FAMILIAL BREAST CANCER

RISK POPULATIONS AND THEIR SURVEILLANCE

Anna von Wachenfeldt
FAMILIAL BREAST CANCER
- RISK POPULATIONS AND THEIR SURVEILLANCE

Anna von Wachenfeldt

Stockholm 2011
ABSTRACT

Women carrying mutations in either BRCA 1 or BRCA2 have a lifetime risk of breast cancer of 80%. As little is known about the risk of other malignancies, apart from ovarian/tubal cancer in mutation carriers, the importance of other malignancies in a family with several cases of breast cancer is hard to evaluate. Women at high risk of breast cancer due to family history are offered genetic counselling and surveillance. Whether women looking for oncogenetic counselling are, in terms of socioeconomic status and health-related quality of life, comparable with women in general is not known. Mammography is a widely used screening method to detect breast cancer and has proven to reduce breast cancer mortality in women older than 50 years. The sensitivity of the method is much lower in women with dense breast and in general young women tend to have denser breast than older women. Most women under surveillance in virtue of family history of breast cancer are younger than 50, thus in a group where mammography alone has not been proved to be effective as a single screening method there is a need for other surveillance methods in women at risk of hereditary breast cancer.

We identified 803 BRCA 1/2-negative families with two or more cases of breast cancer and at least one additional malignancy. The observed proportion of different non-breast cancer in the study families was compared with the percentage distribution of non-breast cancer tumours in Sweden. Tumours in endometrium were seen in a significantly larger proportion in the study group than in the general population and could not be explained by previously known syndromes or other explanations for being overrepresented. Thus we suggest that endometrial carcinoma and breast cancer constitute a new breast cancer syndrome.

In a cross-sectional study aiming to characterize health-related quality of life and socioeconomic status among all healthy women who had ever visited the Oncogenetic Clinic, Department of Oncology, Södersjukhuset in 1998 – 2004, 306 women consented to participate (82.5%). Significantly more women in the study group were cohabiting (74.2 vs. 43.8%), had the highest education level, (56.7 vs. 39.6%) and had the highest household income (36.9 vs. 12.9 %) as compared to the reference population in the same catchment area. Study subjects reported significantly lower levels of health-related quality of life for subscales related to mental health and for general health compared to normative data, but similar levels on subscales related to physical health.

Six-hundred-and-thirty-two women (94%) from one counselling clinic consented to participate in a study aiming to find the most sensitive method to detect breast cancer in women with a familiar risk of the disease. Every woman underwent yearly, and blinded to the other methods, mammography, ultrasound and clinical breast exam. This first report describes the study design and the procedure, and the study cohort regarding hereditary pattern and sociodemographics. Further, the associations between breast density, BMI and other breast-cancer risk factors are elucidated. High breast density was associated with low BMI and young age. However, high density was not associated with increasing risk of breast cancer. Ultrasound and clinical breast examination caused substantially more work-up than MG. The number of detected cancers did not differ from the expected numbers. However, it is too early to draw any conclusion about the sensitivity of the three different modalities.
LIST OF PUBLICATIONS


# TABLE OF CONTENTS

1 GENERAL INTRODUCTION ................................................................. 1
  1.1 Risk factors for breast cancer .................................................... 3
    1.1.1 Hormonal factors ............................................................... 3
    1.1.2 Demographic factors ......................................................... 4
    1.1.3 Breast properties ............................................................... 4
    1.1.4 Daily intake of alcohol ...................................................... 4
    1.1.5 Regular physical exercise ................................................... 5
    1.1.6 Previous ionization radiation .............................................. 5
    1.1.7 Genetic risk factors ............................................................ 5
      1.1.7.1 BRCA 1 and BRCA2 ....................................................... 5
      1.1.7.2 Tumour protein 53 tumour gene .................................... 6
      1.1.7.3 Phosphatase and tensin homologue PTEN ....................... 6
      1.1.7.4 Ataxia-telangiectasia (mutated gene) ATM ..................... 7
      1.1.7.5 E-Cadherin ................................................................. 7
      1.1.7.6 Chek point kinase2, CHEK2 ......................................... 7
      1.1.7.7 Cyclin-dependent kinase inhibitor 2A CDKN2A ............... 7
  1.2 Oncogenetic counselling ............................................................. 8
    1.2.1 Procedure ......................................................................... 8
      1.2.1.1 Awareness ................................................................. 8
      1.2.1.2 Family composition ...................................................... 8
      1.2.1.3 Getting to the counsellor .............................................. 8
      1.2.1.4 Pedigree procedure ...................................................... 9
    1.2.2 Risk assessment ................................................................. 9
      1.2.2.1 Claus tables ............................................................... 9
      1.2.2.2 Autosomal-dominant inherited disease ......................... 9
      1.2.2.3 Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm [BOADICEA] ........... 10
      1.2.2.4 Testing for mutation according to The Swedish National Oncogenetic Group guidelines ................ 10
    1.2.3 Surveillance ....................................................................... 11
      1.2.3.1 Mammography ......................................................... 12
      1.2.3.2 Ultrasound ............................................................... 12
      1.2.3.3 Magnetic resonance imaging .................................... 13
      1.2.3.4 Clinical breast examination ................................... 13
      1.2.3.5 Breast self-examination ............................................ 13
  1.3 Breast imaging methods ............................................................ 12
    1.3.1 Mammography ............................................................... 12
    1.3.2 Ultrasound ................................................................. 12
    1.3.3 Magnetic resonance imaging ........................................ 13
    1.3.4 Clinical breast examination ........................................ 13
    1.3.5 Breast self-examination ................................................ 13
  1.4 Mammographic density ........................................................... 14
  1.5 Attendance at health care surveillance ........................................ 14
    1.5.1 Risk factors for non-attendance at surveillance programmes 14
    1.5.2 Socio - economic factors and breast cancer ....................... 15
  1.6 Psychological factors and oncogenetic counselling ........................ 15
  2 AIMS .............................................................................................. 17
  3 MATERIAL AND METHODS .......................................................... 18
    3.1 PAPER 1 ............................................................................... 18
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADH</td>
<td>Atypical ductal hyperplasia</td>
</tr>
<tr>
<td>ATM</td>
<td>Ataxia-telangiectasia (mutated gene)</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BOADICEA</td>
<td>Breast and ovarian analysis of disease incidence and carrier Estimation Algorithm</td>
</tr>
<tr>
<td>BRCA</td>
<td>Breast cancer gene</td>
</tr>
<tr>
<td>BSE</td>
<td>Breast self-examination</td>
</tr>
<tr>
<td>CBE</td>
<td>Clinical breast examination</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>Cyclin dependent kinase 2A</td>
</tr>
<tr>
<td>CHEK</td>
<td>Chek point kinase</td>
</tr>
<tr>
<td>CR</td>
<td>Close relative</td>
</tr>
<tr>
<td>CS</td>
<td>Cowden’s syndrome</td>
</tr>
<tr>
<td>HRQL</td>
<td>Health related quality of life</td>
</tr>
<tr>
<td>HRT</td>
<td>Hormonal replacement therapy</td>
</tr>
<tr>
<td>LCIS</td>
<td>Lobular cancer in situ</td>
</tr>
<tr>
<td>MG</td>
<td>Mammography</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>PTEN</td>
<td>Phosphatase and tensin homologue</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>SES</td>
<td>Socio-economic status</td>
</tr>
<tr>
<td>SF-36</td>
<td>The Short Form -36 Health Survey</td>
</tr>
<tr>
<td>TP53</td>
<td>Tumour protein 53 tumour gene</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
</tr>
</tbody>
</table>
1 GENERAL INTRODUCTION

Nearly 12.7 million new cancer cases and 7.6 million cancer deaths occurred in 2008 worldwide, and breast cancer is by far the most frequent cancer among women. In 2008 almost 1.4 million females were afflicted with the disease which constitutes almost a quarter of all cancers in women. Despite a wide range of incidence in different regions, with the highest rates in western countries, breast cancer is the most common form in females both in developed and in developing countries. (GLOBOCAN, 2008)

Fig 1 Incidence and mortality in different regions (GLOBOCAN, 2008). Estimated ASR (world) per 100 000

In Sweden like in many developed countries approximately every tenth to eighth woman will be afflicted by breast cancer at some time, and 7380 women were diagnosed with the disease in 2009 (Swedish Cancer Registry, 2009). Most cases occur late in life and are sporadic. A Scandinavian twin study has revealed that hereditary factors are of importance in 27% of all breast cancers (Lichtenstein P, 2000), and 5-10% of cases appear to be the result of autosomal dominant genes (Claus EB, 1991). Familial aggregations of breast cancer have been observed all over the world; in general, early onset and bilateral disease are two important features in these families.
(Claus EB, 1990) thus making heredity, together with gender, age and extensive dense breast tissue on mammogram, the most important risk factor for the disease.

So far BRCA1 and BRCA2 are the only two genes available on a daily clinical basis to be screened in families with clustered breast and/or ovarian cancer. In most families with a pattern of hereditary breast cancer, no mutation can be found and in these families, only a risk of breast cancer is considered. As little is known about the risk of other malignancies, apart from ovarian/tubal cancer in mutation carriers, the importance of other malignancies in a family with several cases of breast cancer is hard to evaluate.

No published data suggests that family clustering of breast cancer of genetic origin is more prevalent in one socioeconomic group than in another. Consequently the need for oncogenetic counselling ought to be evenly distributed in a population. Many studies have revealed low socioeconomic status as a risk factor for non-attendance at health care such as population-screening mammography (Lagerlund M, 2002)(Katz SJ, 2000). Descriptive reports of women attending breast-cancer risk-assessment programmes show that these women tend to be well-educated and of middle or upper income status (Bastani R, 1999) (Watson M, 2005). Numerous studies have explored psychological factors in women attending oncogenetic counselling and in general they report worse scores for anxiety, distress and depression than for women in the general population (Cull A) (Martin W, 2006) (van Dooren S, 2004).

Mammography has long been the basic screening modality for breast cancer surveillance. However, sensitivity is less in women with dense breast and in general young women tend to have denser breasts (Wolfe, 1976) (El-Bastawissi AY, 2000). Also, women attending surveillance programmes tend to be young. At the same time, the relative risk of breast cancer in women with very high breast density is substantially higher than in those with lucent breasts (Chiu SY, 2010) (Heusinger K, 2011)(Wolfe, 1976). Ultrasound of the breast has for several years been used to supplement mammography but is not established for screening on its own. Though widely used and recommended, clinical breast exam has not yet been evaluated as a tool for the surveillance of women at high risk for the disease.

This work reported in this thesis concerned finding women who will benefit the most from oncogenetic counselling and surveillance, and finding the best way of surveillance.
1.1 RISK FACTORS FOR BREAST CANCER

1.1.1 Hormonal factors

Reproductive factors such as early menarche, late menopause, null parity, high age at first partum, no breast feeding all contribute to a longer or more intense fertile period, which is believed to heighten the risk of breast cancer (Veronesi U 2005) (Kelsey JL 1993). Though accounted hormonal risk factors, some of these factors certainly also have genetic/inherited backgrounds.

Other hormonal factors. Current use of hormonal replacement therapy (HRT) is nowadays an established risk factor for breast cancer if used for three or more years. Oral contraceptives also contribute to the risk, but the figures here are not as evident as for HRT. (Veronesi U 2005)

Table 1 Selections of risk factors in breast cancer and corresponding relative risks for women. Adapted from Veronesi, 2005

<table>
<thead>
<tr>
<th>Age</th>
<th>Relative Risk (RR)</th>
<th>High-risk group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&gt;10</td>
<td>Elderly individuals</td>
</tr>
<tr>
<td>Geographical location</td>
<td>5</td>
<td>Developed countries</td>
</tr>
<tr>
<td>Breast density</td>
<td>&gt;5</td>
<td>Extensive dense breast tissue visible on mammogram</td>
</tr>
<tr>
<td>Age at menarche</td>
<td>3</td>
<td>Before age 11 years</td>
</tr>
<tr>
<td>Age at menopause</td>
<td>2</td>
<td>After age 54 years</td>
</tr>
<tr>
<td>Age at first full pregnancy</td>
<td>3</td>
<td>First child after age 40 years</td>
</tr>
<tr>
<td>Family history</td>
<td>≥2</td>
<td>Breast cancer in first-degree relative</td>
</tr>
<tr>
<td>Prev benign breast disease</td>
<td>4–5</td>
<td>Atypical hyperplasia</td>
</tr>
<tr>
<td>Cancer in other breast</td>
<td>≥4</td>
<td>Previous breast cancer</td>
</tr>
<tr>
<td>Socioeconomic group</td>
<td>2</td>
<td>Groups I and II*</td>
</tr>
<tr>
<td>Body-mass index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopause</td>
<td>0·7</td>
<td>High body-mass index</td>
</tr>
<tr>
<td>Postmenopause</td>
<td>2</td>
<td>High body-mass index</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>1·07</td>
<td>7% increase with every daily drink</td>
</tr>
<tr>
<td>Exp. to ionisation radiation</td>
<td>3</td>
<td>Abnormal exposure to young girls after age 10 years</td>
</tr>
</tbody>
</table>

Breastfeeding and parity

Relative risk falls by 4.3% for every 12 months of breastfeeding in addition to a 7% reduction for every birth, Women who do not breastfeed,

Use of exogenous hormone

Oral contraceptives 1·2 Current users
HRT 1·66 Current users
Diethylstilbestrol 2 Use during pregnancy
Anthropometric factors.
Having a low BMI at the age of 18 as well as having a BMI over 25 postmenopausally both raise the risk of breast cancer. The relative risk per height increment of 5 cm has been estimated to 1.02 (95% CI 0.96-1.10) in premenopausal women and 1.07(1.03-1.12) in postmenopausal women (Veronesi U 2005).

1.1.2 Demographic factors
Demographic factors include age, gender and geographic area. Breast cancer incidence in Sweden peaks at the age of 63 years (Swedish Cancer Registry, 2009). Breast cancer in men constitutes 0.5% of all cases. Breast cancer in western countries such as the USA, Scandinavia and the UK will occur in approximately every eighth to tenth woman. In Mediterranean countries e.g. Spain, the incidence is less than half that in the former countries while in China and Japan the corresponding lifetime figure is 2% (GLOBOCAN 2008).

1.1.3 Breast properties.
Breast with a dense mammographic pattern is associated with a relative breast-cancer risk of up to 6 times (N. F. Boyd 1995). A previous biopsy showing a pattern of lobular cancer in situ (LCIS) from either breast gives a risk of 20% of malign tumour in either breast. However, LCIS is looked upon as a marker of increased risk rather than an anatomic precursor of malignancy (Nelia Afonso 2008). Atypical ductal hyperplasia (ADH) constitutes an overall risk of 2.4 of getting a malign tumour later (L M Marshall 1997).

1.1.4 Daily intake of alcohol.
Many studies postulate alcohol as positively associated with breast cancer in postmenopausal women, already elevated with moderate consumption (>10 g/day) (Lew JQ, 2009). Lew et al also conclude that associations are stronger with hormone-positive tumours than with hormone negative ones. They also found an association with long-term use of HRT. In a multicentre prospective study in ten European countries (Lois B. Travis, 2005) of over 250, 000 women, 5% of all incident cases of breast cancer (n=12 589) were attributed to alcohol (Schütze M 2011).
1.1.5 Regular physical exercise

Regular physical exercise is believed to reduce the risk of breast cancer. Numerous studies provide evidence of this in postmenopausal women (Awatef M 2011). In a review of 62 studies Friedenreich et al 2008 found in the majority of these studies an average risk decrease of 25-30% among the most physically active women compared with the least physically active (Friedenreich CM 2010). In contrast, Awatef et al, like in many other studies (Magnusson CM 2005), found no corresponding risk reduction among premenopausal women.

1.1.6 Previous ionization radiation

Previous ionization radiation of the breast region. Numerous studies reveal an increased risk of breast cancer in long-term survivors of Hodgkin’s disease after radiotherapy (Travis LB, 2005). A comparison between long-term survivors treated at a high radiation dose delivered to the breast showed an association with an eightfold increased risk compared with that in patients who received lower doses (Travis LB, 2005).

1.1.7 Genetic risk factors

Close relatives with breast cancer.

As a rule of thumb, the risk gets higher with every case of breast cancer in the close family as well as with an earlier onset for each case.

Autosomal-dominant inherited mutations in certain genes are associated with an increased risk of breast cancer. Breast cancer in association with other tumours constitutes different syndromes in these families.

1.1.7.1 BRCA 1 and BRCA2.

In 1994 and 1995 respectively the two tumour-suppressor genes BRCA 1 (Miki Y, 1994) and BRCA 2 (Tavtigian SV, 1996) were cloned. In spite of enormous efforts in numerous scientific laboratories all over the world, so far BRCA 1 / 2 are the only two genes that could be offered on a daily clinical basis to be screened in families with clustered breast and /or ovarian cancer. Carrying a mutation in either gene involves a substantially elevated risk of breast cancer and ovarian/tubal cancer (Easton, 1995) (King, 2003). Carrying a mutation in either of these suppressor genes entails a 50- 80% lifetime risk of female breast cancer. Being a BRCA1 carrier confers a 30-60% risk of
ovarian/tubal cancer, whereas a carrier of mutation in BRCA2 has a 10-20 % life-time risk off the disease.

Although rare, other inherited syndromes associated with an increased risk of breast cancer and other tumours are known:

1.1.7.2  *Tumour protein 53 tumour gene TP53*

One example is the Li-Fraumeni syndrome, characterised by breast cancer occurring at an exceptionally young age in combination with brain tumour, adrenocortical carcinoma and soft-tissue sarcoma (Li FP, 1969). This syndrome has also been associated with an overrepresentation of tumours in the stomach, colon, rectum, pancreas, and ovary as well as malignant lymphoma, all at an extremely young age and along with the typical tumours constituting a classical Li-Fraumeni syndrome (Nichols KE, 2001). Earlier studies have found mutations in the causative gene TP53, rarely seen in families where breast cancer cases predominate (Zelada-Hedman M, 1997). In contrast, a study by Mouchawar published in 2010 demonstrated a 5% risk of TP53 mutation in women affected with breast cancer at an age under 35 years if they had one or several close relative (CR) with breast cancer. In women below 35 of age and with no family history of breast cancer a 3% risk was demonstrated (Mouchawar J, 2010). This will probably lead to a change in counselling so that that women with breast cancer under the age of 35 tested negative for mutations in *BRCA 1* and *BRCA 2* will be offered mutation analysis in *TP53*. In other respects, carriers of *TP53* mutations are also at substantial risk of malignancies of many other origins and also a substantial risk of malignant tumours during childhood and adolescence. Screening methods for malignancies in other organs than breasts have not been established or evaluated in this context. For this reason, counselling is complicated and the advantage of finding a mutation in *TP53* in a family is not always obvious.

1.1.7.3  *Phosphatase and tensin homologue PTEN.*

In Cowden’s syndrome, females are afflicted with malignant tumours of the breast and the thyroid gland caused by germ line mutations in the suppressor gene PTEN (Liaw D, 1997). PTEN mutations in familial breast cancer outside this syndrome are rare (Chen J, 1998) (Guénard F 2007).
1.1.7.4 *Ataxia-telangiectasia (mutated gene) ATM.*
The role of ataxia-telangiectasia mutated gene heterozygosity in breast cancer has been controversial. Heterozygotes represent increased risk, in particular at older age (Athma P, 1996). Occasional germ line mutations have also been reported in familial breast cancer (Chen J B. G., 1998).

1.1.7.5 *E-Cadherin.*
Germ line mutations in the tumour suppressor gene E-Cadherin are associated with an increased risk of diffuse gastric cancer and to some extent also of lobular breast carcinoma, but are not seen (Keller G, 1999) (Salahshor S, 2001) or very rarely (Schrader KA, 2011) in familial breast cancer without diffuse gastric cancer.

1.1.7.6 *Chek point kinase2, CHEK2*
Some families with the Li-Fraumeni phenotype also bear mutations in the CHEK2 gene (Bell DW, 1999). Studies of families with multiple cases of breast cancer indicate that a certain mutation in the CHEK2 gene, 1100delC, is associated with an increased risk of both sporadic and familial breast cancer (CHEK2 breast cancer case-control consortium, 2004). In a recent study from Finland, 82 well-characterized, high-risk hereditary breast and/or ovarian cancer BRCA1/2-founder negative individuals were screened for germ line mutations in seven breast-cancer-susceptibility genes including CHEK2. Fairly many mutations, 12.2%, were found in CHEK2 among these women, but the finding warrants further segregation analyses to evaluate the clinical significance (Kuusisto KM, 2011).

1.1.7.7 *Cyclin-dependent kinase inhibitor 2A CDKN2A*
An increased familial risk of breast cancer and pancreatic cancer has been described in Swedish families segregating the cyclin-dependent kinase inhibitor 2A, CDKN2A/p16-mutation and showing multiple cases of malignant melanoma (Borg A, 2000).

Other genes such as *BRIP, PLAB2* and *RAD 51C* are even more rarely seen in populations of hereditary-breast-cancer families (Wong MW, 2011).
1.2 ONCOGENETIC COUNSELLING

1.2.1 Procedure

1.2.1.1 Awareness
Awareness of family history of breast cancer as a risk factor is growing in the Swedish general population for several reasons. There is growing awareness of family history of breast cancer as a risk factor among nurses, physicians and others working in the health system. Family members in a counselled family, where an enhanced risk for the disease has been identified, are encouraged to spread information to other family members about the risk. The media have given attention to the issue in different ways, including focus on individual young women affected with hereditary breast cancer. These factors certainly have led to more and more women at high risk of the disease being identified.

1.2.1.2 Family composition
Almost all individuals seeking information about breast cancer risk because one or several close relatives (CR) are affected with the disease, are females. In a study from Stockholm including 306 healthy women, we found that 50% had mothers affected with the disease. The second most common CRs with breast cancer were sisters. Substantially more CRs affected with breast malignancy were of maternal than of paternal origin. Concerning the individual composition of in these women's families, it was equally common for them to have one or more daughters as to have one or more sons and also just as many had sisters as they had brothers. The majority of these women, 57%, sought information about the risk of breast cancer in the family for their own sake in the first place and thereafter for the sake of their daughter/s. Of the 306 participating women five regretted undergoing counselling and in 14 cases there was a missing value. Thus, 287 women (96%) were pleased with counselling and would go through the procedure again had they known at the beginning what they knew afterwards.

1.2.1.3 Getting to the counsellor
First, someone must have been noticed in the family history of breast cancer in the family. It could be the woman herself, a relative or a friend who drew attention to the circumstance. It could also be someone in health care such as a gynaecologist or family doctor. Or it could be a nurse involved in mammography screening, who all are trained to ask invited women about their family history of breast cancer. The woman
could then be referred to the counselling clinic or could contact the clinic herself. At all counselling clinics in the Stockholm region the client will then be invited to contact the clinic for an appointment with the counsellor to start the procedure.

1.2.1.4 Pedigree procedure
At first appointment the counsellor will draw a pedigree including all first-, second- and third-degree family members on both sides that the proband, the person being counselled, is aware of. Year of birth and all known malignancies including age at onset are noted. The procedure to get the malignant diagnoses verified includes getting permission from the affected relative or, if deceased, from her/his closest relative. Most of the diagnoses are histologically verified as shown by medical records or the Swedish Cancer Registry. A few cases originated from the beginning of the twentieth century, before the Registry was established. These are mostly verified by checking the Swedish Cause of Death Registry, to which doctors have been required to report all deaths since 1911. On average 80% of all tumours of breast and gynaecological origin in a family are verified (data from paper 3). Tumours of other origins that could be a part of \textit{BRCA 1} or \textit{BRCA 2} inheritance are also verified to a high degree, as are most other malignancies.

1.2.2 Risk assessment
1.2.2.1 Claus tables
Claus tables (Claus EB, 1994) have been widely used. However they include only two close relatives for estimating lifetime risk of breast cancer.

1.2.2.2 Autosomal-dominant inherited disease
In families with three or more close relatives diagnosed with breast or ovarian cancer, it is assumed that there is an autosomal-dominant inherited disease. In families where mutations in any of the high-penetrance genes \textit{BRCA 1} or \textit{BRCA 2} have been ruled out or could not be tested for, a first-degree female relative of an affected woman in such a family is thought to have a 50% chance of carrying a genetic aberration entailing increased risk of breast cancer. The penetrance for such a heritability is assumed to mimic the effect of mutations in \textit{BRCA 1} or \textit{BRCA 2}; but there is great uncertainty in assessing the risk of breast cancer in these families.
1.2.2.3 *Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm [BOADICEA]*

Since 2009 the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm [BOADICEA] risk estimation model has largely replaced Claus tables in the clinic at the Karolinska University Hospital in Stockholm. The BOADICEA is based on 1484 breast cancer cases and 156 multiple-case families mainly from England (AC Antoniou 2004). The model takes into account unlimited family members, family history of malignancies of breast (unilateral or bilateral) ovarian/tubal, pancreatic and prostate origin. Also taken into account are birth cohort, age at cancer onset, BRCA1/2 screening status and Jewish ancestry. A special web-based risk assessment programme is available so that genetic counsellors can estimate the risk for every woman in a counselled family. The model also predicts the probability for a family member to be a carrier of a mutation in BRCA 1 and BRCA 2. However it is important to evaluate the accuracy of the model’s predictions in independent cohorts, which has not yet been done in data sets from a Swedish population.

1.2.2.4 *Testing for mutation according to The Swedish National Oncogenetic Group guidelines.*

In the counselling situation in Sweden, mutation analysis of the *BRCA1* and *BRCA2* genes is usually offered to families fulfilling any of the following criteria:

1) at least three cases of breast cancer in first-degree relatives, one of whom was under the age of 50 at the time of diagnosis,
2) two first-degree relatives with breast cancer, one before the age of 40 years,
3) one case of breast cancer before 35,
4) any combination of breast cancer and ovarian cancer in a family regardless of age,
5) one case of ovarian cancer before age 45.

Among Swedish families with a pedigree fulfilling these criteria, fewer than one-third segregate mutations in either gene (Einbeigi Z, 2001) (Arver B C. A.-D., 1999). Families with a distribution of tumours typical for the Li-Fraumeni syndrome are offered mutation analyses of p53 but very few germ line p53 mutations have been found.
1.2.3 Surveillance

Women with enhanced risk of breast cancer are recommended surveillance and proposed controls are mainly due to level of risk and of breast properties in these women. In Sweden the following recommendations are suggested to these women:

Lifetime risk of breast cancer according to the BOADICEA model.

- **<17 %**
  - No extra surveillance. Recommend population screening mammography and monthly breast self-examination (BSE).

- **17-19 %**
  - Population screening with mammography, but advance screening (annual mammography and ultrasound) from about five years before the youngest case in the family may be considered, if additional factors indicate increased risk.

- **≥20 %**
  - Yearly diagnostic imaging from about five years before the youngest case in the family was afflicted. At follow-up before age 50 and where there are mammographically dense breasts, supplementary imaging such as ultrasound should be performed for increased sensitivity.

- Mutation carriers BRCA 1 and BRCA2
  - Annual surveillance from 25 years of age, including visit to clinician and breast MRI.

Additionally all women are instructed in self-examinations and also encouraged to contact a cancer clinic if they observe any abnormalities in their breasts (my comments).
1.3 BREAST IMAGING METHODS

1.3.1 Mammography

The Swedish National Board of Health and Welfare advised on population-screening mammography as early as in 1986. This advice was based on early results from Swedish mammography trials and after gradual introduction the surveillance was introduced throughout the country eleven years later. Regular mammography screening followed by diagnosis and treatment leads to a significant reduction in breast cancer mortality, as stated by the EUSOMA (European Society of Mastology) in 1993. Meta-analysis of six randomised controlled trials, including four Swedish ones, found statistically significant evidence for a 22% reduction in breast cancer mortality in women over 50 years of age. On the other hand, population-screening mammography in women under 50 has long been controversial and the efficacy of the method in this age-group has not been demonstrated consistently. Thus, some studies could not demonstrate any reduction in breast cancer mortality (Miller AB, 2002) while others reported non-significant reductions (Moss SM & Group, 2006), (Jonsson H, 2000). Hellquist et al have recently shown a significant reduction of relative risk (RR) of breast cancer mortality of 0.74 (95% CI, 0.66-0.83) among invited compared with not-invited women aged 40-49 years (Hellquist BN, 2011).

1.3.2 Ultrasound

Ultrasound as a breast-imaging method is free from radiation and is a procedure that women in general find less uncomfortable than mammography. The method is frequently used to supplement to MG and, unlike MG, can be used in pregnant women. Few studies have used US as a single screening modality with modern technique. Berg et al concluded, from a study of 2809 women with different types of elevated risk of breast cancer, that US yielded 1.1 to 7.2 extra cancers if added to MG but the method also substantially increased the rate of false positives (Berg WA, 2008). Automated whole-breast ultrasound (AWBU) was judged to be beneficial if added to MG. The authors concluded that the method might be an alternative to the more costly MRI in high-risk women but that further research was warranted (Kelly KM, 2010). In Japan, women in general have denser breasts and breast-cancer incidence is highest between the late forties and the early fifties. In an article from Japan the authors concluded that US was equivalent to MG in detecting breast cancer in women in their forties, and that MG and US are complementary (Tohno E, 2009). However, they also found a lack of evidence for US as a single screening method for reducing mortality in breast cancer.
1.3.3 Magnetic resonance imaging
Numerous studies indicate that the sensitivity of magnetic resonance imaging (MRI) is superior to that of other imaging methods such as MG and US (Warner, 2004) (Kriege, 2004) (Kuhl CK, 2005) (Leach MO, 2005) (Weinstein SP, 2009) (Kuhl C, 2010) especially in young women and in BRCA1-or BRCA2- mutation carriers (Warner E, 2004).

1.3.4 Clinical breast examination
Clinical breast examination (CBE) is a standard procedure in women at high familial risk of breast cancer and is performed in connection with the annual visit to the clinician. Though widely used, this breast examination has not proved to decrease breast-cancer mortality.
In a study by Gui et al., 1500 women at standard risk and 1078 women at moderate/high risk of breast cancer were monitored with annual CBE and MG. A total of 31 cancers were detected, of which 26 were palpable. Fourteen of these palpable cancers were not seen on MG and the authors concluded that CBE was an important component of the monitoring women at increased risk of breast cancer (Gui GP, 2001).

1.3.5 Breast self-examination
Breast self-examination (BSE) is frequently recommended to women in general and is also advocated for those at high risk of the disease. In contrast, the U.S. Preventive Service Task Force recommendation statement (USPSTF, 2010) for breast cancer screening does not recommend clinicians teaching women how to perform BSE. In a “meta analysis”, including two population-based randomised studies in altogether 400 000 women in Russia and Shanghai, the authors concluded that BSE is not an effective surveillance method and that it does not lower mortality from breast cancer. Further, according to the review, BSE is associated with substantially more women seeking medical advice for different symptoms, and having biopsies (Kösters JP, 2003).
1.4 MAMMOGRAPHIC DENSITY

High breast density may confer a mammographic sensitivity of only 30-40%, and it represents an independent risk factor for breast cancer (Mandelson MT, 2000) (Kolb TM, 2002). This risk was first described in the mid-1970s by Wolfe (Wolfe JN, 1976). In a study by Boyd et al. the relative risk in women with very high breast density compared to women with lucent breasts was 6.05 (Boyd NF, 1995) while Chiu et al. found a relative risk of 1.57 (1.18-1.67) in dense breasts compared to fatty breasts (Chiu SY, 2010). In studies of homo- and heterozygote twins, breast density was found to follow hereditary traits (Boyd NF, 2002). However, the increased breast cancer risk demonstrated in carriers of BRCA1 or BRCA2 mutations seems not to depend on mammographic density (Gierach GL, 2010) (Mitchell G, 2006).

1.5 ATTENDANCE AT HEALTH CARE SURVEILLANCE

1.5.1 Risk factors for non-attendance at surveillance programmes

Studies in Sweden and other western countries have revealed several factors as predictive of non-attendance at population mammography screening. Such factors include no offspring and/or living without a partner, low or extra-high education and low income (Katz SJ, 2000) (Lagerlund M, 2002) (Aarts MJ, 2011).

In agreement with these observations, descriptive reports of socioeconomic factors among women attending oncogenetic counselling clinics show that they tend to be well-educated and of middle or upper income status (Bastani R, 1999) (Watson M, 2005). There is, however, no published data suggesting that family clustering of breast cancer of genetic origin is more prevalent in higher socioeconomic groups. Reports of
an association between high socioeconomic status and an elevated risk of breast cancer (with an RR 1.1 – 3.5) are believed to reflect extrinsic factors rather than genetic factors (Braaten T, 2004).

### 1.5.2 Socio-economic factors and breast cancer

According to numerous studies, non-attendance in mammography screening programmes is associated with unfavorable socio-economic status (SES) and moreover to advanced disease (Zackrisson S, 2004). In a recent study from Holland women of low SES were found to have a significantly higher tumour stage at diagnosis than women with high SES. This was equally found among non-attendees, women with screen-detected cancers and women diagnosed with interval cancers (Aarts MJ, 2011). Altogether, studies on the influence of socioeconomic factors on women with breast cancer have demonstrated lower rates of survival among women of low SES (Kaffashian F, 2003). Although Rutqvist and Bern (Rutqvist LE, 2006) observed a higher rate of survival among Swedish women with high income, more skilled work and a high level of education, these differences reflected the distribution of clinical stage of disease when diagnosed rather than a direct effect of socioeconomic variables. In contrast, in a recent study from the United States the difference in survival according to SES, favouring women with the highest level of education and household income, could only be partly explained by screening attendance and early detection (Sprague BL, 2011). The authors of that study conducted in the US concluded that research will be needed to understand the additional factors contributing to the remaining difference between women with differing educational levels.

### 1.6 Psychological factors and oncogenetic counselling

Numerous studies have explored psychological factors in women attending oncogenetic counselling. In general they report worse scores for anxiety, distress and depression in women with a family history of breast cancer than in women in the general population (Cull A, 1999) (Martin W D. L., 2005) (van Dooren S, 2004). In contrast, a Swedish study of women undergoing pre-symptomatic testing for mutations in BRCA 1/2, showed no differences in vitality, mental health, role emotional functioning, social functioning or general health as compared to women in the Swedish population (Arver B, 2004). In addition, in a Norwegian study individuals with hereditary risk of cancer were in better physical shape than, and similar mental shape to, the general population (Carlsson AH, 2004). A Danish study of 213 women concluded that genetic
counselling does not appear to have a negative effect on general anxiety, symptoms of depression or Health related quality of life (HRQL), and that counselling can help ease cancer-specific distress among women with a family history of breast cancer (Mikkelsen EM, 2009).
2 AIMS

PAPER 1

to search for putative breast-cancer-associated syndromes in families with two or more cases of breast malignancies;

PAPER 2

to characterize women at an oncogenetic counselling clinic in terms of socioeconomic status (SES) and health-related quality of life (HRQL),
to compare SES data from the women at the counselling clinic with population-based data for women in the same catchment area,
to compare HRQL in the studied sample with a normative age-matched sample from the general population,
to compare different objective risk groups with respect to SES and HRQL;

PAPER 3

to describe the design and the procedure of a prospective screening study,
to describe the study cohort regarding hereditary patterns and socio-demographics,
to report results of the first mammography, ultrasound and clinical breast examination and
to elucidate the associations between breast density and familial breast cancer risk, BMI and other breast-cancer risk factors.
3 MATERIAL AND METHODS

The work presented in this thesis comprised three different studies with different study populations and methods. Consequently, the material and methods used are presented separately.

3.1 PAPER 1

3.1.1 Material

In 2000 the Swedish National Oncogenetic Group decided to include all families in Sweden that had up to that point ever been subject to oncogenetic counseling at any of the eight counseling clinics in Sweden. Altogether 4000 families were identified. For the purpose of this paper we sorted out the families with identified mutations in the BRCA 1 or BRCA 2 or in any of the genes that causes increased risk of tumours of the colon as well as all families with breast or colon-only malignancies. At that stage 1648 families remained. Approximately 50% of them originated from any of the three counseling clinics in Stockholm and, with descending contribution, clinics in Lund, Umeå, Gothenburg, Uppsala and Linköping contributed to the remaining 50 percent. Excluded families at this point were families with no case of breast cancer or families with only one case of the disease. Thus, the remaining 803 families represents the cohort of families in this paper.

3.1.1.1 Study families

Diagnoses from both maternal and paternal branches were included. Every case of cancer in first- and second-degree relatives, and in first cousins, was recorded. More than one study family could originate from one pedigree, depending on which pedigree branch was chosen. In this way, one case of cancer could be included in two, or sometimes three, study families; but no case of malignancy was ever counted more than once as an observed case of that particular type of tumour. Altogether, the 803 study families originated from 750 pedigrees. Most of the diagnoses were histologically verified from medical records or the Swedish Cancer Register. A few of the cases originated from the beginning of the twentieth century,
before the Cancer Register was established. These cases were mostly verified by checking the Swedish Cause of Death Register.

3.1.1.2 Reference Population
In addition to serving as a means of verifying diagnoses, the Swedish Cancer Register was used as a reference population in this study. In 1958, Sweden had 7.5 million inhabitants of whom 19,324 were reported to the Cancer Registry. In 1999 the corresponding figures were 8.8 million and 45,180.

3.1.2 Method

The proportion of different cancers other than breast cancer in the study families was computed and 95% confidence intervals (CI) were calculated. If the 95% CI did not cover the proportion from 1958 (or 1999) a significant difference from that year could be demonstrated. The population data were obtained from official Swedish statistics for two separate years and comparisons were made using statistics from the first and last years for which Swedish cancer statistics were available. Only malignancies with a significant difference in proportion compared with the general population in 1958 and 1999 were considered over-represented.

To permit comparison of the frequency of non-breast-cancers in our study families with data from Statistics Sweden, we corrected for the fact that 87% of the cancers reported in 1958 and 86% in 1999 were non-breast-cancers.

3.2 PAPER 2

3.2.1 Material

3.2.1.1 Study population

A consecutive series of 373 women attending genetic counselling at the oncogenetic outpatient clinic at Södersjukhuset, Stockholm, Sweden between 1 April 1998 and 1 June 2004 were eligible for inclusion. A total of 306 women (82.5%) participated in the study. The criteria for attending the clinic and thus for eligibility for study, were wide, including at least one relative with breast, ovarian or breast/ovarian cancer. The vast
majority (82.3%) of the women had in their family history at least two close relatives with breast cancer.

Women previously treated for breast and/or ovarian cancer or other malignancies were not eligible. As we chose to include in a consecutive manner following the woman’s first visit to the clinic, some women made only one visit, while others had attended several times when the questionnaires (see below) were sent.

3.2.1.2 Reference population

Socioeconomic status
An age-matched reference population aged 25 to 74 years comprising all women living in the same catchment area at the time of the questionnaires (2004) was used (n= 277 783).

Health related quality of life
The reference population for health-related quality of life consisted of a normative age-matched sample of Swedish women from the general population. The Short Form -36 Health Survey (SF-36) was used.

3.2.2 Method
A questionnaire and an information letter explaining the purpose and the procedure of the study were sent to all 373 women by 1 June 2004. By answering and returning the questionnaire, the woman accepted to participate.

3.2.2.1 Questionnaires and Reference sample

Socioeconomic status
The questionnaire addressing socioeconomic characteristics was developed for a study obtaining HRQL reference values in a large sample of the Swedish population. The questionnaire included three separate indicators of socioeconomic status (SES). Comparisons between data from study subjects and the population were explored using, for the reference population, figures from official statistics from three population-based registers. Each register cover close to 100% of the reference population.

- Marital status comprised four alternatives; “married or cohabiting”, “divorced/separated”, “widowed”, “single”.
Reference data on marital status was obtained through the Register of Total Population 2004. This is updated continuously and provides data on all individuals in Sweden.

- **Education** consisted of three categories; elementary school (=9 years), elementary school + 2-3 years of upper-secondary, elementary school + 4-or more years of upper-secondary.

  - For reference values on education, the National Register of Education 2004 was used. This register covers all individuals living in Sweden aged 16-74 and is updated every year with new data on the highest attained level of education.

- Annual household income was classified in Swedish crowns (SEK) and upgraded compared to the original questionnaire as income brackets <300 000, 300 001-500 000, >500 000 (300 000 - 500 000 SEK = 32 500 -54 200 Euro or 44 000-73 300 USD 15.05.2007).

  - Economic situation for the reference population was obtained from the Register of Total Household Income 2004 which covers all sources of income subject to taxation, and therefore includes social benefits.

Health-related quality of life
The Short Form-36 Health Survey (SF-36), was used for assessing of HRQL. It consists of 36 items constituting eight subscales:
- physical functioning (PF), role limitations as a result of physical problems (RP),
  - general health perception (GH), pain (BP), role limitations as a result of emotional problems (RE),
  - social functioning (SF), vitality (VT) and emotional well-being (MH).
Higher score signifies better health. For each of the eight scales, the scores are summed and transformed to a scale of 0–100. The Swedish version has shown good psychometric properties, and normative data for Swedish women are available.

3.2.2.2 Statistics

Descriptive statistics were generated for the study population regarding age at first visit and characteristics of family and family history. SES data from the study population
and from the reference population were compared using a one-sample test of proportion or a one-sample $t$ test.

Chi-square tests of independence were used for comparing categorical data between the three defined risk groups in the study population. For continuous data, the F test in linear regression models was used. In these regression models risk groups were represented by two dummy variables. Mean scores and 95% confidence intervals were calculated for each of the eight SF-36 subscales for the study population. Expected mean scores were calculated using age-specific mean-scale scores from normative Swedish data and the age distribution in the study population. One-sample $t$ tests were used to compare observed and expected mean scores.

## 3.3 PAPER 3

### 3.3.1 Material

Study participants were consecutively recruited between January 2002 and June 2006 from the Familial Cancer Centre, Oncology Department, Karolinska University Hospital. This is located at three sites in Stockholm: Danderyd Hospital (site 1), Karolinska University Hospital (site 2) and Södersjukhuset (site 3). All the women approached had had oncogenetic counselling and fitted into one of the four inclusion groups. A minor proportion of cancer diagnoses in the study subjects’ families were not verified due to insufficient data or foreign ancestry. Mutation screening of the $BRCA1/2$ genes was offered to families according to Swedish National Oncogenetic Group guidelines. A total of 659 women were approached for the study, 632 (94%) consented to participate.

#### 3.3.1.1 Inclusion criteria – four groups

Suitable for inclusion were healthy women 25-60 years of age with a $\geq 17\%$ lifetime risk of breast cancer. Eligible women had either

1. $\geq 17\%$ lifetime risk of breast cancer according to Claus tables, or

2. a family history indicating an autosomal-dominant disease, with one or more first- or second-degree relatives with breast and/or ovarian cancer, or
3. a personal history of breast or ovarian cancer without signs of recurrence, with one or more first- or second-degree relatives with breast and/or ovarian cancer, or

4. mutation in BRCA1, BRCA2, PTEN or TP53 with or without a personal history of breast or ovarian cancer.

Women with no known mutation in the family could be included from 10 years before the youngest family member was affected with the disease, while mutation carriers were eligible from 25 years of age. A normal mammogram one year before the first screening round was mandatory.

3.3.2 Methods

Study 3, which is still in progress, is a prospective, blinded, intraindividual, comparative surveillance cohort study. The surveillance includes six annual screening rounds for MG, US, MRI, CBE and BSE. The last screening round will be completed in June 2011.

The paper reporting Study 3 is a first report. It includes a description of the main study design and the procedure, the study cohort regarding hereditary pattern and sociodemographics. Results of the first MG, US, and CBE are described. Associations between breast density, BMI and other breast-cancer risk factors are elucidated.

3.3.2.1 Procedure

Fig 3. Procedure – Flowchart for a woman included at site 2

Blinding procedure

No communication on findings between the radiologist and the study subject was permitted. The results from the three imaging modalities were sent in closed envelopes to the clinicians. No communication, regardless of finding, between the radiologists
and/or the clinicians involved in the study, was allowed before the annual CBE. The clinician was not permitted to open the envelope until the CBE was performed and documented. Subsequently all imaging results were disclosed and presented to the study subject. No more action was taken until the next screening round if all exams were normal.

3.3.2.2 Imaging modalities

Mammography
All mammograms were double-read by two of five breast radiologists with at least twenty years experience. Mammography was performed with two views per breast (medi-oblique and craniocaudal) using analogue technique at both sites.

Breast ultrasound
All the US breast radiologists had more than fifteen years experience of clinical breast US examination. Both breasts including the axillas, were systematically examined by one of three breast radiologists.

Magnetic resonance imaging
No study subjects underwent MRI during the first screening round.

Clinical breast examination
The breasts and regional lymphatic areas were examined physically with the study subject sitting as well as lying down. All CBEs were performed by one of three oncologists, who all had more than five years experience of breast cancer diagnostics.

Breast self examination
All the women had been instructed to do monthly BSEs and were encouraged to contact the clinician if they noted any abnormality in the breasts.

3.3.2.3 Breast assessment

- Findings: The MG, US and/or CBE findings were scored on a five-point scale; 1= normal, 2= benign, 3= possibly malignant, 4= probably malignant and 5=
malignant. This is a modified version of a classification of mammographic findings described by Azavedo et al. and frequently used in Sweden (Azavedo, 1989)

- **Density** Mammographic density was estimated according to Wolfe’s division of breast density into four groups of increasing density: N1, P1, P2 and DY.

- **Easy or difficult to interpret** To explore aspects of difficulty in interpreting the results of the imaging modalities, a dichotomized, subjective assessment was introduced. The breast radiologists categorized all images as easy or difficult to interpret.

In case of abnormalities
If there was an abnormality, code three or more on any examination modality, the study subject was mandatorily referred for further work-up to the radiologist who had detected the lesion. From this point on, all data from the examinations were made available to all the physicians involved in the work-up.

Palpable lesions coded 3 or more were referred to a cytologist for fine-needle biopsy. (Referring for cytology was optional for codes below 3). If the diagnosis was still indeterminate after further work-up including additional views, spot compression views and core biopsy, an excision biopsy was performed.

In pregnant or lactating women, only US imaging was used. CBE was done in all women.

### 3.3.2.4 Lifestyle assessment
A questionnaire was developed and captured demographic characteristics, medical history including history of gynaecological and/or breast surgery, reproduction history, external hormonal treatment, menopausal status, height and weight (body mass index), cup size, habits of self-examination and lifestyle factors such as smoking, alcohol habits and physical activity. The questionnaire was self-administered and collected in connection with the visit to the clinician, a procedure that will be repeated every six study rounds.
3.3.2.5 Further risk assessment
Since 2009 the Breast and Ovarian Analysis of Disease Incidence and Carrier
Estimation Algorithm [BOADICEA] risk estimation model has replaced the Claus
tables in the clinical setting. To compare old and new risk estimations, the lifetime risk
(20-80 years of age) for each woman according to BOADICEA (21) was estimated
retrospectively.

3.3.2.6 Statistical analyses
All data analyses were performed with the SPSS for Windows program, version 16.0.
The main functions used for analysing the data were frequency tables, t tests, analyses
of variance (ANOVA) and chi-square test for trend. Breast density as a dichotomous
outcome, high versus low (N1 and P1 = low and P2 and DY = high), was analysed
using binary logistic regression models. The factors used in the analyses were children
yes/no, risk group, premenopausal status yes/no, BMI at inclusion < 25 versus ≥ 25,
and mean age as a continuous variable. Forward stepwise binary logistic analyses were
performed controlling for BMI and age. Approximately 5% of the values were missing
from the variables used in the analyses.
4 RESULTS AND DISCUSSION

4.1 PAPER 1

Breast cancer in connection with other malignancies represents several known breast cancer syndromes such as breast and ovarian cancer syndromes in families carrying mutations in either suppressor gene BRCA1 or BRCA2, or the hereditary pattern seen in families harbouring mutations in p53 or PTEN. However, it is not known whether non-breast cancer malignancies apart from these syndromes in breast cancer families confer a risk on family members.

In the 803 study families we found:

- Breast cancer: 2203 cases = 2.7 cases per family
- 35 different non-breast-cancer diagnoses: 1706 cases = 2.12 cases per family.

Tumours in colon, ovary, endometrium, pancreas and liver, and leukaemia, were represented in an observed proportion with a 95% CI that did not cover the calculated proportion from official statistics in 1958 and 1999. Proportions of all non-breast/ovarian tumours were also calculated and the same five malignancies (ovarian tumours not included), together with malignant tumours in connective tissue and larynx, had an observed proportion with a 95% CI that did not cover the proportions in the general population in 1958 and 1999. All the over-represented types of cancer, except for tumours in the larynx, were also present among the multiple-case families.

Some malignancies were present in a smaller proportion of the study families than in the reference population:
- all cancers of the urinary tract, except for tumours of the kidney,
- all malignancies of the lymphoproliferative system, such as lymphomas and myelomas together.

Syndromes described earlier could, at least in part, explain the higher frequency of ovary and pancreatic tumours in the study families, and leukemia. The excess of ovary and colon cancer could partly be a result of confounding by indication and the excess of
malignancies in the liver could depend on secondary tumours in the study families. However, we conclude that endometrial carcinoma has not been described earlier in that context, suggesting that endometrial carcinoma and breast cancer could constitute a new breast cancer syndrome.

In 2000 Mutter et al stated that decreased function of the PTEN suppressor gene is a marker of precancerous lesions in the uterus and a major gene involved in the pathogenesis of endometroid endometrial adenocarcinoma (Mutter GL, 2000). Accordingly, in the list of operational criteria for the diagnosis of the Cowden Syndrome (CS) established by the International Cowden Consortium, endometrial cancer was added to the major criteria for the syndrome (Eng, 2000). Eng concluded that endometrial carcinoma might be a major CS cancer, and the presence of tumours of endometrial origin might be important in finding mutations in the PTEN gene in counselled families. The risk of endometrial cancer in PTEN mutation carriers is estimated to be 5 -10%. In our data set of 124 families with both breast and endometrial cancer, five also presented one case each of thyroid cancer. However, no further analysis has been undertaken to clarify whether these families could constitute a subgroup of families with Cowden Syndrome in our cohort.

On the other hand, a histological study by Liang et al found certain tumours of the endometrium, endometrial serous carcinoma, to be more prevalent in women previously treated for breast cancer than was endometroid carcinoma of the uterus, especially in women under 50 years. They also cited our suggestion that endometrial cancer together with breast cancer could constitute a new breast- cancer syndrome possibly in accordance with their findings. Further investigation is warranted to categorize phenotypes of endometrial tumours in this subgroup of malignancies in our study sample.

4.2 PAPER 2

4.2.1 Results and Discussion socio-economic status

The characteristics of the study groups with respect to age at the time of the first visit to the clinic, description of family, family history and documented objective risk did not
differ significantly in the majority of cases from the corresponding characteristics of the eligible women who declined participation (n=65, 17.5%). At the same time, the mean values of all three socioeconomic indicators for the study group and other women in the same geographical area did differ significantly.

Table 2. Comparison of women 25-74 years seeking oncogenetic counselling and women from the general population (25-74 years) in the same catchment area.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study group (n=306)</th>
<th>General population</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (95%CI)</td>
<td>44.0 (42.7 to 45.2)</td>
<td>46.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Marital status:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>74.2%</td>
<td>43.8%</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>10.5%</td>
<td>17.7%</td>
<td></td>
</tr>
<tr>
<td>Widowed</td>
<td>1.6%</td>
<td>3.9%</td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>13.7%</td>
<td>34.6%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Education level:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elementary school 7-9 years</td>
<td>9.5%</td>
<td>17.3%</td>
<td></td>
</tr>
<tr>
<td>Elementary school + 2-3 years</td>
<td>33.9%</td>
<td>43.1%</td>
<td></td>
</tr>
<tr>
<td>Elementary school &gt;=4 years</td>
<td>56.7%</td>
<td>39.6%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Household income:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;300 000 Skr</td>
<td>27.2%</td>
<td>57.9%</td>
<td></td>
</tr>
<tr>
<td>300 001-500 000 Skr</td>
<td>36.2%</td>
<td>29.3%</td>
<td></td>
</tr>
<tr>
<td>&gt;500 000 Skr</td>
<td>36.6%</td>
<td>12.9%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*From Chi-square goodness of fit test or one-sample t-test.
*Confidence interval.

Information regarding the general population (n=277 783) refers to the year 2004.

The same differences between our subjects and the reference population were observed when the study group was divided into sub-groups on the basis of risk of developing breast cancer: low (<21%), intermediate (21-39), high (40-80%), youngest age at which breast cancer was diagnosed in another member of the family (<40, 40-49, >49 years) or the death of an immediate relative from this disease.
In a population-based study conducted in the Geneva canton of Switzerland covering all incidents of breast cancer diagnosed during 1990-1999, women with and without a family history of breast cancer were compared regarding detection of breast cancer by surveillance, stage at diagnosis, likelihood of adequate treatment and survival (Verkooijen HM, 2009). The authors demonstrated, somewhat in contrast to our findings, that the presence of a positive FH eliminates differences in access to screening and optimal treatment. However, differences in stage at diagnosis and subsequent mortality were still unfavorable in women of low socioeconomic status (SES) regardless of a present FH or not. However, selection to that study was population-based and did not reflect women at a counselling clinic.

On the other hand, one could speculate that women in that study had a lower barrier to get a mammography than ‘our’ women who first had to have counselling which most often during the pedigree-procedure, includes contact with more or less close relatives – which can be seen as an obstacle. In addition, to reach a counsellor implies making contact with the health and welfare system on one’s own initiative – which in itself can be an obstacle.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Low (n=117)</th>
<th>Intermediate (n=56)</th>
<th>High (n=124)</th>
<th>P-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>42.2 (9.0)</td>
<td>45.5 (10.6)</td>
<td>45.3 (11.3)</td>
<td>0.033c</td>
</tr>
<tr>
<td>Youngest age at diagnosis of breast cancer in the family, mean (SD)</td>
<td>46.1 (10.6)</td>
<td>43.4 (6.6)</td>
<td>44.3 (7.6)</td>
<td>0.11c</td>
</tr>
<tr>
<td>Close relative died of breast cancer</td>
<td>40 (34)</td>
<td>28 (50)</td>
<td>49 (40)</td>
<td>0.14</td>
</tr>
<tr>
<td>Cohabitant</td>
<td>86 (74)</td>
<td>41 (73)</td>
<td>93 (75)</td>
<td>0.95</td>
</tr>
<tr>
<td>Higher education</td>
<td>70 (60)</td>
<td>32 (57)</td>
<td>67 (54)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

aSeven women are excluded from analysis when information regarding age at diagnosis of breast cancer are missing.

bP-value from chi-square test of independence.

cP-value from F-test in a linear regression model. Risk group is represented by two dummy variables in the model.
4.2.2 Results and Discussion Health related quality of life

The study sample scored lower than expected on five of the eight subscales, whereas three subscales relating to physical health showed mean scores close to expected. As age differences were found between the risk groups, this variable was accounted for in the analysis of HRQL (SF-36). No statistical differences in HRQL were found between the risk groups.

![Difference between observed and expected scores](image)

*Fig. 4 Difference between observed and expected scores*

When the study group was divided according to highest level of education, the same differences between all three sub-groups and the reference values were seen (data not shown). This was also the case when the study group was subdivided on the basis of objective estimated risk of developing breast cancer (data not shown). In contrast, there were no significant differences between the sub-groups.

Study subjects’ SF-36 scores were affected neither by highest educational level nor degree of objective risk of breast cancer. However, we cannot conclude anything from our results about why these women sought counselling. Hence, we do not know whether these women were in general worried and anxious and would have had the
same SF-36 scores irrespective of an increased risk of breast cancer. Or whether, before knowing about a possible increased risk, they would have had SF-36 scores in parity with normative data. Nor do we know if their mental health will change over time.

4.3 PAPER 3

A total of 659 women were approached for the study and 632 consented to participate.

**Baseline data**
Baseline data including social status, educational level, health-related factors and hormonal-related factors are presented.

Example:
- Married or living with partners: 471 (77%)
- Had children: 492 (78%)
- Had an academic degree: 398 (63%)

Ages ranged from 25 to 60 years, with a mean age of 44.1. The mean age was statistically significantly higher in Risk Group 3 (see below) than in the other risk groups. No major differences regarding baseline data were found between the four risk groups.

**Risk assessment and defining risk groups**
The pedigrees were defined regarding hereditary pattern and divided into 16 different subgroups. Subsequently these subgroups were classified according to the lifetime risk of breast cancer.

Four risk groups were defined:

1. Moderate risk of breast cancer; 173 women
2. High risk of breast cancer; 387 women
3. Heredity and own history of breast- or ovarian cancer; 26 women
4. Very high risk, mutation carriers +/- breast- or ovarian cancer; 46 women

In the retrospective analyses using the BOADICEA risk estimation model and a cut-off value of ≥17% lifetime risk of breast cancer, 217 (34%) of the women would not have been eligible for surveillance in this study.

**Encoding mammography, breast ultrasound, clinical examination and further work-up**
All the breast MG (n=612), US (n=624) and CBE (n=627) were blinded to the people conducting the other two. No statistically significant differences were found regarding the coding for the different breast examination methods between the risk groups or age groups.
**Mammographic evaluation**
No lesions suspicious for malignancy (codes 4 and 5) were identified by the MG breast radiologists. In 18 cases (17 with code 3 and one with code 2) supplementary mammographic images were recommended and seven of these women needed further investigation with stereotactic biopsy.

**Subjective experience of reading the mammographic images**

**Table 4 Mammography - Easy or difficult to interpret in relation to density pattern**

<table>
<thead>
<tr>
<th>Mammography - Easy or difficult to interpret in relation to density pattern</th>
<th>DY + P2 (%)</th>
<th>P1 + N1 (%)</th>
<th>Total number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difficult</td>
<td>165 / 411 (40.1)</td>
<td>22 / 199 (11.1)</td>
<td>187 / 610 (30.6)</td>
</tr>
<tr>
<td>Easy</td>
<td>246 / 411 (59.8)</td>
<td>177 / 199 (88.9)</td>
<td>423 / 610 (69.3)</td>
</tr>
<tr>
<td>Total number</td>
<td>411</td>
<td>199</td>
<td>610</td>
</tr>
</tbody>
</table>

**Ultrasound evaluation**
The US breast radiologists identified two women with a code-4 lesion and one with a malignant lesion, code 5. In total 75 women were recommended additional work-up with fine-needle biopsy or ultrasound-guided core biopsy.

**Subjective experience of reading the ultrasound images**

**Table 5 Ultrasound - Easy or difficult to interpret in relation to density pattern**

<table>
<thead>
<tr>
<th>Ultrasound - Easy or difficult to interpret in relation to density pattern</th>
<th>DY + P2 (%)</th>
<th>P1 + N1 (%)</th>
<th>Total number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difficult</td>
<td>93 / 411 (22.6)</td>
<td>25 / 197 (12.7)</td>
<td>118 / 608 (19.4)</td>
</tr>
<tr>
<td>Easy</td>
<td>318 / 411 (77.4)</td>
<td>172 / 197 (87.3)</td>
<td>490 / 608 (80.6)</td>
</tr>
<tr>
<td>Total</td>
<td>411</td>
<td>197</td>
<td>608</td>
</tr>
</tbody>
</table>

**Clinical breast examination**
The clinicians referred 89 women for further work-up with cytology. These clinical findings were coded as follows: code 1 – 4 cases, code 2 – 48 cases, code 3 – 36 cases, code 4 – 1 case, code 5 – 0 case. There were 87 benign findings among CBE-referred cytology, one atypia but no malignancy.
Breast self exam

Fig 5 BSE in different age groups

Fig 6 BSE in different risk groups

Breast density and fine-needle referrals

Table 6. Breast density and fine-needle referrals MG

<table>
<thead>
<tr>
<th>Density Category</th>
<th>Total Number</th>
<th>Density</th>
<th>N/fine needle cytology referred from MG</th>
</tr>
</thead>
<tbody>
<tr>
<td>DY</td>
<td>1/60 (1.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>4/355 (1.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>2/169 (1.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>0/30 (0.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Breast density and fine-needle referrals US

<table>
<thead>
<tr>
<th>Density Category</th>
<th>Total Number</th>
<th>Density</th>
<th>N/fine needle cytology referred from US</th>
</tr>
</thead>
<tbody>
<tr>
<td>DY</td>
<td>8/60 (13.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>48/355 (13.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>19/169 (11.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>2/30 (6.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8. Breast density and fine-needle CBE referrals

<table>
<thead>
<tr>
<th>Density Category</th>
<th>Total Number</th>
<th>Density</th>
<th>N/fine needle cytology referred from CBE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DY</td>
<td>8/60 (13.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>59/355 (16.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>18/169 (10.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>2/30 (6.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Results of work-up**

Ten women were referred for surgery, seven with benign lesions and three with malignant tumours.

*Table 9. Breast cancer cases*

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Risk group</th>
<th>MG Code</th>
<th>Density</th>
<th>Easy/Diff</th>
<th>US Code</th>
<th>Easy/Diff</th>
<th>CBE Code</th>
<th>T Code</th>
<th>Tumour size (mm)</th>
<th>Invasive y/n</th>
<th>Grade y/n</th>
<th>ER + y/n</th>
<th>Prolif (%)</th>
<th>Node y/n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>59</td>
<td>2</td>
<td>1</td>
<td>N1</td>
<td>Easy</td>
<td>4</td>
<td>Easy</td>
<td>1</td>
<td>U</td>
<td>9</td>
<td>y</td>
<td>2</td>
<td>y</td>
<td>&lt;2%</td>
<td>n</td>
</tr>
<tr>
<td>Case 2</td>
<td>47</td>
<td>2</td>
<td>1</td>
<td>DY</td>
<td>Diff</td>
<td>5</td>
<td>Diff</td>
<td>2</td>
<td>M</td>
<td>11</td>
<td>Y</td>
<td>3</td>
<td>Y</td>
<td>30%</td>
<td>n</td>
</tr>
<tr>
<td>Case 3</td>
<td>38</td>
<td>1</td>
<td>3</td>
<td>P2</td>
<td>Diff</td>
<td>3</td>
<td>Diff</td>
<td>4</td>
<td>O</td>
<td>22</td>
<td>Y</td>
<td>3</td>
<td>Y</td>
<td>23%</td>
<td>y</td>
</tr>
<tr>
<td>Case 4*</td>
<td>41</td>
<td>1</td>
<td>3</td>
<td>P2</td>
<td>Unknown</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>R</td>
<td>10</td>
<td>Y</td>
<td>3</td>
<td>N (TN)</td>
<td>90%</td>
<td>y</td>
</tr>
</tbody>
</table>

*One woman was diagnosed with breast cancer through the population-based service screening with mammography four weeks before scheduled study examinations.*
Breast density in different risk groups and age groups

To identify any predictors for high density, multivariate analyses were used. The two density groups N1 and P1 were merged and defined as low density (n=199), while P2 and DY were merged and defined as high density (n=413). BMI ≤ 25 and low age were statistically significant predictors of high breast density in both the univariate and the multivariate analyses. Non-significant competing factors were children y/n, risk group, and premenopausal vs. postmenopausal status.

Many studies have reported that there seems to be no association between BRCA1 and BRCA2 carrier status and breast density (Gierach GL 2010) (Mitchell G 2006). Likewise, many studies have reported density to be associated with heredity for breast cancer (Martin LJ 2010)(Boyd NF 2002). As far as we know, ours is the first study to interpret the association between density and increasing risk of familial breast cancer.

To evaluate density we chose Wolfe’s well-established pattern-based method (Wolfe, 1976) which may be less specific than quantitative-based scales for classifying highly dense breast. However, in a recent study by Garrido-Estepa et al both methods were considered to have high reproducibility (Garrido-Estepa M, 2010).

To elucidate the association between difficulty in reading breast images and breast density, a second subjective scale was introduced: all the breast radiologists judged whether the breasts were easy or difficult to read. It is less known whether mammographically dense parenchyma also affects the assessment of ultrasound and/or CBE thereby occasioning a need for further work-up. For breast evaluated as DY or P2, there were numerically higher percentages of referrals for fine-needle biopsies from all three examination methods.

Mammographic breast results evaluated as DY were much more frequently interpreted as difficult to read on MG than on US, 75% vs. 24%. However, it is too early to conclude whether there is any diagnostic relevance in this subjective method.

The study included at least three visits per screening round for the participating women. Despite this, only 6% of the women we approached declined to participate and also, only a few women dropped out because the study was too time-consuming.
Concerning socioeconomic status a majority of the women were well-educated and married or cohabiting, which agrees well with many previous descriptive reports of women who visit oncogenetic counselling clinics (Bastani R 1999) (Watson M 2005) and with previous findings from the Stockholm area (paper 3) (Von Wachenfeldt A, 2009). Paper 3 included patients from two sites, northern and south Stockholm. We are aware that some of the present subjects (from site 3) in this study also participated in paper 2. On the other hand, no statistically significant differences in baseline data between the study sites were found, although income and levels of education differ between these catchment areas in the Stockholm region (www.socialstyrelsen.se 2011). We believe that the high participation in the study permits generalization of the results to other women seeking oncogenetic counselling.

It is difficult and challenging to categorize women into different risk groups regarding risk of breast cancer due to hereditary factors in families with no identified mutation. Claus tables are widely used; however, they include no more than two close relatives for estimating lifetime risk of breast cancer. Many different types of risk estimation and risk categorization have been used in oncogenetic studies (Berg WA, 2008) (Kriege M, 2004) (Weinstein SP, 2009) (Schmutzler, 2006). Our estimation was based on the pedigree pattern, as this risk has been estimated by others (Schmutzler RK, 2006) (Kuhl CK, 2005) while other authors base their estimation on percentage risk from the Claus tables / or the Gail model (Kriege M, 2004) (Weinstein SP, 2009).

A third of our study population was classified as being at moderate risk and 2/3 as being at high risk, which is similar to the risk groups in a study by Kriege et al. (5), who used modified Claus tables (20) for risk estimation. Thus, our risk categorization and Claus tables resulted in approximately the same distribution between risk groups and are hence presumably comparable.

The new BOADICEA risk assessment model has recently been introduced in Swedish clinics. Notably, using this model, 34% of ‘our’ women would not have been eligible if a threshold of 17% lifetime risk had been used. Evaluation of whether BOADICEA is a more appropriate risk assessment method to predict breast cancer in this cohort will be a topic for another report.

The referrals to fine-needle biopsy from US were 77/624 (12.3%) and from CBE 87/627 (13.9). We believe that these considerably high numbers reflect, at least in part,
the limited experience of using these two methods as single screening methods. Berg et al concluded that adding US to MG in a single screening round added sensitivity in terms of breast malignancies being diagnosed, but also substantially increased the number of false positives. The next five rounds of this large, prospective, screening study will show whether this will change over time as the specialists using the two methods get more experienced. The lower number of referrals for cytology from the MG radiologists may be the consequence of their long experience of using MG as a single screening method. Recall rate for MG in this study was 18/ 612 (2.9%) which tallies with rates from the population screening program in Sweden (3%) (SBU2011).

We felt that women who have been alerted to their increased risk of breast cancer could not, for ethical reasons, be denied surveillance. Hence, no reference group was used, which is a limitation of the study. Further, digital technique is more accurate for reading dense breast and breast in women < 50 years of age (Pisano ED, 2005) but at the time of the first screening round, analogue mammographic technique was used – another weakness. During subsequent screening rounds digital technique was being introduced gradually at both MG sites (sites 2 and 3).

In conclusion, high breast density in a population of women with family history of breast and/or ovarian cancer is associated with low BMI and young age but not with increasing familial risk of the disease. The number of detected cancers did not significantly differ from the expected numbers. However, it is too early to draw any conclusion about the sensitivity of the three different test modalities. Ultrasound and clinical breast examination caused substantially more work-up than MG. In addition, the size of the study population and the thorough risk categorization, in combination with the blinded procedure, will enable us to explore strengths and limitations of the breast examination modalities investigated.
5 CONCLUSIONS AND CLINICAL IMPLICATIONS

PAPER 1
We suggest that endometrial carcinoma and breast cancer could constitute a new breast cancer syndrome. 
Further investigation is warranted to categorize phenotypes of both breast and endometrial tumours in this subgroup.

PAPER 2
SES
Women attending public oncogenetic counselling because of breast cancer in one or several close relatives are more often living with spouses/partners, have higher education level and higher household income than age-matched women from the same catchment area.

This indicates a higher awareness of, and/or availability to, oncogenetic counselling among women of high socioeconomic status (SES) than among those with low SES. Efforts should now be made to facilitate for women of lower SES and with increased risk of breast cancer to be considered for counselling and, if indicated, subsequent intensive breast cancer screening.

HRQL
Women attending oncogenetic counselling because of breast cancer in one or several close relatives exhibit lower levels of HRQL for subscales related to mental health and for general health but have levels equal to normative data for all other items that evaluate physical health.

If a woman experiences a moderate, high or very high risk of breast cancer, this does not affect how she answers questions reflecting her mental and general health. These findings require further study to elucidate how information about increased risk of breast cancer affects women over time.

PAPER 3
High breast density in a population of women with family history of breast and/or ovarian cancer is associated with low BMI and young age.

High density is not associated with increasing risk of breast cancer.

The number of detected cancers did not significantly differ from the expected numbers. However, it is too early to draw any conclusion about the sensitivity of the three different test modalities. The next five screening rounds will, thanks to the thorough risk categorization in combination with the blinded procedure and the size of the study population, enable us to explore strengths and limitations of the breast examination modalities investigated in women at familial risk of breast cancer.
6 ACKNOWLEDGEMENTS

This has been a long journey. I want to express my deepest gratitude to all of you who in one way or another have been my companions during this trip:

**Tommy Fornander**, my main supervisor, for sharing your knowledge and your practical wisdom. You have numerous times shed new light upon seemingly unsolvable problems, both in the daily clinic and in breast cancer research, helping me to see the ‘obvious’ solutions;

**Annika Lindblom**, my supervisor, for not giving up on me. You were the one who got me hooked on breast cancer oncogenetics. You were there from the start and I always felt your support;

**Yvonne Brandberg**, my supervisor, for sharing your deep knowledge of the surveillance of women with an increased risk of breast cancer, of quality-of-life and other questionnaires, and for being encouraging and helpful in my work. You have also been invaluable as a colleague in the ABC-team;

**Annelie Liljegren** and **Brita Arver** my co-workers, colleagues, supervisors and friends for good work in late evenings, on weekdays, on Sundays and whenever. Together we form the famous ABA-group!

**All co-authors** in all three articles produced, thank you for contributing so devotedly.

**Mariann Iiristo**, for being such a good head of the breast section at the Department of oncology. Your wonderful flair for organization makes daily work at outpatients’ a lot more than just a job;

**Roger Henriksson**, head of the Department of oncology, Karolinska University Hospital, for having the courage to be our boss

**Lars-Erik Rutqvist**, my former chief at the Department of Oncology, Södersjukhuset for good advice and encouraging support early in my career;

**Ulla Glas**, my first chief at the Department of Oncology, Södersjukhuset for employing me in the first place, thus letting me loose in the field of oncology;

**Ulla Goldman**, my ‘office-mate’, for endless talks without either of us listening, yet always paying attention when the topic gets serious and requires an answer;

**Sara Margolin**, my colleague, both in the broader field of breast cancer oncology and in breast cancer oncogenetics, for being such an enthusiastic and wise doctor;

**Gerard Winblad**, my colleague for so many years, for being such a warm person and empathic doctor;

**Reza Khoshnoud, Ingveldur Björnsdottir, Linda Thorén, Jenny Lundin** my dear colleagues in the breast team at the out-patient clinic with whom I share so many good memories of working in breast cancer and so much laughter. Not forgetting **Asgerdur Sverrisdottir**, my former colleague and present PhD student of Tommy’s.

**All dear colleagues** in the breast team north of Slussen;

**Agneta Holm**, my best laughter-friend ever, for always listening and for sharing so many thoughts about life;

**All other dear colleagues, new and old**, at the Department of Oncology, site Södersjukhuset;

**Aina Johnsson**, welfare officer, of extremely great knowledge and courage, for invaluable help over hassles with sick leave;
Kersti Hjukström, with whom I have shared so many hurried talks, reflections and worries about all “our” mutual patients. Thank you for being such a talented nurse; Lotta Bodell, for being such a caring nurse for all your patients and for the doctors as well; Charlotte, Helene, Katja, Maria N, Maria Ö, Marika, Rauni, Sanna, Tina for invaluable help in the daily care of out-patient breast cancer patients at Södersjukhuset All present and former nurses at the Oncology Department at Södersjukhuset/Karolinska for good cooperation in the care of breast cancer patients; Gunilla, Eva, Lisa, Viveka, Eva and all other dear friends, all of you number one! Sophia for bringing me up right from childhood; and for staying a close friend and dear sister; Ebba my sister for being such a big personality; and for letting me know that I’m always welcome in your and Torleif’s wonderful home at Skeppsta; David my little brother who in a time of desperation became my big brother, with whom I talk about life; and to Paula, my dear sister-in-law, and Emelie and Carl, my lovely nephews; Bibbi my vivid mother who never gives up on life and always sees the glimmering star and the sun even when skies are cloudy; and to my father Göran who never stopped showing me so much love. Mats my former husband for sharing so many years with me and for being such a wonderful father to our daughters; Stina and Clara my babies who have grown up into lovely, wise, smart and beloved daughters.
7 REFERENCES


Azavedo E, Svane G, Auer G ”Stereotactic fine-needle biopsy in 2594 mammographically detected non-palpable lesions.” The Lancet 1989 May 13;1033-1036


SBU. "Datorassisterad granskning inom mammografiscreening (CAD)." sbu alert, 2011.


