The Impact of Pregnancy on Breast Cancer Survival in Women Who Carry a $BRCA1$ or $BRCA2$ Mutation

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science

Institute of Medical Sciences
University of Toronto

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2013

Abstract

Background: Young BRCA mutation carriers with a history of breast cancer often inquire about the impact of pregnancy upon their risks of cancer recurrence and survival.

Methods: We identified 128 BRCA carriers who were diagnosed with breast cancer while pregnant or who became pregnant after breast cancer diagnosis. Women were matched to 269 controls. Women were followed from the date of breast cancer diagnosis until the date of death. The Kaplan-Meier method and a left-truncated Cox proportional hazard model were used to estimate 15-year survival rates.

Results: The adjusted hazard ratio associated with 15-year survival for women diagnosed with breast cancer who were or became pregnant after breast cancer diagnosis, compared to women who did not become pregnant was 0.76 (95% CI 0.31 to 1.91 p = 0.56).

Conclusion: Pregnancy concurrent with or after a diagnosis of breast cancer does not appear to adversely affect survival among BRCA1/2 mutation carriers.
Dedication

I want to dedicate this work to my husband Daniel and my three children Pablo, Andreina and Rafael, for their constant support and encouragement to strive for success.
Acknowledgments

The successful completion of my Thesis would have not been possible without the support of:

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<tr>
<td>PABC</td>
<td>pregnancy-associated breast cancer</td>
</tr>
<tr>
<td>PFBC</td>
<td>pregnancy following breast cancer</td>
</tr>
<tr>
<td>BRCA</td>
<td>Breast Cancer Gene</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Breast Cancer Gene One</td>
</tr>
<tr>
<td>BRCA2</td>
<td>Breast Cancer Gene Two</td>
</tr>
</tbody>
</table>
Glossary of Terms

**Pregnancy-associated breast cancer (PABC):** is defined when breast cancer is diagnosed during a pregnancy or within the first year post-partum.

**Pregnancy following breast cancer (PFBC):** is defined when a pregnancy occurs one year after breast cancer diagnosis or after.

**BRCA1 and BRCA2:** Genes that help to repair DNA and help to control cell growth. A harmful change in either of these genes means a person has Hereditary Breast and Ovarian Cancer syndrome, which causes a significantly increased risk for breast and ovarian cancer.

**Hereditary Breast and Ovarian Cancer Syndrome (HBOC Syndrome):** an inherited condition that raises the risk of developing breast and ovarian cancer associated with mutations on the BRCA1 and BRCA2 genes.

**Germline Mutation:** a gene change in reproductive cells (egg or sperm) that become incorporated into the DNA of every cell in the body of offspring; hereditary mutations are passed on from parents to offspring.

**BRCA-associated breast cancers:** refers to the histological and pathological characteristics of this particular breast cancer type. BRCA-associated cancers are commonly of the basal type (ER-PR- Her2-, EGFR + or CK5/6+).

**Pregnancy-associated breast cancers:** refers to the histological and pathological characteristics of this particular breast cancer type. Pregnancy-associated breast cancers are more likely to be large tumors, lymph nodes positive, high histological grade, and hormone receptor negative.

**Penetrance:** in genetics is the proportion of individuals carrying a particular variant of a gene (allele or genotype) that also express an associated trait (phenotype). In medical genetics, the penetrance of a disease-causing mutation is the proportion of individuals with the mutation who exhibit clinical symptoms. For example, if a mutation in the gene responsible for a particular autosomal dominant disorder has 95% penetrance, then 95% of those with the mutation will develop the disease, while 5% will not.
Chapter 1
1 Introduction and Thesis Overview

1.1 Introduction

Breast cancer is the most common cancer among women worldwide (1). Although the peak age of breast cancer diagnosis is 61 years of age, approximately 25% of breast cancers present in women during their reproductive life (2). The current tendency of women to delay childbearing until their thirties and forties increases the likelihood of being diagnosed with breast cancer during a pregnancy or becoming pregnant after a diagnosis of breast cancer. Today, the diagnosis of breast cancer during a pregnancy or within one year postpartum is estimated to be between one in 3,000 to one in 10,000 pregnancies (3–9).

The impact of pregnancy on breast cancer prognosis varies, depending upon if it occurs prior to, during or after a diagnosis of breast cancer (10,11). Recent literature on pregnancy and breast cancer are using consistent definitions. If breast cancer is diagnosed during a pregnancy or within the first year post-partum it is defined as a pregnancy-associated breast cancer (PABC) (12,13) and if a pregnancy occurs one year after breast cancer diagnosis or thereafter, it is defined as a pregnancy following breast cancer (PFBC) (14).

Pregnancy-associated breast cancer and pregnancy following breast cancer are more likely to occur in women who develop breast cancer at a young age, such those who carry a mutation in either of the breast cancer susceptibility genes, BRCA1 and BRCA2. Women who carry a mutation in the BRCA1 or BRCA2 genes have a lifetime risk of developing breast cancer of 80% (15,16).
Clinicians are often approached by BRCA mutation carriers with a personal history of breast cancer who wish to know what impact a pregnancy may have on their health. Because BRCA carriers are recommended to undertake risk reduction surgeries (prophylactic mastectomy or oophorectomy) and chemoprevention to reduce their breast and ovarian cancer risk, discussing issues related to fertility and family planning is of paramount importance. Among their concerns when they consider having a baby are the risk of having a recurrence and psychosocial issues such as leaving a child without a parent and passing the BRCA mutation to their offspring (17).

In this thesis we are going to focus only on risk of recurrence and survival.

The process of understanding the impact of pregnancy-associated breast cancer and pregnancy-following breast cancer on breast cancer recurrence and survival in BRCA1 and BRCA2 mutation carriers has been hampered by the paucity of data. To our knowledge, there is only one study that examined the influence of pregnancy and the risk of developing breast cancer in carriers. In this study the number of patients was small and not matched. This study reported that BRCA1 carriers had a significantly higher likelihood of having a pregnancy-associated breast cancer than BRCA2 carriers [BRCA1 HR = 3.9 (95% CI = 1.4–10) vs. BRCA2 HR = 1.9 (95% CI = 0.5–7.0)] and recommended that women who carry a BRCA1 mutation should be monitored closely during and after pregnancy for breast cancer (18).

The goal of this thesis is to examine the influence of the surge of gestational hormones of women who carry a BRCA1/2 mutation diagnosed with breast cancer at the time of a pregnancy or who become pregnant after breast cancer diagnosis on survival and disease-free survival. The information obtained from this study will help assist physicians in clinical decision making and will allow BRCA mutation carriers to make informed decisions when planning their families.
1.2 Thesis Overview

This thesis is comprised of five chapters. The first chapter includes the introduction and thesis overview. The second chapter is the background. It summarizes relevant issues of the epidemiology of BRCA1 and BRCA2 associated breast cancer. It explores various factors involved in the development of mammary glands in BRCA mutation carriers and their influence on the risk of breast cancer in this population. It discusses some aspects of breast cancer of young premenopausal women and it discusses the literature on pregnancy-associated breast cancer and pregnancy-following breast cancer.

Chapter three includes the objectives, the hypotheses, methods and the conceptual framework for the research study. Chapter four presents the result of the survival analysis and multivariate analysis. Chapter five includes the discussion, highlights the limitations of the study, conclusions and future directions.
Chapter 2
2 Background and Literature Review

2.1 Hereditary Breast Cancer

Hereditary breast cancer represents about 5 to 10% of all breast cancers. Women who carry a germline mutation in either of the \textit{BRCA1} or \textit{BRCA2} gene face a lifetime risk of breast cancer of 70-80\% (15). Once diagnosed with breast cancer, their risk of a second primary breast cancer or ovarian cancer is very high (2\% per year) in carriers (19–24) compared to non-carriers (0.5\% per year) (25,26).

2.2 The \textit{BRCA} Mutation and Breast Cancer

In 1994 and 1995, the discovery of the breast cancer one and two (\textit{BRCA1} or \textit{BRCA2}) genes started a new phase in the understanding of genetic epidemiology of breast cancer (21,27–29). Genetic testing became available in developed countries to screen women with a strong family history of breast and ovarian cancer. The detection of a \textit{BRCA} mutation in high-risk women raised awareness of breast cancer risk and helped physicians to formulate breast cancer-risk reduction strategies in this population. The lifetime risks of developing breast cancer in \textit{BRCA1} and \textit{BRCA2} mutation carriers are similar (70\%); however, women with a \textit{BRCA1} mutation have a greater risk of ovarian cancer (40\% vs. 20\%) (20).
*BRCA1* and *BRCA2* are tumor suppressor genes involved in the process of DNA damage repair and cell cycle control (29). These genes are located on chromosomes 17 and 13, respectively. The *BRCA1* gene interacts with several proteins involved in various cellular pathways, including cell cycle progression, gene transcription regulation, DNA damage response, apoptosis and ubiquitylation (30–32). The *BRCA2* gene is also involved in the DNA repair pathways that involve homologous recombination, chromatin remodeling and cell cycle checkpoint control (29).

*BRCA1* and *BRCA2* mutations are inherited in an autosomal dominant fashion. Recurrent mutations in the *BRCA1* and the *BRCA2* genes have been found in some populations and it might reflect the geographic isolation of these areas and/or the inbreeding among its habitants (33). For example, in the *BRCA* carriers of Ashkenazi Jewish descent, three founder mutations have been found, two in the *BRCA1* gene (185delAG, 5382insC) and one in the *BRCA2* gene (6174delT) (34,35). Similarly, recurrent founder mutations have been found in the French Canadian population [*BRCA1* (C4446T) and *BRCA2* (8765delAG)] (33), the Polish population [*BRCA1* (5382insC, C61G, and 4153delA)] (36), and more recently in the Bahamas [*BRCA1* (T5443G, 4730insG, IVS13+1G>A, 943ins10 and 185delAG)] (37). Founder mutations have been seen in several island populations, such as Iceland (*BRCA2* 995del5) (38,39), Greenland (*BRCA1* p.Cys39Gly)(40), and Cyprus [*BRCA1* c.1840A>T (K614X), c.5310delG (5429delG)] [*BRCA2* c.3531-3534delCAGC (3758del4), c.8755delG (8984delG)] (41).

In the cross-sectional study of 237 families with at least four cases of breast cancer, the Breast Cancer Linkage Consortium found the prevalence of mutations of 52% in the *BRCA1* gene, 32%
in the \textit{BRCA2} gene (19). The frequency of \textit{BRCA1} mutations was higher (81\%) if the women had a family history of both breast and ovarian cancers.

\subsection*{2.3 Mammary Gland Development in \textit{BRCA} Mutation Carriers}

The mammary lobules in the breast tissue of women who carry a \textit{BRCA1} or a \textit{BRCA2} mutation depict a distorted branching architectural pattern unlike the breast tissue of non-\textit{BRCA} mutation carriers. The mammary lobules are of three subtypes based on the developmental pattern: 1) L1 or type 1 lobules, 2) L2 or type 2 lobules, and 3) L3 or type 3 lobules. The L1 or type 1 lobules are less developed while the L3 or type 3 lobules are the most differentiated lobules and possess the highest number of ducts per lobular unit. It is believed that the L1 lobules are the most predominant type in young nulliparous women as well as in women who carry a \textit{BRCA} mutation and are the site of origin of ductal carcinomas (42).

Both \textit{BRCA1} and the \textit{BRCA2} genes are involved in proliferation and differentiation of the mammary gland (42). This has been demonstrated by the up-regulation of \textit{BRCA1} and the \textit{BRCA2} mRNA expression during rapid proliferation and differentiation of breast epithelial cells through breast development in young women and also during different stages of pregnancy (43). It has been suggested that the increased expression of \textit{BRCA1} and \textit{BRCA2} mRNA during breast development in puberty and pregnancy may be associated with an increased risk of breast cancer at an early age and during pregnancy in women with a strong family history of breast cancer (44).
2.4 Reproductive Factors and the Risk of Breast Cancer

Both local and circulating hormones influence breast cancer risk and progression. Hormones may also explain the effects of early menarche, late menopause, parity, and breastfeeding on breast cancer risk (29,42,45) Other reproductive factors such as age at the first childbirth, use of oral contraceptive or hormone replacement therapy also play an important role on breast cancer risk. In the general population, young age at first birth appears to decrease breast cancer risk (46,47). In contrast, BRCA1 carriers, having children early does not appear to reduce their breast cancer risk substantially, and in BRCA2 carriers, pregnancy may increase the risk (48–50). One study reported that women with a BRCA1 mutation who had four or more children had a 38% decrease in breast cancer risk when compared to nulliparous BRCA1 women; whereas among BRCA2 carriers, women with two or more children were at approximately 1.5 times the risk of breast cancer as nulliparous BRCA2 women (51). A summary of the breast cancer-risks and reproductive factors is presented in Table 2.1.

Table 2.1 Breast Cancer Risk and Reproductive Factors in Carriers and Non-Carriers

<table>
<thead>
<tr>
<th></th>
<th>Early Menarche</th>
<th>Late Menopause</th>
<th>Multi-parity</th>
<th>Early Age at the first Childbirth</th>
<th>Breast-feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1 carriers</td>
<td>≠</td>
<td>≠</td>
<td>↓</td>
<td>≠</td>
<td>↓</td>
</tr>
<tr>
<td>BRCA2 carriers</td>
<td>≠</td>
<td>≠</td>
<td>↑</td>
<td>≠</td>
<td>≠</td>
</tr>
<tr>
<td>Non-carriers</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
</tbody>
</table>

≠ no effect; ↑ increase in cancer risk; ↓ decrease in cancer risk
Lambe et al. reported that pregnancy has a dual effect on the risk of breast cancer: it transiently increases the risk after childbirth but reduces the risk thereafter (52). Deleterious effects of pregnancy are associated with the rise of gestational hormones that leads to breast cells hyperplasia and hypertrophy (52,53). The protective effects are probably due to the differentiation of the remaining stem cells of the breast (which are the cells more vulnerable to carcinogenesis), into a final developed gland (42).

The effect of breast-feeding may vary between carriers and non-carriers. High prolactin levels have been associated with increased breast cancer risk in non-carriers. Studies in the BRCA population have demonstrated that breastfeeding has a protective effect against breast cancer in BRCA1 carriers (for each month of breast-feeding, OR = 0.98, 95% CI = 0.97 to 0.99; P < .001). Women with BRCA1 mutations who breast-fed for more than 1 year were less likely to have breast cancer than those who never breast-fed (OR = 0.55, 95% CI = 0.38 to 0.80; P < .001), but not association was found in BRCA2 carriers (OR = 0.95, 95% CI = 0.56 to 1.59; P < .83) (50,54).

2.5 Pathological features of BRCA-associated Breast Cancers

Based on immunohistochemistry, there are several breast cancers subtypes with each distinct biological phenotypes and clinical implications. Normal-like breast cancer is associated with no expression of immunostaining (ER-/PR-/Her2-/EGFR-). Luminal A is characterized for having ER and PR positive and Her2 negative, luminal B have ER/PR/Her2 positive. Basal-like is characterized for not expressing any receptor (ER-/PR-/Her2-) also known as “triple negative”
and also associated with the expression of cytokeratins 5/6 and epidermal growth factor receptor (EGFR)(55–61).

Women with BRCA mutations are more likely to present with early-onset breast cancer and to develop bilateral breast cancer than non-carriers (21,62). The breast cancers in BRCA1 carriers are more likely to be invasive ductal carcinomas, hormone receptor-negative (ER-negative, PR-negative, and Her2 negative), have a high histological grade, and have P53 mutations than non-hereditary breast cancers (7,63–65). Triple-negative breast cancers, those that express epithelial keratins ck5 or ck6 or EGFR, also known as ‘basal’ phenotype, are seen more often in BRCA1-associated cancers (65). BRCA2 histological characteristics are very similar to those found in sporadic breast cancers; therefore BRCA2 carriers have lesser adverse pathological features than BRCA1 mutation carriers (91).

2.6 Management of Breast Cancer Risk in Carriers

Breast cancer risk management for women with a BRCA mutation consists of breast screening (66,67)(clinical breast exam, mammography and magnetic resonance imaging (MRI)), chemoprevention with tamoxifen, and risk reduction surgeries, which include prophylactic bilateral mastectomy and bilateral salpingo-oophorectomy (68).

The Ontario Government in 2011 approved funding to expand the Ontario Breast Screening Program (OBSP) to screen women at high risk for breast cancer. Cancer Care Ontario expect that the introduction of combined mammography and MRI screening into the OBSP for women aged
30 to 69 who are at high risk for breast cancer will improve their quality of care, ensuring that they receive the benefits of screening and promoting the early detection of breast cancer (69,70). Chemoprevention with tamoxifen in women with a BRCA mutation has shown to reduce the risk of contralateral breast cancer by 50% (71) as well as the risk of primary breast cancer (72).

Bilateral prophylactic radical mastectomy is the most effective preventive strategy to date for women with a BRCA mutation, reducing the risk of breast cancer by more than 90% (73,74).

Management options for women at high risk for ovarian and fallopian tube cancer consist of screening with transvaginal ultrasound and CA125 blood test, chemoprevention through the use of oral contraceptives, and surgical reduction of risk through tubal ligation or prophylactic salpingo-oophorectomy (75).

Prophylactic salpingo-oophorectomy has been shown to reduce not only the risk of ovarian and fallopian tube cancer by 75-96% (76–80), but also reduces the risk of breast cancer by up to 50% if performed prior to menopause.

2.7 Breast Cancer in Young Women

Approximately 25% of breast cancers are diagnosed prior to menopause (2). Definitions of breast cancer in young women vary in the literature. Some define it as breast cancers diagnosed before 35 years of age, others subdivided into those breast cancers diagnosed before 40 and those diagnosed between 40 and 50 years.
Despite the lack of consensus on its terminology, the critical relevance of young women affected with breast cancer lies in the high risk of dying compared to their post-menopausal counterparts even if diagnosed at an early stage and receiving intense treatment (81). Baharat, A et al reported that women 40 years of age or younger are 52% more likely to die from breast cancer than women older than 40 years of age (59).

Breast cancer in young women also has a higher incidence of contralateral breast cancer; higher proportion of estrogen and/or progesterone receptor-negative, Her-2 receptor-positive and TP53 mutations (82,83). All of these features are related to an aggressive course.

2.8 Pregnancy and Breast Cancer

2.8.1 Epidemiology

In 2009, the mean age of women at first birth varied significantly across countries from 21.3 years of age in Mexico to 30.5 years in New Zealand (OECD)(84). In North America it has increased from 24.9 years in 2000 (85) to 27.8 years in 2009 (OECD)(84). Birth rates for women from 20 through 39 years declined, but the rate for women 40 to 44 years continued to rise. —The birth rate for women aged 40 to 44 rose 3% from 9.8 in 2008 to 10.1 live births per 1,000 women in 2009 —the highest rate reported since 1967 (NVSS) (86)(Figure 2-1).
Approximately 40% of breast cancers in \textit{BRCA} mutation carriers are diagnosed before age 40 and the majority of breast cancers are diagnosed when the woman is still fertile (i.e., prior to menopause). The peak incidence of breast cancer in \textit{BRCA1} carriers is estimated to be at age of 40 (62), and in \textit{BRCA2} carriers at age of 48 (19). In contrast, the mean age of diagnosis of breast cancer in the general population in women in North America is around 61 years of age (1).

Based on an analysis of our cohort study of \textit{Risk Factor Analysis of Breast and Ovarian Cancer in Women Carriers of BRCA1 and BRCA2} which is the parent cohort study from which the patients for the current study were drawn, we found that over 50% of breast cancers in carriers are diagnosed in women in their thirties or forties. There is a large degree of overlap between the
ages of cancer diagnosis and the ages of birth in BRCA mutation carriers participating in this cohort (Figure 2-2). The early age of breast cancer diagnosis amongst most hereditary kindreds and the increasing frequency of childbearing delay observed in contemporary women make pregnancy-associated or pregnancy following breast cancer a particularly relevant clinical dilemma for this patient population. The characteristics of the parent cohort study are presented in section 3.52).

Figure 2-2 Age Distribution for Birth and Breast Cancer in Women who carry a BRCA1 or a BRCA2 mutation
2.8.2 Timing of Pregnancy and Breast Cancer

The impact of a pregnancy diagnosed at the time of breast cancer should be distinguished from a pregnancy that occurs after breast cancer diagnosis. For non-carriers, the longer the length between breast cancer diagnosis and pregnancy, the better the prognosis (10,11). The former - pregnancy-associated breast cancer - involves the surge of gestational and lactating hormones, which accelerate proliferation, and spreading of breast cancer cells whereas a pregnancy following breast cancer involves the potential re-activation of tumour cells. Furthermore, a pregnancy-associated breast cancer is probably due to a chance while a pregnancy following breast cancer is more likely to be planned. Therefore, in this thesis I will refer to them as a two separate entities.

2.8.2.1 Pregnancy-associated Breast Cancer

Breast cancer diagnosed during a pregnancy or within one year after childbirth is defined as pregnancy-associated breast cancer. Overall, the incidence of pregnancy-associated breast cancer is estimated to be one in 3,000 to one in 10,000 pregnancies (3–9). However, this incidence is expected to increase due to the nowadays tendency of women to delay motherhood, especially women from the Western world. In Sweden, 1,161 cases of pregnancy-associated breast cancer were identified among 16,620 women diagnosed with breast cancers from 1963 to 2002. In this study, the incidence of pregnancy-associated breast cancer increased from 16.0 to 37.4 per 100,000 deliveries (9). This incidence is similar to that reported in a review conducted at the
Memorial Sloan-Kettering Cancer Center in the United States in 2000 where the overall incidence of pregnancy-associated breast cancer was 10 to 39 per 100,000 deliveries (13).

Breast tissue during pregnancy undergoes cell hypertrophy and differentiation, augmentation of the lymphatic and blood vessel supplies. Progesterone increases more than 1000-fold, estrogens increase more than 100-fold, corticosteroids increase between two- and threefold, and insulin and prolactin are also significantly elevated compared with the non-pregnant levels (53). Breast tumors diagnosed during pregnancy are larger in size and therefore, more likely to present with increased axillary lymph node involvement and lympho-vascular invasion. High-grade invasive ductal carcinoma is the most frequent histological type of breast tumor in pregnant women. These tumors are often hormonal receptor (estrogen receptor and progesterone receptor) negative and Her2 receptor positive (82,83). The similarities in clinical pathological features of pregnancy-associated breast cancer, BRCA-associated breast cancer and the breast cancer in young women make it difficult to distinguish between these three entities on the basis of pathology and immunohistochemistry (Table 2-2).

<table>
<thead>
<tr>
<th>Pathological Characteristics</th>
<th>BRCA-associated Breast Cancers</th>
<th>PABC-associated Breast Cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Size</td>
<td>≠</td>
<td>Large</td>
</tr>
<tr>
<td>Histological Grade</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Axillary Lymph Nodes</td>
<td>Often involved</td>
<td>Often involved</td>
</tr>
<tr>
<td>Histological Type</td>
<td>Ductal</td>
<td>Ductal</td>
</tr>
<tr>
<td>Estrogen Receptor</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Progesterone Receptor</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Her2</td>
<td>Non-expressed</td>
<td>Expressed</td>
</tr>
<tr>
<td>------</td>
<td>---------------</td>
<td>-----------</td>
</tr>
<tr>
<td>EGFR</td>
<td>Positive</td>
<td>≠</td>
</tr>
<tr>
<td>Lympho-vascular invasion</td>
<td>Often present</td>
<td>Often present</td>
</tr>
<tr>
<td>Ki67</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>ck5, ck6</td>
<td>Mutated</td>
<td>≠</td>
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The most common clinical presentation of breast cancer during pregnancy is a palpable mass (87,88). Ultrasound of breast is the initial imaging test of choice for the assessment of palpable mass in pregnant and lactating women (89,90). The breast ultrasound is usually complemented with mammogram to accurately detect microcalcifications otherwise hardly visible by breast ultrasound alone. The sensitivity and specificity of combined use of breast ultrasound and mammogram is fairly high during pregnancy (negative predictive value of 100%) (90). Robbins and colleagues reviewed the imaging reports of women with pregnancy-associated breast cancer. A total of 164 abnormalities were detected among 155 women. Of these lesions, 12 were evaluated by mammography alone, 49 were evaluated by ultrasound alone, and 73 were evaluated by both techniques. Of those 85 abnormalities evaluated with mammogram the sensitivity was 100%, specificity was 93%, positive predictive value was 40% and, negative predictive value was 100%, while of the 122 abnormalities evaluated by ultrasound the sensitivity was 100%, specificity was 86%, positive predictive value was 19% and, negative predictive value was 100% (90).

The use of magnetic resonance imaging (MRI) to diagnose a breast mass during pregnancy or lactation is still in debate and is not routinely recommended (91). Gadolinium crosses the
placenta. Therefore, contrast enhanced magnetic resonance imaging with gadolinium has not been approved to evaluate breast mass during pregnancy (92).

### 2.8.2.2 Pregnancy Following Breast Cancer

Pregnancy following breast cancer is defined as a pregnancy that occurs one year after breast cancer diagnosis or thereafter. The majority of research studies about pregnancies following breast cancer treatment report better survival and prolonged disease free survival (30,58,93–99). There are several possible explanations for these outcomes. First, they may have had less aggressive cancers that did not require chemotherapy; second, healthy women with no recurrence, or with low-risk cancers are more likely to attempt to become pregnant, “healthy mother effect” (14); and third, in multiparous women, fetal antigens and a fully developed mammary gland occasioned from previous pregnancies might confer a protective immunization effect and a less vulnerable breast tissue for carcinogenesis (42,100).

Fortuny et al studied the opinion of *BRCA1/2* carriers regarding childbearing in 77 women who underwent genetic testing. Seventy percent of them had personal history of cancer and 57% had a university degree. They found that 36% will not have children regardless of their mutation status, 12% will not have children if they tested positive, 55% will consider prenatal diagnosis, 48% will consider pre-implantation genetic diagnosis and 30% will consider adoption (17). We can infer that knowing they carry a *BRCA1/2* mutation, significantly affects women’s decision on family planning.
2.8.3 **Management and treatment**

The management of pregnancy-associated breast cancer is influenced by clinical, ethical, emotional and cultural considerations. To maximize care, a multidisciplinary approach is optimal whereby the obstetrician, medical oncologist, radiation oncologist, surgical oncologist, genetic counselor and the patient are involved. The goal of the management is to provide the best care to the mother and deliver a healthy baby.

A histological evaluation of breast tissue is warranted to confirm the diagnosis of breast cancer if a palpable focal mass is not detected with either breast ultrasound or mammogram. Breast tissue can be obtained by two techniques: 1) fine needle aspiration biopsy and, 2) core biopsy. The fine needle aspiration is a technique operator-dependent and thus, inadequate samples and inability to distinguish between invasive and non-invasive breast cancers are major disadvantages (sensitivity = 98%, specificity = 97%, false-negative rate = 0% to 32%) (101) (102). Breast tissue during pregnancy and lactation results in cellular changes that lead to false-positive or false-negative result with fine needle aspiration (103).

A more definitive means of obtaining breast tissue is to perform a ultrasound guided or stereotactic core biopsy under local anesthesia (104). The sensitivity of core biopsy has been reported around 90% (105). The core biopsy can be safely performed during pregnancy. Complications of core biopsy are rare and include milk fistula, abscess and mastitis.

Current recommendations for the management and treatment of pregnancy-associated breast cancer from the American Society of Cancer Oncology (ASCO) (83,106) as well as from the European Society for Medical Oncology (ESMO) (107) are consistent.
In most cases, the treatment of pregnancy-associated breast cancer does not differ from the treatment of breast cancer in non-pregnant women. Studies suggest that stage I to III breast cancer can be treated with surgery and chemotherapy during the second and third trimester of pregnancy without compromising the life of fetus (92,108,109). Cyclophosphamide, methotrexate and fluorouracil (CMF), and cyclophosphamide, adriamycin and fluorouracil (CAF) are most common chemotherapeutic regimens used to treat pregnancy-associated breast cancer (92). Hahn and colleagues in a prospective cohort of 57 pregnant women who were treated with 5-fluorouracil, doxorubicin and cyclophosphamide (FAC) during the second and third trimesters reported few short-term complications for the majority of children exposed to chemotherapy in utero (4,110). Taxanes have been described in literature in around 30 pregnant breast cancer patients (111–113). There is no evidence that they increase the risk of pregnancy complication. Thus, they remain the second best option in case anthracyclines are contraindicated for any reason. Pant et al. studied 15 pregnant women with breast cancers that expressed Her2 were treated with trastuzumab and found that 50% of the treated women developed oligohydramnios (114). Trastuzumab interferes with the production of amniotic fluid leading to a severe oligohydramnios and thus it should be avoided to treat pregnant women with breast cancer. If trastuzumab needs to be given, it is advisable to inform the mother about the risks and restrict the treatment to just one trimester. Hormonal therapy with tamoxifen is contraindicated during any trimester of pregnancy due to a considerable risk of fetal congenital anomalies (115). Total mastectomy is the preferred surgical approach during pregnancy because breast-conserving surgery (lumpectomy) requires post-surgical radiotherapy, which is not feasible in pregnant women.
The clinical management of a pregnancy following breast cancer does not differ from women who have never had cancer. There is no evidence of differences in pregnancy outcomes or perinatal complications in breast cancer survivors with a pregnancy following breast cancer treatment nor contraindication to breastfeeding (49,116).

The Health Canada's Steering Committee recommendations for women considering pregnancy following a diagnosis of breast cancer include informing women that despite the limited data on the effect of pregnancy on outcomes such as breast cancer recurrence and survival, most of the studies have shown no evidence that subsequent pregnancy adversely affects survival (66,67).

The safe timing of a pregnancy will arise when discussing subsequent pregnancy in a breast cancer survivor. The fact that the impact of a subsequent pregnancy on breast cancer survival is uncertain partly explains why most of these women decide not to take the risk.

The Society of Obstetricians and Gynaecologist of Canada (SCOG) guidelines recommends postponing pregnancy for three years after breast cancer diagnosis but if lymph nodes are involved, is prudent to extend this period to five years (III-C level of evidence) (117). Azim et al, after a sensitivity analysis, reported that pregnancy two years after breast cancer diagnosis conferred a high mortality reduction with no evidence of heterogeneity compared to women with personal history of breast cancer and no subsequent pregnancy (pooled RR:0.55; p for heterogeneity= 0.16)(118). Azim et al, in a subsequent study examined disease free survival of women with a pregnancy following breast cancer based on estrogen receptor (ER) status. He found that the disease free survival of women with ER-positive or ER-negative breast cancers and a subsequent pregnancy did not differ when compared with non-pregnant women (ER
positive women HR = 0.91; 95% CI = 0.67-1.24; P = .55) (ER negative women HR= 0.75; 95% CI = 0.51-1.08; P = .12). Furthermore, women with a pregnancy associated breast cancer have a better overall survival than non-pregnant women (HR = 0.72; 95% CI = 0.54-0.97; P = .03), independently of ER status (P = .11) (58).

Saphner et al studied the long-term risk of recurrence for breast cancer (119). In this study, the peak hazard of recurrence occurred in the first two years irrespective of the lymph node status (-/+), decreases in the interval of two to five years but beyond five years, decreases slowly through to year 12. The average hazard of recurrence between years five and 12 in was 4.3% per year. Patterns of recurrence of breast cancer also vary according to hormonal receptor status (119,120). In a recent study, Lin et al. (120) examined 15,204 women who presented to National Comprehensive Cancer Network centers with stage I/II/ III breast cancers. Seventy percent of women (n = 2569) had triple negative breast cancers, 17% of women (n = 2602) had Her2 positive breast cancers, and 66% had HR positive/Her2 negative (n = 10,033) breast cancers. Compared to HR positive/Her2-negative tumours, triple-negative tumours were associated with a greater risk of brain or lung metastases; and women with triple-negative tumours had worse breast cancer-specific and overall survival, even after adjusting for important co-variables (age, stage, race, grade and, chemotherapy) (adjusted HR = 2.72; 95% CI = 2.39-3.10; P < .0001). The risk of death for HR negative/Her2 negative tumours was higher within the first 2 years after diagnosis than for the other subtypes (overall survival for 0-2 years: OR, 6.10; 95% CI = 4.81-7.74).
The time frame of two years seems reassuring according to these studies results. The higher incidence of tumour recurrence has passed after two years alongside the ovarian function had time to recover from the gonadotoxic effects of chemotherapy (119).

In summary, according to the literature, pregnancy in non-carriers does not carry an adverse outcome if the pregnancy occurs at least two years after the diagnosis. A woman must carefully consider her personal risk of recurrence, based upon her tumor biology, consider her desire for children and discuss fully these issues with her oncologist and obstetrician. Although there are not clear guidelines for women who carry a BRCA1 or a BRCA2 mutation, their management and treatment does not differ from their counterparts.

Yet, whether the two years’ time frame is applicable to women who carry a BRCA1 or BRCA2 mutation is uncertain. Carriers have an increased risk of developing a second primary breast cancer or an ovarian cancer and delays in cancer risk-reducing surgeries may worsen these women’s prognosis. In a recent study from our group, we found that BRCA1 mutation carriers with breast cancer who had a prophylactic oophorectomy experienced a mortality reduction of 70%. This striking result is the result of a dual effect: oophorectomy reduces deaths from ovarian cancer but also reduces deaths from a second primary breast cancer (Metcalfe K, 2013 submitted to JCO).

2.8.4 Tamoxifen and Pregnancy

Tamoxifen is an important agent that decreases the risk of recurrence of cancer in premenopausal and postmenopausal patients with ER positive tumors. Tamoxifen is recommended for a period
of five years and pregnancy is contraindicated during treatment (121). Tamoxifen has known teratogenic risk to a fetus, based on controlled animal studies (category D). The AstraZeneca Safety Database documented 11 newborns with congenital malformations of 44 live human births, which represents a frequency of one malformation for every four live births (122). In contrast, Clark et al reported 85 women who became pregnant while receiving prophylactic tamoxifen as part of a trial in healthy women at high risk for breast cancer and found no fetal abnormalities (123).

According to the American Society of clinical Oncology (ASCO) guidelines, women should be advised not to become pregnant while taking tamoxifen or within two months of discontinuing tamoxifen and should use barrier or non hormonal contraceptive measures if sexually active (124). According to the literature the malformations associated with tamoxifen are as follow:

- Ambiguos genitalia (Tewari K, 1997): The newborn presented with clitoral hypertrophy and urethral opening could not be identified. There was one common perineal opening for urethra and vagina. There were also labioscrotal folds fused. Internally, uterus and ovaries were present (125).

- Goldenhar’s syndrome (Cullins, 1994): right side microtia, preauricular skin tags and hemifacial microsomia. It is thought to be a nonspecific field defect involving development of the first and second branchial arches resulting in abnormalities of the eyes, ears, and vertebrae. Most cases are sporadic. The cause of this syndrome is unknown and likely heterogeneous (126).
- Pierre Robin Sequence (Berger, 2008): this syndrome is defined as the triad of small mandible (micrognathia) glossoptosis and cleft palate. It is believed that tamoxifen affects the early mandible development for an unknown mechanism. This malformations are also seen when fetuses are exposed to isotretinoin, thus is hypothesized that the craniofacial defects are due to distortion of the cranial neural crest cell migration into the first pharyngeal arch (127).

Hormone receptor positive patients treated with tamoxifen have had a 50% reduction in recurrence rate during years 0 to 4 after treatment and 30% during years 5 to 9. Overall the recurrence rate reduction average 39% (RR = 0.61 p = <0.00001) for any recurrence, and the mortality rate reduction average 30% (RR = 0.71 during years 0-4 p = < 0.00001) (128). Two recent randomized clinical trials, aTTom (adjuvant Tamoxifen Treatment offers more) (129) and ATLAS (Adjuvant Tamoxifen Longer Against Shorten) (130), have evaluated continuing treatment with tamoxifen beyond 5 years. Both studies have demonstrated that 10 years of treatment with tamoxifen showed an additional 25% reduction in breast cancer mortality 10 years and beyond compared to 5 years of treatment (28 vs. 32% HR = 0.85 P = .003). In ER negative breast cancers, tamoxifen had little or no effect on breast cancer recurrence or mortality (121).

2.8.5 Chemotherapy effects on fertility

In addition to advancing age, chemotherapy may impair a woman’s reproductive capability. Chemotherapy damages the ovarian tissue and leads to premature ovarian failure and menopause (131–135). The effects of chemotherapy depend on the patient’s age, on the chemotherapy agent
used and on the number of cycles of chemotherapy. Alkylating agents are among the most 
gonadotoxic. Their effects are dose-dependent and include direct destruction of the oocytes, 
depletion of the primordial follicles, damage to the intra-ovarian blood vessels and fibrosis of the 
ovarian cortex (132). Petrek et al found that breast cancer patients younger than 35 recovered 
their menstrual cycle in approximately 85%, women between 35 and 40 years had 45% to 61% 
recovery rate and women over 40 years had a high rate of permanent amenorrhea (136). As a 
result of these effects, many women who wish to have children after a diagnosis of breast cancer, 
particularly if aged 40 years or more, will require fertility treatment, or choose another 
alternative such as preservation of their own eggs, use of donor’s eggs or adoption (137,138).

Another issue for mutation carriers is the lower reserve of primordial follicles in the ovaries of 
women who carry a BRCA1 or a BRCA2 mutation (137,139–141). The data suggests that 
carriers are lower responders to fertility treatment than non-carriers thus; BRCA carriers who opt 
for in-vitro assisted fertilization strategies may have reduced chances to succeed. If carriers have 
a lower reserve of follicles, chemotherapy could deplete the ovaries of follicles and cause 
premature ovarian failure in carriers than in non-carriers (140). However, in a recent study from 
our research group, we found no statistical differences in the age of onset of chemotherapy-
induced amenorrhea among carriers versus non-carriers (Valentini et al, submitted to JCO Jan 
2013). In this study chemotherapy-induced amenorrhea was more likely to occur in women aged 
37 years or older. There is a natural progressive decline in fertility that starts at age 35 and the 
addition of chemotherapy may diminish that further.
2.8.6 **Prognosis**

2.8.6.1 Prognosis of Pregnancy-associated Breast Cancer

*BRCA*-associated breast cancer and pregnancy-associated breast cancer share several adverse pathological features. These pathological features have shown to have prognostic and predictive significance. A prognostic factor correlates with the natural history of the disease while a predictive factor is associated with a tumor response to a given therapy (142). Prognostic and predictive factors associated with poor outcome include increased tumor size, lymph node involvement, high histological grade, hormone receptor negative and Her2 gene overexpressed (142). The most significant prognostic factor in breast cancer is axillary lymph node involvement (143,144). Five-year survival rates varied from 45.5% for tumors equal to or greater than 5 cm with positive nodes to 96.3% for tumors less than 2 cm with no involved nodes (143). Pregnancy-associated breast cancers have a preponderance of large tumors, lymph nodes positive, high histological grade, and hormone receptor negative (145) and *BRCA*-associated cancers are commonly of the basal type (ER- PR- Her2-, EGFR + or CK5/6+) (65). Therefore, the coexistence of adverse pathological features of pregnancy-associated breast cancer and carrying a *BRCA* mutation might affect survival and disease free survival in *BRCA* carriers who become pregnant during or after breast cancer diagnosis.

The recommendations from physicians on gestational breast cancer varied widely in the past. In 1993, Saunders et al, conducted a survey on 15 colleagues including general practitioners, surgeons and obstetricians in the United Kingdom, in regards to how they would manage gestational breast cancer. General practitioners and surgeons but not obstetricians thought that
pregnancy confers a worse prognosis. Views concerning therapeutic abortion varied but most
thought that it was unnecessary. They were also asked if they thought that pregnancy increases
the risk of breast cancer recurrence and 83% of the general practitioner and 45% of the
obstetricians answered that they did not know. When asked what advice they would give to a
patient in regards to a later pregnancy most answered to wait 2 years, although 50% of general
practitioners answered they would seek specialist advice (8). This survey was conducted in 1993
and our understanding of a pregnancy-associated breast cancer has evolved to some extent.

In a case-control study, Zemlickis and colleagues compared 119 cases (women diagnosed breast
cancer during pregnancy or one year after) with 269 controls (non-pregnant women with breast
cancer) matched on prognostic factors. They observed that pregnant women were 2.5 times more
likely to present with metastatic disease than non-pregnant women (95% confidence interval =
1.1 to 5.3, p = 0.02) (146). In another case series, Beadle and colleagues reported on 668 breast
cancers in women aged 35 years or younger. After ten years, the women with pregnancy-
associated breast cancer had similar rates of loco-regional recurrence (23.4% vs. 19.2%, p =
0.47), distant metastasis (45.1% vs. 38.9%, p = 0.40) and overall survival (64.6% vs. 68.8%, p =
0.07) as to their non-pregnant counterparts (147).

Azim and colleagues reported on 65 pregnant women with breast cancer who were compared
with non-pregnant women with breast cancer and were matched for age, year of surgery, stage
and chemotherapy. They found a significantly inferior disease-free survival (hazard ratio 2.3,
confidence interval 1.3-4.2) and overall survival (hazard ratio 2.6, confidence interval 1-6.5) in
women who were diagnosed with breast cancer while they were pregnant. Others have also
suggested that the pregnancy is an independent poor prognostic factor for overall survival in
cases when compared to stage-matched controls (148–150). Murphy and colleagues, in a recent retrospective study performed at the Memorial Sloan Kettering Cancer Centre, compared 99 patients with pregnancy-associated breast cancer (36 women were diagnosed during and 63 women were diagnosed after breast cancer) with 186 non-pregnant women. They found that although the pregnancy group tumors were more advanced (p = 0.03) had high histological grade (p = 0.01), ER and PR receptor negative (p = < .0001) and lymph node positive (p = 0.01), the overall survival did not differ from controls (p = 0.08) (145).

2.8.6.2 Prognosis of Pregnancy-following breast cancer

According to a recent metanalysis by Azim et al, women with a pregnancy following breast cancer had a 41% reduced risk of death compared to women who did not get pregnant (118). Many other studies have reported that pregnancy following breast cancer diagnosis does not have adverse effects on survival or breast cancer recurrence, and some studies have reported a long term protective effect of pregnancy on recurrence (93,97,98,100,151–153).

Verkooijen et al, in a large population-based study of 492 patients who became pregnant a year or more from diagnosis had a lower 15-year mortality rate than the comparison group (non-pregnant women after breast cancer) (16.8% versus 40.7% respectively). In this study, the mortality rate was calculated using the time elapsed from breast cancer diagnosis to death. Women who gave birth within one to two years had 3-fold higher chance of dying compared with women who deliver four or more years after breast cancer diagnosis (104).
Another study that compared 107 women with one or more pregnancies following breast cancer were compared to 344 controls (no pregnancy after diagnosis) found similar results (154). Women were matched by breast cancer stage and length of survival. This study found a non-significant difference in the risk of breast cancer recurrence or death among pregnant and non-pregnant groups after a follow up of 12 years.

In a study of 438 women with pregnancy associated breast cancer and 2,775 controls, Mueller and colleagues reported a mortality ratio of 0.54 (95% CI: 0.41-40.71) for women who gave birth after 10 months of a diagnosis of breast cancer, compared with women who did not have a pregnancy (97). This implies that pregnancy after breast cancer might have a protective effect on survival.

In summary, the majority of research studies involving women who became pregnant after breast cancer have reported that pregnancy one year or more following breast cancer does not adversely affect survival. However, the literature related to pregnancy-associated breast cancer is inconsistent. Some authors do not consider pregnancy as a prognostic factor for survival (5,94,97,118,131,145,147,154–161) while others consider that pregnancy is a poor prognostic factor for survival (104,148,162–167) (Appendix 1).

2.8.7 Limitations of Previous Studies

Studies conducted on pregnancy-associated breast cancer have several limitations. Firstly, prospective studies are difficult to undertake because the small number of women who chose to
become pregnant after breast cancer. Thus, sample sizes are small because pregnancy at the time of breast cancer and pregnancy after breast cancer are rare events.

Secondly, inconsistencies in defining pregnancy groups were problematic in the past. Recent studies have come to a consensus in this regard. Defining the time from pregnancy to breast cancer and breast cancer to pregnancy is essential as it can influence the outcomes. The risk of breast cancer recurrence is higher the first two years after diagnosis and the surge of gestational hormones have its highest impact during the time the woman is pregnant.

Thirdly, the range of age also varies among the literature. Some publications include women less than 35, some include women less than 40 years and some include any premenopausal women regardless her age.

Three common biases are described: self-selection bias, treatment effect and survivorship bias.

The “healthy mother effect” is a self-selection type of bias where it is more likely that women who are healthy will try to conceive. They have had probably early stage breast cancer. To avoid this bias in our study we have matched cases and controls by tumour characteristics, age, place of residency and BRCA mutation type. We also adjusted for histopathological covariates (tumour characteristics, stage and grade) in order to minimize the potential factors that are inherently present in a self-selected cohort.

Bias due to treatment effects are represented by the effects of cancer risk-reducing strategies such chemoprevention and salpingo-oophorectomy and by breast cancer treatment such as chemotherapy and endocrine therapy. To overcome bias due to treatment effect we adjusted for chemotherapy (yes/no), oophorectomy (yes/no), radiotherapy (yes/no) and tamoxifen (yes/no).
Cofactors that influence both, the exposure (pregnancy) and the outcome (death) are known as confounders. Oophorectomy greatly improves survival in BRCA carriers (outcome), but also abolishes their capacity to conceive (pregnancy or exposure). Chemoprevention and treatment with Tamoxifen improve survival by 40% (outcome) but its anti-estrogenic effects interferes with fertility (exposure) (122,168). Likewise, chemotherapy agents given to premenopausal women may induce premature ovarian failure (169). There is a benefit of chemotherapy on survival but it can also decrease fertility of breast cancer survivors.

Most important is survivorship bias. By definition, women are alive at the time of enrolment, genetic testing, breast cancer diagnosis and childbirth. However, the time from diagnosis to pregnancy, the time from diagnosis until genetic testing, the time from diagnosis to enrolment varies widely. For instance, women enrolled in this study have opted for BRCA1 and BRCA2 genetic testing. Genetic testing for BRCA1/2 was only available after 1995 but women included in this study were diagnosed from 1985 and onwards. For some subjects 10 years may have elapsed from diagnosis to testing. This is very common in clinical practice. Women survived several years before genetic testing; this is referred to as survivorship bias. If the survival analysis only included for women who have survived till the time of enrolment, there are missing those women who died prior to enrolment.

Women who became pregnant several years after diagnosis represent another source of survival bias. If the study analyzes the data from pregnancy (childbirth) as start point to death as end point; the time that the women survived from breast cancer diagnosis to pregnancy is censored.
To control for survivorship bias, we used a left truncation analysis and three different start points: from breast cancer diagnosis to death, from childbirth to death and from the date of ascertainment to death (a detailed explanation of our methodology is presented in section 3.8).
Chapter 3
3 Methods

3.1 Research Question

There is paucity of data available on pregnancy during or after breast cancer addressing specifically women who carry a BRCA1 and BRCA2 mutation. Because women who carry these mutations develop early onset breast cancer, pregnancy and breast cancer is more likely to coexist. Carriers, who survived breast cancer and wish to conceive, are often seen in our genetic clinic inquiring about the risk and impact of a pregnancy on their health. By examining the survival experience of women who carry a BRCA1 or BRCA2 mutation and become pregnant during or after a breast cancer diagnosis, a rational approach to their management and counseling can be developed. To properly answer these important clinical questions, more scientific evidence is needed. Therefore, we have formulated the following research question:

**Does pregnancy influence the risk of breast cancer recurrence or survival in carriers of BRCA1 and BRCA2 mutations with a history of breast cancer?**

3.2 Hypothesis

Pregnancy at time of breast cancer diagnosis has a deleterious impact on recurrence and survival of women who carry a BRCA1 or BRCA2 mutation.
3.3 Objectives

The aims of this study are:

1. To determine the impact of pregnancy at the time of breast cancer diagnosed (pregnancy-associated breast cancer) on breast cancer recurrence and survival of women who carry a \textit{BRCA1} or \textit{BRCA2} mutation.

2. To determine the impact of pregnancy following breast cancer treatment (minimum of one year) on the risk of breast cancer recurrence and impact on survival of women who carry \textit{BRCA1} or \textit{BRCA2} mutation.

3.4 Outcomes

Primary endpoint:

The primary end point of this study was breast cancer-specific mortality.

Secondary endpoint:

The secondary endpoint for this study was distant breast cancer recurrence diagnosed after one year of breast cancer diagnosis.
3.5 **Study Design**

3.5.1 **Setting**

This is an international, multicenter, historical cohort study of women known to carry a *BRCA1* or *BRCA2* mutation and diagnosed with invasive breast cancer. The study was based at the Familial Breast Cancer Unit at Women’s College Research Institute in Toronto and included 57 centers across Europe and North America (*Appendix 2*).

The research team has been working in the field of Familial Breast and Ovarian Cancer research for over 15 years. Patient consent to be invited for further studies as well as the authorization to release medical information forms have been obtained in advance from the parent cohort study (see section 3.5.2).

3.5.2 **Parent Cohort Study Description**

The parent cohort study, from which this current study is drawn, is the largest long-term study of women who carry a mutation in one of the two breast cancer genes (*BRCA1/BRCA2*). It started in 1995 and includes over 80 study centers across North America, Asia and Europe (S Narod, PI). The purpose of the cohort study is to better understand the prevention and treatment of hereditary breast and ovarian cancers. Participants enrolled in the parent cohort study are required to complete a baseline questionnaire at the time of the on study entry. The baseline questionnaire (*Appendix 3*) collects information on demographics, family history, medical illness, gynecological and obstetrical history (age of menarche, age of menopause, parity,
breastfeeding, hormone replacement therapy, fertility treatments, gynecological and abdominal surgeries and, contraception methods), first and second (if applicable) breast and ovarian cancer information (date of diagnosis, mode of detection, surgical and adjuvant treatment), other cancer types, breast and ovarian cancer screening (CA125 test, mammography, MRI), and lastly cancer prevention (surgical: oophorectomy and prophylactic mastectomy and chemoprevention: tamoxifen and other therapies).

Every two years a follow up questionnaire (Appendix 4) is collected to gather information on incident breast or ovarian cancer, breast or ovarian recurrence, other cancers (skin, peritoneal, fallopian tube) and update information on various hormonal, reproductive, and lifestyle factors that may be associated with the development of breast and ovarian cancer in high-risk families. This study continues to accrue subjects at the rate of approximately 500 subjects per year. Every woman enrolled in the parent cohort study has completed a baseline questionnaire. The rate of completion of the follow-up questionnaires is around 77.2% ± 3%. At the time of the statistical analysis, the parent cohort study had 13,334 women, all known carriers of a BRCA1 or BRCA2 mutation. Approximately 50% of women have no personal history of breast cancer and they are undertaking genetic screening programs at each corresponding center, 35% of women have been diagnosed with breast cancer and 15% have been diagnosed with ovarian cancer. The goal is to accrue 20,000 women.

3.5.3 Study Population

All women participating in this current study are known to carry a BRCA1 or a BRCA2 mutation.
Genetic Testing:

Mutation detection was performed using several techniques and all mutations were confirmed by direct sequencing. All mutations were considered deleterious. The molecular techniques used to identify carriers of mutations varied from laboratory to laboratory and included the protein truncation test (PTT), multiples denaturing gradient gel electrophoresis (DGGE), high-pressure liquid chromatography (dHPLC) and direct sequencing. The techniques varied depending on whether or not the family mutation was known or not, and whether or not the subject was a member of a distinct ethnic group with characterized founder mutations (Ashkenazi Jewish, French-Canadian, Dutch). Mutation analysis was conducted by provincial laboratories in British Columbia, Saskatchewan Manitoba and Ontario, by Myriad Genetics in the United States and by research laboratories in Poland, Norway and Austria. Dr. Narod’s Laboratory at Women’s College Hospital also provided mutation analysis to women in North America that qualified for the parent cohort study.

3.5.4 Recruitment

The data collection for this study began in September 2010 and finished in June 2012. Pregnancy status was defined as the number of live childbirths or stillborn prior and after breast cancer diagnosis. The selection criteria used were as follow:
3.5.4.1 Selection Criteria

Patients were drawn from the parent cohort to the current study if they met the following inclusion criteria:

Common Inclusion Criteria:

- Carrier of a BRCA1 or BRCA2 mutation
- Personal history of breast cancer, stage I/II/III
- Age 20 to 45 at the time of breast cancer diagnosis
- Diagnosed with breast cancer from 1985 to 2011
- Alive or deceased in 2010

Inclusion Criteria for Control Subjects:

- No pregnancy at the time of breast cancer diagnosis or after

Inclusion Criteria for Case Subjects:

- Breast cancer diagnosed during a pregnancy or within the first year post-partum.
- Pregnancy following one year of breast cancer diagnosis or after

Common Exclusion Criteria:

- Diagnosed with breast cancer stage IV
- Breast cancer recurrence (distant) detected within one year from date of breast cancer diagnosis.
- Less than two years of follow-up
- Hysterectomy
- Natural, chemical or surgical menopause
- Diagnosed with ovarian or any other cancer (except skin)

Exclusion Criteria for Control Subjects:

- Oophorectomy in the matched control patient prior to pregnancy in the exposed patient
- Recurrence in the matched control patient prior to pregnancy in the exposed patient
Exclusion Criteria for Case Subjects:

- Pregnancy outcome resulted in miscarriage, therapeutic abortion or spontaneous abortion

3.5.5 **Ascertainment of study subjects**

Of the 13,334 subjects in the database, we excluded: 7,042 (53%) subjects who were unaffected (no invasive breast cancer diagnosis); of the 7,042 subjects 6,180 have never been diagnosed with breast cancer and 862 have been diagnosed with Ductal Carcinoma In Situ (DCIS) or Lobular Carcinoma In Situ (LCIS). We also excluded 1,050 (8%) subjects who had less than two years of follow up, 1,576 (12%) subjects who were over 45 years or younger than 20 years old at the time of breast cancer diagnosis, 590 (4%) subjects who were diagnosed with breast cancer prior to 1985, 450 women who pregnancy outcome were either miscarriage, therapeutic or spontaneous abortions (3%), 201 (2%) subjects who had natural or surgical menopause (hysterectomy ± salpingo-oophorectomy), 64 (.4%) had an oophorectomy prior to breast cancer, 173 (1%) subjects who had stage IV breast cancer, 131 (1%) subjects who developed ovarian cancer or other non-skin cancer and 76 (0.5%) subjects whom information was missing or incomplete (i.e. date of birth, date of breast cancer diagnosis or breast cancer treatment) or patients were lost to follow-up. The reason why we excluded stage IV breast cancer cases is because they may represent patients too ill to undergo biopsy or to become pregnant; the exclusion of these short survival cases therefore tends to bias survival upwards. In total, 11,353 subjects were excluded (Figure 3-1).
Figure 3-1 Ascertainment of Subjects

High Risk Factor Study of Women with Hereditary Breast and Ovarian Cancer \( n=13,334 \) carriers of BRCA1/2

13,334
11,353-

= 1,981
Eligible Participants

6,180 subjects are unaffected (No breast Cancer) and 862 had DCIS/LCIS = 7,042

1,050 subjects had less than two years of follow-up

+ 3,061 excluded for other reasons

Other Exclusion steps

<table>
<thead>
<tr>
<th>Exclusion Criterion</th>
<th>No of participants</th>
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<tr>
<td>Diagnosed age &lt; 20 or &gt; 45 y.o</td>
<td>1576</td>
</tr>
<tr>
<td>Diagnosed before 1985</td>
<td>590</td>
</tr>
<tr>
<td>Miscarriage and abortions</td>
<td>450</td>
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<tr>
<td>Menopause prior to breast cancer</td>
<td>201</td>
</tr>
<tr>
<td>Oophorectomy prior to breast cancer</td>
<td>64</td>
</tr>
<tr>
<td>Stage IV breast cancer</td>
<td>173</td>
</tr>
<tr>
<td>Ovarian cancer any time</td>
<td>131</td>
</tr>
<tr>
<td>Missing information</td>
<td>76</td>
</tr>
</tbody>
</table>
From the parent cohort study, we identified 1,981 women eligible for the study. We included all women diagnosed with invasive breast cancer between the years of 1985 to 2010, of these 450 women were diagnosed with breast cancer at the time of a pregnancy or who became pregnant after breast cancer. Pregnancy outcomes included miscarriage, spontaneous or therapeutic abortions, live birth and still born. We then restricted are sample to pregnancy outcomes that included live birth or still born (preterm and term pregnancies). Of 450 women, 161 had live birth or stillborn and were classified as case subjects. 289 women were not classified as case subjects because they had miscarriages, therapeutic or spontaneous abortions and were excluded. The rationale for excluding abortions and miscarriage is that according to the literature an interrupted pregnancy or a spontaneous abortion does not have any biological effect on survival of women with breast cancer (170,171). Thus, accounting for them might be another source of survivorship bias to the analysis.

Of 1,981 eligible subjects, 1,820 were control subjects and 161 were case subjects. The case subjects included women who were diagnosed with breast cancer during a pregnancy or within 1 year postpartum (group of pregnancy-associated breast cancer - PABC) and, women who became pregnant following a year of diagnosis of breast cancer (group of pregnancy-following breast cancer – PFBC). A collection data sheet was completed for each case. Dates of breast cancer diagnosis and childbirth, pregnancy outcome (live birth/still born), parity and a graphic representation of the pregnancy sub-study group were recorded (Appendix 5). The control subjects included patients with no pregnancy associated or following a diagnosis of breast cancer (controls). Of the 161 cases, 83 had PABC, 68 had PFBC. Ten women had two pregnancies, one associated breast cancer and other following breast cancer. We assigned these ten women to the
PABC group (Figure 3-2). We did this for two reasons. First, as per other studies the highest impact of pregnancy on survival and disease-free survival is seen in the PABC group compared to the PFBC group (118,172). Second, the number of women with both PABC and PFBC were too small to study it as a sub-group.

**Figure 3-2 Ascertainment of Subjects after Matching**
3.6 **Power Calculations:**

In the initial planning of this study we estimated that 300 study subjects would be necessary in order to detect a difference in mortality of 30%, based on a power of 80% and a type one error of 5%. Due to difficulties in collecting all data for cohort members we were able to enrol 165 women into the cohort and therefore the power to detect a moderate difference in survival was compromised.

Moreover, the powered calculation was based on merging the two groups and this is not ideal. Each of these may have different prognoses and we only had sufficient power to study the patients group as a whole.

3.7 **Matching strategy**

The key point of matching is to control for differences in prognostic factors. The selected controls should have the same *a priori* risk of becoming pregnant and of dying from breast cancer as the cases. We attempted to identify up to three controls for each case subject. The rationale for matching a case with more than one control is to increase the power of the study. Also, it is usually not possible to match for more than a few variables because of practical difficulties in finding patients who meet all of the matching criteria. Therefore, from our original 161 cases and 1,820 controls we went to 128 cases and 269 controls after the matching. Case and control subjects were matched by age (± 2 years), *BRCA* mutation type (*BRCA1* vs. *BRCA2*), country of residency, date of breast cancer diagnosis (± 2 years) and date of completion of baseline questionnaire (date of study entry ± 2 years). Cases could not have had a hysterectomy
or oophorectomy or have undergone natural menopause prior to diagnosis. They could not have experienced a distant recurrence prior to the date of pregnancy (if they experienced a local recurrence they remained eligible). To be eligible to be matched to a given case, the control had not to have had an oophorectomy or experienced a distant recurrence prior to the date of birth for the index pregnancy in the matched case (Figure 3-3).

In addition to the matching strategy we analyzed the effects of relevant co-variables on survival including oophorectomy, chemotherapy, tumour size, lymph nodes and estrogen receptor. To consider the effects of all this variables simultaneously we used a multivariable analysis. Cox proportional model is a type of multivariable analysis used in survival analysis. It is described in the data analysis section.

**Figure 3-3 Diagram to Illustrate Eligibility for Matching**

After matching the 1,981 eligible subjects, of the 161 cases we identified 128 matched sets (128 cases) and of the 1,820 controls we identified 269 matched controls (269 controls). We matched
1 to 3 controls per case. Of the 128 matched cases, 75 case subjects were diagnosed with a pregnancy-associated breast cancer and 53 case subjects were diagnosed with a pregnancy following breast cancer. We retrieved pathology reports for 55% (n=70) of the 128 cases and for 53% (n=143) of the 269 matched controls.

3.8  Data Collection

The data for this study were obtained from three sources: 1) the study questionnaires, 2) the medical chart and 3) the surgical pathology report.

Study questionnaires:

The data regarding subject eligibility were compiled from study questionnaires completed at the time of enrolment in the parent cohort study. From the baseline questionnaire, we collected date of birth, country of residence, type of BRCA mutation, date of genetic testing, obstetrical and gynaecological history breast cancer screening and breast cancer history (date of diagnosis, primary surgery, adjuvant treatment, recurrence and reconstructions). From the follow-up questionnaire, we collected details of new cancers, second primary cancers and cancer recurrences (local/distal/regional and date). Subsequent pregnancies and fertility treatment were also recorded.
Pathology reports:

Pathology reports were requested from the corresponding research centres or directly from the hospital where the surgery was performed, using a signed authorization of release of information form. Pathology reports included the macro- and microscopic description of the tumour (type of carcinoma, grade, involvement of surgical margins, angiolymphatic invasion, *in-situ* component, micro-calcifications, involvement of nipple and skin, sentinel lymph nodes, number of lymph nodes removed, number of lymph nodes involved and hormone receptor status).

Medical Chart Review:

Staff at the local centres performed the medical chart review. The following variables were abstracted from the medical charts: obstetric history, including date of delivery, gestational age, pregnancy outcome, duration of lactation, type of breast cancer surgery [lumpectomy/ mastectomy (ipsilateral and/or contralateral)], salpingo-oophorectomy, chemotherapy (yes/no), radiotherapy (yes/no), tamoxifen (yes/no), date and cause of death and, breast cancer recurrence (distant/local).

Detailed information on the stage of the cancer at diagnosis, pathology details and on treatments received was documented. Signed consent forms as well as authorization of release of medical information form were obtained from the parent cohort study. Some centers contacted their participants directly and provided us with anonymous data including pathology report, and follow-up questionnaire. If the subject identified has died, the next of kin was contacted and asked to sign an authorization to release medical information. This is being done to try to reduce
selection bias. In the event of loss to follow-up, additional family members, if available, were asked to help locate the study subject.

The study lead investigator (AV) was responsible for communicating with the other centers, data collection and completion, data management and storage and quality control. The data was entered into a Microsoft Access database designed for this purpose. Ethics approval through the Ethics Committee at WCRI was obtained before the study commenced (Appendix 6).

3.9 Data Quality

Three issues pertaining to data quality were considered: disease definition, completeness of data collection, and quality of follow-up for vital status, incident cancers and pregnancies. Disease definition was described in several sections of this thesis. Pregnancy-associated breast cancer (PABC) is defined as breast cancer diagnosed during a pregnancy or within the first year post-partum. Pregnancy-following breast cancer (PFBC) is defined as pregnancy that occurs one year after breast cancer diagnosis or after. Date of pregnancy is defined as the date of childbirth and Date of breast cancer diagnosis is defined as the date of the biopsy. Both dates, date of breast cancer diagnosis and date of pregnancy most have at least the month and the year in order to exactly estimate the time in month from diagnosis to pregnancy and classify a subject as PABC, PFBC or control. Uncompleted date of pregnancies or date of breast cancer diagnosis was addressed by looking for more information into the follow up questionnaires, contacting the subject (if alive), reviewing the medical chart or contacting the Centre. We created a data collection form with a timeline graph (Appendix 5) to plot the dates of pregnancy and diagnosis.
and to carefully categorize each participant into its corresponding sub-group. The date of death was obtained from the death certificate and study questionnaire. The death certificate must specify the cause of death. If the cause of death were unknown or other than breast cancer the subject were excluded from the analysis. If any dates were uncertain, not consistent or missing, the subject was excluded from the study.

Completeness of data collection: data on obstetrical history, breast cancer characteristics and treatment were fully obtained and consistent from the three data sources. However, original pathology reports were retrieved only in half of the cases and half of the control subjects. We compare the data collected on pathological characteristics of breast cancer from the parent cohort study questionnaires with the original pathology reports. For inconsistencies we accessed the electronic medical records for patients in Ontario, Canada. For cases outside of Canada we contacted the Institution where the primary surgery was performed to retrieve the medical chart or in some cases the centre provided information recorded in their registry.

Quality of follow-up: Follow-up rate for the parent cohort study was estimated in 77.8%. Incident breast or ovarian cancer cases affects survival estimates if the unregistered cases have different prognoses to the registered cases. Difficulties in ascertaining the vital status of subjects generally result in an overestimation of survival as deaths are missed (subjects erroneously considered alive can introduce bias). Follow-up difficulties may be due to confidentiality constraints, lack of access to medical records, lengthy follow-up, or linkage failure with registry records. It is expected that events would be observed more frequently in patients with longer follow-up times than in patients with a short follow-up (178). Our research team has been devoted to achieve the greatest possible completeness of the data. For this reason, the parent
cohort study database is more than the sum of its contributing centres, and its value as a resource for hereditary breast and ovarian cancer syndrome is considerably greater.

3.10 Data Analysis

Mortality from breast cancer was the primary endpoint and distant breast cancer recurrence (if diagnosed after one year of breast cancer) was the secondary endpoint. The data were analyzed using SAS statistical software for survival analysis (Kaplan-Meier method and Cox-proportional hazards model) (173). We estimated the adjusted hazard ratio associated with pregnancy as well as with other tumour characteristics and treatments (chemotherapy yes/no) and oophorectomy (yes/no). In all survival analyses, oophorectomy was treated as a time-dependent covariate.

Cancer survival is expressed as the percentage of patients alive at a certain point in time after diagnosis. Survival analysis takes the survival times of a group of subjects and generates a survival curve. However, at the end of study some subjects are still alive. The length of time they are still alive or when did they die is unknown. These subjects are also known as censored. Kaplan-Meier survival analysis is a method designed to account for censoring individuals by which the survival rate is calculated every time a patient dies.

Covariates are factors that influence survival, such as age and breast cancer stage. To account for the effect of each covariate or predictor in a survival analysis we used the Cox proportional hazards model. This method calculates a coefficient for each covariate, which will be reflected on the survival curve, where 0 (HR = 1) means no effect on the curve and positive (HR > 1) or negative (HR = < 1) values means that the covariate is associated with increased or reduced
mortality. In survival analysis, the **hazard ratio** (HR) is the ratio of how often a particular event happens in one group compared to how often it happens in a comparison group over time. Hazard ratios differ from relative risk ratios in that relative risk is a cumulative over the study period using a defined endpoint. Hazard ratio instead, represents instantaneous risk over the study time period. Hazard ratios suffer somewhat less from selection bias with respect to the endpoints chosen, and can indicate risks that happen before the endpoint. If the hazard ratio is 1, it means that there is no difference in survival between the two groups. If the hazard ratio is greater than 1, it means that survival is poorer in the exposed group but if the hazard ratio is less than 1, it means that the survival is better in the exposed group (173). For example, if there are two groups A and B, HR = 4.5 for survival means that the risk death for group B is 4.5 times greater than for group A.

### 3.10.1 Kaplan-Meier Survival Analysis

Survival for breast cancer was calculated using the **Kaplan-Meier method**. The Kaplan-Meier method, also known as product-limit-method, estimates the survival of a cohort over time. On the vertical axis of the survival curve is represented the estimated probability of surviving, and on the horizontal axis is represented the period of time following the beginning of observation. Kaplan-Meier method poses several advantages over other methods to evaluate survival. First, Kaplan-Meier method uses the exact time of death to calculate survival. Second, Kaplan-Meier method calculates the survival of subjects every time the outcome occurs. Thus, some data points are closer in the time-axis, whereas others are spread far apart. This will be reflected in the shape of the curve along the X-axis or time axis, as each step of the curve will have different lengths.
When a patient dies, the probability of surviving at that moment is calculated (number of patient surviving / number at risk of dying at that time). Those who have already died or are lost to follow up upon that point are not at risk of dying and thus, are not used in the calculations. The curve displays steps that correspond to the death of each subject in the cohort. If the number of subjects were increased, the size of the steps would diminish. Lastly, Kaplan-Meier accounts for subjects who are lost to follow-up or censored. Subjects who are lost to follow up are still at risk of dying and therefore, they should be included in the calculations (174).

The log-rank test is used to test whether the difference between survival times between two groups is statistically different or not, however this test does not allow the evaluation of the effect of the other independent variables.

3.10.2 **Left Truncated Survival Analysis**

Left truncated analysis is a technique useful for controlling delayed study entry or uncompleted nature of the observation (175). Left truncation is present, for instance, in studies of disease mortality where survival from the time of diagnosis is the outcome of interest even though the subjects have been diagnosed several years prior to the enrolment in the study (176).

We may distinguish two types of truncation:

- Left truncation: the case when only those who have survived more than some minimum amount of time are included in the observation sample (small survival times are not observed). In survival analysis, left truncation occurs when cancer survivors interviewed
some time after the diagnosis and who are still alive at baseline are followed over time (175–177) (Figure 3-4). As an example of left censoring, we may follow up a patient for any infectious disorder from the time of his or her being tested positive for the infection. We may never know the exact time of exposure to the infectious agent (178).

- Right truncation: this is the case when only those persons who have experienced the exit event (death) by some particular date are included in the sample, and so relatively long survival times are systematically excluded (those who still alive at the end of the study, or are lost to follow-up, also known as censoring) (173).
Figure 3-4 Left Truncated Diagram

(A) Observational epidemiological study with follow-up data, (B) survival analysis ‘at risk’ set. (A) Study recruitment starts at R and ends at C. Date of diagnosis and event are indicated by Dx and E respectively. (B) Eligible cases are aligned by Dx on y axis of time since diagnosis. Dashed lines indicate unobserved time.

In the parent cohort study, subject’s eligibility is based on whether or not a woman has tested positive for a $BRCA1/2$ mutation regardless of her personal history of being diagnosed or not with breast cancer. Only after a confirmation of the $BRCA$ mutation, the woman is enrolled in the study. A participant may develop or not breast cancer at different points in time that ranges from several years prior to or after $BRCA$ testing and, prior to or after the date of enrollment. In our Pregnancy study, left truncation analysis has been applied to censor for the time period between diagnosis and the date of enrollment (or completion of the baseline questionnaire). The value of a left truncated analysis on this particular study derives from the fact that survival cohorts often comprise a mix of prevalent cancers and incident cancers (177). Prevalent cancer refers to those women diagnosed with breast cancer prior to the study entry and who are still alive at the start of follow-up. The primary concern about prevalent cancers is that they may represent a healthy subset of women who were diagnosed prior to follow-up. If subjects enrolled into the study survived long enough to allow disease to occur but some develop the disease while other did not then, we must consider differences in susceptibility among survivors (175–177). Therefore, one of the enigmas is whether differences in susceptibility influences differences observed between incident and prevalent cancers. The $BRCA$ population is a mixture of subjects with varying degrees of susceptibility or penetrance (see glossary) to develop breast cancer; $BRCA$ carriers are at high-risk of developing breast cancer but some carriers may never develop it (15).

In addition, prevalent cancers are associated with women that may have died prior to enter the study. The absence of this subset of prevalent cases that fail to survive until the sampling date results in a study group biased towards favorable survival (179).

Studies comprising only incident cancers (inception cohorts) also have limitations (180). First, the potential bias inherent of prevalent cancers may also be seen in incident cancers because, as
time in follow-up increases, more susceptible women will be more likely to experience the disease than less susceptible women (180). If we wanted to avoid prevalent cancers biases and only study incident cancers this also may introduce bias. First, it may reduce statistical power due to a decrease in the time to develop the disease or a reduction in the age range at baseline, having fewer women eligible. In addition, the length of follow-up may be insufficient to develop the disease, to develop a recurrence or to cause death (177). Although a prospective study of an inception cohort may sometimes be feasible, a retrospective inception cohort of all women diagnosed after the start of follow-up may be more reasonable (177).

We analysed the data by using three start points: breast cancer diagnosis, childbirth and date of ascertainment. We performed six survival analyses.

Primary Survival Analysis:

The primary analysis was an estimate of survival following breast cancer diagnosis. The patient was followed from the date of diagnosis (determine by the date of the biopsy) until death from breast cancer, date of last follow-up or death from another cause. As (by definition) patients were alive at the time of completion of the baseline questionnaire, we used a left-truncated survival analysis (175), whereby the time period between diagnosis and completion of the baseline questionnaire was censored.

Second Survival Analysis:

In the second analysis, we followed the patients from the date of ascertainment (baseline questionnaire completion) until the date of death.

Third Survival Analysis:
In the third analysis, we followed the patients from the date of childbirth of the index case (and the same date in the matched control) until death. This was done to control for survivorship bias, because all cases and matched controls were alive at the date of parturition of the index case. We first conducted a survival analyses for all case and control subjects and then we evaluated separately the subgroups of subjects with pregnancy-associated breast cancer and pregnancy following breast cancer and their matched controls.

Fourth Survival Analysis:

We also performed a survival analysis using breast cancer recurrence as endpoint. We considered only regional and distant recurrences. Local recurrences were not considered as an end point, because 55% of the cases in our study had a mastectomy and if we included women with local recurrences we were going to omit 45% of the subjects (almost a half of the sample size).

We followed the patients from the date of breast cancer diagnosis until the date of the breast cancer recurrence, using a left-truncated analytic approach described above.

Fifth Survival Analysis:

In the fifth survival analysis we followed the patients from the date of ascertainment until the time of breast cancer recurrence (distant recurrence).

Sixth Survival Analysis:

In the sixth survival analysis we followed the patients from the date of childbirth of the index case (and the same date in the matched control) until the date of breast cancer recurrence (distant recurrence).
3.10.3 **Cox Proportional Hazard Model Analysis**

By using the Cox proportional hazard model, we examined the influence of covariates on the relative risk of dying of breast cancer for the cases (pregnancy-associated breast cancer and pregnancy following breast cancer) and compare to the control group (no pregnancy during or after breast cancer diagnosis). Possible differences in the baseline characteristics of the cases and comparison group and treatments received, age at diagnosis, tumour size (<2, 2-5, >5 cm), lymph node status (positive, negative, missing), ER status (positive, negative, missing), use of chemotherapy (yes/no) and oophorectomy (yes/no, time-dependent-variable) were adjusted for in the models. The result from the univariate (crude) and multivariate (adjusted) Cox’s model is presented using an end point for survival of breast cancer specific mortality cut at 15 years and left censored at the date of baseline. In addition to the four survival analyses, we performed two additional analyses of covariates using Cox proportional hazard model. The first Cox proportional analysis studied the covariates in all subjects (n=397); the second Cox proportional analysis studied the covariates only of case subjects (n=128). The aim of the second Cox proportional analysis was to compare outcomes between pregnancy-associated breast cancer and pregnancies following breast cancer. We have used Cox proportion hazard model to evaluate more than one independent variable at the time on survival of the two groups (pregnant and non-pregnant).

The conclusion of this thesis is based upon the primary outcome. The additional survival analyses are meant to contribute to the conclusion and to control for bias. A summary of the survival analyses is presented in Table 3-1.
Table 3-1 Summary of Survival Analyses

<table>
<thead>
<tr>
<th>Kaplan-Meier Method</th>
<th>Start Point</th>
<th>End Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Survival Analysis for Pregnant- and Non Pregnant</td>
<td>Date of Breast Cancer diagnosis</td>
<td>Breast Cancer Specific Mortality</td>
</tr>
<tr>
<td>group (Figure 4-1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First Survival Analysis for Pregnant Sub-groups and Non</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant (Figure 4-3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second Survival Analysis (Figure 4-4)</td>
<td>Date of Ascertainment</td>
<td></td>
</tr>
<tr>
<td>Third Survival Analysis (Figure 4-5)</td>
<td>Date of Childbirth</td>
<td></td>
</tr>
<tr>
<td>Fourth Survival Analysis (Figure 4-6)</td>
<td>Date of Breast Cancer diagnosis</td>
<td>Breast Cancer Recurrence</td>
</tr>
<tr>
<td>Fifth Survival Analysis (Figure 4-7)</td>
<td>Date of Ascertainment</td>
<td></td>
</tr>
<tr>
<td>Sixth Survival Analysis (Figure 4-8)</td>
<td>Date of Childbirth</td>
<td></td>
</tr>
<tr>
<td>Additional Survival Analysis</td>
<td>Co-Variables</td>
<td>Start Point</td>
</tr>
<tr>
<td>Cox Proportional Analysis including all subjects (n=397)</td>
<td>Age at diagnosis Tumor size (&lt;2, 2-5 &gt;5 cm) Lymph node status (+/-) Estrogen receptor (+/-) Chemotherapy (Yes/No) Oophorectomy (Yes/No)</td>
<td>Date of Breast Cancer Diagnosis</td>
</tr>
<tr>
<td>(Table 4-6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cox Proportional Analysis including only case subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=128) (Table 4-7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter 4
4 Results

In this chapter, the results of survival analyses for PABC and PFBC are presented. We have hypothesized that women who carry a BRCA1 or a BRCA2 mutation that are pregnant at the time of breast cancer diagnosis or become pregnant following a breast cancer diagnosis have a worse survival than women who do not become pregnant during or after breast cancer. The hypothesis is based on the potential that pregnancy hormones could accelerate cancer cell growth. Each of the six survival analyses had two end points. Breast cancer specific-mortality was our primary end point and distant recurrence was our secondary end point. The data were analyzed from three starting points: date of breast cancer diagnosis, date of enrolment and date of parturition. The primary survival analysis was estimated from the date of breast cancer diagnosis to the date of death from breast cancer. Breast cancer survival will be the proportion of patients alive at certain times after diagnosis (Table 4-1).

The influence of prognostic factors, such as breast cancer histopathological features, on pregnancy-associated and pregnancy following breast cancer were analyzed by Cox proportional methods. The results of each investigation are followed by a brief interpretation.

4.1 Patient Characteristics

Patient characteristics are presented in Table 4-1. After matching, a total of 397 women were eligible for the study. Of these, 128 women were cases (pregnancy-associated breast cancer or pregnancy-following breast cancer) and 269 were controls (no pregnancy after breast cancer
Of 128 cases, 75 (58.6%) women had pregnancy-associated breast cancer and 53 (41.2%) women had pregnancy-following breast cancer.

Subjects were matched by age (± 2 years), BRCA mutation type (BRCA1 vs. BRCA2), country of residency, date of breast cancer diagnosis (± 2 years) and date of completion of baseline questionnaire (date of study entry ± 2 years). For each case subject, we identified between one to three matched controls (mean 2.1).

The mean age of diagnosis of breast cancer was 32.5 years for the case subjects and was 33.8 years for the control subjects. The age of breast cancer diagnosis of women in the pregnancy group was 1.3 years earlier than non-pregnant women (p = 0.009). For all subjects, the mean year of breast cancer diagnosis was 1997 (range 1985-2011). Noteworthy, treatment options for breast cancer after 1985 have changed patterns of breast cancer progression and prognosis. The study entry dates range from 1996 to 2011. The majority of the subjects reside in North America (cases 75% and controls 78%) (Appendix 2).

One hundred and six cases (81.3%) and 227 (84.4%) matched controls carry a BRCA1 mutation. Twenty-four cases (18.8%) and 42 (15.6%) matched controls carry a BRCA2 mutation. Women with a BRCA1 mutation were more numerous than women with a BRCA2 mutation. This difference might be attributed to the earlier age of onset of BRCA1-breast cancers than BRCA2-breast cancers. Although BRCA2-breast cancers still present at lower age than average, BRCA2-breast cancer resembles breast cancers of the general population and thus, they present at later ages.
All case subjects and 200 (71%) controls had experienced at least one birth. The mean age at first childbirth for the case subjects was 30.7 years and for the control subjects was 25.6 years (p = <0.0001). The mean age at last childbirth was 35.0 years for case subjects and was 28.7 years for the control subjects (p = <0.0001). This difference is statistically significant and it might express that women in the case group have not yet completed childbearing and thus, they have chosen to become pregnant at later ages.

The mean time elapsed from breast cancer diagnosis to pregnancy in the case group was 2.4 years (range 0 to 13 years). The meantime from last pregnancy to breast cancer in the comparison group was 5.8 years (range from 1 to 21) (p < 0.0001). As expected this difference was statistically significant. Women in the pregnancy group will have a shorter time from pregnancy to diagnosis. It has been hypothesized that the shorter the time from pregnancy to diagnosis, the worse the prognosis is, of which those whose interval is less than 2 years had the worse prognosis. This finding will be discussed in the next chapter in more detail.

Approximately one-half of the cases and matched controls underwent a prophylactic salpingo-oophorectomy at some time after childbirth in the index case. We selected our comparison group to ensure that no control had an oophorectomy prior to the date of the delivery of the index case. Oophorectomy is a risk-reducing surgery recommended to women who carry a BRCA1 or a BRCA2 mutation after the age of 35. It reduces the risk of breast cancer by up to 50% and the risk of ovarian cancer by 80-90% (76–80). This striking effect on risk reduction requires attention when analyzing survival in this high-risk population. We have assessed the influence of this important co-variable by using the Cox proportional hazard model (Table 4-6).
Table 4-1 Characteristics of Cases and Controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases</th>
<th>Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Date of birth (mean) (range)</strong></td>
<td>1965.2 (1947-82)</td>
<td>1964.5 (1946-81)</td>
<td>0.44</td>
</tr>
<tr>
<td><strong>Age at breast cancer diagnosis (range)</strong></td>
<td>32.5 (25-42)</td>
<td>33.8 (26-44)</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>Date of baseline questionnaire (range)</strong></td>
<td>2003.5 (1996-2011)</td>
<td>2003.7 (1996-2012)</td>
<td>0.84</td>
</tr>
<tr>
<td><strong>Mutation n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1</td>
<td>106 (81.3%)</td>
<td>227 (84.4%)</td>
<td></td>
</tr>
<tr>
<td>BRCA2</td>
<td>24 (18.8%)</td>
<td>42 (15.6%)</td>
<td></td>
</tr>
<tr>
<td><strong>Place of residence</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North America</td>
<td>96 (75.0%)</td>
<td>210 (78.1%)</td>
<td></td>
</tr>
<tr>
<td>Poland</td>
<td>20 (15.6%)</td>
<td>43 (16.0%)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>12 (9.4%)</td>
<td>16 (6.0%)</td>
<td></td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0</td>
<td>78 (29.5%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>128 (100%)</td>
<td>191 (71.0%)</td>
<td></td>
</tr>
<tr>
<td>Mean Parity</td>
<td>2.1(1-5)</td>
<td>2.0 (1-4)</td>
<td>0.62</td>
</tr>
<tr>
<td><strong>Age of menarche (years)</strong></td>
<td>12.8 (9-17)</td>
<td>12.8 (10-21)</td>
<td>0.73</td>
</tr>
<tr>
<td><strong>Age at first birth (years)</strong></td>
<td>30.7 (18-42)</td>
<td>25.6 (18-33)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Age at last birth (years)</strong></td>
<td>35.0 (27-44)</td>
<td>28.7 (20-38)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Time from diagnosis to last childbirth (years)</strong></td>
<td>2.4 (0 to 13)</td>
<td>-5.9 (-21 to -1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Salpingo-oophorectomy after breast cancer diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>55 (43.0%)</td>
<td>127 (47.2%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>73 (57.0%)</td>
<td>142 (52.8%)</td>
<td>0.43</td>
</tr>
<tr>
<td><strong>Vital Status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>120 (92.8%)</td>
<td>246 (91.5%)</td>
<td></td>
</tr>
<tr>
<td>Dead</td>
<td>8 (6.3%)</td>
<td>23 (8.6%)</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>Cause of death</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer</td>
<td>7</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>
4.2 Breast Cancer Characteristics

Table 4-2 presents breast cancer pathological characteristics of cases and controls. We retrieved pathology reports for 213 (54%) subjects. Difficulties in getting pathology reports were related to several factors: the numbers of Institutions, the primary surgery was done in another Institution (some centres are genetic clinics and do not perform surgeries) and, the time to retained medical records was exceeded (some Institutions retained medical records for a maximum of 10 years). However, the number of pathology reports retrieved was balanced between cases and controls. Overall, breast cancer characteristics were similar among all subjects who had pathology reports available. Based on these reports, the mean tumour size among case subjects was 24.6 mm and among controls was 27.7 mm (p = 0.54). The proportion of subjects with positive lymph nodes was similar in cases and controls (40 % of cases vs. 43 % of controls). In both groups (pregnant and non-pregnant), the majority of tumours were estrogen receptor negative (ER- = 68.9% of control subjects and 78.9% of case subjects; p = 0.19). None of these differences were statistically significant.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n = 128)</th>
<th>Controls (n = 269)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 cm</td>
<td>30 (52.6%)</td>
<td>62 (57.3%)</td>
<td>0.93</td>
</tr>
<tr>
<td>2-5 cm</td>
<td>23 (40.4%)</td>
<td>54 (43.2%)</td>
<td></td>
</tr>
<tr>
<td>&gt; 5 cm</td>
<td>4 (7.0%)</td>
<td>9 (7.2%)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>71</td>
<td>144</td>
<td></td>
</tr>
<tr>
<td>Mean size (mm)</td>
<td>24.6 (4-150)</td>
<td>27.7 (4-520)</td>
<td>0.64</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>36 (61.0%)</td>
<td>75 (57.3%)</td>
<td>0.63</td>
</tr>
<tr>
<td>Positive</td>
<td>23 (39.0%)</td>
<td>56 (42.8%)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>69</td>
<td>138</td>
<td></td>
</tr>
<tr>
<td>Receptor status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-</td>
<td>41 (78.9%)</td>
<td>73 (68.9%)</td>
<td>0.19</td>
</tr>
<tr>
<td>ER+</td>
<td>11 (21.2%)</td>
<td>33 (31%)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>76</td>
<td>163</td>
<td></td>
</tr>
</tbody>
</table>

Breast cancer pathological characteristics among pregnancy-associated and pregnancy-following breast cancer and controls are presented in Table 4-3. The tumour mean size was 23.7 mm for pregnancy-associated, 25.9 mm for pregnancy following breast cancer and 27.7 mm for women in the comparison group.

Lymph nodes were positive in 28.6% of women with pregnancy associated breast cancer, 54.2% of women with pregnancy following breast cancer and 42.8% of control subjects (p = 0.13).

Although this difference did not achieve statistical significance, tumors found during pregnancy in our patients tend to have lesser involvement of the lymph nodes than tumors of women who became pregnant after breast cancer. Six tumors of pregnancy-associated breast cancer (19.4%),
33 of pregnancy following breast cancer (31.1%) and five of control subjects (23.8%) were ER positive (p = 0.40). Overall, we found no statistical significant differences between tumor characteristics of women with pregnancy-associated breast cancer and pregnancy-following breast cancer. However, tumors of pregnancy following breast cancer group in our study were slightly more advanced than tumors of pregnancy-associated breast cancer. Pregnancy following breast cancers in our study were smaller, but lymph node positive and ER negative.

Table 4-3 Breast Cancer Pathological Characteristics among Pregnancy sub-groups and Controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n = 128)</th>
<th>Controls (n = 269)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PABC (n=75)</td>
<td>PFBC (n=53)</td>
<td></td>
</tr>
<tr>
<td>Tumour size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 cm</td>
<td>16 (47.1%)</td>
<td>14 (60.9%)</td>
<td>62 (49.6%)</td>
</tr>
<tr>
<td>2-5 cm</td>
<td>16 (47.1%)</td>
<td>7 (30.4%)</td>
<td>54 (43.2%)</td>
</tr>
<tr>
<td>&gt; 5 cm</td>
<td>2 (5.9%)</td>
<td>2 (8.7%)</td>
<td>9 (7.2%)</td>
</tr>
<tr>
<td>Missing</td>
<td>41</td>
<td>30</td>
<td>144</td>
</tr>
<tr>
<td>Mean Size (mm)</td>
<td>23.7 (10-83)</td>
<td>25.9 (4-150)</td>
<td>27.7 (4-520)</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>25 (71.4%)</td>
<td>11 (45.8%)</td>
<td>75 (57.3%)</td>
</tr>
<tr>
<td>Positive</td>
<td>10 (28.6%)</td>
<td>13 (54.2%)</td>
<td>56 (42.8%)</td>
</tr>
<tr>
<td>Missing</td>
<td>40</td>
<td>30</td>
<td>138</td>
</tr>
<tr>
<td>Receptor status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-</td>
<td>25 (80.9%)</td>
<td>16 (76.2%)</td>
<td>73 (68.9%)</td>
</tr>
<tr>
<td>ER+</td>
<td>6 (19.4%)</td>
<td>5 (23.8%)</td>
<td>33 (31.1%)</td>
</tr>
<tr>
<td>Missing</td>
<td>17</td>
<td>32</td>
<td>163</td>
</tr>
</tbody>
</table>

Breast cancer characteristics and treatments used among study subjects are presented in Table 4-4. Unilateral breast cancer presented in 97 (75.8%) of cases and 214 (79.6%) of controls.
Bilateral breast cancer was seen in 31 (24.2%) case subjects and 55 (20.1%) of controls (p = 0.39). Similar proportion of subjects underwent breast conserving surgery (44.9% cases and 51.3% of controls) and mastectomy (53.9% cases and 47.2%). The majority of study subjects received adjuvant treatment with chemotherapy (83.5% of cases and 82.4% of controls p = 0.79). Half of the study subjects received radiotherapy (52.8% of cases and 54.9% of controls p = 0.69). Treatment with tamoxifen was taken by 16.7% of cases and 24.7% of controls (p = 0.07). The low proportion of women treated with tamoxifen might be due to the low proportion of estrogen positive tumours (21% for cases and 31% for controls p = 0.19). In general no significant differences were found between breast cancer controls, pregnancy-associated breast cancers and pregnancies following breast cancer.
Table 4-4 Breast Cancer Characteristics and Treatment used among Study Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases $n = 128$</th>
<th>Controls $n = 269$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>97 (75.8%)</td>
<td>214 (79.6%)</td>
<td>0.39</td>
</tr>
<tr>
<td>Bilateral</td>
<td>31 (24.2%)</td>
<td>55 (20.1%)</td>
<td></td>
</tr>
<tr>
<td>Primary surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumpectomy</td>
<td>57 (44.9%)</td>
<td>136 (51.3%)</td>
<td>0.37</td>
</tr>
<tr>
<td>Unilateral Mastectomy</td>
<td>69 (53.9%)</td>
<td>125 (47.2%)</td>
<td></td>
</tr>
<tr>
<td>Bilateral Mastectomy</td>
<td>1 (0.8%)</td>
<td>4 (1.5%)</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>21 (16.5%)</td>
<td>47 (17.6%)</td>
<td>0.79</td>
</tr>
<tr>
<td>Yes</td>
<td>106 (83.5%)</td>
<td>220 (82.4%)</td>
<td></td>
</tr>
<tr>
<td>Radiotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>60 (47.2%)</td>
<td>120 (45.1%)</td>
<td>0.69</td>
</tr>
<tr>
<td>Yes</td>
<td>67 (52.8%)</td>
<td>146 (54.9%)</td>
<td></td>
</tr>
<tr>
<td>Tamoxifen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>105 (83.3%)</td>
<td>201 (75.3%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Yes</td>
<td>21 (16.7%)</td>
<td>66 (24.7%)</td>
<td></td>
</tr>
</tbody>
</table>

4.3 Breast Cancer Survival Analysis

After a mean follow-up of 12.2 years (range 2 to 26 years) from diagnosis to death a total of 26 subjects died of breast cancer. Seven of the 128 case subjects (5.5%) and 19 of the 269 matched controls (7.1%) had died of breast cancer. The 15-year actuarial survival rates were 91.5% for the pregnant case subjects and 88.6% for the non-pregnant controls. For those who died, the mean time from breast cancer to death was 7.4 years (range 0.7 to 16.1 years). There were no differences among cases and controls. Thirty-one subjects experienced a distant recurrence of breast cancer. Of these, we observed 11 of the 128 case subjects (8.6%) and 20 of the 269
matched controls (7.9%) that experienced a distant breast cancer recurrence. The mean time from breast cancer diagnosis to distant recurrence was 8.1 years (range 0.7 to 17.6 years). There were no differences among cases and controls (Table 4-5).

Table 4-5 Breast Cancer Survival and Disease-free Survival among Cases and Controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases n =128</th>
<th>Controls n = 269</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer recurrence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>117 (91.4%)</td>
<td>249 (92.6%)</td>
<td>0.69</td>
</tr>
<tr>
<td>Yes</td>
<td>11 (8.6%)</td>
<td>20 (7.9%)</td>
<td></td>
</tr>
<tr>
<td>Breast cancer-specific mortality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>121 (94.5%)</td>
<td>250 (92.2%)</td>
<td>0.55</td>
</tr>
<tr>
<td>Yes</td>
<td>7 (5.5%)</td>
<td>19 (7.1%)</td>
<td></td>
</tr>
</tbody>
</table>

4.3.1 Kaplan-Meier Survival Curves

For this study, survival curves were examined in three ways: from the date of breast cancer diagnosis, date of enrolment and date of childbirth to death as a primary end point and breast cancer recurrence as a secondary end point (Table 3-1).

The Kaplan-Meier survival curve from breast cancer diagnosis to death is presented in Figure 4-1. Nineteen subjects in the non-pregnant group and seven of the pregnant group died at 15-years of follow-up. There was not statistical significance (p=0.76).
Figure 4-1 Breast Cancer-specific Survival for Pregnant and Non-Pregnant Women: from date of Breast Cancer Diagnosis to Death

Figure 4-2 shows the breast cancer-specific survival curve for pregnant (cases) and non-pregnant women (controls) from date of diagnosis to death with its corresponding confidence intervals.
The pregnant group (green line) confidence interval range from CI = 0.89 to 0.99. The non-pregnant group (red line) confidence intervals range from 0.90 to 0.98. Confidence Intervals provide information about a range in which the true value lies with a certain degree of probability, as well as about the direction and strength of the demonstrated effect. This enables conclusions to be drawn about the statistical plausibility and clinical relevance of the study findings (181).

**Figure 4-2 Breast Cancer-Specific Survival Curve showing the Confidence Interval for pregnant and non-pregnant women (from the date of breast cancer diagnosis to death)**
Figure 4-3 shows the survival curve from date of diagnosis to death for each of the pregnancy subgroups. Four subjects with a pregnancy prior to one year of diagnosis died compared to only one with a pregnancy after one year of diagnosis. Women with a pregnancy-associated breast cancer have an inferior survival compared to women with a pregnancy following breast cancer diagnosis. However, this difference was not statistically significant (p = 0.49).

Figure 4-3 Breast Cancer-Specific Survival for Pregnant Sub-groups and Non-Pregnant Women: from Date of Diagnosis to Death
The Kaplan-Meier survival curve from the date of ascertainment to death is presented in Figure 4-4. Nineteen women in the non-pregnant group and seven of the pregnant group died at 15-years of follow-up. At 15 years after the date of ascertainment, the 15-year survival rate was 86.0% for the pregnancy-associated case subjects and was 86.9% for the matched controls. This result was not statistically significant (p = 0.51).

**Figure 4-4 Breast Cancer-Specific Survival for Pregnant and Non-Pregnant Women: from Ascertainment to Death**
The Kaplan-Meier survival curve from the date of parturition to death is presented in Figure 4-5. At 15 years after the birth of the index child, the 15-year survival rate was 90.0% for the pregnancy-associated case subjects and was 87.1% for the matched controls. Sixteen women died in the non-pregnancy group and six died in the pregnancy group and the difference was not statistically significant (p = 0.68).

**Figure 4-5 Breast Cancer-Specific Survival in Subjects with and without a pregnancy after Breast Cancer: from Date of Last Childbirth to death**

Likewise, when the end point was recurrence, the Kaplan-Meier survival curves did not show a significant difference for pregnant and non-pregnant women (Figure 4-6). From the date of
breast cancer diagnosis to distant recurrence we observed 17 distal recurrences in the non-pregnant group and five distal recurrences in the pregnant group. At 15 years from the date of breast cancer, 94.6% of the pregnant cases and 91.9% of the comparison group were free of regional or distant recurrence (p = 0.22).

**Figure 4-6 Breast Cancer Recurrence-Free Survival in Pregnant and Non-Pregnant Women from Date of Diagnosis to Distant Recurrence**

![Breast Cancer Recurrence-Free Survival Graph]

*Probability of survival vs. Years after follow-up*

- **P = 0.22**
- No parity after dx, 1/248
- Had parity after dx, 5/124
The Kaplan-Meier survival curve again did not show a significant difference when constructed from the date of ascertainment as a start point and until the date of distant recurrence as end point (Figure 4-7). We observed eight distant recurrences in both groups, pregnant and non-pregnant (p = 0.22). At 15-year survival rate was 85.0% for the pregnancy group and was 92.1% for the matched controls.

**Figure 4-7 Breast Cancer Recurrence-Free Survival in Pregnant and Non-Pregnant Women from Date of Ascertainment to Distant Recurrence**
The Kaplan-Meier survival curve from the date of childbirth of the index case until the date of distant recurrence did not show a significant difference (Figure 4-8). We observed seven distant recurrence in the pregnancy group and 14 in the non-pregnant group (p=0.85).

Figure 4-8 Breast Cancer Recurrence-Free Survival in Pregnant and Non-Pregnant Women: from Date of Last Childbirth to Distant Recurrence
4.3.2 Cox Proportional Analysis

Unadjusted Analysis

The 15-year unadjusted hazard ratio for breast cancer-specific mortality from the date of breast cancer diagnosis was 0.91 (95% CI 0.38 to 2.18; \( p = 0.83 \)) for all subjects in the pregnancy group when compared to non-pregnant matched controls. The 15-year unadjusted hazard ratio was 0.89 (95% CI 0.30 to 2.65; \( p = 0.83 \)) in women with pregnancy-associated breast cancer and was 0.93 (95% CI 0.27-3.17 \( p = 0.91 \)) in women with pregnancy following breast cancer, compared to matched controls. The unadjusted hazard ratio was 0.81 for \( BRCA1 \) carriers (95% CI 0.32 to 2.05). There were no deaths among \( BRCA2 \) carriers.

Adjusted Analysis

After adjustment for age at breast cancer diagnosis, chemotherapy use (yes/no), tumour size (<2 cm, 2-5 cm, >5 cm), lymph node status (-/+), estrogen receptor status and oophorectomy (-/+), the hazard ratio for mortality from the time of breast cancer diagnosis was 0.76 (95% CI 0.31 to 1.91) for pregnant versus non-pregnant cases. The adjusted hazard ratio was 0.72 for \( BRCA1 \) carriers pregnant versus non-pregnant cases (95% CI 0.27 to 1.90). The adjusted hazard ratio was 0.79 (95% CI 0.25 to 2.44) for the pregnancy-associated breast cancer sub-group and was 0.73 (95% CI 0.21 to 2.68) for the pregnancy following breast cancer sub-group (Table 4-6).
The mortality curves for women with a pregnancy associated breast cancer; pregnancy following breast cancer and, no pregnancy are compared in Figure 4-3.

We also looked at prognostic factors for breast cancer-specific survival in the entire study population. In a multivariate analysis tumour size and bilateral salpingo-oophorectomy were significant prognostic factors (Table 4-6). Those women with a tumour of size larger than 5.0 cm had a much greater risk of death than women with breast cancers of size less than 2.0 cm (adjusted HR = 8.98; 95% CI 1.32 – 61.6; p = 0.02). Women who underwent bilateral salpingo-oophorectomy had a much lower risk of death than women who had two ovaries intact (adjusted HR = 0.20; 95% CI 0.06 – 0.62; p = 0.006). Women who underwent bilateral salpingo-oophorectomy who received chemotherapy were less likely to die of their disease than women who did not receive chemotherapy (adjusted HR = 0.39; 95% CI 0.14 to 1.09), but his was not statistical significant (p = 0.07).
<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th></th>
<th>Multivariate**</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>(95% CI)</td>
<td>p-value</td>
<td>HR</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>Pregnancy after diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td></td>
<td>0.91</td>
<td>(0.38-2.18)</td>
<td>0.83</td>
</tr>
<tr>
<td>Yes</td>
<td>0.91</td>
<td>(0.38-2.18)</td>
<td>0.83</td>
<td>1</td>
<td>(0.31-1.91)</td>
</tr>
<tr>
<td>Pregnancy after diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Pregnancy</td>
<td>1</td>
<td></td>
<td>0.89</td>
<td>(0.30-2.65)</td>
<td>0.84</td>
</tr>
<tr>
<td>Pregnancy associated</td>
<td>0.89</td>
<td>(0.30-2.65)</td>
<td>0.84</td>
<td>0.79</td>
<td>(0.21-2.68)</td>
</tr>
<tr>
<td>Pregnancy following</td>
<td>0.93</td>
<td>(0.27-3.17)</td>
<td>0.91</td>
<td>0.73</td>
<td>(0.21-2.68)</td>
</tr>
<tr>
<td>Age at diagnosis (trend per year)</td>
<td>0.91</td>
<td>(0.82-1.01)</td>
<td>0.08</td>
<td>0.92</td>
<td>(0.82-1.04)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td></td>
<td>0.53</td>
<td>(0.22-1.29)</td>
<td>0.16</td>
</tr>
<tr>
<td>Yes</td>
<td>0.53</td>
<td>(0.22-1.29)</td>
<td>0.16</td>
<td>0.39</td>
<td>(0.14-0.09)</td>
</tr>
<tr>
<td>Bilateral salpingo-oophorectomy *</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td></td>
<td>0.19</td>
<td>(0.06-0.57)</td>
<td>0.003</td>
</tr>
<tr>
<td>Yes</td>
<td>0.19</td>
<td>(0.06-0.57)</td>
<td>0.003</td>
<td>0.20</td>
<td>(0.06-0.62)</td>
</tr>
<tr>
<td>Tumour size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 cm</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-5 cm</td>
<td>1.78</td>
<td>(0.51-6.33)</td>
<td>0.37</td>
<td>2.08</td>
<td>(0.55-7.96)</td>
</tr>
<tr>
<td>&gt; 5 cm</td>
<td>5.70</td>
<td>(1.03-31.4)</td>
<td>0.05</td>
<td>8.98</td>
<td>(1.32-61.6)</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1.67</td>
<td>(0.51-5.48)</td>
<td>0.40</td>
<td>1.79</td>
<td>(0.50-6.44)</td>
</tr>
<tr>
<td>Receptor status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER+</td>
<td>0.42</td>
<td>(0.05-3.39)</td>
<td>0.41</td>
<td>0.39</td>
<td>(0.04-3.63)</td>
</tr>
</tbody>
</table>

HR, Hazard Ratio; CI, Confidence Interval
* Oophorectomy is time dependent; ** all variables used in the regression.

When looking at prognostic factors at 15 years for breast cancer-specific survival in case subjects only (Table 4-7), the multivariate analysis of all co-variables (age at diagnosis, chemotherapy (yes/no), oophorectomy (yes/no), tumour size (<2, 2-5, >5 cm), lymph node status and estrogen receptor status (ER=/>ER-) did not achieve statistical significance.
**Table 4-7** Hazard Ratios at 15 years of Breast Cancer-Specific Mortality, Case Subjects ONLY

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th></th>
<th></th>
<th>Multivariate**</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>(95% CI)</td>
<td>p-value</td>
<td>HR</td>
<td>(95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age at diagnosis (trend per year)</td>
<td>0.98</td>
<td>(0.80-1.18)</td>
<td>0.80</td>
<td>0.95</td>
<td>(0.78-1.15)</td>
<td>0.57</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>(0.13-4.70)</td>
<td>0.80</td>
<td>1</td>
<td>(0.05-5.55)</td>
<td>0.60</td>
</tr>
<tr>
<td>Yes</td>
<td>0.79</td>
<td>(0.02-1.97)</td>
<td>0.17</td>
<td>1</td>
<td>(0.01-1.94)</td>
<td>0.15</td>
</tr>
<tr>
<td>Oophorectomy *</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>(0.06-7.74)</td>
<td>0.77</td>
<td>1</td>
<td>(0.07-25.5)</td>
<td>0.86</td>
</tr>
<tr>
<td>Yes</td>
<td>0.69</td>
<td>(0.00-NA)</td>
<td>1.00</td>
<td>1.30</td>
<td>(0.00-NA)</td>
<td>1.00</td>
</tr>
<tr>
<td>Tumour size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 cm</td>
<td>1</td>
<td>(0.09-14.1)</td>
<td>0.90</td>
<td>1</td>
<td>(0.10-37.9)</td>
<td>0.67</td>
</tr>
<tr>
<td>2-5 cm</td>
<td>1.13</td>
<td>(0.14-16.9)</td>
<td>0.74</td>
<td>3.51</td>
<td>(0.19-64.6)</td>
<td>0.40</td>
</tr>
<tr>
<td>&gt; 5 cm</td>
<td>1.51</td>
<td>(0.00-NA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HR, Hazard Ratio; CI, Confidence Interval

* Oophorectomy is time dependent; all variables used in the regression.
Chapter 5
5 Discussion

5.1 Summary

The results of this study are that survival of women who carry a BRCA1 or BRCA2 mutation and who become pregnant at the time of, or after a breast cancer diagnosis is not affected by the pregnancy. After adjustment for histopathological prognostic factors, the survival experiences of the non-pregnant and pregnant sub-groups in our study were similar. We anticipated that the high hormonal levels of pregnancy (53),(182) might lead to an inferior outcome in women known to carry a BRCA mutation with breast cancer and a pregnancy. However, the increment of gestational hormones does not appear to have stimulated the growth of breast cancer cells (171).

The patients in this study were all young (mean age of diagnosis 33.9 years); age of onset before age 40 is an adverse prognostic factor for breast cancer per se in the general population (183), but it is not clear if age of onset before age 40 is also a risk factor for mortality after breast cancer in the BRCA population (Huzarski, T 2013 submitted JCO).

Among pregnant women in this study, the mean tumour size was 2.5 cm; 40% were node-positive and 79% were ER-negative. Despite the high prevalence of adverse histopathological prognostic features, the 15-year survival rate for these patients was 93%. Women who have low-risk breast cancers may choose to become pregnant more often than women with poor prognostic cancers (‘healthy mother effect’) (14) but the distribution of prognostic features of
the cancers in the women who did and who did not become pregnant after breast cancer was similar.

The cancer patients in the study are a highly selected group. By definition, the women had to be alive and free of distant recurrence at the time of parturition in order to be eligible, and this will more likely select for survivors, which explains in part our excellent fifteen-year survival rate (91.5% for controls and 92.8% for cases). On average, 2.4 years had passed from diagnosis to birth. However, we also selected our controls to be alive and recurrence-free at the time of delivery of the baby in the matched case, so the survivorship bias should apply equally to cases and controls. We adjusted for potential survivorship bias by using a left-truncated survival analysis, wherein we only considered person-years in the follow-up period after the date of ascertainment (183). Finally, we compared survival three ways: from the date of breast cancer, from the date of parturition and from the date of ascertainment and for all three analyses the mortality experiences of the cases and comparison groups were similar with either endpoint (breast cancer-specific mortality or breast cancer recurrence).

5.2 Breast Cancer Characteristics

Pregnancy-associated breast cancer has no specific subtype, albeit according to the literature, they present more often with ER/PR negative, Her2+, high histological grade and lympho-vascular invasion. We observed similar pathological features in the pregnant and non-pregnant groups. We attributed this finding to the fact that all our subjects were known to carry a BRCA1/2 mutation. However, the pathological features of pregnancy-associated breast cancers
and BRCA-associated breast cancers overlap and, it is difficult to distinguish if pathological features are due to the gestational hormones or to the BRCA mutation per se.

Contrary to what is reported in the literature (55), the cancers detected in women who were pregnant at the time of breast cancer diagnosis (pregnancy-associated breast cancer) in our study were smaller. The tumour mean size was 23.7 mm for pregnancy-associated, 25.9 mm for pregnancy following breast cancer and 27.7 mm for women in the comparison group. According to the literature, tumours found during pregnancy are larger in size than non-pregnant women. This might be due to delays in diagnosis. The increased density of the breast tissue during pregnancy interferes with clinical examination and diagnostic image (146). Although differences in tumor size in our study did not achieve statistical significance (p = 0.12) we could attribute this finding to an earlier and frequent cancer screening routines practice in carriers than in non-carriers. Knowing to carry a BRCA1 and BRCA2 mutation raise awareness of breast cancer risk and promote compliance with breast and ovarian cancer screening strategies in both, patients and health care providers (69,184).

Thirty nine percent of case subjects and 43% of controls in our study had lymph nodes positive tumours (p = 0.63). Other studies have also reported no significant difference on the lymph node involvement between pregnant and non-pregnant groups. Shousha et al reported a high incidence of lymph node positive tumors in pregnant and non-pregnant women (78% and 90% respectively) and Genin et al, reported lymph node involvement in 59% of the non-pregnant group vs. 52% of the pregnancy-associated breast cancer group (55,56).

Twenty nine percent of women in the pregnancy-associated breast cancer subgroup presented with lymph node positive tumors in comparison with 54.2% of women in the pregnancy
following breast cancer subgroup \((p = 0.13)\) (**Table 4-3**). Although, the lower proportion of lymph node positives in women with pregnancy-associated breast cancer in our study did not achieve statistical significance, one could expect that this particular group, which is under the influence of high gestational hormones, would be more likely associated with lymph node positive than women who became pregnant a year after diagnosis. The number of case subjects was too small to draw any conclusion and this result may be due to chance.

In the multivariate analysis, breast cancer pathological characteristics examined in our study (tumour size, lymph node and estrogen receptor) among pregnancy subgroups (**Table 4-7**) did not achieve statistical significance and the confidence intervals obtained were wide, suggesting again that the sample size is too small. More research needs to be done on breast cancer pathological features among pregnancy subgroups.

Only 21% of the cases in our series had ER positive tumors. This percentage is slightly lower than that found in young women in the general breast cancer population. Maru et al. found that 45% of the young women (< 30 years old) had ER-positive tumors (185). ER positivity is strongly associated with later age at diagnosis and is more characteristic of postmenopausal women (56).

Elledge et al. evaluated the histology of pregnancy-associated breast cancers. Contrary to others studies, they reported half of tumors to be ER positive, 83% PR positive and 58% Her-2/neu positive. Here, hormonal receptors were detected more often by immunohistochemistry than by ligand-binding assay on the same tumor specimens. The researchers concluded that high circulating estrogens typical of pregnancy might interfere with the ligand-binding assay (60).
We found a higher proportion of \textit{BRCA1} mutation carriers than \textit{BRCA2} mutation carriers (81\% vs. 19\%). Johansson et al. reported that women with \textit{BRCA1} mutations were 3.9 times more likely to present with a pregnancy-associated breast cancer [95\% CI 1.4–10.8] than \textit{BRCA2} mutation carriers (OR =1.9 [95\% CI 0.5–7.0]) (18). This might explain the similarity on histopathological findings between \textit{BRCA1}-breast cancers and pregnancy-associated breast cancers. Pathological features of \textit{BRCA2}-breast cancers, instead, are comparable to breast cancers seen in the general population. It is also known that \textit{BRCA2}-associated breast cancers develop at later age than \textit{BRCA1}-associated breast cancers whereby fertility capability has diminished and a pregnancy coexisting with a diagnosis of breast cancer is less likely to occur.

5.3 Breast Cancer Survival

We found no evidence for an effect of pregnancy on mortality after a diagnosis of breast cancer in \textit{BRCA1} or \textit{BRCA2} carriers. Former studies on mortality after pregnancy-associated breast cancer in the general population were inconsistent (Appendix 1). Breast cancer diagnosed shortly after delivery has been associated with a worse prognosis (148), but if a pregnancy occurs long after diagnosis it is associated with a neutral or a protective effect (10,11,172).

However, three important published meta-analysis aimed to study the impact of pregnancy-associated breast cancer and pregnancy following breast cancer on survival have elucidated this uncertainty in the general population (57,118,172). The first meta-analysis included 30 studies aimed to examine only the impact of pregnancy-associated breast cancer (breast cancer diagnosed during pregnancy or within one year postpartum) on survival and risk of breast cancer.
recurrence (172). They found that pregnancy-associated breast cancer has a poor prognosis and a higher risk of recurrence compared to controls (women with no pregnancy after breast cancer diagnosis). Furthermore, women diagnosed during puerperium had poorer overall survival (pHR 1.81) than those diagnosed during pregnancy (pHR 1.30). This difference has been attributed to physiological changes of the breast tissue microenvironment during breast involution (186). The second meta-analysis included 14 studies conducted exclusively in women with pregnancies following breast cancer (pregnancy occurring one year after breast cancer diagnosis). By contrast, the researchers reported that pregnancy two or more years after diagnosis conferred a high mortality reduction (pooled RR: 0.55) compared to controls (women with no pregnancy after breast cancer diagnosis) (118). The third meta-analysis examined nine studies on both pregnancy-associated breast cancer and pregnancy following breast cancer. This study found a statistically higher overall survival in women with a pregnancy following breast cancer compared to non-pregnant women (controls): fixed effect model estimated pooled hazard ratio for death 0.51 (95% confidence interval: 0.42–0.62). No study heterogeneity was observed: $Q = 10.4$, $P = 0.17$; $I^2 = 48\%$. These pooled studies indicate that pregnancy occurring at least 10 months after diagnosis does not affect prognosis and that sometimes may improve survival (57).

The recommendations to BRCA carriers in regards to whether it is prudent to become pregnant after breast cancer and regarding the management of pregnancy-associated breast cancers are not established. To date, there are no specific recommendations for women who carry a $BRCA1$ or $BRCA2$ mutation; thus, recommendations for carriers are the same as for non-carriers. The Society of Obstetricians and Gynaecologist of Canada (SCOG) recommends to postpone
pregnancy for three years after diagnosis but if lymph nodes are involved, they extend this period to five years (117).

In this study, we found that carriers in the pregnancy group who underwent oophorectomy had a significant reduction in the risk of dying of their breast cancer (HR = 0.20; 95% CI: 0.06 – 0.62 p = 0.006). Carriers also have an increased risk of a second primary breast cancer and of a primary ovarian cancer and therefore, salpingo-oophorectomy are now considered a standard of care for women with breast cancer and a BRCA mutation. In support of this, we recently reported that BRCA1 mutation carriers with breast cancer in Poland who had an oophorectomy (before or after diagnosis) experienced a relative mortality reduction of 70%. Oophorectomy reduces deaths not only from a second primary breast cancer but also from ovarian cancer (Metcalf K, 2013 submitted to JCO). Because an oophorectomy renders a woman infertile, it is important to discuss the risks of delaying oophorectomy with breast cancer patients who wish to have a baby. We also found a strong reduction in mortality associated with chemotherapy in the study group (HR 0.39 CI = 0.14-0.09 p = 0.07) and therefore we recommend that women who wish to become pregnant after a diagnosis of breast cancer in a BRCA1 carrier do not forego chemotherapy or delay it. In a recent, paper we reported that 90% of women less than 35 years of age with a BRCA mutation who received chemotherapy resumed menses (Valentini A, 2013 submitted to JCO). In the present study, 83.5% of the women who became pregnant after breast cancer had received chemotherapy and this proportion was similar to that of women who did not become pregnant (82.4%).
5.4 Significance

BRCA mutation carriers often develop breast cancer at early age. This group of women may have a higher risk of presenting with a pregnancy-associated breast cancer or a pregnancy following breast cancer. Many breast cancer survivors want to have a baby after breast cancer treatment. They wish to know what is the risk of their cancer recurring or the impact of delaying risk-reducing strategies. Indeed, within our genetic clinics these queries are very common.

Our study has shown that pregnancy has not had an adverse effect on survival of women who carry a BRCA1/2 mutation. The results of this study will help to provide more accurate information on breast cancer risk and survival to those carriers in childbearing age with a personal history of breast cancer as well as assist them in the process of decision-making and family planning. The information will be directly relevant to patients and physicians (see section 5.6).

5.5 Limitations of the Study

There are several limitations to our study. First our results are based on retrospective information obtained from women who opted for BRCA1 and BRCA2 genetic testing. A randomized design is not feasible because of the limited number of women who chose to become pregnant after breast cancer and because some women become pregnant by chance. Genetic testing for BRCA1/2 was only available after 1995. Women who survived treatment and feel healthy are more willing to carry a pregnancy. Using matching technique and adjusting for covariates such as tumour characteristics (size, lymph node status and estrogen receptor), and a left-truncated adjusted
survival analysis to censor for those women who entered the cohort after the date of breast cancer diagnosis have all been used to reduce potential survivorship bias.

Secondly, the sample size of exposed women was small (n = 128 pregnancy associated and pregnancy following breast cancer) but considering that very few breast cancer patients carry a \textit{BRCA} mutation and even fewer are associated with pregnancy, this combination is a very rare event and ours is the only study to date to address the association. We hypothesized that pregnancy during or after breast cancer increase mortality in \textit{BRCA} carriers however we did not find a statistically significant difference. Subgroups were small and we could be at risk of a false-negative result (Type II error). We increased the power of the study for the main analyses by combining women with \textit{BRCA1} mutations and \textit{BRCA2} mutations and women with pregnancy-associated breast cancers and pregnancy following breast cancers.

Thirdly, we were able to retrieve pathology reports for only one-half of the index cases and this limited our ability to adjust for cofactors, but an analysis of the samples with available pathology data indicated that the cancer features were similar for case subjects and comparison subgroups. Missing pathology reports may reduce the representation of our sample and can lead to distorted inferences about the population. Imputation is a statistical approach use to prevent missing data from interfering with the confidence of the study results.

Fourthly, subjects were enrolled from 57 countries, and treatment was not standardised. Chemotherapy regimens vary among Institution. The use of different chemotherapy agents, dosage and regimes could affect survival and fertility (187). Some obtain a greater survival benefit by increasing the intensity of the dose, whereas others lower it to diminish toxicity (188). Wood et al. conducted a randomized trial using three different levels of doses and dose intensity
(dose per unit of time) of adjuvant chemotherapy in 1572 women with node-positive, stage II breast cancer with CAF (cyclophosphamide, doxorubicin, and 5-FU). They found that women treated with high or moderate dose intensity had significantly longer disease-free survival (P < 0.001) and overall survival (P = 0.004) than those treated with low dose intensity. Increasing dose intensity regimens like this are not widely accepted due to the risk of bone-marrow toxicity. In another study, comparing adjuvant chemotherapy with cyclophosphamide, methotrexate, and fluorouracil (CMF) and surgery alone, chemotherapy dose was reduced if signs of toxicity appeared or in older patients. They found that patients receiving <85% of the dose had poorer survival (189). The Early Breast Cancer Trialists' Collaborative Group (EBCTCG) presented a meta-analysis of treatment with polychemotherapy where randomized studies suggest that CMF alone compared to anthracycline based regimens resulted in lower risk of recurrence and mortality (69% vs 72% 5-year survival) in the anthracycline group (188).

5.6 **Strengths of the Study**

This is the first study which aimed to examine the impact of pregnancy-associated breast cancer and pregnancy following breast cancer exclusively in women who carry a *BRCA*1 and *BRCA*2 mutations. The parent cohort study (where our participants were drawn from) is one of the few multicenter international cohorts in the world capable to provide sufficient number of carriers to analyze this population in particular.
The study design carefully controlled for self-selection and survivorship bias. We achieved a well-matched cohort with almost no differences among pregnant and non-pregnant groups. We used a left-truncated approach and three-ways start points to control for survivorship bias.

The results of this study will provide more robust data on the effect of pregnancy on survival and disease-free survival to health care providers involved in the care and management of this high-risk population. Information released on important clinical questions such as “Can I get pregnant after breast cancer?” or “What are my chances of breast cancer relapse?” will now be based on scientific evidence from a study performed exclusively in carriers and not by translating outcomes of studies conducted in the general population.

5.7 **Recommendations**

1.) Pregnancy following breast cancer in women who carry a *BRCA1* or a *BRCA2* mutation does not appear to affect survival. *BRCA* mutation carriers in childbearing age who wish to have a baby can safely pursue a pregnancy.

2.) *BRCA* mutation carriers with a personal history of breast cancer who are considering becoming pregnant should also consider the impact of delaying the benefits of breast cancer therapy. Oophorectomy reduces the risk of breast cancer recurrence up to approximately 80%. Women with breast cancer should be advised to have their ovaries removed as soon as childbearing has been completed.
3.) Primary caregivers, Obstetricians, Medical Oncologist and Genetic Counselors should be aware of the impact of a pregnancy in a woman who carries a BRCA mutation. They might inform carriers that, according to the best available evidence, having children after breast cancer will not decrease their survival. Also, they should ensure appropriate timelines of risk-reducing strategies are taken to improve prognosis of breast and ovarian cancer.

5.8 Conclusion

In conclusion, pregnancy at the time of breast cancer diagnosis or after breast cancer diagnosis does not appear to adversely affect survival among BRCA1/2 mutation carriers. If the woman chooses to retain her ovaries after breast cancer treatment, then she should not be advised against pregnancy. However, patients and physicians must also consider the potential therapeutic benefit of oophorectomy in this context.

5.9 Recommendations for Further Research

The inability to conduct a randomized clinical trial combined with the rareness of a pregnancy-associated or pregnancy following breast cancer in carriers creates difficulties to reach a sufficient sample size to strengthen the confidence of our results. We have one of the few databases in the world with a sufficient number of BRCA carriers to answer this important clinical question. To optimize outcomes, future research should focus on increasing the sample size, perhaps by merging with other databases or applying other statistical resources. Imputation
deals with missing data by accounting for it (190). We did not apply an imputation approach but it could be an option for future research.

We hope this study will open insight to further research in order to provide better care to this vulnerable patient population. Fertility preservation in carriers prior to and after breast cancer treatment, survival of pregnancy-associated breast cancer in carriers compare to non-carriers, evaluation of physicians’ opinion and understanding of gestational breast cancer in women at high-risk of pregnancy-associated breast cancer are an example of unexplored themes.

5.10 Dissemination Strategy

While the study was ongoing, the graduate student monitored published and unpublished information on pregnancy in BRCA1/2 carriers. The graduate student and the principal investigator attended the following meetings:


2. The annual conference of FORCE (Facing Our Risk of Cancer Empowered), Florida US. September 2012. Oral presentation


5. 10th Annual Graduate Student Research Day Women’s College Hospital. Proposal discussion. University of Toronto May 16th, 2011. **Oral Presentation**


FORCE is a non-profit organization dedicated to inform and update the cancer genetic community, scientists, physicians, genetic counsellors, and the general public in regards to cancer genetics and hereditary breast and ovarian cancer.

As a graduate student I have presented the background and preliminary results of this study in seminars, presentations and posters at Women’s College Hospital and the University of Toronto. Following the thesis defense, the results will be published in a peer-reviewed journal. The study results will also be presented at international meetings / conferences to inform the wider genetic, medical and scientific community.
References or Bibliography


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126. Cullins SL, Pridjian G, Sutherland CM. Goldenhar’s syndrome associated with tamoxifen given to the mother during gestation. JAMA ☐ : the journal of the American Medical


115


<table>
<thead>
<tr>
<th>Publication (Author)</th>
<th>Year</th>
<th>Study design</th>
<th>Population age</th>
<th>Sample size</th>
<th>Definition of PABC/PFBC</th>
<th>Outcome</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ali SA et al. (Magee-Women’s Hospital of University of Pittsburgh Medical Center; UPMC, USA) Ref (163)</td>
<td>2012</td>
<td>Retrospective Case-control Study</td>
<td>Women of reproductive ages. Mean age 33 years</td>
<td>40 cases and 40 controls</td>
<td>Women diagnosed breast cancer during pregnancy and 48 weeks post partum. Abortions were included</td>
<td>OS DFS</td>
<td>PABC vs. non-PABC OS = 4.9 vs. 6.0 years (p = 0.02). DFS = 2.7 vs. 5.1 years (p = 0.01). Significant shorter OS and DFS in PABC compared to non-PABC</td>
</tr>
<tr>
<td>Anderson BO et al. (Memorial Sloan-Kettering Cancer Center (MSKCC), NY, USA) Ref (109)</td>
<td>1996</td>
<td>Retrospective Case-control Study</td>
<td>Women &lt; 30 years. Mean age 28 years</td>
<td>22 cases and 205 controls</td>
<td>PABC: standard definition Breast cancer diagnosed during pregnancy or 1 year post-partum.</td>
<td>OS DFS</td>
<td>DFS was decreased in PABC compared to non-PABC (p = 0.0004). OS for stage I-II were the same among PABC and non-PABC (73% vs. 74% 10-year survival) but stage IIIA had shorter DFS and OS when associated with pregnancy (0% vs. 35% 10-year survival).</td>
</tr>
<tr>
<td>Azim HA Jr. et al. (European Institute of Oncology (IEO) in Milan, Italy) Ref (191)</td>
<td>2011</td>
<td>Retrospective Case-control Study</td>
<td>Women &lt; 50 years of age. Mean age 36 years</td>
<td>65 cases and 130 controls</td>
<td>Women diagnosed breast cancer during pregnancy. Does not include post partum. Abortions were included</td>
<td>OS DFS BR specific DFS</td>
<td>DFS (5-year, 52.1% vs. 74.3%; p = 0.01) and BRDFS (5-year, 56.6% vs. 74.3%; p = 0.04). OS (5-year OS, 79.6% vs. 88.4%; p = 0.17). DFS and BRDFS were statistically significant but OS did not achieve statistical significance</td>
</tr>
<tr>
<td>Azim HA Jr. et al. (European Institute of Oncology [Milan], Jules Bordet Institute [Brussels], Vall D’Hebron University Hospital [Barcelona], Macerata Hospital and La Paz University Hospital [Madrid], Danish Breast Cancer Cooperative</td>
<td>2012</td>
<td>Multicenter Retrospective Cohort</td>
<td>Women younger than 50 years old. Mean age 32 years</td>
<td>333 cases and 874 controls</td>
<td>Women diagnosed BC that became pregnant any time after diagnosis. Mean time from BC to Pregnancy: 4.7 years in cases and controls</td>
<td>DFS OS according to ER status</td>
<td>No difference in DFS was observed in ER-positive BC (HR = 0.91; P = 0.55). All patients’ irrespective ER status (HR= 0.84; 95% P = 0.14). Pregnant group showed a better OS (HR = 0.72; P = 0.03), with no interaction with ER status (P = 0.11)</td>
</tr>
<tr>
<td>Group</td>
<td>Authors and Source</td>
<td>Year</td>
<td>Study Design</td>
<td>Population</td>
<td>Cases/Controls</td>
<td>Inclusion Criteria</td>
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</tr>
<tr>
<td>-------</td>
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<tr>
<td>Beadle BM et al. (M.D Anderson Cancer Centre, USA) Ref (147)</td>
<td>2009</td>
<td>Retrospective Case-control Study</td>
<td>Included only women ≤ 35 years. Mean age 26 years</td>
<td>104 cases, 564 controls</td>
<td>PABC: standard definition Breast cancer diagnosed during pregnancy or 1 year post-partum</td>
<td>OS, LRR, Distant metastasis</td>
<td></td>
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<tr>
<td>Bladstrom A et al. (University Hospital, Sweden) Ref (165)</td>
<td>2003</td>
<td>Population-based Study</td>
<td>Women ≤ 45 years. Mean age 34 years</td>
<td>Of 14,693 BC 49 PABC cases</td>
<td>Women diagnosed breast cancer during a pregnancy (9 months). Women with BC before a pregnancy were excluded</td>
<td>OS</td>
<td></td>
</tr>
<tr>
<td>Blakely LJ et al. (M.D Anderson Cancer Centre, USA) Ref (93)</td>
<td>2003</td>
<td>Retrospective Case-Control Study</td>
<td>Women ≤ 35 years. Mean age not specified.</td>
<td>47 cases and 323 controls</td>
<td>Pregnancy after BC treatment. Time elapsed from BC to pregnancy was not specified. Abortions were included</td>
<td>HR</td>
<td>RFS</td>
</tr>
<tr>
<td>Bonnier P et al. (Marseille Public Hospital System, France) Ref (148)</td>
<td>1997</td>
<td>Retrospective Multi-Institutional Case-Control Study</td>
<td>Women in reproductive ages. Mean age 34.2 years</td>
<td>154 cases and 308 controls</td>
<td>PABC: breast cancer diagnosed during pregnancy or 6 months post-partum</td>
<td>OS</td>
<td>DFS</td>
</tr>
<tr>
<td>Cardonick E et al. (Cooper University Hospital, University of Medicine and Dentistry of New Jersey, Camden, USA) Ref (155)</td>
<td>2010</td>
<td>Prospective Cohort Study</td>
<td>Mean age 34.8 years</td>
<td>130 cases</td>
<td>PABC: standard definition Breast cancer diagnosed during pregnancy or 1 year post-partum</td>
<td>OS</td>
<td>DFS</td>
</tr>
<tr>
<td>Cordoba O et al. (Service of Gynecology, Hospital Vall d’Hebron, Barcelona, Spain) Ref (94)</td>
<td>2011</td>
<td>Retrospective Case-Control Study</td>
<td>Women &lt; 36 years. Mean age 35 years</td>
<td>18 cases (8 out the 18 were</td>
<td>Pregnancy after breast cancer treatment mean time from BC to OS</td>
<td>DFS</td>
<td>5-years survival rate 100% in the pregnant group and 80% in the non-pregnant group. 5-years DFS was 94% and 64%, respectively (P=0.009). Pregnancy does not adversely affect prognosis</td>
</tr>
<tr>
<td>Study</td>
<td>Year</td>
<td>Study Design</td>
<td>Cohort Characteristics</td>
<td>Outcomes</td>
<td></td>
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<tr>
<td>Cooper DR et al. (Women’s Medical College of Pennsylvania, USA) Ref(192)</td>
<td>1970</td>
<td>Retrospective Case-Control Study</td>
<td>Women &lt; 40 years. Mean age not specified; 32 cases and 64 controls, Women who became pregnancy within 5 years after mastectomy</td>
<td>OS DFS 5-years OS for PABC was 75% and for non-PABC was 61%</td>
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<tr>
<td>Ezzat A et al. (King Faisal Specialist Hospital and Research Centre, Riyadh, Kingdom of Saudi Arabia Ref(161)</td>
<td>1996</td>
<td>Retrospective Case-Control Study</td>
<td>Women between 20 to 45 years; 28 cases and 84 controls, Women with primary breast cancer diagnosed during pregnancy who had no distant metastases</td>
<td>OS DFS There was non-significant difference in OS (P = 0.86) and relapse-free survival (P = 0.48) between the two groups. PABC does not adversely affect survival.</td>
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<tr>
<td>Garcia-Manero M et al (University of Navarra, Pamplona, Spain) Ref(193)</td>
<td>2008</td>
<td>Retrospective Descriptive Study</td>
<td>Women between 20 to 46 years. Mean age 34 years; 22 cases, Women diagnosed breast cancer during pregnancy and lactation</td>
<td>Fetal and Maternal outcome Perinatal Mortality 0%. Maternal Mortality 22.7% (5/22). All 5 died of breast cancer.</td>
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<tr>
<td>Gelber S et al. (Dana-Farber Cancer Institute, USA) Ref(131)</td>
<td>2001</td>
<td>Retrospective Case-Control Study</td>
<td>Women’s age not specified; 94 cases and 188 controls, Women diagnosed breast cancer during pregnancy or thereafter</td>
<td>OS 5- and 10-year OS for cases were 92% and 86% respectively, 5- and 10-year OS for the matched comparison group were 85% and 74% respectively (HR = 0.44; 95% CI = 0.21 - 0.96; P = .04). Pregnancy does not adversely affect survival.</td>
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<tr>
<td>Guinee VF et al. (M.D Anderson Cancer Centre, USA) Ref(162)</td>
<td>1994</td>
<td>Retrospective Case-Control Study</td>
<td>Women &lt; 30 years; 66 PABC 86 PFBC and 139 controls, PABC and PFBC standard definitions</td>
<td>OS DFS The RR of dying of the pregnant group is 3.26 compare to non-pregnant group. Increased risk of mortality in breast cancer patients with concurrent or recent previous pregnancy</td>
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<tr>
<td>Halaska M et al. (University in Prague, Prague; FN Motol, Prague, Czech Republic; Ioannina University Hospital, Ioannina, Greece) Ref(156)</td>
<td>2009</td>
<td>Retrospective Case-Control Study</td>
<td>Median age 33.7 years (range from 25 to 41 years); 32 cases and 32 controls, PABC standard definition. Abortions were included.</td>
<td>OS DFS Overall survival was similar in PABC and non-PABC patients (p = 0.45). DFS was shorter in women with a pregnancy within a year after delivery (p = 0.0178)</td>
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</table>

"abortion" and "controls" indicate the number of cases and controls in each study.

"Pregnancy 44.5 months (range:10-84)" indicates the average pregnancy duration.

"5-years OS for PABC was 75% and for non-PABC was 61%" indicates the survival rate for different groups.

"There was non-significant difference in OS (P = 0.86) and relapse-free survival (P = 0.48) between the two groups. PABC does not adversely affect survival." indicates the statistical analysis results.

"Pregnancy does not adversely affect survival." indicates the conclusion drawn from the study.

"Overall survival was similar in PABC and non-PABC patients (p = 0.45). DFS was shorter in women with a pregnancy within a year after delivery (p = 0.0178)" indicates additional findings from the study.
<table>
<thead>
<tr>
<th>Study Reference</th>
<th>Year</th>
<th>Study Design</th>
<th>Sample Description</th>
<th>Key Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibrahim EM et al. (King Faisal Specialist Hospital and Research Centre, Saudi Arabia) Ref(157)</td>
<td>2000</td>
<td>Retrospective Case-Control Study</td>
<td>Women with the diagnosis of breast cancer</td>
<td>72 cases</td>
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<tr>
<td>Ishida T et al. (Gunma University School of Medicine, Japan) Ref(166)</td>
<td>1992</td>
<td>Retrospective Case-Control Study</td>
<td>Women with the diagnosis of breast cancer</td>
<td>192 cases and 191 controls</td>
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<tr>
<td>Ives A et al. (University of Western Australia, Australia) Ref(95)</td>
<td>2006</td>
<td>Population-based study</td>
<td>Women &lt; 45 years old (15-44), PABC median age 31, PFBC median age 35</td>
<td>Of 2,539 BC, 123 were pregnant during or after BC diagnosis</td>
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<tr>
<td>Johansson A et al. (University of Massachusetts Medical School, Worcester, USA; Karolinska University Hospital, Stockholm and Regional Oncologic Center, Uppsala, Sweden) Ref(167)</td>
<td>2012</td>
<td>Population-base Cohort Study</td>
<td>Women between 15-44 years. Mean age</td>
<td>1,110 cases and 14,611 controls</td>
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<tr>
<td>Kranick, JA (Columbia University, NY; and Kaiser Permanente Northern California, Oakland, CA; USA) Ref(154)</td>
<td>2010</td>
<td>Retrospective Case-Control Study</td>
<td>Mean age of diagnosis 32 for cases and 34 for controls</td>
<td>107 cases and 344 controls</td>
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<tr>
<td>Kroman N et al. (Statens</td>
<td>1997</td>
<td>Population-based study</td>
<td>Women ≤ 45</td>
<td>Of 5,725 Women gave birth</td>
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<tr>
<td>Study</td>
<td>Design</td>
<td>Eligible Women</td>
<td>Number</td>
<td>PFBC: Pregnancy after treatment of breast cancer (median time = 39 months). Abortions included</td>
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<tr>
<td>Serum Institut and Rigshospitalet, Copenhagen Denmark) Ref (46)</td>
<td>base Cohort Study</td>
<td>years</td>
<td>women with BC 173 had PFBC</td>
<td>10 months before diagnosis and after. Abortions were included</td>
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<tr>
<td>Kroman N et al. (Statens Serum Institut and Rigshospitalet, Copenhagen Denmark) Ref(96)</td>
<td>Population-base Cohort Study</td>
<td>Women ≤ 45 years</td>
<td>Of 10,236 women with BC 371 had PFBC</td>
<td>PFBC: pregnancy after treatment of breast cancer (median time = 39 months). Abortions included</td>
</tr>
<tr>
<td>Largent A et al. (Epidemiology Division, Department of Medicine, University of California, Irvine, Irvine, California, USA) Ref (194)</td>
<td>Population-based, Case–Case study</td>
<td>Women ≤ 35 years</td>
<td>Pregnancy in women with early-onset breast cancer</td>
<td>OS</td>
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<tr>
<td>Largillier R et al. (Centre Azuréen de Cancérologie, France) Ref (158)</td>
<td>Retrospective case-control, cohort</td>
<td>Women ≤ 35 years. Mean age 32 years</td>
<td>Total 908 cases and 708 controls, PFBC = 118 cases and 762 controls</td>
<td>Pregnancy within 1 year before the breast cancer diagnosis, and subsequent pregnancy corresponded to a pregnancy occurring after the treatment period.</td>
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<tr>
<td>Lethaby AE et al. (Departments of Endocrinology and Oncology, Auckland Hospital, Auckland, New Zealand) Ref (99)</td>
<td>Case-Control Study</td>
<td>Women ≤ 45 years</td>
<td>835 cases 430 pregnant at the time of diagnosis, “Pregnancy status at diagnosis” and “lactation status at diagnosis” were defined as pregnant or breast-feeding at</td>
<td>OS</td>
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<tr>
<td>Study, Reference</td>
<td>Year</td>
<td>Study Design</td>
<td>Eligibility Criteria</td>
<td>Number and Type of Participants</td>
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<tr>
<td>Mignot L et al. (Société Française de Gynécologie)</td>
<td>1986</td>
<td>Case-Control Study</td>
<td>Women in childbearing age</td>
<td>68 cases and 136 controls</td>
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<td>Moreira (Clinical Oncology Service at Hospital da Santa Casa de Misericordia, Belo horizonte, Minas Gerais, Brazil)</td>
<td>2010</td>
<td>Retrospective Case-Control Study</td>
<td>Women ≤ 45 years</td>
<td>87 cases and 252 controls</td>
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<tr>
<td>Mueller BA et al. (University of Washington, USA)</td>
<td>2003</td>
<td>Retrospective Case-Control Study</td>
<td>Women &lt; 45 years</td>
<td>438 cases and 2775 controls</td>
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<tr>
<td>Murphy CG et al. (Memorial Sloan-Kettering Cancer Center, NY, USA)</td>
<td>2012</td>
<td>Retrospective Case-Control Study</td>
<td>Women ≤ 48 years</td>
<td>99 cases and 186 controls</td>
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<td>Nugent (Kaiser Permanente Medical Center, Los Angeles, USA)</td>
<td>1985</td>
<td>Hospital-based</td>
<td>Women ≤ 40 years of age</td>
<td>19 cases and 155 controls</td>
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<td>Petrek JA et al. (Memorial Sloan-Kettering Cancer Center, USA)</td>
<td>1991</td>
<td>Retrospective case-control, cohort</td>
<td>Not specified</td>
<td>56 cases</td>
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<td>Rippy EE et al. (Royal Surrey County Hospital, UK)</td>
<td>2009</td>
<td>Retrospective Case-Control Study</td>
<td>Women ≤ 45</td>
<td>304 cases</td>
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<tr>
<td>Authors</td>
<td>Year</td>
<td>Design</td>
<td>Population</td>
<td>Cases</td>
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<tr>
<td>Rodriguez AO et al. (University of California, USA) Ref (150)</td>
<td>2008</td>
<td>Retrospective case-control, population</td>
<td>Women ≤ 55 years</td>
<td>797 cases</td>
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<tr>
<td>Sankila R et al. (Finnish Cancer Registry, Helsinki, Finland) Ref (14)</td>
<td>1994</td>
<td>Population-based matched survival study</td>
<td>Women &lt; 40 years old</td>
<td>91 cases and 471 controls</td>
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<tr>
<td>Smith LH et al. (University of California, USA) Ref (198)</td>
<td>2003</td>
<td>Population-based Retrospective Cohort Study</td>
<td>Women in childbearing age</td>
<td>935 (246 during pregnancy and 663 postpartum) BC (423 cases, 0.13 per 1000)</td>
</tr>
<tr>
<td>Stensheim H et al. (Norwegian Radium Hospital, Rikshospitalet University Hospital, Montebello and Faculty Division the Norwegian Radium Hospital, University of Oslo, Oslo, Norway) Ref (3)</td>
<td>2009</td>
<td>Population-based Cohort Study</td>
<td>Women age 16 to 49</td>
<td>Total 43,900 59 during pregnancy 46 lactating 138 post-cancer 13,211 non-pregnant (controls)</td>
</tr>
<tr>
<td>Tretli S et al. (The Cancer Registry of Norway, Institute for Epidemiological Cancer Research, Montebello,</td>
<td>1988</td>
<td>Retrospective Case-Control, Study</td>
<td>Women ≤ 45 years</td>
<td>20 Pregnant during BC, 15 lactating</td>
</tr>
<tr>
<td>Study</td>
<td>Year</td>
<td>Study Design</td>
<td>Methodology</td>
<td>Number</td>
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<tr>
<td>Oslo, and The Norwegian Radium Hospital, Montebello, Oslo, Norway. Ref (149)</td>
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<tr>
<td>Velentegas P et al. (University of Washington, USA) Ref (98)</td>
<td>1999</td>
<td>Retro and Prospective Cohort Study</td>
<td>Women ≤ 40 years</td>
<td>53 cases and 265 matched controls</td>
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<tr>
<td>Verkooijen HM et al. (The Singapore Birth Register National University of Singapore and Karolinska Institute and Swedish Multi-Generation Register) Ref (199)</td>
<td>2010</td>
<td>Population-based Cohort Study</td>
<td>Women ≤ 40 years</td>
<td>492 cases 8529 controls</td>
</tr>
<tr>
<td>von Schoultz E et al. (Karolinska Hospital, Stockholm, Sweden) Ref (152)</td>
<td>1995</td>
<td>Retrospective Case-Control, Study</td>
<td>Women ≤ 45 years</td>
<td>173 before BC and 50 after BC</td>
</tr>
<tr>
<td>Zemlickis D et al. (The Research Institute of the Hospital for Sick Children, Toronto, ON, Canada). Ref (146)</td>
<td>1992</td>
<td>Retrospective Case-Control, Study</td>
<td>Women 23 to 47 years</td>
<td>102 cases and 269 controls</td>
</tr>
</tbody>
</table>

Abbreviations: BC, breast cancer; PABC, pregnancy-associated breast cancer; PFBC, pregnancy-following breast cancer; OS, overall-survival; DFS, disease-free survival; RFS, recurrence-free survival; PFS, progression-free survival; LCC, loco-regional recurrence; SMRs standardized mortality ratios

1 Pregnancy-associated breast cancer, standard definition: Breast cancer diagnosed during or within one years of pregnancy.
2 Pregnancy-following breast cancer, standard definition: Pregnancy after the treatment of breast cancer
<table>
<thead>
<tr>
<th>Centre</th>
<th>[PI]</th>
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<th>%</th>
<th>Centre</th>
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<tbody>
<tr>
<td>1 Pomeranian Medical University, Szczecin, Poland [Jan Lubinski]</td>
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<td>63</td>
<td>15.8</td>
<td>30 Dana Farber Cancer Institute, Boston, MA, USA [Judy Garber]</td>
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<td>2 Creighton University School of Medicine, Omaha, NE, USA [Henry Lynch]</td>
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<td>23</td>
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<td>31 Los Angeles, Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, Beverly Hills, CA, USA [Beth Karlan]</td>
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<tr>
<td>3 The Ohio State University Medical Center Columbus, OH, USA [Leigha Senter]</td>
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<td>20</td>
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<td>32 Strang Cancer Prevention Centre, New York, NY, USA, [Michael Osborne]</td>
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<td>4 Montreal General Hospital, QC, Canada [William Foulkes]</td>
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<td>5 Women's College Hospital, Toronto, ON, Canada [Steven Narod]</td>
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<td>6 London Regional Cancer Program, London ON, Canada (LRCC) [Peter Ainsworth]</td>
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<td>35 New York, Beth Israel Medical Center, New York, NY, USA [Mary Kay Danby]</td>
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<td>7 British Columbia Cancer Agency, Vancouver, BC, Canada [Charmaine Kim-Sing]</td>
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<td>37 UT Southwestern Medical Center, Dallas, TX, USA [David Euhus]</td>
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<td>9 Princess Margaret Hospital, University of Toronto, ON, Canada [Barry Rosen]</td>
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<td>41 North York General Hospital, Toronto, ON, Canada [Wendy Meschino]</td>
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<td>13 Juravinski Cancer Centre, Hamilton, ON, Canada [Louise Bordeleau]</td>
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<td>42 Harvard Vanguard Medical Associates, Boston, MA, USA [Bita Tabesh]</td>
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<td>14 Genomic Medicine Institute and Center for Personalized Genetic Healthcare, Cleveland Clinic, Cleveland, OH, USA [Charis Eng]</td>
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<td>15 University of Chicago, Chicago, IL, USA [Olufunmilayo Olopade]</td>
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<td>44 Facing our Risk of Cancer Empowered (FORCE), FL, USA [Susan Friedman]</td>
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<td>Queen Elizabeth Health Sciences Centre, Halifax, Nova Scotia, Canada [Daniel Rayson]</td>
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<td>Mayo Clinic, Rochester, MN, USA [Fergus Couch]</td>
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<td>Mt. Sinai Hospital, Toronto, ON, Canada [Seema Panchal]</td>
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<td>Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria [Christian Singer]</td>
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<td>Moffitt Cancer Centre, Tampa, FL, USA [Tuya Pal]</td>
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<td>St. Mary Regional Cancer Center, PA, USA [Josephine Costalas]</td>
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<td>Institut Curie Hôpital, Institut Curie Centre de Recherche, Paris, France [Dominique Stoppa Lyonnet]</td>
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<td>The University of Hong Kong, Hong Kong [Ava Kwong]</td>
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<td>The Susanne Levy Gartner Oncogenetics Unit, Chaim Sheba Medical Center and the Sackler School of Medicine, Tel-Aviv University, Israel [Eitan Friedman]</td>
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<td>28</td>
<td>Northwestern University, Chicago, IL, USA [Taya Fallen]</td>
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<tr>
<td>29</td>
<td>Long Beach Memorial, Long Beach, CA, USA [Carey Cullinane]</td>
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**Total** 397 100
Appendix 3 Baseline Questionnaire

RESEARCH QUESTIONNAIRE FOR
A STUDY OF BREAST AND OVARIAN CANCER
IN HIGH RISK FAMILIES

This questionnaire is part of a research study to improve our understanding of the prevention and treatment of hereditary breast and ovarian cancers.

Name: _________________________

Date of Birth: ___/___/______   Age: ___

Date Completed: ___/___/______

133
SECTION I – Fertility History
We would like you to describe your pregnancy history, as this has been found to affect the risk of cancer in some women.

1. Have you ever been pregnant?
   □ No       →       Go to question 6.
   □ Yes

2. Please consider all pregnancies, in order, from first to last. Give year of pregnancy. Place an ‘X’ in the appropriate column for the outcome of each pregnancy. For live-born children try to recall for how long you breast-fed each child and indicate months of breast-feeding in the right column (if not breast-fed, enter ‘0’).

<table>
<thead>
<tr>
<th>PREGNANCY OUTCOMES</th>
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</tbody>
</table>

*(for twins, enter ‘T’ at the corresponding cell)*

3. How many of these pregnancies were caesarean sections? _________

Circle which pregnancies (1-9 in chart above): 1 2 3 4 5 6 7 8 9

4. Did you ever have difficulty breast-feeding?
   □ No
   □ Yes       →       Reason: __ poor milk production __ pain
                   __ premature infant __ mastitis (breast inflammation)
                   __ other (please specify): __________________
Circle which child (1-9 in chart above) you experienced this with:

1 2 3 4 5 6 7 8 9

5. Have you ever taken medication to stop milk production?
   - [ ] No
   - [ ] Yes → Name of medication (if known): _________________________
   Method: ___ injections or ___ pills
   Circle with which child (1-9 in preceding chart) you took this medication.

1 2 3 4 5 6 7 8 9

6. How old were you when you had your first menstrual period? _____ YEARS OLD

7. How would you describe your menstrual cycle? By regular we mean that the start of your period was predictable within 5 days. (Please check one)
   - [ ] My periods are/were always regular.
   - [ ] My periods are/were usually regular.
   - [ ] My periods are/were never regular.

8. How many days apart are your menstrual periods? That is, from the start of one period to the start of the next period. For example, many women have cycles of 28-32 days. (Please check one)
   __ 28 days   __ 29 days   __ 30 days   __ 31 days   __ 32 days   Other → ___ days

9. Do you currently have menstrual periods? That is, have you had a menstrual period within the last year? Please answer “Yes” if you are currently pregnant, breast-feeding or taking hormones which temporarily stop your menses (Mirena IUD or birth control pills).
   - [ ] No
   - [ ] Yes

10. Have your periods stopped completely?
    - [ ] No → Go to question 13.
    - [ ] Yes

11. How old were you when your periods stopped completely? _____ YEARS OLD

12. What was the reason your periods stopped? (Select one only)
    - [ ] Natural Menopause (change of life)
    - [ ] Hysterectomy (uterus removed/ovaries not removed)
    - [ ] Uterus and ovaries removed
☐ Oophorectomy (ovaries removed/uterus not removed)
☐ Medication / Chemotherapy
☐ Other (please specify): ________________________________

13. Have you ever seen a doctor for a problem of difficulty in getting pregnant or in carrying a pregnancy, such as several miscarriages?
   ☐ No → Go to question 16.
   ☐ Yes → What reason did the doctor give to explain why you had trouble getting or staying pregnant? (Please check all that apply.)
   __ no problem was found  __ problem with cervix problem
   __ problem with ovaries  __ partner has fertility problem
   __ problem with fallopian tubes  __ endometriosis
   __ other (please specify): ________________________________

14. Have you ever taken medication to increase your chances of becoming pregnant?
   ☐ No
   ☐ Yes → Name of medication(s): ________________________________
       For how many months did you take this medication? _____ months
       What years did you take this medication? ______, ______

15. Have you ever received fertility treatment such as in vitro fertilization/Embryo Transfer (IVF/ET) to help you get pregnant?
   ☐ No
   ☐ Yes → What type of treatment did you receive?
       ________________________________

16a. Have you ever used birth control pills, Norplant (implants), or Depo-Provera (injections) to prevent pregnancy or for any other reason?
   ☐ No → Go to question 17.
   ☐ Yes → Can you describe the times?

<table>
<thead>
<tr>
<th>Name of Medication</th>
<th>Starting Year</th>
<th>Ending Year</th>
<th>Length of time used</th>
<th>Method</th>
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<td>4</td>
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</tbody>
</table>
16b. Are you currently using birth control pills, Depo-Provera or Norplant?
- No
- Yes

17a. Have you ever taken hormone replacement therapy for menopause (i.e. estrogen, progesterone)?
- No  →  Go to question 18.
- Yes  →  Complete table below:

<table>
<thead>
<tr>
<th>Name of Hormone</th>
<th>Starting Year</th>
<th>Ending Year</th>
<th>Length of time used (Years)</th>
<th>Method</th>
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17b. Are you currently taking hormone replacement therapy?
- No
- Yes  →  Name of hormone: ___________________________  dose? ______________

SECTION II – Breast Cancer Diagnosis

18. Have you ever been diagnosed with breast cancer?
- No  →  Go to question 32 (Section III).
- Yes  →  Year of diagnosis: _________

Check one  →  I have had cancer in
- one breast
- both breasts
We would like to discuss your first diagnosis.

What year was it? __________

Month (if known)? __________

Which breast? □ Left □ Right

Type of Cancer (check one):
□ It was an invasive cancer.
□ It was a non-invasive cancer (early stage breast cancer).
   → Was it Ductal Carcinoma In-situ (DCIS)? □ Yes □ No
   → Was it Lobular Carcinoma In-situ (LCIS)? □ Yes □ No
□ It was both invasive and non-invasive.
□ I’m not sure.

Treatment
Did you have surgery for the first breast cancer?
40. □ No
 □ Yes → Year of surgery: __________
   What type of surgery did you have?
   Check one → □ Lumpectomy (tumour removed, breast remains)
   □ Mastectomy (one breast removed)
   □ Bilateral mastectomy (both breasts removed)

41. Detection
How was the lump first detected?
   Check one → □ Only by mammography screening
   □ Did you feel a lump?
   □ Did the doctor feel a lump?
   □ MRI screening
   □ Other: _______________

19. Did you receive chemotherapy for the first breast cancer?
□ No
 □ Yes

20. Did you receive radiation therapy for the first breast cancer?
□ No
 □ Yes

21. Did you receive Tamoxifen (Nolvadex) for the first breast cancer?
□ No
 □ Yes → Number of pills per day: __________
   From: __________ (year) to __________ (year)
22. Did you receive any other drugs for the first breast cancer?
   [ ] No
   [ ] Yes → Which drug?
   [ ] Femara (letrozole)
   [ ] Aromasin (exemstane)
   [ ] Arimidex (anastrazole)
   [ ] Other: ________________________
   Number of pills per day: ______
   From: __________ (year) to __________ (year)

23. Have you had a recurrence of your first breast cancer?
   [ ] No
   [ ] Yes → Date: __ __ / __ __ / ______ (mm / dd / yyyy)
   Site of recurrence: ________________________________

24. Have you had further surgery in the affected breast (such as complete mastectomy or reconstruction)?
   [ ] No
   [ ] Yes → Date: __ __ / __ __ / _____ (mm / dd / yyyy)
   Type of surgery: ________________________________

IF YOU DID NOT HAVE A SECOND BREAST CANCER, GO TO SECTION III QUESTION 32.

25. We would like to discuss your second diagnosis (This does NOT include a recurrence, i.e. a cancer in the same breast as the previous breast cancer).

   What year was it? __________
   Month (if known)? __________
   Which breast? [ ] Left [ ] Right

   **Type of Cancer (check all that apply):**
   [ ] It was an invasive cancer.
   [ ] It was a non-invasive cancer (early stage breast cancer).
      → Was it Ductal Carcinoma In-situ (DCIS)? [ ] Yes [ ] No
      → Was it Lobular Carcinoma In-situ (LCIS)? [ ] Yes [ ] No
   [ ] It was both invasive and non-invasive.
   [ ] I'm not sure.

   **Treatment**
   Did you have surgery for the second breast cancer?
☐ No  ☐ Yes → Year of surgery: _________
What type of surgery did you have?
Check one →  ☐ Lumpectomy (tumour removed, breast remains)
☐ Mastectomy (one breast removed)
☐ Bilateral mastectomy (both breasts removed)

42. Detection
How was the lump first detected?
Check one →  ☐ Only by mammography screening
☐ Did you feel a lump?
☐ Did the doctor feel a lump?
☐ MRI screening
☐ Other: _______________________

26. Did you receive chemotherapy for the second breast cancer?
☐ No  ☐ Yes

27. Did you receive radiation therapy for the second breast cancer?
☐ No  ☐ Yes

28. Did you receive Tamoxifen (Nolvadex) for the second breast cancer?
☐ No  ☐ Yes → Number of pills per day: _________
From: _________ (year) to _________ (year)

29. Did you receive any other drugs for the second breast cancer?
☐ No  ☐ Yes → Which drug?
☐ Femara (letrozole)
☐ Aromasin (exemstane)
☐ Arimidex (anastrazole)
☐ Other: _______________________
Number of pills per day: _________
From: _________ (year) to _________ (year)

30. Have you had a recurrence of your second breast cancer?
☐ No  ☐ Yes → Date: ___ / ___ / ___ ___ (mm / dd / yyyy)
Site of recurrence:
__________________________________________
31. Have you had further surgery in the affected breast (such as complete mastectomy or reconstruction)?
   
   □ No
   □ Yes → Date: ___ / ___ / ___ ___ (mm / dd / yyyy)
   Type of surgery: __________________________

SECTION III – Breast Cancer Screening/Prevention

32. Have you ever taken any drugs for the prevention of breast cancer (i.e. drugs taken before any diagnosis of breast cancer)?
   
   □ No
   □ I’m not sure
   □ Yes → Name of Drug: ___ Tamoxifen (Nolvadex) ___ Raloxifene (Evista)
   ___ Aromasin (Exemestane) ___ Femara (Letrozole)
   ___ Arimidex (Anastrozole)
   □ Other (please name): __________________________
   Number of pills per day: __________________
   Date started: ___ / ___ ___ (mm / yyyy) to ___ / ___ ___

33. Have you ever participated in a clinical trial for the prevention of breast cancer?
   
   □ No
   □ Yes → Which one? ___ Tamoxifen Trial (Tamoxifen vs. placebo)
   ___ STAR Trial (Tamoxifen vs. Raloxifene)
   ___ Other: __________________________
   ___ Unknown
   Do you know which drug you took? □ Yes □ No
   If yes, which one? __________________________

34. Have you ever had breast implants or breast reconstruction?
   
   □ No
   □ Yes → Type: ___ Saline ___ Silicone ___ TRAM-flap
   ___ Other __________________________
   Year of surgery: ___ ___ ___

35. Have you ever had breast reduction?
36. Have you undergone surgery at any time in order to prevent breast cancer (e.g., preventive removal of the breasts)? **NOTE: The surgery is only preventive if you’ve never previously been diagnosed with cancer in that breast.**
   - No
   - Yes → What year? _________
   - Which breast? ___ Left ___ Right ___ Both
   - Procedure: ___ Total mastectomy (nipple and areola removed)
   - ___ Subcutaneous mastectomy (nipple and areola preserved)

37. Have you ever had a breast biopsy?
   - No
   - Yes → Number of biopsies: _________
   - Month/Year of last biopsy: ______/___________
   - What was the result of the biopsy? (Check all that apply)
     - __ normal
     - __ DCIS
     - __ atypical hyperplasia
     - __ cancer
     - __ LCIS
     - __ fibroadenoma
     - __ other (please specify): ______________________________

38. Approximately how many mammograms have you had in your lifetime? I have had ______ mammograms. If you do not recall the exact number, please provide a range. Age at first mammogram? _________ years old.
    Age at last mammogram? _________ years old.

39. Have you ever had any abnormalities detected by mammogram?
   - No
   - Yes → Please describe the abnormality:
     ______________________________
     ______________________________
     Month/Year of abnormality: ______/___________

40. Have you ever had MRI screening of your breasts?
   - No
   - Yes → Age at first MRI: _________
     How many MRIs have you had? ___________________
Where were they done:

____________________________________________

Were there any abnormalities detected by MRI? □ Yes
□ No

If yes, please describe:

___________________________________

Year of abnormality: __ __ __ __
What year was your last MRI done? __ __ __ __

SECTION IV – Ovarian Cancer

41. Have you ever had ovarian cancer?
□ No → Go to question 46 (Section V).
□ Yes → Year of diagnosis: __________

42. Did you have surgery for your ovarian cancer?
□ No
□ Yes → Check one → ___ One ovary was removed
       ___ Both ovaries were removed

43. Did you receive chemotherapy for your ovarian cancer?
□ No
□ Yes

44. Did you receive radiation therapy for your ovarian cancer?
□ No
□ Yes

45. Have you had a recurrence of your ovarian cancer?
□ No
□ Yes → Date: ___ / ___ / ___ (mm/dd/yyyy)
       Site of recurrence: ________________

SECTION V – Reproductive/Abdominal Surgeries

46. Have you had one or both of your ovaries removed (oophorectomy) for reasons other than ovarian cancer? (e.g. preventative measures, fibroids, cyst, scar tissue, or pain)
□ No
☐ Yes  →  Year of surgery: __________
    Reason for the surgery: ____________________________
    Number of ovaries removed:  ___ One  ___ Two

47. Have you ever had a tubal ligation (fallopian tubes tied)?
   ☐ No
   ☐ Yes  →  Year: __________

48. Have you had surgery performed on your reproductive organs: including ovaries,
    fallopian tubes, or uterus? (e.g. hysterectomy, cervix removed, myomectomy, D & C)
   ☐ No
   ☐ Yes  →  Year: __________
    What operation was performed? ____________________________
    Reason for the surgery: ____________________________

49. Have you ever had another operation on your abdomen? (e.g. gall bladder, appendix,
    laparoscopy, hernia, etc)
   ☐ No
   ☐ Yes  →  1. Type of surgery: ____________________________ Year: __________
   ☐ Yes  →  2. Type of surgery: ____________________________ Year: __________
   ☐ Yes  →  3. Type of surgery: ____________________________ Year: __________
Today’s Date: ________________
Month – Day – Year

Date of Birth: ________________
Month – Day – Year

Place of Birth: ____________________________________________________________
City Province/State

Current Residence: _________________________________________________________
City Province/State

Ethnic Background: _________________________________________________________

50a. What is the major ancestry of your father? (Please circle one option.)

African or African American Irish
  (country of origin: ________) Italian
Ashkenazi Jewish Native American (Amer. Indian)

Asian/Pacific Islander Polish/Slavic/Eastern
  (country of origin: ________) Russian

Dutch Scandinavian (Swedish/Finnish/
  English Norwegian/Dane)

European Bloc countries Scot-Irish or Scottish
French Canadian Sephardic Jewish
German Other (specify: ____________)
Hispanic Unknown
  (country of origin: ________________)

50b. What is the major ancestry of your mother? (Please circle one option.)

African or African American Irish
  (country of origin: ________) Italian
Ashkenazi Jewish Native American (Amer. Indian)

Asian/Pacific Islander Polish/Slavic/Eastern
  (country of origin: ________) Russian

Dutch Scandinavian (Swedish/Finnish/
  English Norwegian/Dane)

European Bloc countries Scot-Irish or Scottish
French Canadian Sephardic Jewish
German Other (specify: ____________)
Hispanic
(country of origin: _____________)

51. What is your: current weight? _______ pounds
current height? _______ feet _____ inches

Think back to when you were 18 years old, about the time you graduated from high school.
How much did you weigh then? _____ pounds
at age 30? _____ pounds
at age 40? _____ pounds
What is the most you have ever weighed (exclude pregnancy)? _____ pounds
How old were you when you weighed the most? _____ years old

52. Do you know how much you weighed when you were born?
   □ No
   □ Yes → _____ pounds _____ ounces OR _____ grams

53. What is your mother’s year of birth? ______________

54. What is your birth order (i.e. first-born, second-born, third-born etc.)? ______________

55. Were you part of a multiple birth (i.e. twin, triplet)?
   □ No
   □ Yes → (Please check one)
       ____ twin → Are you an identical twin? □ Yes □ No
       ____ triplet
       ____ other (please specify): __________

SECTION VII – Lifestyle

56. Have you ever smoked cigarettes regularly?
   □ No
   □ Yes → From: __________ (age first started) to __________ (age last used)
   On average, how many packs do/did you smoke per week?
   __________
   Do you still smoke? □ Yes □ No
57. Do you or did you ever drink coffee regularly?
   □ No
   □ Yes  →  From: __________ (age first started) to __________ (age last used)
   On average, how many cups do/did you drink a day?
   Caffeinated: _____ cups  Decaffeinated: _____ cups  Total: _____ cups
   □ Yes  □ No

58. Do you drink alcoholic beverages?
   □ No
   □ Yes  →  From: __________ (age first started) to __________ (age last used)
   On average, how many alcoholic drinks do/did you have per week?
   (Please check one)
   □ 0-3  □ 4-9  □ 10-20
   □ 20 or more
   What type of alcoholic beverages do/did you drink? (Check all that apply)
   □ beer    □ wine    □ hard liquor

59. Have you ever been a regular user of talcum powder?
   □ No
   □ Yes  →  Did you apply it directly to the vaginal area?  □ Yes
   □ No
   Did you apply it to sanitary napkins?  □ Yes
   □ No
   Other use of talcum powder?  (describe)
   __________________________
   What age did you start using talcum powder? __________ years old

SECTION VIII – Other Illnesses

60. Are you taking or have you taken any medications (prescriptions or over-the-counter) on a regular basis?
   □ No
   □ Yes  →  Complete:
   1. Drug: _____________________________________________
      From: ______ (year) to ______ (year)
      Reason: ___________________________________________
   2. Drug: _____________________________________________
From: ______ (year) to ______ (year)
Reason: ___________________________________________

3. Drug: ____________________________________________
From: ______ (year) to ______ (year)
Reason: ___________________________________________

61. Have you been diagnosed with any cancer other than breast or ovarian?
   □ No
   □ Yes → What type? ____________________ Year of Diagnosis?
       __________

62. Please describe briefly any medical problems that you have had in the past, especially those that may have required hospitalization.
   __________________________________________________
   __________________________________________________

63. Have you received your genetic test results?
   □ No
   □ Yes → Date you received your results: __ __ / __ __ / __ __ __ __ (mm / dd / yyyy)

Thank you for taking the time to complete this questionnaire. Should the need arise, may we call you again?
   □ No
   □ Yes → Telephone number ____________________

E-mail address ____________________

Mailing address ____________________
In the case that we are unable to reach you at this number, please provide a telephone number of a relative that we can call to obtain your new contact information:

Name: ____________________________ Relationship: ____________________________

Telephone number: ____________________________
FOR OFFICE USE (to be completed by Genetic Counsellor):

Participating centre: ______________________

Contact person: ______________________

Interviewer (if by phone): ______________________

Family Number: ______________

Individual Identification No: ______________

Date questionnaire sent: ______________________
Month – Day – Year

Date questionnaire received: ______________________
Month – Day – Year

Information received by mail _____ or by telephone _____ or in clinic _____

<table>
<thead>
<tr>
<th>Genetic Test Result Disclosed to Patient?</th>
<th>Yes</th>
<th>No</th>
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<tbody>
<tr>
<td>Date Test Result Disclosed:</td>
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<tr>
<td>Month – Day – Year</td>
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<td>Mutation: BRCA___: ______________________</td>
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FOllOW-UP QUESTIONNAIRE FOR

A STUDY OF BREAST AND OVARIAN CANCER

IN HIGH RISK FAMILIES

Date Completed: ________________________(mm / dd / yyyy)
Name: _______________________________________________________
Date of Birth: __________________________(mm / dd / yyyy)
Fam/Ind#: _________________________________________________
Telephone #: _______________________________________________
Date of Previous Questionnaire: ______________________(mm / dd / yyyy)
Centre: ___________________________________________________
SECTION I - REPRODUCTIVE UPDATE

1. Have you had any pregnancies since __ __ / __ __ __ __ (mm / yyyy) (including still born, miscarriage, abortion)?
   - No
   - Yes  →  Year of pregnancy: __ __ __ __  Date of birth: __ __ / __ __ / __ __ __ __ (mm/dd/yyyy)
     Pregnancy outcome (please check one):
     - ___ Liveborn
     - ___ Stillborn
     - ___ Abortion
     - ___ Miscarriage
     - ___ Other: ____________________

2. Have you ever had any difficulty breastfeeding?
   - I have never breastfed
   - No
   - Yes  →  Reason: _____________________________

3. Are you still having menstrual periods? That is, have you had a menstrual period within the last year? Please answer “Yes” if you are currently pregnant, breast-feeding or taking hormones which temporarily stop your menses (Mirena IUD or Birth control pills.)
   - Yes
   - No  →  At what age did they stop? ______________
     Reason they stopped? (Please check one)
     - ___ Ovaries removed (uterus remained)
     - ___ Medication/Chemotherapy
     - ___ Uterus removed (ovaries remained)
     - ___ Natural Menopause
     - ___ Both uterus and ovaries removed
     - ___ Other ______________

4. Have you ever taken birth control medication?
   - No
   - Yes  →  (i)  From: __ __ __ __ to __ __ __ __ (year)
     Length of time used: _________ years _________ months
     Name of medication: __________________________
     Method (please check one):  ___ Pill  ___ Injection
     ___ Implant  ___ Other

   (ii)  From: __ __ __ __ to __ __ __ __ (year)
     Length of time used: _________ years _________ months
     Name of medication: __________________________
     Method (please check one):  ___ Pill  ___ Injection
Are you still using birth control medication?  □ Yes  □ No

5. Have you ever taken hormone replacement therapy (HRT)?
   □ No
   □ Yes → (i) From: __ __ __ __ to __ __ __ __ (year)
   Length of time used: __________ years __________ months
   Name of medication: ________________________________
   Dose (mg/day): ________________________________
   Method (please check one): ___ Pills ___ Cream ___ Gel
   ___ Patch
   ___ Vaginal Suppositories ___ Other _______
   From: __ __ __ __ to __ __ __ __ (year)
   Length of time used: __________ years __________ months
   Name of medication: ________________________________
   Dose (mg/day): ________________________________
   Method (please check one): ___ Pills ___ Cream ___ Gel
   ___ Patch
   ___ Vaginal Suppositories ___ Other _______

   Are you still using HRT?  □ Yes  □ No

SECTION II - LIFESTYLE

6. Have you ever smoked cigarettes?
   □ No
   □ Yes → From: __________ (age first started) to __________ (age last used)
   On average, how many packs do/did you smoke per week?
   ____________________
   Do you still smoke?  □ Yes  □ No

7. Have you ever drank coffee?
   □ No
   □ Yes → From: __________ (age first started) to __________ (age last used)
   On average, how many cups do/did you drink a day?
   Caffeinated: _____ cups  Decaffeinated: _____ cups  Total: _____ cups
   Do you still drink coffee?  □ Yes  □ No
8. Have you ever drank alcohol?

☐ No
☐ Yes → From: _________ (age first started) to _________ (age last used)

On average, how many alcoholic drinks do/did you have per week?

(Please check one)

___ 0-3  ___ 4-9  ___ 10-20  ___ 20 or more

What type of alcoholic beverages do/did you drink? (Check all that apply)

___ beer  ___ wine  ___ hard liquor

Do you still drink alcohol?  ☐ Yes  ☐ No

9. What is your current weight: _________ pounds  or  _________ kgs

10. What is your highest level of education? (please check one)

___ No Schooling
___ Attended elementary school  ___ Graduated from elementary school
___ Attended high school  ___ Graduated from high school
___ Attended college/university  ___ Graduated from college/university
___ Attended graduate school  ___ Graduated from graduate school

SECTION III – BREAST CANCER SCREENING/PREVENTION

11. Have you ever had a mammogram?

☐ No
☐ Yes → Age at first mammogram: _________

How many mammograms have you had? _________

Were there any abnormalities detected by mammogram?

☐ Yes  ☐ No

If yes, please describe: ____________________________________
Year of abnormality: __ __ __ __

What year was your last mammogram done? __ __ __ __

12. Have you ever had MRI screening of your breasts?

☐ No

☐ Yes  → Age at first MRI: _______

How many MRIs have you had? __________________

Where were they done:

__________________________________________

Were there any abnormalities detected by MRI?  ☐ Yes  ☐ No

If yes, please describe: ________________________________

Year of abnormality: __ __ __ __

What year was your last MRI done? __ __ __ __

13. Have you ever had a breast biopsy (this includes needle and core biopsies)?

☐ No

☐ Yes  → Number of biopsies: ______________________

Date of first biopsy: __ __ / __ __ / __ __ __ __ (mm / dd / yyyy)

Date of last biopsy: __ __ / __ __ / __ __ __ __ (mm / dd / yyyy)

Result of biopsies: ________________________________

__________________________________________

14. Have you undergone preventive removal of your breasts?
No

Yes → Date: ___ / ___ / ___ ___ (mm / dd / yyyy)

Which breast was removed? ___ Left ___

Right ___ Both

Procedure: ___ Subcutaneous Mastectomy (nipple and areola preserved)

___ Total Mastectomy (nipple and areola removed)

___ Other:

15. Have you ever had breast implants or breast reconstruction?

No

Yes → Type: ___ Saline ___ Silicone ___ TRAM-flap

___ Other __________________________________________

Year of surgery: ___ ___ ___

16. Have you ever had breast reduction?

No

Yes

Year of surgery: ___ ___ ___

17. Have you ever taken any drugs for the prevention of breast cancer (i.e. drugs taken before any diagnosis of breast cancer)?

No
☐ Yes → Name of Drug: ___ Tamoxifen (Nolvadex)   ___ Raloxifene (Evista)  
   ___ Aromasin (Exemestane)   ___ Femara (Letrozole)  
   ___ Arimidex (Anastrozole)   ___ Other (please name):  

__________  
Dosage (mg/day): _______________ 
Date started: __ __/ __ __/ __ __ __ __ (mm/dd/yyyy) to __ __/ __ __/ __ __ __  
__ (mm/dd/yyyy) 

18. Have you ever participated in a clinical trial for the prevention of breast cancer?  
   ☐ No  
   ☐ Yes → Which one?   ___ Tamoxifen Trial (Tamoxifen vs. placebo)  
   ______ STAR Trial  
(Tamoxifen vs. Raloxifene)  
   ____ Other:  

__________________________  
____ Unknown  
Do you know which drug you took? ☐ Yes   ☐ No  
If yes, which one? __________________________
19. Have you ever had a trans-vaginal ultrasound (internal ultrasound done through the vagina)?

☐ No

☐ Yes → Age at first ultrasound: ____________________

Number of ultrasounds: ____________________

Abnormalities detected by ultrasound: ☐ Yes ☐ No

**If Yes:** Describe: ____________________

Year of abnormality: __ __ __ __

Year of last trans-vaginal ultrasound: __ __ __ __

20. Have you ever had a blood test for CA125 (marker for ovarian cancer)?

☐ No

☐ Yes → Age at first CA125: _____________

Number of CA125 tests: _________

Abnormalities detected: ____ No ____ Elevated ____ Don’t Know

Year of abnormality: __ __ __ __

Year of the last CA125: __ __ __ __
21. Have you had your ovaries removed for reasons **other than ovarian cancer**?

[ ] No

[ ] Yes → Year: __ __ __ __ Number removed: [ ] 1 [ ] 2

Year: __ __ __ __ (if ovaries removed in two separate surgeries)

Reason: ___ Abnormal CA125 test ___ Preventative

___ Cyst ___ Other _____________________

---

**SECTION V – BREAST CANCER (PREVIOUS DIAGNOSIS)**

*Did you have breast cancer at the time of or prior to the date of the last questionnaire?*

[ ] NO → Go to Question 30 (Section VII).

[ ] YES → Please complete this section.

**Year of previous cancer:** __ __ / __ __ __ __ (mm / yyyy)

**Side:** ___ Left ___ Right ___ Both

**Type (check all that apply):** ___ Invasive

___ Non-invasive → ___ Ductal Carcinoma In-situ (DCIS)

___ Lobular Carcinoma In-situ (LCIS)
Treatment:  ____ Surgery: ____________  ____ Radiation  
    ____ Chemotherapy  ____ Hormone

Therapy

22. Have you ever taken any drugs for the treatment (and to prevent a recurrence) of your breast cancer?

□ No

□ Yes → Name of Drug:  ____ Tamoxifen (Nolvadex)  ____ Raloxifene (Evista)  
    ____ Aromasin (Exemestane)  ____ Femara (Letrozole)  
    ____ Arimidex (Anastrozole)  ____ Other:  
    ________________________________

Dosage (mg/day): _____________________

Date started: ___ / ___ / ___ (mm / yyyy) to ___ / ___ / ___ (mm / yyyy)

23. Have you had a breast cancer recurrence?

□ No

□ Yes → Date: ___ / ___ / ___ / ___ / ___ / ___ (mm / dd / yyyy)

   Site:

   ________________________________

   Mode of detection:  ____ Mammogram  ____ X-ray  ____ Doctor  
   ____ Self-exam  ____ Other:  
   ________________________________

   Treatment: ________________________________
24. Have you had any further surgery on the affected breast(s)?

☐ No

☐ Yes → Type of surgery: _____ Lumpectomy (part of breast removed)

Which breast? ___ Left ___

Right ___ Both

Date of lumpectomy: ___ / ___ / ___ (mm/dd/yyyy)

_____ Mastectomy (whole breast removed)

Which breast? ___ Left ___ Right ___ Both

Date of mastectomy: ___ / ___ / ___ (mm/dd/yyyy)

_____ Reconstruction

Which breast? ___ Left ___ Right ___ Both

Date of reconstruction: ___ / ___ / ___ (mm/dd/yyyy)

SECTION VI – OVARIAN CANCER (PREVIOUS DIAGNOSIS)

Did you have ovarian cancer at the time of or prior to the date of the last questionnaire?
25. Have you had an ovarian cancer recurrence?

- **No**
- **Yes** → Date: __ __ / __ __ / __ __ __ __ (mm / dd / yyyy)

  Site: ____________________________________________

  Mode of detection: ___ CA125 test ___ Trans-vaginal ultrasound
  ___ Doctor ___ Other _______________

  Treatment: ____________________________________________

26. Do you still have your ovaries?

- **No**
- **Yes** → How many?  □ 1  □ 2

---

**SECTION VII – NEW BREAST CANCER DIAGNOSIS**

27. Have you been diagnosed with breast cancer since ____ / ________ (mm / yyyy)?

- **No** → Go to Question 34 (Section IX).
- **Yes** → Date of diagnosis: ___ __ / ___ __ / ___ __ __ __ (mm / dd / yyyy)
Which breast? ___ Left   ___ Right   ___ Both

**Type of Cancer (check all that apply):**

☐ It was an invasive cancer.

It was a non-invasive cancer (early stage breast cancer).

→ Was it Ductal Carcinoma In-situ (DCIS)?    ☐ Yes    ☐ No

→ Was it Lobular Carcinoma In-situ (LCIS)?    ☐ Yes    ☐ No

☐ I’m not sure.

**Treatment:** (i) Surgery?    ☐ Yes    ☐ No

If Yes: ____ Mastectomy (whole breast removed)

Which breast? ___ Left   ___ Right   ___ Both

Date of mastectomy:  __ __ / __ __ / __ __ __ __ (mm / dd / yyyy)

____ Lumpectomy (part of breast removed)

Which breast? ___ Left   ___ Right   ___ Both

Date of lumpectomy:  __ __ / __ __ / __ __ __ __ (mm / dd / yyyy)

____ Axillary Node Dissection (lymph nodes removed)

Number removed: _____
Were any found to have cancer?  □ Yes  □ No

How many? _____

(ii) Chemotherapy?  □ Yes  □ No

If yes, name of drug: ____________________

(iii) Radiation therapy?  □ Yes  □ No

(iv) Other treatment drugs (e.g. Tamoxifen, Femara, etc.)?  □ Yes  □ No

If yes, name of drug: ____________Dosage (mg/day):

_____

Date started: __ __ / __ __ ___to__ __ / __ __ ___ (mm / yyyy)

Name of hospital: ______________________ City: ______________________

How the cancer was first detected? ________________________________

If a lump was felt, was a mammogram done to confirm the cancer? □ Yes  □ No

If yes, was an abnormality detected? □ Yes  □ No

28. Have you had a breast cancer recurrence?

□ No

□ Yes  → Date: __ __ / __ __ / __ __ ___ (mm / dd / yyyy)
Site of recurrence: ______________________
Treatment: ______________________

SECTION VIII – NEW OVARIAN CANCER DIAGNOSIS

29. Have you been diagnosed with ovarian cancer since _____ / _______ (mm / yyyy)?

☐ No → Go to Question 36 (Section X).

☐ Yes → Date of diagnosis: __ __ / __ __ / __ __ __ __ (mm / dd / yyyy)

Treatment: (i) Ovaries removed? ☐ Yes

☐ No

If Yes: How many? 1 2

Date of surgery: __ __ / __ __ / __ __ __ __ (mm / dd / yyyy)

(ii) Chemotherapy? ☐ Yes ☐ No

If Yes: Name of drug: _____________________

(iii) Radiation therapy? ☐ Yes ☐ No

Name of hospital: ___________________________ City: _________________

How was the cancer first detected? _____________________________________
Was a trans-vaginal ultrasound done?  □ Yes  □ No
  
  If yes, were any abnormalities detected?  □ Yes  □ No

Was a CA125 test done?  □ Yes  □ No
  
  If yes, were any abnormalities detected?  □ Yes  □ No

30. Have you had an ovarian cancer recurrence?
  
  □ No

  □ Yes → Date: __ __ / __ __ / __ __ __ __ (mm / dd / yyyy)

  Site of recurrence: ______________________________

  Treatment: ______________________________

SECTION IX – OTHER CANCERS

31. Have you been diagnosed with any cancer other than ovarian or breast?

  □ No

  □ Yes → Date of diagnosis: __ __ / __ __ / __ __ __ __ (mm / dd / yyyy)

  Type: ______________________________

  Treatment: ______________________________

SECTION X – MEDICAL HISTORY
32. Have you ever had any of the following conditions or allergies?

☐ No

☐ Yes \(\rightarrow\) Check ALL that apply.

☐ Asthma Age at onset: _________

☐ Eczema Age at onset: _________

☐ Hay fever Age(s) at onset: _________

What medications and/or treatments have you taken for these conditions?

(Please include Start/End Dates and Dosage)

______________________________________________________________________

______________________________________________________________________

33. Have you ever been diagnosed with arthritis?

☐ No

☐ Yes \(\rightarrow\) What type of arthritis? Check ALL that apply.

☐ Osteoarthritis Age at onset: _________

☐ Rheumatoid arthritis Age at onset: _________

☐ Other: _______________ Age at onset: _________

What medications and/or treatments have you had for arthritis?

(Start/End Dates and Dosage)

______________________________________________________________________

______________________________________________________________________
34. Have you ever been told that you have osteoporosis?

☐ No

☐ Yes → Age at diagnosis: _________

What medications and/or treatments have you had for osteoporosis?
(Start/End Dates and Dosage)

____________________________________________________________________

____________________________________________________________________

35. Have you ever been diagnosed with thyroid problems?

☐ No

☐ Yes → Check ALL that apply.

☐ Hyperthyroid (overactive) Age at onset: _________

☐ Hypothyroid (underactive) Age at onset: _________

☐ Other: _______________ Age at onset: _________

What medications and/or treatments have you had for thyroid problems?
(Start/End Dates and Dosage)

____________________________________________________________________

____________________________________________________________________

36. Have you ever been told that you have high cholesterol?

☐ No

☐ Yes → Age(s) at diagnosis: _________
What medications and/or treatments have you taken for high cholesterol?

(Start/End Dates and Dosage)

______________________________________________________________________

______________________________________________________________________

37. Have you ever been told that you have high blood pressure?

☐ No

☐ Yes → Age at onset: _________

What medications and/or treatments have you taken for high blood pressure? (Start/End Dates and Dosage)

______________________________________________________________________

______________________________________________________________________

38. Have you ever been diagnosed with diabetes?

☐ No

☐ Yes → Check ALL that apply

☐ Type I diabetes (autoimmune)  Age at onset: _________

☐ Type II diabetes  Age at onset: _________

☐ Gestational diabetes  Age(s) at onset: _________

What medications and/or other treatments have you taken for diabetes?

☐ Insulin  Brand: _____________  Dosage: ______________

☐ Gliclazide (Diamicron)  Dosage: ______________

☐ Metformin (Glucophage)  Dosage: ______________
Rosiglitazone (Avandia) Dosage: _________________
Pioglitazone (Actos) Dosage: _________________
Glyburide (Diabeta) Dosage: _________________
Other: _____________________ Dosage: _________________
Other: _____________________ Dosage: _________________

39. Have you ever been diagnosed with heart disease?

☐ No
☐ Yes → What type of heart disease? *Check ALL that apply.*

☐ Coronary heart disease Age at onset: ________
☐ Cardiomyopathy (heart muscle weakness) Age at onset: ________
☐ Angina Age at onset: ________
☐ Heart attack (myocardial infarction) Age(s) at onset: ________
☐ Other: _____________________ Age(s) at onset: ________

What medications and/or treatments have you had for heart disease?
(Start/End Dates and Dosage)
____________________________________________________________________
____________________________________________________________________

40. Have you ever had a blood clot (thromboembolism)?

☐ No
☐ Yes → Age(s) at diagnosis: ________

Where was the clot? ____________________________
41. Have you ever had a stroke?

☐ No

☐ Yes  →  Age(s) at diagnosis: _________

42. Have you ever been diagnosed with an autoimmune disease?

☐ No

☐ Yes  →  Check ALL that apply.

☐ Lupus (SLE)  Age at diagnosis: _________

☐ Multiple sclerosis  Age at diagnosis: _________

☐ Psoriasis  Age at diagnosis: _________

☐ Other: _____________  Age at diagnosis: _________

What medications and/or treatments have you taken for your autoimmune disease?

(Start/End Dates and Dosage)

________________________________________________________

________________________________________________________

43. Have you ever been diagnosed with an eye condition?

☐ No

☐ Yes  →  Check ALL that apply.

☐ Cataracts  Age(s) at onset: _________

☐ Glaucoma  Age at onset: _________

☐ Detached retina  Age(s) at onset: _________
☐ Other: ___________________  Age(s) at onset: _________

**What medications and/or treatments have you taken for your eye condition(s)?**

*(Start/End Dates and Dosage)*

______________________________________________________________________

______________________________________________________________________

44. Have you ever had any gastrointestinal (GI) problems?

☐  No

☐  Yes  →  *Check ALL that apply.*

☐  Gall stones  Age(s) at onset: _________

☐  Lactose intolerance  Age at onset: _________

☐  Crohn’s disease  Age at onset: _________

☐  Ulcerative colitis  Age at onset: _________

☐  Other: _______________  Age(s) at onset: _________

**What medications and/or treatments have you taken for gastrointestinal (GI) problems?**

*(Start/End Dates and Dosage)*

______________________________________________________________________

______________________________________________________________________

45. Have you ever been diagnosed with polycystic ovarian syndrome (PCOS)?

☐  No

☐  Yes  →  Age at onset: _________

**What medications and/or treatments have you taken for PCOS?**

*(Start/End Dates and Dosage)*
46. Have you ever had any urinary and/or genital problems?
   □ No
   □ Yes → Check ALL that apply.
   □ Cystocele (fallen bladder) Age(s) at onset: _________
   □ Urinary tract infection (UTI) Age(s) at onset: _________
   □ Other: ___________________ Age(s) at onset: _________

What medications and/or treatments have you taken for urinary or genital problems?
(Start/End Dates and Dosage)

______________________________________________________________________
______________________________________________________________________

47. Have you ever suffered from depression and/or anxiety?
   □ No
   □ Yes → □ Depression Age(s) at diagnosis: _________
   □ Anxiety Age(s) at diagnosis: _________

What medications and/or treatments have you had for depression and/or anxiety?
(Start/End Dates and Dosage)
SECTION XI – OTHER MEDICAL HISTORY

48. Have you received your genetic test results?
   - [ ] No
   - [ ] Yes → Date you received your results: ____ / ____ / ____ (mm / dd / yyyy)

49. Have you had any surgery since ____ / _______ (mm / yyyy) not previously mentioned?
   - [ ] No
   - [ ] Yes (Complete table below)

<table>
<thead>
<tr>
<th>Date of Surgery</th>
<th>Type of Surgery</th>
<th>Reason for Surgery</th>
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50. Have you taken any other regular prescription since ____ / _______ (mm / yyyy) not previously mentioned?
   - [ ] No
   - [ ] Yes (Complete table below)

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<thead>
<tr>
<th>Drug 1</th>
<th>Drug 2</th>
<th>Drug 3</th>
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<tr>
<td>Date Started</td>
<td>Date Ended</td>
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51. Do you take dietary supplements or vitamins (i.e. multi-vitamins, calcium supplement etc.)?

☐ No

☐ Yes → ___ Multi-vitamins for _______ years ________ months

___ Supplements (including herbal supplements)

Type (e.g. Evening primrose oil, calcium etc):

1. _______________________ for ________ years ________ months

2. _______________________ for ________ years ________ months

3. _______________________ for ________ years ________ months

52. Please describe briefly any other medical problems that you have had in the past, especially those that may have required hospitalization.

_____________________________________________________________________________

_____________________________________________________________________________

________________________________________

___
SECTION XII – PEDIGREE UPDATE

54. Have there been any new cancers diagnosed in your family since _____ / _______ (mm / yyyy)?
   □ No  □ Yes  (Complete table below)

<table>
<thead>
<tr>
<th>Name</th>
<th>Relationship</th>
<th>Type of Cancer</th>
<th>Year of Diagnosis</th>
<th>Age at Diagnosis</th>
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55. Have there been any births or deaths in your family since _____ / _______ (mm / yyyy)?
   □ No  □ Yes  (Complete table below)

<table>
<thead>
<tr>
<th>Name</th>
<th>Relationship</th>
<th>Date of Birth</th>
<th>Date of Death</th>
<th>Cause of Death</th>
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Thank you very much for taking the time to complete this questionnaire.

Should the need arise, may we contact you again?  □ No  □ Yes

Telephone number: ________________  Alternate contact: ________________

E-mail address: ____________________

Mailing address: __________________________________________________________

                        CITY: __________________  PROVINCE: ________________  POSTAL CODE: __________
Appendix 5 Data Collection Form

DATA COLLECTION FORM

Check Date: ________________ Individual Number: ________________
Center Name: ________________ Date of Birth: ________________
Family Number: ________________ Baseline Date: ________________

<table>
<thead>
<tr>
<th>PREGNANCY YEAR</th>
<th>BRCA DIAGNOSIS DATE</th>
<th>INTERVALE</th>
<th>PABC/PFBC</th>
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PREGNANCY YEAR:  病理诊断日期：  诊断间隔：  PABC/PFBC

- Delivery Date
- Pregnancy Associated BrCa
- Pregnancy Following BrCa
- Time of diagnosis of BrCa
- Baseline Date
- Death
- Lost to FU/Do not contact
Appendix 6 Women's College Hospital Research Ethics Board Letter of Approval

Notification of REB Continued Approval

Date: 14 February, 2012
To: Dr. Steven Narod
Re: 2010-0064-E

The impact of pregnancy during and after breast cancer on survival in carriers of BRCA1 and BRCA2 mutations

Sponsor: Canadian Breast Cancer Foundation -

REB Initial Approval Date: 11 February, 2011
REB Annual Renewal Approval Date: 14 February, 2012
REB Expiry Date: 11 February, 2013
Documents Approved: Annual Review - 2012 Annual Renewal Application Form ver: 02/08/2012
Consent Form - Consent form ver: 10/08/2010

The above named study has received continued approval by the Women’s College Hospital Research Ethics Board until the expiry date noted above. If, during the course of the research, there are any serious adverse events, confidentiality concerns, changes in the approved project, or any new information that must be considered with respect to the project, these should be brought to the immediate attention of the REB. In the event of a privacy breach, you are responsible for reporting the breach to the WCH REB and the WCH Corporate Privacy Officer (in accordance with Ontario health privacy legislation – Personal Health Information Protection Act, 2004). Additionally, the WCH REB requires reports of inappropriate/unauthorized use of the information.

If the study is expected to continue beyond the expiry date, you are responsible for ensuring the study receives re-approval. The REB must be notified of the completion or termination of this study and a final report provided. As the Principal Investigator, you are responsible for the ethical conduct of this study.


Sincerely,

Alexandra Chappell, Acting Research Ethics Coordinator, Women’s College Hospital Research Ethics Board

for

Dr. Miriam Shuchman, Chair, Women’s College Hospital Research Ethics Board