.11, p=0.762).

DISCUSSION

In this Dutch population-wide prospective cohort study we estimated influence of well established prognostic factors in 173,797 female primary breast cancer patients in two time cohorts; 1999-2005 and 2006-2012. Median age at breast cancer diagnosis was 59 years, with a peak around menopause; age cohort 50-59 years. Tumors diagnosed in the most recent cohort, were smaller, more often lymph node negative, and more often low grade than tumors from the first time period.

Table 3 Cox univariable and multivariable analyses of clinicopathological variables for overall survival in invasive breast cancer patients in the Netherlands Cancer Registry from 1999-2005*

	Univariable Analyses			Multivariable Analyses†			
Clinicopathological variables	**HR**	**95% CI**	**P value**	**Number**	**HR**	**95% CI**	**P value**
Pathological tumor category							
T1a	Ref			2,393	Ref		
T1b	1.07	0.97-1.18	0.195	9,589	1.09	0.99-1.20	0.098
T1c	1.50	1.37-1.64	**<0.001**	29,100	1.40	1.27-1.53	**<0.001**
T2	2.74	2.50-3.00	**<0.001**	26,597	1.91	1.74-2.10	**<0.001**
T3	4.17	3.76-4.61	**<0.001**	2,710	2.60	2.34-2.89	**<0.001**
T4	7.59	6.88-8.38	**<0.001**	2,856	2.77	2.50-3.07	**<0.001**
Pathological node category							
N0	Ref			45,280	Ref		
N1	1.04	1.01-1.07	**0.018**	18,993	1.35	1.30-1.39	**<0.001**
N2	1.78	1.72-1.85	**<0.001**	5,981	2.19	2.08-2.30	**<0.001**
N3	3.02	2.89-3.17	**<0.001**	2,991	3.48	3.28-3.69	**<0.001**

*Cox univariable and multivariable proportional hazard models were developed to estimate hazard ratios with 95% confidence intervals (CI) for overall survival. Median follow-up was 9.8 (0-15) years. In multivariable analysis all clinicopathological relevant variables and variables with a P value <0.05 in univariable analysis were included. The assumption of proportional hazards was found to be valid by graphically plotting the log-log survival curves. A two-sided P value ≤0.05 was considered statistically significant. Missing values were analyzed as separate unknown group within the same variable. CI= Confidence Interval, N0= no pathologically assessed regional lymph nodes with metastasis/isolated tumor cells, N1= metastasis in 1-3 regional lymph nodes, N2= metastasis in 4-9 regional lymph nodes, N3= metastasis in ≥10 regional lymph nodes, Ref= reference category, T1a= ≤0.5 cm (including micro invasion), T1b= >0.5 cm and ≤1 cm, T1c= >1 cm and ≤ 2 cm, T2= >2 cm and ≤5 cm, T3= >5 cm, T4= any size with direct extension to chest wall and/or to skin.

†Corrected for age, tumor grade and morphology, breast surgery, axillary lymph node dissection, chemotherapy, hormonal therapy, targeted therapy, radiotherapy, and second primary breast cancer.

Five-year relative survival rates improved in the recent cohort in all tumor stages; to 100% in all tumors ≤1 cm and to 98% for tumors between 1-2 cm, and improved increasingly with larger tumor size.

In univariable and multivariable analyses both tumor stage and lymph node status were of significant influence on overall survival in both cohorts (p<0.001). Importance of early detection is dual as with increasing tumor size also lymph node positivity increases.[25] We determined influence of stage corrected for both tumor biology and therapy. There was no difference in hazard rate for breast cancers sized 1 cm or smaller, not with long-term follow-up, nor in recent times (2006-2012 T1b versus T1a, p=0.677). When node negative, these patients do not regularly get adjuvant therapy in the Netherlands even when ER negative. With 100% 5-year relative survival rates it seems justified to simplify the next edition of

Table 4 Cox univariable and multivariable analyses of clinicopathological variables for overall survival in invasive breast cancer patients in the Netherlands Cancer Registry from 2006-2012*

	Univariable Analyses			Number	Multivariable Analyses		
Clinicopathological variables	**HR**	**95% CI**	**P value**		**Hazard Ratio**		**P value**
Age at diagnosis in years	1.07	1.07-1.07	**<0.001**	83,191	1.04	1.04-1.05	**<0.001**
Pathological tumor category							
T1a	Ref			3,840	Ref		
T1b	0.93	0.79-1.09	0.339	12,207	1.04	0.88-1.22	0.677
T1c	1.61	1.39-1.85	**<0.001**	34,156	1.54	1.33-1.78	**<0.001**
T2	3.53	3.07-4.07	**<0.001**	27,937	2.17	1.87-2.52	**<0.001**
T3	5.61	4.81-6.55	**<0.001**	3,212	2.78	2.36-3.27	**<0.001**
T4	14.64	12.57-17.06	**<0.001**	1,839	3.32	2.83-3.90	**<0.001**
Pathological node category							
N0	Ref			53,223	Ref		
N1	1.01	0.96-1.06	0.734	21,851	1.25	1.17-1.32	**<0.001**
N2	1.66	1.56-1.78	**<0.001**	5,396	2.36	2.16-2.58	**<0.001**
N3	3.19	2.97-3.41	**<0.001**	2,721	4.02	3.66-4.42	**<0.001**
B&R grade							
Grade 1	Ref			17,327	Ref		
Grade 2	1.42	1.33-1.53	**<0.001**	32,662	1.18	1.10-1.27	**<0.001**
Grade 3 (including anaplastic)	2.49	2.33-2.67	**<0.001**	22,263	1.69	1.56-1.82	**<0.001**
Unknown	6.17	5.77-6.61	**<0.001**	10,939	1.68	1.54-1.83	**<0.001**
Morphology							
Ductal carcinoma or ductal mixed	Ref			66,104	Ref		
Lobular carcinoma	1.20	1.13-1.27	**<0.001**	9,127	0.91	0.86-0.97	**0.003**
Other	1.64	1.55-1.73	**<0.001**	7,960	0.94	0.89-1.00	0.040
Estrogen receptor status							
Negative	Ref			13,873	Ref		
Positive	0.55	0.53-0.58	**<0.001**	67,967	0.71	0.66-0.77	**<0.001**
Unknown	1.21	1.08-1.37	**0.001**	1,351	1.00	0.85-1.18	0.972
Progesterone receptor status							
Negative	Ref			26,261	Ref		
Positive	0.58	0.56-0.60	**<0.001**	53,535	0.81	0.77-0.85	**<0.001**
Unknown	0.93	0.85-1.01	0.069	3,395	0.81	0.72-0.90	**<0.001**
HER2 status							
Negative	Ref			67,393	Ref		
Positive	1.06	1.00-1.12	**0.047**	10,897	1.00	0.93-1.08	0.933
Unknown/inconclusive	2.88	2.73-3.04	**<0.001**	4,901	0.94	0.88-1.00	**0.045**

Table 4 Cox univariable and multivariable analyses of clinicopathological variables for overall survival in invasive breast cancer patients in the Netherlands Cancer Registry from 2006-2012* (continued)

	Univariable Analyses			Number	Multivariable Analyses		
Clinicopathological variables	**HR**	**95% CI**	**P value**		**Hazard Ratio**		**P value**
Breast surgery							
Mastectomy	Ref			34,421	Ref		
Breast conserving therapy	0.39	0.37-0.41	**<0.001**	44,117	0.87	0.81-0.93	**<0.001**
No surgery	7.57	7.23-7.92	**<0.001**	4,653	4.12	3.78-4.49	**<0.001**
Axillary lymph node dissection							
No	Ref			52,353	Ref		
Yes	1.18	1.14-1.23	**<0.001**	30,838	1.29	1.21-1.38	**<0.001**
Chemotherapy							
No	Ref			48,417	Ref		
Yes	0.52	0.50-0.54	**<0.001**	34,774	0.86	0.80-0.92	**<0.001**
Hormonal therapy							
No	Ref			37,931	Ref		
Yes	1.21	1.17-1.26	**<0.001**	45,260	0.64	0.61-0.68	**<0.001**
Targeted therapy†							
No	Ref			75,797	Ref		
Yes	0.55	0.51-0.60	**<0.001**	7,394	0.58	0.52-0.65	**<0.001**
Radiotherapy							
No	Ref			29,569	Ref		
Yes	0.35	0.34-0.37	**<0.001**	53,622	0.69	0.64-0.73	**<0.001**
Second primary breast cancer							
No	Ref			79,889	Ref		
Yes	1.12	1.03-1.23	**0.010**	3,302	1.01	0.93-1.11	0.762

*Cox univariable and multivariable proportional hazard models were developed to estimate hazard ratios with 95% confidence intervals (CI) for overall survival. Median follow-up was 3.9 (0-8) years. In multivariable analysis all clinicopathological relevant variables and variables with a P value <0.05 in univariable analysis were included. The assumption of proportional hazards was found to be valid by graphically plotting the log-log survival curves. A two-sided P value ≤0.05 was considered statistically significant. Missing values were analyzed as separate unknown group within the same variable. B&R= Bloom & Richardson, CI= Confidence Interval, HER2= human epidermal growth factor receptor 2, N0= no pathologically assessed regional lymph nodes with metastasis/isolated tumor cells, N1= metastasis in 1-3 regional lymph nodes, N2= metastasis in 4-9 regional lymph nodes, N3= metastasis in ≥10 regional lymph nodes, Ref= reference category, T1a= ≤0.5 cm (including micro invasion), T1b= >0.5 cm and ≤1 cm, T1c= >1 cm and ≤ 2 cm, T2= >2 cm and ≤5 cm, T3= >5 cm, T4= any size with direct extension to chest wall and/or to skin.
†Mainly trastuzumab.

the pathological tumor classification by combining T1a and T1b into one extremely favorable category.

Patients diagnosed between 2006-2012 underwent breast conserving therapy more often and axillary lymph node dissection less often (p<0.001), due to more favorable tumor stage and the increasing use of sentinel lymph node biopsies throughout the years.[14] Even though tumor stage was more favorable in patients diagnosed between 2006-2012 uptake of all forms of (neo) adjuvant systemic therapy was increased (p<0.001), due to extended indication in Dutch guidelines from 2005 and on.[18]

Surgery is of prime importance for survival, and breast conserving therapy had a favorable survival compared to mastectomy despite correction for stage and adjuvant therapies (HR 0.87 95% CI 0.81-0.93, p<0.001). In the 2006-2012 cohort axillary lymph node dissection, advised only for patients with positive lymph nodes confirmed by sentinel node biopsy or cytology, decreased overall survival in multivariable analysis (HR 1.29, 95% CI 1.21-1.38, p<0.001).

In multivariable analyses chemotherapy decreased hazard of death with approximately 14% in the 2006-2012 cohort. HER2 status was known for the 2006-2012 cohort, but adjusted for targeted therapies, like trastuzumab, HER2 positive status was no longer significantly associated with overall survival. Apparently, trastuzumab is so effective that, with its current use in tumors >1 cm, effect of HER2 positivity on survival becomes negligible. The unknown HER2 status group, contained a large number of patients with inconclusive HER2 status, who might well be HER2 positive, but who did not all receive targeted therapy. This group had significantly higher hazard rates, which endorses the necessity of targeted therapy in HER2 positive patients.

Due to the relatively favorable survival rates of breast cancer patients long follow-up and large groups of patients are needed to have sufficient power to detect differences in overall survival. A recent Lancet publication with global data showed age standardized net breast cancer survival of 80% or more in 34 countries and an increase worldwide, but had no data on factors influencing it.[1] Our more recent results of 2006-2012 even indicated a 5-year relative survival rate of 96%. Another recent study compared breast cancer recurrence and outcome patterns in 3,589 patients treated from 1986-1992 matched 1:1 with patients from 2004-2008.[3] The authors describe a lower hazard rate of breast cancer relapse and a lower hazard rate of death between the two time periods, but similar outcome patterns by ER and HER2 status. Their study was not designed to identify current prognostic factors. This study is difficult to compare to ours, as it differs with respect to time frames chosen for the cohorts, the matched design, and the much lower number of patients.

The nationwide registration in the Netherlands of breast cancer incidence, pathology, and treatment data by the Netherlands Cancer Registry, combined with linkage to the municipal administration for vital status verification provides unique

and reliable population-based data. However, our study does have limitations. Due to the nature of our research question, there is a large difference in follow-up between the two cohorts. Furthermore, between 1999-2005 it was not standard of care in the Netherlands to evaluate hormone and HER2 status, making adjustment for these factors in our oldest cohort impossible. Since our main interest was to identify and quantify effect of traditional prognostic factors in the current era, we solved these limitations partly by analyzing both time cohorts separately, without comparative analyses. The 1999-2005 cohort gives insights on impact on long-term survival. Finally, although we have extensive clinicopathological data, no data was available regarding patients' co-morbidity which likely influences both primary outcome (survival) and type of therapy, and some influence on tumor stage and lymph node stage at detection cannot be excluded.

In conclusion, our population-wide study in 173,797 female patients is the first to assess effect of traditional prognostic factors like tumor size and nodal status on survival using such recent data. Our results can aid physicians in clinical decision making and informing patients about their prognosis. Furthermore, our data are of special importance for research trials, especially screening trials and modeling studies, that often use prognosis per tumor stage as primary outcomes. Tumor size and nodal status were of significant and major influence independent of tumor biology, also in the current era of more conservative surgery and new, more effective, and more widely applied systemic (neo-)adjuvant therapies. Finally, our results emphasize the importance of early breast cancer diagnosis as it greatly affects overall survival.

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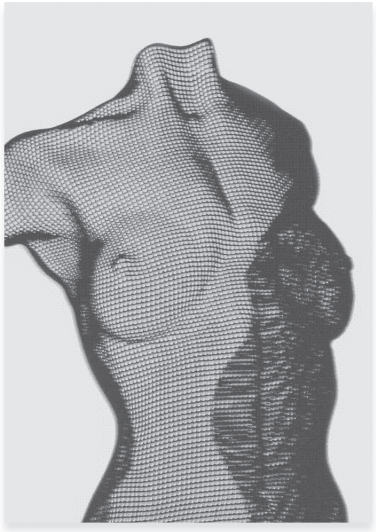
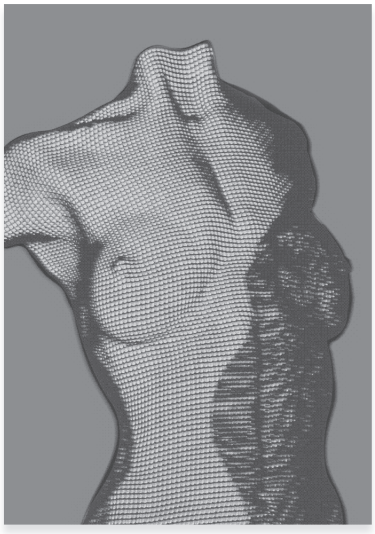
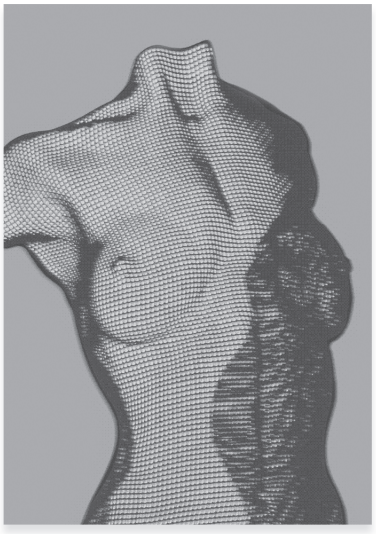

Chapter 10

Summary and general discussion

MAIN FINDINGS

Breast cancer screening for women with a *BRCA1/2* mutation or familial risk is generally accepted as an effective secondary preventive measure. However, what the most optimal screening strategy is; method and frequency of screening, and appropriate age group, is under discussion. Current screening strategies in the Netherlands are exemplified by three patient cases in **chapter 2**. Furthermore, whether earlier breast cancer detection by screening translates in actual survival benefit in these women is until now unknown for this specific group. This has to do with the favorable breast cancer survival rates, currently a relative 5-years survival rate of more than 90%,[1] and consequently the large numbers of patients and long follow-up needed to demonstrate survival differences by early detection through breast cancer screening.

In **chapter 3**, we described long-term survival, median follow-up of 9 years, of the MRI Screening Study (MRISC) patients, with *BRCA1/2* gene mutations or familial risk, compared with controls, matched for risk group, year of diagnosis, and age at diagnosis. MRISC patients were screened with annual MRI and mammography, and controls were unscreened below 50 years, or participated in the national breast cancer screening program with biennial mammography screening if above 50 years. Screening with annual MRI and mammography improved metastasis free breast cancer survival substantially for women with a *BRCA1* mutation or familial risk. MRISC patients were almost three times less likely to develop breast cancer metastases compared to controls. The difference was non-significant in *BRCA2* mutation carriers, most likely due to the small numbers in this specific subgroup. For ethical reasons a randomized controlled trial with a non-screened group is not likely to be ever performed, and the best available alternative is comparison with matched controls. However, due to the non-randomized design the study does have some limitations. The most important one being lead time bias; the time by which diagnosis has been advanced by screening.[2] We corrected for this bias and estimated lead time with MISCAN (micro simulation screening analysis), a well-validated micro simulation model,[3] calibrated earlier for the MRISC *BRCA1* and *BRCA2* cohort,[4] the MRISC familial risk cohort,[5] and the Dutch national breast cancer screening program.[6]

With MISCAN we also estimated cost-effectiveness of different screening strategies in MRISC patients with a familial risk, but without a *BRCA1/2* gene mutation, by simulating a cohort of 5 million women with a family history of breast cancer in **chapter 4**. The cost per life year gained (LYG) was €102,164 (3.5% discounted) for the MRISC screening scheme (screening with annual MRI and mammography and biannual clinical breast examination), approximately 2.5 times higher than the screening scheme currently advised in the Netherlands with annual mammography and clinical breast examination, but estimated mortality reduction rose from 17% to 25%. However, MRI screening was not cost-effective in women with a familial

risk in our study. The main limitation of our cost-effectiveness study in comparing mammography screening with additional MRI screening is the fact that MRISC data, on which the MISCAN model was calibrated, comes from a non-randomized cohort study. Furthermore, breast density, which may influence results,[7] was not taken into account in this study. To overcome these limitations a randomized controlled trial comparing mammography and MRI screening, and assessing breast density is needed. In **chapter 5**, we describe the protocol of a study addressing exactly these issues; the Familial MRI Screening Study (FaMRISC). In the FaMRIsc, a multicenter study currently executed in the Netherlands, women with a breast cancer cumulative lifetime risk (CLTR) of ≥20% due to their family history are included and randomized in two groups. Group 1 receives annual mammography screening and clinical breast examination and group 2 is screened with annual MRI screening, clinical breast examination, and biennial mammography. An automated breast density measurement is done on raw data of the first mammography of all participants. Results are expected in 2017.

Additional MRI screening has been found cost-effective for *BRCA1/2* mutation carriers, and is a generally accepted screening strategy in the ages 25-60 years. However, with MRI sensitivity being much higher than mammography sensitivity especially in *BRCA1* mutation carriers (67% versus 25%), but also in *BRCA2* mutation carriers (69% versus 62%),[8] there are growing doubts about whether mammography still contributes to early breast cancer detection in these groups.[9] Moreover, radiation may have negative effects, and may even induce breast cancers particularly in *BRCA1/2* mutation carriers,[10] the very thing screening was started for in the first place.

In **chapter 6**, we compared sensitivity and specificity of MRI, mammography, and the combination of the two tests. In *BRCA1/2* mutation carriers of all ages mammography next to MRI screening did not increase breast cancer sensitivity significantly. The contribution to breast cancer sensitivity of mammography in addition to MRI screening was different for women with *BRCA1* and women with *BRCA2* mutation. In *BRCA1* mutation carriers overall adding mammography to MRI screening increased sensitivity by roughly 4%. In the *BRCA1* ≤40 years group addition of mammography increased sensitivity by 9.3%. In order to detect one breast cancer missed by MRI in *BRCA1* mutation carriers overall 641 screens with mammography are needed. With that large number, it is questionable if the benefit of possible earlier breast cancer detection by adding mammography to MRI can really outweigh the radiation harms and risk of false positive results.

For *BRCA2* mutation carriers overall mammography increased sensitivity by 13%, but for women aged 40 years or below without additional mammography one third of breast cancers would not have been detected. Unfortunately, our numbers are small in this specific subgroup, and therefore this large and clinically relevant difference is not statistically significant. In *BRCA2* mutation carriers overall only 156 mammography screens are needed to detect a breast cancer missed with MRI. Con-

tribution of mammography appears to be more relevant in young *BRCA2* mutation carriers. Balancing the contribution and disadvantages of mammography should lead to different screening guidelines for *BRCA1* and *BRCA2* mutation carriers.

Above the age of 60 often less intensive screening is advised for *BRCA1/2* mutation carriers. In **chapter 7**, we demonstrated in a prospectively assembled national cohort over 70% of 60-year old Dutch *BRCA1/2* mutation carriers had breast(s) at risk, i.e. had not undergone therapeutic or risk-reducing bilateral mastectomy, with still a 20-30% risk of breast cancer. In 148 breast cancers in *BRCA1/2* mutation carriers ≥60 year 53% were detected in an unfavorable tumor stage with biennial mammography, versus 21% with annual mammography. Therefore continuation of annual breast cancer screening of *BRCA1/2* mutation carriers ≥60 seems worthwhile, when life expectancy is good. This is relevant for the majority of *BRCA1/2* mutation carriers.

In search for new prognostic parameters in **chapter 8**, we assessed the value of cell adhesion molecules (CAMs); E-cadherin, carcinoembryonic antigen (CEA), N-cadherin, and epithelial CAM (Ep-CAM). CAMs play an important role in the process of metastasis. Tissue Microarrays (TMAs) of 574 breast cancer patients with a median follow-up of 19 years were immunohistochemically stained for all markers, and expression was microscopically determined. Breast cancer patients with high intensity expression of CEA had a 3.6 times higher risk of relapse, compared to patients with below median CEA expression and above median E-cadherin expression. An interaction was found between these two CAMs, suggesting a biological association.

Finally, in **chapter 9**, we investigated the value of long established prognostic parameters, like tumor size, lymph node involvement, and grade in the current era of new systemic therapies. Also in multivariable analysis survival of invasive breast cancers decreased significantly with increasing tumor size from 1 cm and larger. Survival decreased independently with progressing number of positive lymph nodes. Also in contemporary times with more effective systemic therapies tumor stage at breast cancer diagnosis influences overall survival significantly and therefore early detection continues to be of great importance.

In summary, annual screening with mammography and MRI improves breast cancer specific distant metastasis free survival for women with a familial risk or *BRCA1* mutation. However, this screening strategy is cost-effective for *BRCA1/2* mutation carriers, but not for women with a familial risk. Possibly additional MRI screening is cost-effective in a subgroup of women with familial risk with dense breasts. This is currently being investigated in the Dutch randomized controlled multicentre trial: FaMRIsc. Contribution of mammography to breast cancer screening sensitivity when combined with MRI seems very little for *BRCA1* mutation carriers, but may be relevant for young *BRCA2* mutation carriers. For *BRCA1/2* mutation carriers continuation of annual screening also above 60 years seems relevant and worthwhile. Well established prognostic factors, and therefore also early breast cancer

detection, are still relevant in the current era of improved systemic therapy, but the search for new biological markers might also be fruitful, an example of this is the combination of E-cadherin and CEA tumor cell expression.

METHODOLOGICAL CONSIDERATIONS

The main limitation in our studies is the non-randomized design. Due to the non-randomized design some effects of bias could be present when comparing a screened group with an unscreened group. The most important biases in screening are: lead time bias, length bias, overdiagnosis, and selection bias.[2] We tried to correct for lead time bias by extracting lead time as estimated by the well-validated MISCAN model from metastasis free survival. We countered length bias, screening detecting tumors with a more favorable prognosis e.g. slower growing,[2] by also including all interval cancers in our analyses.

Overdiagnosis, screening detecting breast cancers that would never become symptomatic,[2] is unlikely to be a large problem in our population of mainly young women, and *BRCA1/2* mutation carriers with very fast growing tumors.[11] We dealt with selection bias, screening attracting healthier women with a higher socio-economic status, who might have a better prognosis,[2] by using a control group not aware of their mutation status. Controls aged above 50 at diagnosis all did participate in the Dutch national breast cancer screening program.

However, some limitations of the non-randomized design cannot be resolved or corrected for. Since in the MRISC study all patients received both MRI and mammography screening and were treated for breast cancer when detected with one of both modalities, one can never know when the cancer would be visible on the other screening modality, and if this delay in detection would be clinically relevant. The only way to determine this is by a randomized controlled trial comparing mammography screening with MRI screening. The Familial MRI Screening Study is the study to answer these questions. Results are expected in 2017.

Furthermore, familial risk is a very broad concept and CLTRs are estimated based on modified tables of Claus,[12] which only take familial risk into account. Other models incorporate additional risk factors. However, risk estimates for the same woman vary greatly with different models.[13] Current risk models have wide confidence intervals, when estimating risk at the personal level and even in large groups as shown in the MRISC study. We therefore did not divide familial risk groups in different sub categories, and when possible we performed sensitivity analyses using different CLTRs.

The MISCAN survival outcomes were calibrated based on data of patients that were not treated with new targeted therapies like trastuzumab.[14-16] Stage specific survival rates might have changed with new systemic therapies. Moreover, both

increasing experience with MRI screening[17] and recent advances in MRI technology and methods may have improved current MRI screening results.

FUTURE DIRECTIONS

Breast cancer awareness was already very present in the Western world, but with famous *BRCA1* mutation carrier Angelina Jolie writing an editorial in the New York Times on her risk-reducing mastectomy (RRM), attention has increased even further, and this has led to an increase in family cancer center referrals, *BRCA1/2* DNA testing, and may even lead to an increase in RRM numbers.[18] The increasing attention and testing may lead to an increase in screening, while the increase in RRM numbers may diminish this effect. Until now most screening studies published recommendations for *BRCA1* and *BRCA2* mutation carriers combined, as if they are one group with comparable tumor and patient features. However, it has been very clear for some time now that these mutations lead to two different breast cancer entities. *BRCA1* mutation carriers have breast cancers with different pathological characteristics,[19] they respond differently to neo-adjuvant therapy,[20] and mammography screening sensitivity is much lower than for *BRCA2* mutation carriers.[8] More and more evidence is piling up that these mutations lead to very different phenotypes, and patients carrying these mutations should be screened or treated according to their mutation type. Following results of this thesis and work of other groups a randomized controlled trial for *BRCA1* mutation carriers comparing combined annual MRI and mammography screening with annual MRI screening only is justified to find a definite answer to whether mammography can be omitted for these women. For *BRCA2* mutation carriers mammography might still be worthwhile. On the other hand for both *BRCA1* and *BRCA2* mutation carriers in the future annual screening should be continued after 60 years. Life expectancy in relatively good health is increasing, and age limits in treatment guidelines for other cancers are also stretched. Treatment guidelines for older breast cancer patients in general might change, as older breast cancer patients receive less aggressive treatment and experience higher mortality from breast cancer,[21,22] and treatment decisions are based on tools that might not be accurate in older patients specifically.[23]

For *BRCA1* and *BRCA2* mutation carriers annual mammography and MRI screening is now advised, but other promising screening tools like digital breast tomosynthesis are not yet studied in women with a familial or hereditary risk and might be a valuable addition or replacement,[24] although radiation effects increase,[25] and special care is warranted for *BRCA1/2* mutation carriers. In the Netherlands screening for women with a familial risk starts from 35 or 40 years depending on how high the CLTR is estimated. Considering the broad confidence interval of the estimation of CLTRs it is questionable if this division in risk groups is justified.[13] Results of the FaMRIsc are expected to give a definite answer as to whether or not

additional MRI screening is needed and cost-effective, and if so if this depends on a woman breast density.

Currently, another study, the DENSE-trial,[26] is investigating the relation of dense breasts and cost-effectiveness of additional MRI screening in the national breast cancer screening program in the Netherlands. Results of this study might lead to implementation of MRI screening for a much larger group of women. This will influence MRI prices and availability in the Netherlands. If MRI screening is implemented in the national breast cancer screening program it could be a possibility to transfer screening of high risk women, like *BRCA1/2* mutation carriers and women with a familial risk, to this program. This would lead to a decrease in costs of this screening.

Independent of the DENSE-trial's results reorganization of breast cancer screening care in high risk women by transferring this care to the national breast cancer screening program is worth considering. Nowadays most high risk women in the Netherlands are screened in (academic) hospitals. Screening of this generally healthy population is coordinated by highly specialized surgeons and medical oncologists, who give patients screening results and perform clinical breast examination annually. Most hospitals have diminished costs by transferring screening care to less expensive, but also specialized nurse-practitioners or physician assistants who work directly under the surgeon or medical oncologist. However, by integrating screening care of high risk women into the national breast cancer screening program costs could be reduced significantly. There are two options for transferring screening care to the national breast cancer screening program; either all care of all risk groups including the *BRCA1/2* gene mutation carriers, which requires performing and reading MRIs in the national breast cancer screening program. This option is logistically very challenging, since nowadays MRI screening is not performed in the national breast cancer screening program, and there is a lack of both MRI equipment as well as trained staff able to perform and read MRI images. The second option would be more easy and would entail implementing care of the familial risk group specifically into the national breast cancer screening program. Since Dutch guidelines advise screening with annual mammography only for this specific risk group, and performing and reading mammograms is already standard of care in the national breast cancer screening program, little extra logistical efforts are needed for this option. Both these options are probably going to lower costs, although the magnitude of cost reduction differs.

Costs of MRI screening might decrease in the future, for instance by performing a rapid breast MRI of 3 minutes that seems comparable to the standard 21 minute study, and that can be read by an expert radiologist in less than 30 seconds.[27] This method seems promising; MRI screening can become much cheaper with this quick method. When MRI costs decrease cost-effectiveness increases, and MRI screening might become cost-effective for all women, even in the general population. A multicenter prospective cohort study in which results of this fast MRI are validated

is eagerly awaited, although to the best of my knowledge such a trial is not yet running.

Screening in the future should be as personalized as possible. At least separate breast cancer screening strategies for *BRCA1* and *BRCA2* mutation carriers, and women with a familial risk should be developed. Better risk models should be developed making it possible to select women for whom screening is relevant more adequately. One way to do this might be by looking at specific risks of certain groups of *BRCA* gene mutations, instead of looking at gene mutations as a *BRCA1* or *BRCA2* gene mutation. Another way could be to include environmental factors in the risk calculations. Taking breast density in account when choosing for a screening modality could also be a way. Currently, screening ages are determined for specific risk groups as a whole. Inevitably, some women are screened "unnecessarily" for many years before they develop breast cancer. Age of onset of breast cancer might be a family trait,[28] and might be an interesting direction for further research in planning preventive measures.

The familial risk group, the largest group of women screened outside the national breast cancer screening program, comprises women with a very wide range of CLTR, and their risk is probably caused by a combination of multiple gene mutations with low penetrance for breast cancer and environmental factors.[29,30] The exact breast cancer risk these multiple low penetrance genes entail, the aggressiveness of breast cancers they cause, and age of onset of these breast cancers is unknown. With whole exome sequencing costs decreasing rapidly, genetic testing soon will be available for general clinical use.[31] This will lead to rapid unraveling of more variants within the genome that can be used to predict disease onset, affect progression, and modulate drug response. Ideally, results of whole genome sequencing of an individual will be incorporated into specialized risk models that will predict breast cancer CLTR, but also age of onset of breast cancer accurately. However, such a model does not yet exist and cautiousness is warranted since interpretation of this bulk of genomic data and their meaning for individual patients is difficult. The whole genome sequencing technique is developing faster, than our knowledge on interpretation of its results, leading to information of genetic alterations of unknown value.

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

- Tumor stage at breast cancer detection influences overall survival significantly. Early breast cancer detection continues to be of great importance.
- Screening with annual mammography and MRI improves breast cancer specific distant metastasis free survival in *BRCA1* mutation carriers and women with a familial risk.

- MRI screening improves breast cancer sensitivity in *BRCA1* and *BRCA2* mutation carriers and is cost-effective from 25-60 years.
- From 60 years onwards annual screening should be continued, however whether this should be with MRI or mammography is yet to be studied.
- A randomized controlled trial for *BRCA1* mutation carriers comparing combined annual MRI and mammography screening with annual MRI screening only should ideally give a definite answer to whether mammography can be omitted for these women. Second best would be use of current available data in a micro simulation model, like MISCAN, to weigh screening harms and benefits of additional mammography.
- There is until now too little evidence to justify a randomized controlled trial comparing annual MRI only with additional annual mammography screening for *BRCA2* mutation carriers, at least under the age of 40 years.
- Regular MRI screening is currently not cost-effective for all women with a familial breast cancer CLTR $\geq$20.
- Outside of screening trials women with a familial CLTR $\geq$20 in the ages of 35-60 years should be screened with annual mammography.
- Whether MRI screening is cost-effective for women with a CLTR $\geq$20 and high breast density is now being investigated in the FaMRIsc.

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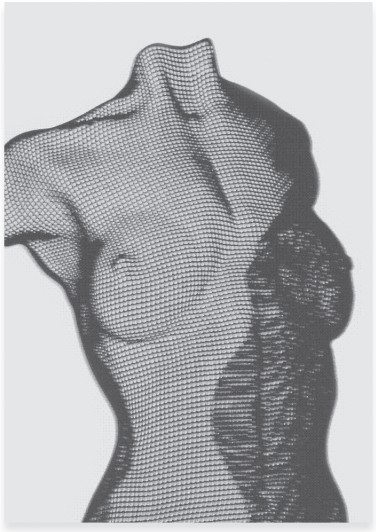
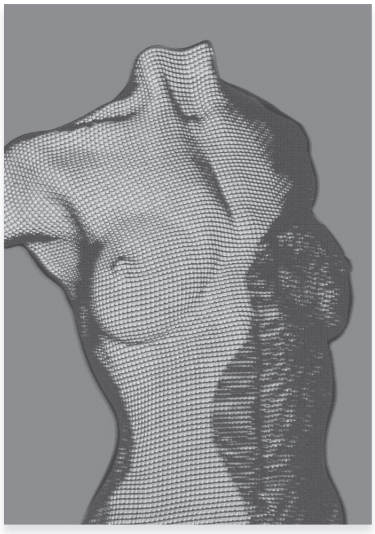
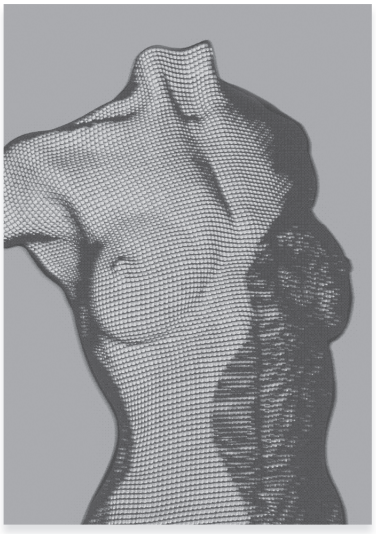

Chapter 11

Samenvatting

SAMENVATTING

Borstkankerscreening voor vrouwen met een *BRCA1/2*-mutatie of met een familiaire belasting is algemeen geaccepteerd als effectieve preventieve methode. Maar wat de meest optimale screening strategie is; methode, frequentie en leeftijdscategorie van screening is onderhevig aan debat. Huidige screening strategieën en de Nederlandse richtlijn worden aan de hand van 3 patiënten casus toegelicht in **hoofdstuk 2**. Of vroegdetectie door screening ook daadwerkelijk leidt tot een overlevingswinst is nog maar de vraag voor deze specifieke groep vrouwen. Dit heeft alles te maken met de relatief gunstige 5-jaarsoverleving, van meer dan 90%[1] en daardoor de grote groepen patiënten en lange follow-up nodig om een overlevingsverschil door vroegdetectie bij screening aan te tonen.

In **hoofdstuk 3**, beschrijven we de lange-termijn overlevingsresultaten, met een mediane follow-up van 9 jaar, van patiënten uit de MRI Screening Studie (MRISC) met een *BRCA1/2*-mutatie of een familiaire belasting vergeleken met gematchte controles. MRISC-patiënten waren gescreend met jaarlijks MRI en mammografie en controles waren niet gescreend onder de 50 jaar, of gescreend in het Nederlands borstkanker bevolkingsonderzoek met tweejaarlijks mammografie indien 50 jaar of ouder. Screening met jaarlijkse MRI en mammografie verbeterde de borstkankerspecifieke metastasevrije overleving substantieel voor *BRCA1*-draagsters en vrouwen met een familiaire belasting. MRISC-patiënten hadden 3x minder kans op borstkankermetastasen in vergelijking met de controles. Het verschil was niet statistisch significant voor *BRCA2*-draaagsters, meest waarschijnlijk, omdat in deze specifieke subanalyse de groep patiënten te klein was. Uit ethische overwegingen is het niet waarschijnlijk dat een onderzoek wordt uitgevoerd met een niet-gescreende controle groep. Het best mogelijke alternatief is daarom deze vergelijking met gematchte controles. Echter door het niet-gerandomiseerde design van de studie kent de studie wel enkele beperkingen. De meest belangrijke is lead-tijd bias,[2] ofwel de tijd die borstkankerdiagnose vervroegd wordt door screening. We corrigeerden voor deze bias en bepaalden lead-tijd met MISCAN (micro simulatie screening analyse), een goed gevalideerd microsimulatie model,[3] eerder gecalibreerd voor het MRISC *BRCA1* en *BRCA2* cohort[4] en familiaire cohort[5] en het Nederlands Borstkanker Bevolkingsonderzoek.[6]

Met MISCAN simuleerden we tevens een cohort van 5 miljoen vrouwen met een familiaire belasting voor borstkanker, maar zonder *BRCA1/2* mutatie, om zo de kosteneffectiviteit van verschillende screening strategieën in MRISC-patiënten te bepalen in **hoofdstuk 4**. De kosten per gewonnen levensjaar waren €102.164,- (3,5% discounted) voor het MRISC screening schema met jaarlijks MRI, mammografie en halfjaarlijks lichamelijk onderzoek. Dit was ongeveer 2,5 keer zoveel als het screening schema dat tegenwoordig in Nederland wordt geadviseerd; jaarlijkse screening met mammografie en lichamelijk onderzoek. Het MRISC-schema leidde echter wel tot een hogere mortaliteitsreductie van 25% tegenover 17% van de

huidige richtlijn. Toch is door de hoge kosten per gewonnen levensjaar MRI niet kosteneffectief in deze vrouwen met een familiaire belasting. Wel is het zo dat de studie enkele tekortkomingen heeft, alle data komt namelijk van de MRISC-studie en deze studie was niet-gerandomiseerd. Daarnaast is borstdichtheid, de verhouding klierweefsel t.o.v. de verhouding vetweefsel, een belangrijke risicofactor voor borstkanker en met een grote invloed op mammografiesensitiviteit,[7] niet meegenomen in deze studie.

Om deze tekortkomingen op te lossen is een gerandomiseerde studie opgezet die mammografie en MRI-screening vergelijkt en borstdensiteit hierbij betrekt. In **hoofdstuk 5** beschrijven we het studieprotocol van deze studie; de Familiaire MRI Screening Studie (FaMRIsc). In de FaMRIsc-studie, een multicenter studie die op dit moment in Nederland loopt, worden vrouwen met een borstkanker cumulatieve lifetime risico van ≥20% ten gevolg van hun familiaire belasting gerandomiseerd in 2 groepen. Groep 1 wordt gescreend met jaarlijkse mammografiescreening en lichamelijk onderzoek en groep 2 wordt gescreend met jaarlijkse MRI-screening, lichamelijk onderzoek en tweejaarlijkse mammografie. Een geautomatiseerde borstdensiteit meting wordt gedaan op de ruwe data van de eerste mammografie van alle deelneemsters. Resultaten worden in 2017 verwacht.

Aanvullende MRI-screening is kosteneffectief voor *BRCA1/2*-mutatiedraagsters en is over het algemeen de geaccepteerde screening strategie voor *BRCA1/2*-mutatiedraagsters in de leeftijd van 25-60 jaar. Omdat de sensitiviteit van MRI-screening veel hoger is dan die van mammografie, vooral in *BRCA1*-draagsters (67% versus 25%), maar ook in *BRCA2*-draagsters (69% versus 62%),[8] zijn er groeiende twijfels of mammografie überhaupt nog iets toevoegt aan borstkankerdetectie in deze groepen.[9] Bovendien heeft de straling van mammografie negatieve bijwerkingen en kan deze zelfs borstkanker veroorzaken, vooral in *BRCA1/2*-mutatiedraagsters,[10] de hele reden waarom in de eerste plaats met screening was begonnen.

In **hoofdstuk 6**, vergelijken we de sensitiviteit en specificiteit van MRI, mammografie en de combinatie van beide testen. In *BRCA1/2*-mutatiedraagsters van alle leeftijden verhoogde mammografie de borstkankersensitiviteit niet significant. De toegevoegde waarde op borstkankersensitiviteit van additionele mammografie bij MRI-screening was verschillend voor *BRCA1*- en *BRCA2*-mutatiedraagsters. In *BRCA1*-draagsters verhoogde de additionele mammografie de sensitiviteit met ongeveer 4%. In de *BRCA1*-draagsters ≤40 jaar was de verhoging van de sensitiviteit door de toevoeging van mammografie grofweg 9%. Anders gezegd, om 1 door MRI gemiste borstkanker te detecteren zijn 641 mammografieën nodig. Met dat enorme aantal is het natuurlijk de vraag of het voordeel van die ene borstkankerdetectie wel opweegt tegen de stralingsrisico's en risico's op vals positieve uitslagen van de mammografie. Voor *BRCA2*-mutatiedraagsters verhoogde de mammografie sensitiviteit met 13%, maar als we kijken naar de vrouwen specifiek van 40 jaar of jonger zouden zonder additionele mammografie een derde van de borstkankergevallen niet gedetecteerd zijn. Helaas, zijn onze aantallen erg

klein in deze specifieke subgroep en daarom is dit grote en klinische relevante verschil niet statistisch significant. Voor *BRCA2*-draagsters in het algemeen zijn slechts 156 mammografiescreening nodig om een door MRI gemiste borstkanker te detecteren. Het lijkt er daarom op dat mammografiescreening met name relevant is voor jonge *BRCA2*-draagsters. Het afwegen van de voor- en nadelen van mammografie zou dan ook tot verschillende screening richtlijnen voor *BRCA1*- en *BRCA2*-mutatiedraagsters moeten leiden.

Boven de leeftijd van 60 jaar wordt minder intensieve screening geadviseerd voor *BRCA1/2*-mutatiedraagsters. In **hoofdstuk 7**, tonen we in een prospectief verzamelde nationale cohort studie aan dat meer dan 70% van 60-jarige Nederlandse *BRCA1/2*-draagsters nog borsten heeft met het dientengevolge risico op borstkanker. Anders gezegd deze vrouwen hebben nog niet om therapeutische of preventieve redenen beiderzijds de borsten laten verwijderen en hebben daarom nog een risico van 20-30% op het ontwikkelen van borstkanker. In 148 borstkankergevallen in *BRCA1/2*-mutatiedraagsters $\geq$60 jaar werd 53% van de kankers gedetecteerd in een ongunstig tumorstadium met tweejaarlijkse mammografie, in vergelijking met 21% van de kankers met jaarlijkse mammografie. Het lijkt er daarom op dat het continueren van jaarlijkse screening voor *BRCA1/2*-mutatiedraagsters van 60 jaar of ouder de moeite waard is, mits de levensverwachting voldoende is. Deze resultaten zijn van groot belang voor het merendeel van de *BRCA1/2*-mutatiedraagsters.

In **hoofdstuk 8** analyseren we de prognostische waarde van cel adhesie moleculen (CAM); E-cadherine, carcinoembryonisch antigen (CEA), N-cadherine en epitheliale CAM (Ep-CAM). CAMs spelen een grote rol in het metastaseringsproces. TMA's van 574 borstkankerpatiënten met een mediane follow-up van 19 jaar werden gekleurd met immunohistochemie en expressie microscopisch bepaald. Borstkankerpatiënten met een hoge intensiteit expressie van CEA hadden een 3,6 keer hogere kans op terugval in vergelijking met patiënten met onder de mediaan CEA expressie en boven de mediaan E-cadherine expressie. Tussen deze twee CAMs werd een interactie gevonden, waarmee een biologisch verband waarschijnlijk is.

Uiteindelijk hebben we in **hoofdstuk 9** de waarde van lang bestaande en in de medische wereld gevestigde prognostische parameters, zoals tumorgrootte, lymfeklier betrokkenheid en tumorgraad, bepaald in de huidige tijd van nieuwe effectievere systemische therapie. Ook in multivariabele analyse verminderde overleving significant met het toenemen van tumorgrootte vanaf 1 cm. Overleving verminderde ook onafhankelijk met toename van het aantal positieve lymfeklieren. Ook in de huidige tijd van meer effectieve systemische therapieën wordt overleving dus significant beïnvloed door tumorstadium bij borstkankerdiagnose en daarom blijft vroegdetectie van borstkanker van groot belang.

Samenvattend verbetert jaarlijkse screening met mammografie en MRI borstkankerspecifieke afstandsmetastase vrije overleving voor vrouwen met een familiaire belasting of *BRCA1*-genmutatie. Maar hoewel deze screening strategie kosteneffectief is voor *BRCA1/2*-mutatiedraagsters, is dat niet zo voor vrouwen met een

familiaire belasting zonder een genetische predispositie. Mogelijk is aanvullende MRI-screening wel kosteneffectief voor een subgroep van de vrouwen met een familiaire belasting met zeer dicht borstklierweefsel. Dit wordt momenteel onderzocht in de Nederlandse multicenter gerandomiseerde trial FaMRIsc. Mammografie lijkt weinig aanvullende winst op te leveren voor borstkankerdetectie wanneer gecombineerd met MRI-screening in *BRCA1*-genmutatiedraagsters, maar lijkt wel relevant voor jonge *BRCA2*-mutatiedraagsters. Voor *BRCA1/2*-mutatiedraagsters lijkt het continueren van jaarlijkse screening boven de 60 jaar relevant en de moeite waard. Tot slot blijven traditionele prognostische factoren hun waarde behouden in het huidige tijdperk van verbeterde systemische therapie en daarmee het belang van vroegdetectie, maar de zoektocht naar nieuwe biologische tumormarkers kan zeker leiden tot aanvullende prognostische factoren.

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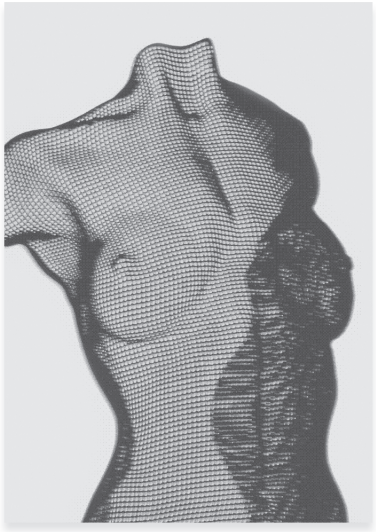
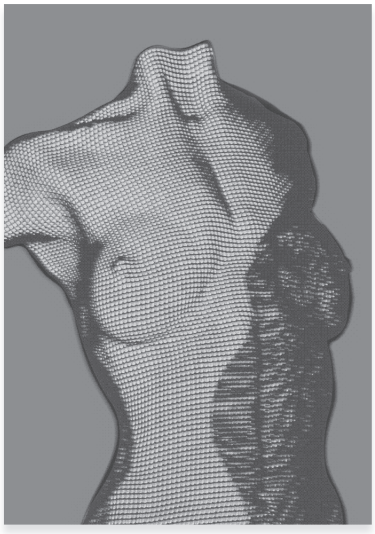
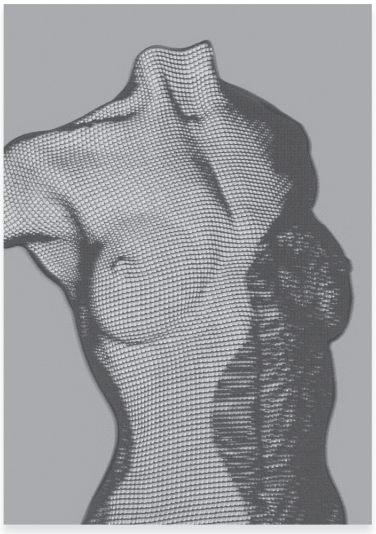

Chapter 12

List of publications

Curriculum Vitae

PhD portfolio

Dankwoord

LIST OF PUBLICATIONS

1. **Saadatmand S**, Bretveld R, Siesling S, et al. Influence of tumor stage at breast cancer detection on survival in modern times: a population based study in 173,797 patients. Under review.
2. Phi XA, **Saadatmand S**, De Bock GH, et al. Quantifying the additional value of mammography to MRI screening in *BRCA1* and *BRCA2* mutation carriers by *BRCA* status and age: an individual patient data meta-analysis. Under review.
3. **Saadatmand S**, Obdeijn IM, Rutgers EJ, et al. Survival benefit in women with *BRCA1* mutation or familial risk in the MRI Screening Study (MRISC). International journal of cancer Journal international du cancer 2015.
4. Zeestraten EC, Reimers MS, **Saadatmand S**, et al. Combined analysis of HLA class I, HLA-E and HLA-G predicts prognosis in colon cancer patients. British journal of cancer 2014;110:459-68.
5. **Saadatmand S**, Vos JR, Hooning MJ, et al. Relevance and efficacy of breast cancer screening in *BRCA1* and *BRCA2* mutation carriers above 60 years: a national cohort study. International journal of cancer Journal international du cancer 2014;135:2940-9.
6. Reimers MS, Zeestraten EC, van Alphen TC, Dekker JW, Putter H, **Saadatmand S**, et al. Combined analysis of biomarkers of proliferation and apoptosis in colon cancer: an immunohistochemistry-based study using tissue microarray. International journal of colorectal disease 2014;29:1043-52.
7. Zeestraten EC, Speetjens FM, Welters MJ, **Saadatmand S**, et al. Addition of interferon-alpha to the p53-SLP(R) vaccine results in increased production of interferon-gamma in vaccinated colorectal cancer patients: a phase I/II clinical trial. International journal of cancer Journal international du cancer 2013;132:1581-91.
8. **Saadatmand S**, Tilanus-Linthorst MM, Rutgers EJ, et al. Cost-effectiveness of screening women with familial risk for breast cancer with magnetic resonance imaging. Journal of the National Cancer Institute 2013;105:1314-21.
9. **Saadatmand S**, Obdeijn IM, Koppert LB, Tilanus-Linthorst M. [Choices for women at risk of hereditary breast cancer]. Nederlands tijdschrift voor geneeskunde 2013;157:A6625.
10. **Saadatmand S**, de Kruijf EM, Sajet A, et al. Expression of cell adhesion molecules and prognosis in breast cancer. The British journal of surgery 2013;100:252-60.
11. **Saadatmand S**, Rutgers EJ, Tollenaar RA, et al. Breast density as indicator for the use of mammography or MRI to screen women with familial risk for breast cancer (FaMRIsc): a multicentre randomized controlled trial. BMC cancer 2012;12:440.

CURRICULUM VITAE

Sepideh Saadatmand was born September 23, 1985 in Teheran, Iran. When she was 4 years old the family moved to the Netherlands, where she spent her childhood in Delft. In 2003 she obtained her Gymnasium high school degree at the Christelijk Lyceum Delft. After that she studied Medicine at the University of Leiden. A very pleasant time in which she also became an active member of the student association Augustinus from 2005 onward. She started law school the same year to suit her wide range of interests. She obtained her Bachelor of Laws in 2008, a year later her Medical Degree followed. She followed her research internship and several medical internships abroad; in Indonesia and Surinam. During her medical internships she found a passion for surgery and also discovered an interest in research. Therefore, she combined studying Biomedical Sciences with research in the surgical laboratory of the Leiden University Medical Center at first, and from 2011 onward with her work as research fellow at the surgical oncology department of the Erasmus Medical Center. She obtained the Biomedical Sciences Master of Science degree in 2012. Guided by her supervisors Dr. Tilanus-Linthorst, professor Dr. Verhoef, and professor Dr. de Koning she set up a large nationwide randomized controlled trial with 12 participating medical centers in 2011. Effectiveness of mammography and MRI for breast cancer screening in women with familial risk is compared, also considering breast density. Results of the study are expected in 2017. In 2014 she temporarily interrupted her work as research fellow to experience life as a surgical intern at the Reinier de Graaf Gasthuis in Delft. In 2015 she continued her work as a research fellow, resulting in this PhD thesis.

CURRICULUM VITAE (NL)

Sepideh Saadatmand werd geboren op 23-09-1985 in Teheran, Iran. Op 4-jarige leeftijd emigreerde zij met haar gezin naar Nederland waar zij opgroeide in Delft. In 2003 behaalde zij het Gymnasium diploma aan het Christelijk Lyceum te Delft en startte met de studie geneeskunde aan de Universiteit van Leiden. Een ontzettend leuke tijd, waarin ze vanaf 2005 ook actief lid was van de studentenvereniging Augustinus. In dat jaar begon ze vanwege haar brede interesse ook aan de studie rechten, waarvan zij in 2008 de bachelor behaalde. Een jaar later, in 2009, volgde het artsexamen. Voor haar wetenschapsstage en een aantal coschappen ging ze op avontuur in Indonesië en Suriname. Tijdens de coschappen ontdekte zij haar passie voor de chirurgie, tegelijkertijd was de interesse voor wetenschappelijk onderzoek gewekt. Daarom combineerde ze de studie biomedische wetenschappen eerst met onderzoek in het chirurgische lab van het LUMC en vanaf 2011 met een promotietraject bij de afdeling chirurgische oncologie van het ErasmusMC. In 2012 behaalde zij haar Master of Science diploma. Onder begeleiding van copromotor Dr. Tilanus-Linthorst en promotoren professor Dr. Verhoef en professor Dr. De Koning zette ze vanaf 2011 een grote landelijke gerandomiseerde studie op met 12 deelnemende centra. Onderzocht wordt of MRI of mammografie de meest effectieve methode voor borstkanker screening van vrouwen met een familiaire belasting is en of dit beïnvloed wordt door borstdensiteit. De eerste resultaten worden in 2017 verwacht. In 2014 werd het traject een jaar onderbroken om ervaring op te doen als ANIOS (arts-assistent niet in opleiding) heelkunde in het Reinier de Graaf Gasthuis te Delft. In 2015 continueerde zij haar promotieonderzoek, resulterend in dit proefschrift.

PHD PORTFOLIO

Summary of PhD training and teaching activities

Name PhD student: S Saadatmand
Promotors: Prof.dr. CJ Verhoef & Prof.dr. HJ de Koning
Supervisor: Dr. MMA Tilanus-Linthorst

Erasmus MC Department: Surgical Oncology
PhD period: 2011-2015

1. PhD training	Year	ECTS
General academic skills		
- Biomedical English Writing and Communication	2011	4
- BROK 'Basiscursus Regelgeving Klinisch Onderzoek'	2012	1.5
Research skills		
- Biostatistical Methods I: Basic Principles	2011	5.7
In-depth courses (e.g. Research school, Medical Training)		
- NVVO Basiscursus Oncologie	2012	1.5
- NVvCO Scholingscursus mammacarcinoom	2012	0.5
Presentations oral		
- NVvH Chirurgendagen, Veldhoven	2013	1.0
- European Society of Surgical Oncology (ESSO) Valencia	2012	1.0
- European Breast Cancer Congress (EBCC), Wenen	2012	1.0
Presentations poster		
- European Breast Cancer Congress (EBCC) Glasgow	2014	1.0
- The European Cancer Congress (ECCO) Amsterdam	2013	1.0
- San Antonio Breast Cancer Symposium (SABCS) San Antonio	2012	1.0
Seminars & workshops		
- Journal club	2011-2014	3.0
- Hebon Annual Workshop	2011-2014	3.0
2. Teaching activities	**Year**	**ECTS**
Lecturing		
- Monthly breast cancer lectures for medical students	2011-2015	4.0
- Breast cancer lecture General Practitioners	2013	1.0
Supervising practicals and excursions		
Examination of Basic Life Support (EHBO) of medical students	2013	1.0
Supervising Master's theses		
- Graduate student	2013-2014	5.0
Other		
- Reviewer Breast Cancer Research	2015	1.0
- Reviewer British Journal of Cancer	2014	1.0
- Reviewer BMC Cancer	2014	1.0

DANKWOORD

"No one can whistle a symphony.
It takes an orchestra to play it."

H.E. Luccock

De mensen die mij goed kennen, weten dat ik, tot mijn eigen grote verdriet, helemaal niet kan fluiten, dus dan weet je het wel. Veel mensen hebben mij geïnspireerd, begeleid en geholpen op allerlei mogelijke manieren. Prettig dat ik nu zwart op wit die mensen de credits kan geven die zij verdienen. Natuurlijk zijn het er te veel om op te noemen, dus voor een ieder die ik hier niet heb genoemd, je hulp ben ik zeker niet vergeten.

Lieve mama en papa, natuurlijk begin ik bij jullie. Jullie stonden immers aan het begin van mijn leven. Lieve mama, wat hebben wij samen mooie en ook spannende avonturen beleefd. In voor jou moeilijke tijden, zoals ons begin in Nederland met zijn drietjes; Salar, jij en ik, deed je alles in je vermogen om ons een gelukkige jeugd te geven en ons niets te laten merken van de zorgen die je zeker had. Je hebt altijd een rotsvast geloof in mij en steunt me in alles. Geen plan was te gek. Bedacht ik om op pad te gaan om alleen rond te reizen in Zuid-Amerika dan sliep jij mijn maandenlange reis slecht, maar tegenhouden deed je me niet. Lachte de juf in groep 5 me uit, omdat ik vond dat ik uit de Bosatlas aan het 'studeren' was, vertelde jij me dat je trots op me was en dat het met dat studeren nog wel goed kwam. Mijn grootste fan! Dank je wel voor het altijd luisterend oor, je eindeloze vertrouwen en liefde. Ik hou van jou! Lieve papa, wij leerden elkaar kennen toen ik 5 jaar was en meteen waren wij de beste vriendjes. Vol enthousiasme werd jij papa van mij en Salar en later van Anoushka. Je werkte hard, liet het ons aan niets ontbreken en geen rapportgesprek heb je gemist. Met jou begonnen ook de reisjes en tripjes, de ontelbare barbecues en het een 'echte papa' hebben. Dank je wel!

Lief zusje Anoushka, lieve grote broer Salar, bedankt voor het altijd voor mij klaar staan, voor het voorbeeld dat jullie voor mij hebben gesteld, voor het aanhoren van mijn geklaag. Bedankt voor alle telefoongesprekken, etentjes, gezelligheid en het vertrouwen.

Beste Dr. Tilanus-Linthorst, beste Madeleine, ik weet ons sollicitatiegesprek nog heel goed. Wat was ik onder de indruk. Ik wist zeker dat ik ongelooflijk veel van je kon leren en wilde niets liever dan de baan. En wat heb ik inderdaad veel van je geleerd; van kennis over het mammacarcinoom, kritisch analyseren en betrouwbaar data verzamelen, tot liefde voor het onderzoek, nieuwsgierigheid, doorzettingsvermogen en strijdlust. Ik ben ongelooflijk trots dat ik je eerste PhD-student

mocht zijn. Maar behalve copromotor was je mijn mentor in de ruimste zin van het woord. Als het even niet liep zoals ik wilde kwamen daar opbeurende woorden en als het nodig was nam je het voor me op. Altijd heb je me de ruimte gegeven voor kritische vragen en discussies over het onderzoek. Ik heb ontzettend veel respect voor alles wat je hebt gedaan en bereikt. Weinigen doen het je na. Hartelijk dank voor het zijn van een inspiratie. Beste professor Tilanus, het inspiratiebron zijn zit denk ik bij jullie in de familie, want ook u bent dat voor mij! De afscheidspresentaties op de dag van uw emeritaat motiveerden mij enorm. Maar hoe kan een mens dat allemaal in één leven doen? Over de schouder van uw echtgenote volgde u mijn bewogen onderzoeksleven op de voet. Als ik u tegenkwam, gaf u me altijd eerlijk en wijs advies. Tijdens een onderzoeksdip lag daar een vriendelijk kaartje in de bus, precies op het moment dat ik het nodig had. Bedankt hiervoor.

Beste professor Verhoef, beste Kees, een voorbeeld hoe je het Daniel team met veel humor coacht als een hecht basketbalteam. In dat opzicht nog altijd een topsporter in je hart, als je de liters satésaus die tijdens de lunch opgingen, niet meerekent natuurlijk. Indrukwekkend hoe je tijdens de overdracht statistieken van alle kankervormen tevoorschijn toverde, hoeveel getallen kan een mens onthouden? Ik hoop dat ik ooit zo luisterrijk en met zoveel humor kan presenteren als jij en zo goed met mensen leer omgaan. Je liet iedereen altijd in zijn waarde. Een enorme gave! Dank je wel voor je begeleiding, je steun en alle grapjes. Hopelijk houden we ook in de toekomst contact.

Beste professor de Koning, beste Harry, omdat we op twee locaties zaten, zagen we elkaar niet vaak. Maar alles wat ik naar je mailde werd razendsnel beantwoord. Knap hoeveel waanzinnige onderzoeksprojecten je begeleidt en hoeveel belangrijk werk je verricht op populatieniveau in Nederland en wereldwijd. Trots dat ik je promovenda mag zijn. Bedankt!

Geachte leescomissie, professor Rutgers, professor Lemmens en professor Uyl-de Groot heel hartelijk dank voor jullie inspanningen en het beoordelen van mijn proefschrift. Wat een eer dat jullie in mijn leescommissie wilden deelnemen. Alle overige leden van de promotiecommissie ook u hartelijk dank voor het kritisch lezen en beoordelen van mijn proefschrift. Hartelijk dank dat u als opponent plaats wilde nemen.

Alle co-auteurs en dat zijn er veel, hartelijk dank voor alle input in de artikelen. Alle onderzoekers, artsen, nurse-practitioners, physician assistants, deelneemsters en anderen die hebben bijgedragen aan de FaMRIsc-studie hartelijk dank hiervoor.

Beste afdeling chirurgische oncologie, te veel mensen om op te noemen, maar dank voor alle uitleg, alle keren lunchen, de kletspraatjes en interesse.

Beste onderzoeksgenootjes; Elvira, Victorien, Wijnand, Stijn, Jan, Ninos en anderen, bedankt voor de gezelligheid, het mariokarten, de MRI-avonden die jullie van me hebben overgenomen, de extreem belangrijke voetbalkennis die ik noodgedwongen heb opgedaan, de tips voor sollicitatietraining, de borrels, de eindeloze keren lunchen op het dakterras, de illegale barbecues op datzelfde dakterras en nog veel meer!

Beste Corine, beste Ada, beste Melanie, altijd in voor een praatje, altijd vrolijk, maar vooral ook steun en toeverlaat. Corine, altijd kon ik bij je terecht met alle vragen. Alles wist je binnen een mum van tijd te regelen en wat heb ik vaak met je gelachen. Leuk! Beste Ada, wat werk jij hard voor de FaMRIsc. Wat ben ik je dankbaar dat ik met een gerust hart een aantal studietaken aan jou kan toevertrouwen. Fijn!

Beste Werkgroep Erfelijk Tumoren hartelijk dank voor al jullie hulp bij de verschillende onderzoeken en ook de kennis die jullie aan me hebben overgedragen. In het bijzonder Petra en Ellen voor alle database activiteiten. Beste Annette, wat baal ik dat ik jouw promotie heb gemist. Ik had het zo graag willen meemaken. Je hebt het vast geweldig gedaan! Wat een onderzoeksplannen hadden wij samen, jammer genoeg is het er nooit van gekomen. Zoveel ideeën en toch zo weinig tijd. Wie weet komt het ervan in de toekomst en anders gewoon zonder excuus een kop koffie met slagroom?

Lieve oud-collega's van het Reinier de Graaf, wat heb ik onwijs veel van jullie geleerd. Wat vond ik het 'dokter zijn' leuk. Wat heb ik veel zin om dat weer te gaan doen. Jullie waren een topteam! Heel veel dank! Beste Dr. Van der Elst, in het bijzonder ook veel dank aan u, wat bent u een fijne opleider, ook voor de ANIOS!

Lieve paranimfen, Eline en Janneke, beiden ontmoet op Bali, dat kan geen toeval zijn! Wat heb ik veel lol met jullie, wat hebben wij veel pret. De vele reizen, wijntjes, feestjes, festivals, balletvoorstellingen, etentjes, de mooie en de verdrietige momenten van het leven hebben we samen gedeeld. Wat fijn en geruststellend dat jullie vandaag naast mij willen staan. Lieve Eline, wat een eer dat ik afgelopen jaar ook jouw paranimf mocht zijn. Je was fantastisch! Lieve Janneke en Eline, ik weet zeker dat we nog heel veel avonturen gaan beleven, zin in!

Lieve vrienden en vriendinnetjes, dank jullie wel voor de aandacht, de afleiding, het vertrouwen, het plezier. Jullie maken mijn leven een feestje!

Lieve Ringo, mijn eigen 'Starr', wat ben ik gek op jou. Toen wij elkaar 5 jaar terug zagen waren we meteen verliefd en 'best friends for life'. Bedankt voor het brengen van de kunst in mijn leven. Bedankt voor de prachtige voorkant, het elke dag

voor me klaarstaan, het zijn van mijn bondgenoot en mijn beste maatje. Woorden schieten per definitie te kort om te omschrijven hoeveel ik om je geef, hoewel dat me niet tegenhoudt om het toch dagelijks te proberen. We zijn het beste team ooit!