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13. ABSTRACT *(Maximum 200 Words)*

This project investigated the influence of reproductive history on risk of breast cancer and its prognosis by taking advantage of very large linkages between population-based health and demographic registries in Denmark. Many of these registries have existed for several decades and contain mandatorily reported information, making very complete and long-term follow-up studies possible. A total of 18 studies were conducted during the study period. In 13 studies, the influence on breast cancer risk of induced abortion, age at birth, number of births, gender of child, multiple births, weight of child, alpha-fetoprotein level during pregnancy and family history was investigated in great detail. Breast cancer was not only investigated as a single outcome but also according to type, size of tumor at time of diagnosis, nodal spread, etc. Five studies dealt with factors that might influence the prognosis of breast cancer. Low age at first birth but not parity was associated with poor survival. A diagnosis of breast cancer less than 2 years after a child birth gave particularly poor survival. Young women had particularly poor survival but only those diagnosed with low-risk disease not receiving adjuvant cytotoxic therapy,-indicating that they would benefit from being given cytotoxic therapy in the future. A pregnancy subsequent to breast cancer did not aggravate the prognosis. Finally, we found a 20-30% difference in chance of survival depending on what quadrant in the breast the tumor was first diagnosed. Survival was most poor for tumors in the lower two quadrants, indicating that a more aggressive treatment approach might increase survival of those tumors.

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FOREWORD

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INTRODUCTION

The general aim of this project was by use of the high quality and population-based nature of registries in Denmark to study reproductive risk factors for breast cancer and its prognosis. This final report comprises results from 18 studies undertaken under this grant. The studies described in detail in the following have been organized according to whether they belong to section I (abortion and breast cancer), II (reproductive history and breast cancer), or III (factors influencing the risk of breast cancer) of the general aims of this grant. The background and specific objectives of each of the studies will be presented in this chapter followed by a comprehensive description of materials, methods and results in the following chapter (body). Conclusions will be presented in the end (conclusions). A specific reference list for each study is supplied at the end of the document. Under the heading "Reportable Ourcomes" a list has been provided which specifies where 16 of the 18 studies have been published or will appear in the near future. The two most recent studies were recently submitted for publication.

I. Abortion and breast cancer

Induced abortion and the risk of breast cancer (Study 1,11,15.)

Reproductive factors are important in breast cancer development, but their exact influence has not been established. A full-term pregnancy has been shown to increase the short-term risk of breast cancer, possibly through growth-enhancing properties of pregnancy-induced estrogens. By contrast it decreases the long-term risk, perhaps by inducing terminal differentiation of the susceptible mammary tissue.[1-5] Based primarily on animal studies, the potential for terminal differentiation of breast cells appears to be significantly lower for a pregnancy terminated by abortion compared to a full-term pregnancy. This observation led Russo et al.[3] to suggest that complete differentiation of the breast cells conveyed by a full-term pregnancy has to be achieved to provide protection against carcinogenic effects. An interrupted pregnancy, on the contrary, might increase the risk of breast cancer because proliferation of breast cells will take place without the protective effect of subsequent differentiation.

Epidemiologic studies on the association between abortion and subsequent breast cancer risk have shown inconsistent results, with risk estimates ranging from moderately elevated to significantly lowered values.[6-23] In their recently published case-control study, Daling et al. found an indication of an elevated risk in women with induced abortion between 9 to 12 weeks' gestation but this finding was based on very limited numbers.[7] In the present study, we took advantage of the mandatory registration in Denmark of gestational age-specific induced abortion history and complete reproductive history to evaluate the hypothesis by Russo.[3]

II. Reproductive history and breast cancer

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Reproductive risk factors for breast cancer by receptor status, histology, laterality and location (Study 5)

It is well established that a woman's reproductive history influences her risk of breast cancer (Kelsey and Gammon, 1993), but the mechanisms behind are unknown. Hormonal changes induced by a pregnancy could play a role, and because cells in the breast may respond differently to hormone stimuli, the effect of reproductive history on the incidence of breast cancer has been suggested to vary by subtypes of breast cancer.

Until today investigations have pursued this idea by examining whether there are differences in effect of reproductive factors according to oestrogen receptor status. The majority have found nulliparity and late age at first birth only to influence the development of oestrogen receptor positive tumours, but not oestrogen receptor negative tumours (Habel *et al.*, 1993, Stanford *et al.*, 1986, Yoo *et al.*, 1997, Potter *et al.*, 1995).

We extended this line of pursuit investigating in more detail the importance of not only receptor status, but also histology, laterality and location of the tumour using a large population-based cohort of Danish women which was linked to a tumour registry with detailed information on breast tumour characteristics.

Preterm delivery and risk of breast cancer (Study 6)

Major hormones influence the development, proliferation, and differentiation of the human breast (Rebar 1994). Based primarily on animal studies, it has been shown that mammary cells proliferate in the first and second trimester of pregnancy and differentiate in the last trimester (Russo et al, 1980). This led Russo and Russo to hypothesize that complete differentiation of the breast cells conveyed by a full-term pregnancy has to be achieved to provide protection against carcinogenic effects. Earlier termination of pregnancy, on the contrary, might increase the risk of breast cancer because proliferation of the breast cells will take place without subsequent differentiation (Russo et al, 1980).

Breast cancer risk in women with a history of a short-term pregnancy has primarily been investigated in relation to spontaneous and induced abortions (Adami et al 1990; Calle et al, 1995; Daling et al, 1994; Kvåle et al, 1987; Michaels et al, 1995; Newcomb et al, 1996; Melbye et al, 1997) which cover the early period of pregnancy. In particular, the large prospective studies have not found such women to be at increased risk of breast cancer (Calle et al, 1995; Kvåle et al, 1987; Melbye et al, 1997). In contrast, few studies have addressed the late period of pregnancy and whether a preterm delivery is associated with an increased risk of breast cancer (Brinton et al, 1983; Rao et al, 1994; Choi et al, 1978; Polednak et al, 1983).

Maternal risk of breast cancer and birth characteristics of offspring by time since birth (Study 7)

Hormonal levels during pregnancy may influence the maternal risk of breast cancer.[1] We investigated this hypothesis by studying the association between certain birth characteristics of the latest offspring and the subsequent maternal risk of breast cancer. The birth characteristics showed (birth weight, gender of offspring and multiple births) are related to the hormonal level during pregnancy.[2-8]

Multivariate competing risks in a poisson regression model: An application with two correlated characteristics of breast cancer (Study 8)

Studies addressing incidence and risk factors for site-specific cancers often operate with only one ultimate cancer diagnosis. However, a more differentiated outcome, i.e. specific subtypes of the cancer, may often be of interest. In practice, such differentiated analyses can be performed with follow-up data applying Cox or Poisson regression analysis on each subtype separately, but in many situations it is desirable to study whether the risk factors have the same effect on the incidence of different subtypes, the purpose being either to study whether the subtypes have the same aetiology or to obtain a better understanding of the causal pathway behind the risk factors. Such an analysis can be performed as a competing risks analysis testing for identical effects of a risk factor for all or some of the subtypes as discussed for the Cox model by Andersen et al[1] p493ff and for Poisson regression by Pierce and Preston[2].

Sometimes more than one subtype classification is studied. If two such classifications are correlated, one may speculate whether differences in the effect of a risk factor according to one classification simply may be an effect of differences according to the other correlated classification. To evaluate such a hypothesis, we introduce in this paper the new concept of *multivariate competing risks*.

The plan of study 8 is as follows: We will in section two introduce an analysis taken from a breast cancer study as a motivating example for the concept of multivariate competing risks which we, subsequently describe in section three. The method will be illustrated on the example in section four, and finally other applications of the method will be discussed in section five.

Reproductive history and stage of breast cancer (Study 9)

It is well established that a woman's reproductive history influences her risk of breast cancer. In particular, parity and age at first birth are considered strongly related to the risk of breast cancer (1). Studies addressing these issues have, however, almost exclusively dealt with breast cancer as one single entity. Thus, little is known about the possible effect of these reproductive factors on tumor biology (tumor progression, metastatic potential etc.) as reflected in stage of the disease at diagnosis.

We hypothesized that parity and age at first childbirth not only are related to the risk of developing breast cancer, but in addition are associated with the stage of the breast cancer at diagnosis. We used a large population-based cohort with detailed information on reproductive history and characteristics of the breast cancer to evaluate whether parity and age at first birth are related to the tumor size or axillary nodal spread at diagnosis.

Gender of offspring and maternal breast cancer risk (Study 12)

A childbirth induces a short-term increase and a long-term decrease in a mother's breast cancer risk (Lambe et al, 1994; Albrektsen et al, 1995a). Hormonal levels during pregnancy may influence both effects. This has been investigated by looking at the maternal breast cancer risk following a pregnancy with characteristics associated with elevated hormonal levels (Enger et al, 1997; Troisi et al, 1998; Wohlfahrt and Melbye, 1999). The maternal breast cancer risk according to gender distribution of offspring has for the same reason, attracted interest as observations have been made of gender differences in the maternal level of serum alfa-fetoprotein, human chorionic gonadotrophin (hCG) and sex hormone-binding globulin that might also be related to maternal breast cancer risk (reviewed in Hsieh et al, 1999). A large Norwegian cohort study with 3,937 cases found no association between breast cancer risk and gender distribution of offspring (Albrektsen et al, 1995b), but recently a Swedish case-control study including 2,328 cases found that only deliveries of male offspring had a protective effect (Hsieh et al, 1999). The mechanisms behind the hormonal influence on the short-term increase and long-term decrease in breast cancer risk following a childbirth are believed to be different (Adami et al, 1998), and it is therefore important to investigate the effects separately. We have recently in a large cohort study including 9,495 cases found no modification by gender of offspring of the short-term effect (Wohlfahrt and Melbye, 1999). In the present study we investigated whether this is also true for the long-term effect of a childbirth.

Age at any birth is equally important for breast cancer risk (Study 13)

It is well established that childbirth affects a woman's breast cancer risk. Traditionally, the timing of the first birth has been considered to be of particular importance, i.e. early age at first birth reduce the risk of breast cancer. This thinking derives from the assumption that breast cells are particularly prone to carcinogenic stimuli prior to a first pregnancy and that maturation and protection of breast cells takes place at time of first pregnancy.[1] However, high parity among parous women may further reduce the risk of breast cancer, which is attributed to a maturation of breast cells not affected by the first birth. This reduction in risk following subsequent births can be substantial,[2,3] and it is therefore intriguing that the timing of the first birth should be the most critical compared with the timing of subsequent births. Presently, little is known on this subject because only few datasets are large enough to estimate the effects of age at each birth simultaneously. In the present study we take advantage of the possibilities to create a large cohort of women with data from population based national registries in Denmark to investigate the influence of the age at first relative to subsequent births on the development of breast cancer.

Alphafetoprotein levels during pregnancy and maternal breast cancer incidence (Study 14)

Reproductive factors, in particular the number and timing of births, are well-established risk factors for breast cancer (1,2,3). The biological mechanisms by which a full-term pregnancy might affect maternal breast cancer risk are not fully understood. While the most popular hypothesis relates to a differentiation of mammary gland cells induced during the late stages of a pregnancy (4), alternative explanations have to be considered. A pregnancy is accompanied by a steep rise in estrogen's (5) and alpha-fetoprotein in maternal serum (MS-AFP) (6). Alpha-fetoprotein is a glycoprotein that is produced by the fetal liver and yolk sack (7). Fetal AFP is transmitted into the maternal circulation via the placenta (8) and via the amniotic fluid and its membranes (9). Thus, the increased production of fetal AFP is followed by a rise in MS-AFP. In the animal model, both naturally occurring and recombinant human AFP has been found to bind estradiol (10,11,12,13) and to suppress estrogen supported growth of breast cancer cells (14,15). While estradiol might promote tumor growth in humans, high levels of AFP would inhibit estrogen-dependent breast cancer growth and thus, AFP might possess biologically important anti-carcinogenic properties (,16,17).
We studied the relation between MS-AFP levels during pregnancy and subsequent maternal risk of breast cancer in a cohort of 42,057 Danish women who gave birth between 1978 and 1996.

Risk of late stage breast cancer following a childbirth (Study 16)

It is well established that the birth of a child decreases a mother's long-term risk of breast cancer (1). However, several studies have found that her risk of breast cancer may be elevated in the immediate years following childbirth (2,3,4,5). This latter observation has been thought explained by a growth enhancing effect of the hormonal changes occurring during pregnancy on malignant or premalignant cells (4). If correct, the pregnancy promoted tumors should have particularly rapid growth and therefore be likely on average to be diagnosed at a later stage, i.e. the transient increase should be especially pronounced for the rate of late stage breast cancer. To evaluate this hypothesis of pregnancy-induced rapid growth of occult tumors we studied the overall rate of breast cancer in the years following a birth and in particluar, the rate of late stage tumors taking advantage of the detailed registration of breast cancer characteristics in Denmark

Breast cancer risk after a childbirth in young women with a family history (Study 18)

Women with a family history of breast cancer (FHBC) have an increased risk of breast cancer (Pharoah et al, 1997). A potential way to modify this risk could be by early childbirths. To investigate this possibility previous studies have focused on the protective effect of many childbirths and young age at first birth (see discussion for references). However, in the last decade there has been a growing acknowledgment of the increased risk of breast cancer in the first 5-10 years after a birth (Lambe et al, 1994; Albrektsen et al, 1995). This effect collide with the period where women with FHBC have a relative higher breast cancer risk, i.e.

before the age of 40 years (Pharoah *et al*, 1997), and the adverse effect might therefore be stronger in women with FHBC. To investigate the short-term effect of a childbirth in women with FHBC we used population-based register data from the Danish population with information on family history. By using register based information on family history we avoided differential recall in cases that could otherwise cause bias.

III. Factors influencing the prognosis of breast cancer

Time since childbirth and the prognosis of breast cancer (Study 2)

An early first delivery and a large number of childbirths are among the best established factors conferring a low risk of breast cancer [1]. Recent studies have described a dual effect of full-term pregnancy on the risk of breast cancer with a transiently increased risk immediately after childbirth followed by a long-term reduction in the risk [2-4]. Although these findings relate to the risk of breast cancer development, they could very well also have implications for the prognosis of this disease. An established breast cancer prior to or during pregnancy might accelerate its growth under the influence of high concentrations of pregnancy hormones, primarily estrogens. However, the available literature on this point is conflicting [5-7], probably as a result of problems with small study sizes or the lack of adjustment for relevant tumour characteristics and reproductive history.

In the present study we took advantage of three nationwide Danish registries, one containing detailed information on tumour characteristics, treatment regimes, and clinical outcome and two others containing complete parity information, to address the question of a possible influence of reproductive history on breast cancer survival.

Parity, age at first birth and the prognosis of breast cancer (Study 3)

It is well-established that reproductive factors influence the risk of breast cancer development (McPherson *et al.*, 1994). Based on animal studies, it has been hypothesized that pregnancy induces differentiation and maturation of the breast cells and that the cells subsequently become less vulnerable to carcinogenic stimuli (Russo *et al.*, 1990). Parous women and in particular multiparous women are known to be at low risk of breast cancer compared with nulliparous women. Women having their first childbirth at a young age seems to experience a particular risk reduction (MacMahon *et al.*, 1970; Ewertz *et al.*, 1990).

Factors influencing the development of breast cancer might also affect its course, but studies on the *prognostic* influence of reproductive factors have been contradictory (Schouten *et al.*, 1997; von Schoultz *et al.*, 1995; Palmer *et al.*, 1982; Guinee *et al.*, 1994; Mason *et al.*, 1990; Lees *et al.*, 1989; Lehrer *et al.*, 1992; Wang *et al.*, 1985; Orr and Fraher, 1995; Mohle Boetani *et al.*, 1988; Korzeniowski and Dyba, 1994; Black *et al.*, 1983; Papatestas *et al.*, 1980). We took advantage of the population-based registration of breast cancer patients established by the Danish Breast Cancer Cooperative Group (DBCG) and a database containing complete information on parity to evaluate the possible importance

of childbirth history and age at first birth as prognostic factors in primary breast cancer.

Should women be advised against pregnancy after breast cancer treatment? (Study 4)

Much attention has been given to the importance of endocrine factors on breast cancer development and prognosis since Beatson one hundred years ago first reported on the positive effect of oophorectomy in women with breast cancer [1]. A woman's reproductive history strongly influences her risk of later developing breast cancer and one of the most well-known associations is the protective effect of having a large number of children, preferable at a young age [2,3]. Whereas childbearing may overall reduce the risk of breast cancer, there is accumulating evidence that childbirth at least in some situations may have a negative effect on the prognosis of breast cancer. Thus, more studies suggest that breast cancer diagnosed during or in the first years after childbearing is associated with a poor prognostic outcome [4-6].

An outstanding question has been whether a pregnancy subsequent to breast cancer treatment may worsen the prognosis. The present literature on this subject seems to indicate that contrary to expectations, there is no negative effect of pregnancy after treatment of breast cancer. However, the evidence is weak and based on small studies which for the most part have lacked the ability to adequately adjust for important confounders [7-16]. Another important obstacle in the study of this question has been that the group of women who decide to have a child subsequent to breast cancer diagnosis is considered to be highly selected [4]. In the Western world, the median age at first childbirth has increased over the last decades. Since motherhood is generally postponed, more patients are seeking medical advice concerning pregnancy after treatment of breast cancer. In the present study we addressed the question of the prognostic influence of pregnancy subsequent to breast cancer treatment based on a linkage analysis between the population-based Danish Breast Cancer Cooperative Group (DBCG) registry and other national registries. Detailed information on stage of disease allowed us to address specifically the potential problem of selection bias.

Factors influencing the effect of age on prognosis in breast cancer: population based study (Study 10)

Women diagnosed with breast cancer in their twenties and thirties appear to have a poorer prognosis than women diagnosed in middle age [1-7]. The reason for this somewhat unusual pattern is unclear. It has been shown that young women with breast cancer are more likely to be diagnosed with lymph node involvement, oestrogen receptor negative status, and with tumours which are large and with a high grade of anaplasia [1-3]. Thus, the poorer outcome could conceivably be due, at least in part to differences in these important prognostic factors but many although not all studies retain a negative effect after adjustment for such confounding factors [1,8-19]. It is unknown to what extent adjuvant cytotoxic treatment might influence this association.

We addressed the effect of age on breast cancer survival adjusted for expected mortality taking advantage of large and very complete population-based registries which included detailed information on clinical presentation, postoperative therapy, and follow-up status on Danish women with breast cancer. Our main objectives were to determine whether the poor prognosis reported among very young women was independent of common prognostic factors and to what extend this pattern might be affected by treatment.

Influence of tumor location on axillary nodal status and breast cancer prognosis (Study 17)

Axillary lymph node status is the single most important prognostic factor in primary breast cancer and the significance of a proper axillary dissection both with regard to staging and local tumor control is well established (1). Recent efforts to optimize the existing staging system with the sentinel node lymphadenectomy have put renewed focus on the prognostic importance of nodal status in breast cancer (2-5). From anatomical studies it is known that lymphatic drainage from the breast goes not only to the axillary lymph nodes, but also to the internal mammary, the supraclavicular nodes, and to lymph nodes outside these locations (6,7). Today's emphasis on axillary nodal status raises an important clinical question as to whether some women with breast cancer are misclassified as low-risk patients because axillary dissection does not reveal spread of the disease to the lymphatic system. Recently, Zucali et al. (8) reported that women with medially located tumors were less likely to be classified as having node positive disease compared with other women with breast cancer. In spite of this, these women had a reduced chance of survival compared with women with lateral tumors. In the present study we extended this line of investigation on the prognostic effect of tumor location based on a large and very detailed population-based registration of breast cancer patients in Denmark.

BODY

I. Abortion and breast cancer

Induced abortion and the risk of breast cancer (Studies 1,11,15)

Material and methods
For the purpose of the present study we performed a linkage of data from the Civil Registration System (CRS) with the National Registry for Induced Abortions, and the Danish Cancer Registry. Before initiating the study we obtained permission from the National Scientific Ethics Committee and the Data Protection Board.

Since April 1, 1968, the CRS has assigned a unique identification number to all citizens in Denmark which permits accurate linkage of information from different registries. The CRS also keeps updated files on dates of livebirths and documents demographic variables such as emigration and death. Since 1939, reporting of induced abortions has been mandatory to the National Board of Health. In 1973, legal rights to induced abortion up to and including 12 weeks of gestation were established for women with residence in Denmark. Permission to have induced abortion after week 12 stated indicators such as medical, ethical (e.g. rape), eugenic, social and special personal conditions that would greatly interfere with proper handling of the newborn child. Since 1973, information on all induced abortions has been computerized in the National Registry of Induced Abortions making the information easily accessible. This registry contains information on exact date and gestational age at time of the induced abortion.[24] The methodology used for the induced abortions included in this analysis (period 1973 to 92) represented almost exclusively surgical removal. The Danish Cancer Registry contains cancer diagnoses from the entire country back to 1943. Independent reporting is taking place from clinicians, pathologists, clinics, radiotherapy units, and hospitals.[25]

A research database was established from the CRS comprising all Danish women born between April 1, 1935, and March 31, 1978, with information on live-born children. Based on the person-identifiable CRS-number a linkage was performed with the National Registry of Induced Abortions supplying information to the database on date of any induced abortion, and the gestational age of the aborted fetus. Subjects were subsequently linked with the Danish Cancer Registry to identify those diagnosed with invasive breast cancer. All women entered the follow-up for breast cancer on April 1, 1968, or on their 12th birthday, whichever came last. The period at risk continued until a breast cancer diagnosis, death, emigration, disappearance, or December 31, 1992 (at which date the cancer registry was considered complete), whichever occurred first. The possible impact of the duration of the pregnancies that ultimately ended as induced abortions was investigated in a log-linear Poisson regression model.[26] Gestational age-specific person-years at risk were calculated in groups for induced abortions that took place at <7, 7 to 8, 9 to 10, 11 to 12, 13 to 14, 15 to 18, and >18 weeks of gestation. Women with more than one induced abortion were in the period between the first and second abortion considered under risk according to the gestational age of the first induced abortion and after the second but before the third according to the gestational age of the second induced abortion,

etc. Adjustment was made for attained age in 1-year intervals and calendar period in 5-year intervals, parity (0,1,2,3,4,5,6,7+), and age at first birth (12 to 19, 20 to 24, 25 to 29, 30 to 34, >34). In an exploratory analysis we also categorized calendar time and age at first birth in 1-year intervals but this had no effect on the results arguing against residual confounding. Age of the woman is denoted age of the woman at diagnosis for clarification. Trend tests were performed treating the grouped gestational age as a continuous variable with each group represented by the mean gestational age. The linear assumption in the trend test was checked by a likelihood ratio test against the model with gestational age as a categorical variable. Estimation of breast cancer incidence rate ratios was performed using the SAS procedure PROC GENMOD.[27] These rate ratios are called relative risks in the following.

Results
Overall, 1,529,512 women were included in the cohort. Of these, 280,965 (18.4 percent) had a total of 370,715 induced abortions distributed as follows: 215,902 women (76.8 percent) had one induced abortion, 47,906 women (17.1 percent) had two, and 17,157 women (6.1 percent) had three or more induced abortions. The gestational age-specific distribution of the number of induced abortions was as follows: <7 weeks: 3.1 percent, 7 to 8 weeks: 37.1 percent, 9 to 10 weeks: 41.9 percent, 11 to 12 weeks: 15.7 percent, >12 weeks: 2.3 percent. Women without induced abortion represented 25,850,000 person-years of follow-up. In this group 8,908 cases of breast cancer were observed. In comparison, women with a history of induced abortion comprised a total of 2,697,000 person-years of follow-up and 1,338 cases of breast cancer.

Overall, the risk of breast cancer in women with induced abortion was not different from that of women without a history of induced abortion after taking into account potential confounding by age, parity, age at first birth, and calendar time (relative risk 1.00; 95 percent confidence interval 0.94 to 1.06). Table 1 presents in more detail the association between variables related to the abortion history and the risk of breast cancer. Both a "crude" relative risk (adjusted for age, parity, calendar time, and age at first birth) and an adjusted multivariate relative risk (adjusted also for the other variables presented in the table) was calculated. As it appears the adjustment did barely change any of the risk estimates. Although age at the induced abortion did not significantly influence the overall risk, there was a tendency towards higher risks of breast cancer in women who were very young, i.e. between 12 and 19 years of age (relative risk 1.29, 95 percent confidence interval 0.80 to 2.08). Neither the number of induced abortions nor live birth history (induced abortion in a nulliparous or before/after a lifebirth) significantly influenced the breast cancer risk. We also looked at the time interval between the induced abortion and breast cancer diagnosis but found no indication of a differential effect (<1 year: RR=0.97; 1-4 years: RR=0.99; 5+ years: RR=1 (ref.)) (Table 1 in paper 1).

There was no effect modification by age of the women at diagnosis of the association between induced abortion and breast cancer risk (12 to 34 years: RR=0.95 (0.78 to 1.14); 35 to 39 years: RR=0.99 (0.87 to 1.14); 40 to 44 years: RR=1.01 (0.91 to 1.12); 45 to 49 years: RR=1.00; 50+ years: RR=1.03 (0.88 to 1.21), P=0.97). Also, there was no effect modification by calendar period (P=0.17) or by calendar period at induced abortion (P=0.83). However, with each week's

increase in gestational age, a 3 percent increase was observed in the risk of breast cancer. The relative risk increased from 0.81 (95 percent confidence interval, 0.58 to 1.13) in women with a gestational age of latest abortion of less than 7 weeks to 1.38 (95 percent confidence interval, 1.00 to 1.90) in women with a gestational age of more than 12 weeks at abortion (P_{trend}= 0.02). We acknowledge the small number of cases in the group above 12 weeks but further evaluated this period and found the following relative risks: weeks 13 to 14: 1.13 (0.51 to 2.53); weeks 15 to 18: 1.23 (0.76 to 2.00); weeks >18: 1.89 (1.11 to 3.22) (P_{trend} =0.016).

Discussion
Our population-based cohort study uncovered no overall increased risk of breast cancer in women with a history of induced abortion. This is very much in line with previous retrospective cohort studies.[9,10,15,16] Two of these studies rather suggested a decreased risk.[10,15] However, all previously published retrospective cohort studies have lacked detailed information on gestational length of the abortion. Results from case-control studies have been inconsistent.[6-8,11-14,17-23] Several reports, particularly those focusing on induced abortions, have documented an increased risk.[7,8,13,21-23]

An almost inevitable concern with the results obtained in these case-control studies is the potential problem with differential misclassification. Even after legislation of abortion the issue continues to be sensitive and it is most likely that women with serious diseases such as breast cancer report induced abortions more completely than other women. Based on a Swedish study which compared registry information with interview data regarding induced abortion, an increase in risk of breast cancer of between 16 and 50 percent could be attributed to differential misclassification in interview data.[28,7] The concern with reporting misclassification led Newcomb et al. to conclude that studies which do not rely on interviews with cases and controls are necessary to resolve the issue adequately.[8] In the present study, all information both with respect to dates and number of induced abortions, reproductive history, and cancer diagnoses was obtained from national registries with mandatory reporting covering the entire population. Follow-up included complete knowledge on death and emigration and was performed through computerized linkage of registry information by means of person-identifiable registration numbers. We therefore conclude that some of the major methodological problems in previous studies were overcome in the present study.

A limitation of our research database was that information on induced abortions was only computerized since 1973. Therefore, for some of the oldest women in the cohort we might have obtained an incomplete history of induced abortions. However, according to the present data, women with a history of induced abortion did not experience a risk of breast cancer different from that of women without such a history. Furthermore, we did not find any indication that the number of induced abortions had any bearing on the breast cancer risk. Therefore, we consider it very unlikely that missing information about abortions prior to 1973 should have any influence on the results of our analysis.

Whereas induced abortion had no overall effect on the risk of breast cancer, we documented a significantly increasing risk with increasing gestational age of the

abortion. The fact that such an increase did not affect the overall result of no association clearly indicates that it is based on small numbers and as such should be considered with caution. We have no explanation as to why a very early induced abortion was associated with a slightly, although insignificant, risk decrease. However, the significantly increasing trend was also apparent after excluding this category of the very early induced abortions. The increased risk in second trimester abortions find biological support from rat experiments and is in line with the hypothesis by Russo.[3]

We were concerned that women who were diagnosed with breast cancer during pregnancy would be advised to have an induced abortion and that this situation would not be equally distributed by gestational age of the abortion. However, the time at risk was only calculated up to the diagnosis of breast cancer in the study, and therefore only later occurring induced abortions that were misclassified as occurring prior to the cancer diagnosis could represent a potential problem. However, a stratified analysis of the risk of breast cancer according to time since induced abortion showed no differential risk and in particular no increased risk within the first year after abortion.

Induced abortions taking place at a gestational age of more than 12 weeks were primarily performed on medical or social indications. This group of women could have a higher breast cancer risk which might explain the elevated relative risks observed for women with late induced abortions. However, we are not aware of any medical condition associated with both a high breast cancer risk and with late induced abortion. We specifically tested whether women with a diagnosed trisomy 21 pregnancy, who also tend to be commonly found among those having a late induced abortion, should have an increased risk of breast cancer. Based on a cohort study of 1335 mothers with this condition (16,022 person-years of follow-up) we found no increased breast cancer risk in this group compared to other parous women (data not shown). It is possible that women with drinking problems delay the interruption of an unwanted pregnancy. Thus, alcohol intake has been associated with increased breast cancer risk but the associations have been weak and inconsistent.[29] Another social indication for late induced abortion would, if anything, tend to yield an overrepresentation of women of low socio-economic status. However, breast cancer risk is associated with high social status and thus we would expect the observed relative risks to be underestimated rather than the opposite.

Nulliparous women with a history of induced abortion did not differ from parous women in risk of breast cancer. In the group of nulliparous women it is irrelevant to consider confounding by lactation and effect of later births. We are therefore very confident that neither of these variables had any confounding potential that influenced our overall result.

II. Reproductive history and breast cancer

Reproductive risk factors for breast cancer by receptor status, histology, laterality and location (Study 5)

Material and methods

Population Registries
Since April 1, 1968, the Civil Registration System (CRS) in Denmark has assigned an individually unique national registration number to all citizens. This number permits accurate linkage of information from different registries. The Civil Registration System also keeps updated information on dates of live births, emigration and vital status.

In 1977, the Danish Breast Cancer Cooperative Group (DBCG) started a series of national prospective studies to systematically evaluate breast cancer treatment programmes. A detailed description of this registry has been given elsewhere (Andersen and Mouridsen, 1988, Kroman et al., 1997). The DBCG collects detailed information on the breast cancer diagnosis including size, nodal status, receptor status, histology, laterality, location and tumour size. The histological subtypes were categorised according to the WHO classification. The location of a tumour was determined on the basis of an indication, received from the surgical departments, of the location of the tumour on a figure of the four quadrants and the central part of the right and left breasts, respectively. When a tumour was located in the borderline between two areas the tumour was assigned to one of the two (or three) adjacent areas by randomisation.

The presence of oestrogen receptors in the breast cancer tissue was determined by quantitative methods (Thorpe, 1988, Thorpe et al., 1986) or by a semiquantitative method (Andersen et al., 1990). Receptor status was defined by a level of receptor ≥10 fmol/mg cytosol protein for the quantitative assays and/or by staining of ≥10% cells in semiquantitative method. Cases considered oestrogen receptor positive by at least one of the assays are considered as receptor positive.

Through a linkage between the DBCG and the Danish Cancer Registry, the DBCG was found to contain information on 94% of all breast cancer patients reported to the Danish Cancer Registry. The Danish Cancer Registry is considered close to complete regarding incident cases of malignant neoplasms diagnosed in Denmark since 1943 (Storm, 1991)

Study cohort
A research parity database was established from the CRS including all women born between April 1, 1935, and March 31, 1978, as earlier described (Westergaard et al., 1997, Melbye et al. 1997). Based on the person-identifiable CRS number, a linkage was performed with the DBCG giving information on registered invasive primary breast cancers in the period from January 1, 1978, to September 30, 1994.

Statistical analyses
The possible impact of reproductive history on the incidence of different types of breast cancer was investigated in a follow-up study analysed by log-linear Poisson regression models (Breslow and Day, 1987). All women entered the follow-up for

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each type of breast cancer on January 1, 1978, or on their 12-year birthday, whichever came last. The period at risk continued until a first time diagnosis of breast cancer (regardless of type), death, emigration, or September 30, 1994, whichever occurred first. Incidence rate ratios are referred to as relative risks. Adjustment was made for age using quadratic splines (with knots: 30,35,40,45,50,55) (Greenland, 1995), calendar period (1978-1982,1983-1988,1989-1992,1993-1994), age at first birth (nulliparous,<20,20-24,25-29,30-34,≥35) and parity (nulliparous, 1,2,3,4+). Splines were used in age adjustment in order to reduce the number of parameters in the type-specific analysis. If there was a relatively small number of cases of a specific subtype, fewer knots were used. All variables were treated as time-dependent. Differences in the asssociation between reproductive history and the incidence fo different subtypes were evaluated by competing risks analysis adjusting for type-specific effects of confounders. P-values for these tests have indices indicating the subtypes compared. For some of the subtypes, the associations with number of births and age at first birth could not be statistically modelled solely by a log-linear trend. All tests and confidence intervals are therefore based on categorised variables. However, in order to describe the overall trends and ease the comparison between subtypes, we have also chosen to give the average risk increase, but without confidence intervals. The average risk increases were estimated with the categorised continuous variables included in the model as continuous variables using the median value within each category as the category score. Traditionally, the risk of breast cancer in nulliparous women is compared with the risk in parous women, disregarding that the parous women have an inhomogenous risk profile according to their reproductive history. To ease the comparison with other studies, we followed this tradition, but in the notes of the tables we compare the risk in nulliparous women with the more homogenous group of parous women with only one birth at the age of 20 to 24 years. All calculations were performed using the SAS procedure PROC GENMOD (SAS Institute Inc, 1996).

Results
In total 1,529,512 women were included in the cohort. Of these, 1,000,276 (65.3%) women had 2,071,415 births before follow-up as follows: 254,694 (25.5 %) had one birth, 494,697 (49.5%) two, 193,250 (19.3%) three, and 57,635 (5.6%) four or more births. A total of 10,790 primary invasive breast cancers were detected in this co-hort during 22.3 million person-years of follow-up. Number of cases according to reproductive history, average age at diagnosis, percentage that were oestrogen receptor positive and percentage of tumours that were larger than 2 cm are shown for each type of breast cancer in Table I.

Reproductive history and the risk of breast cancer
Compared with nulliparous women, parous women had a 13% (8%-18%) lower risk of breast cancer. In parous women the risk of breast cancer increased by 10% by each 5-year postponement of the first birth (age(years) at first birth: 12-19: 0.99 (0.93-1.05), 20-24: 1 (ref), 25-29: 1.19 (1.13-1.24), 30-34 1.27 (1.17-1.37),

35+: 1.33 (1.14-1.55)), and there was a 10% decrease in risk by each additional birth: 1 childbirth: 1 (ref), 2 childbirths: 0.97 (0.92-1.02), 3 childbirths: 0.88 (0.82-0.94), 4+ childbirths: 0.70 (0.63-0.77). The association with reproductive history was not significantly modified by age. In women under 45 years of age, parous women had a significant 10% reduced risk compared with nulliparous, on average an 8% decreased risk per each additional birth, and an 11% increased risk per each 5-year postponement of the first birth. In women over 45 years of age parous women had an 18% reduced risk compared with nulliparous, on average a 12% decreased risk per each additional birth and a 9% increased risk per 5-year postponement of the first birth.

Reproductive history and receptor status
Oestrogen receptor status was available on 6,044 (56%) cases. Of these 68% were oestrogen receptor positive with an average age of 46.5 years at time of diagnosis, whereas patients with oestrogen negative tumours were on average 45.0 years at diagnosis (Table I).

Table II shows the association betwen reproductive history and the incidence of oestrogen receptor negative and positive tumours, respectively. Parous women had a 13% (0%-24%) lower risk of an oestrogen receptor negative tumour compared with nulliparous women and on average a 10% decreased risk by each additional birth. The woman's age at first birth was not significantly associated with her risk of developing oestrogen receptor negative tumours.

Compared with nulliparous women, parous women had a 24%(17%-31%) lower risk of developing a receptor positive tumour. The risk decreased on average by 12% by each additional birth, but was 12% higher by each 5-year increase in age of the woman at her first birth. The association between reproductive history and the incidence of oestrogen receptor positive tumours was not statistically different from the association with the incidence of oestrogen receptor negative tumours, although especially a late age at first birth tended to be stronger related to the risk of receptor positive tumours (12% increase compared with 4%, $p_{ER+ vs ER-}$=0.07).

The pattern was the same when restricting to women under 45 years of age and women aged 45 years or more. In women under 45 years of age the risk of estrogen positive and negative tumours decreased by 6% and 5%, respectively, by each additional birth ($p_{ER+ vs ER-}$=0.81), but was 17% and 8% higher by each 5-year increase in age of the woman at her first birth ($p_{ER+ vs ER-}$=0.17). In women aged 45 years or more the risk of estrogen positive and negative tumors decreased by 11% and 17% by each additional birth ($p_{ER+ vs ER-}$=0.17), but was 10% and 2% higher by each 5-year increase in age of the woman at her first birth ($p_{ER+ vs ER-}$=0.11).

Reproductive history and histological subtype

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Patients diagnosed with ductal carcinomas were on average 44.6 years at diagnosis compared with 46.1 years in patients diagnosed with lobular carcinomas (Table I).

Table III shows the association between parous status, number of births, age at first birth and the incidence of six histological subtypes. As more than 80% of the tumours were ductal carcinomas, the association between reproductive history and the incidence of this subtype was as expected almost identical to the association with the overall incidence of breast cancer. The incidence was 14% lower in parous compared with nulliparous women, the risk decreased on average by 11% by each additional birth and increased by 9% by each 5-year postponement of the first birth (Table III).

The incidence of lobular carcinomas followed a different pattern (Table III). There was no association with parous status nor number of births. However, by each 5-year postponement of the first birth the risk increased on average by 22%. The association between parous status ($p_{lobular\ vs\ ductal}$ =0.10), number of births ($p_{lobular\ vs\ ductal}$=0.09) and the risk of lobular carcinomas was not significantly different from the association with the incidence of ductal carcinomas, but age at first birth was found to have a significantly stronger association with the incidence of lobular carcinomas compared with ductal carcinomas ($p_{lobular\ vs\ ductal}$=0.01).

The risk of developing a mucinous carcinoma was 64% (47%-76%) lower in parous compared with nulliparous women. There was no significant association with number of births but a tendency to an association with a late age at first birth, with a 29% increased risk by each 5-year postponement of first birth (p=0.06). Compared with the associations with the incidence of ductal carcinomas, the association with parous status was significantly stronger ($p_{mucinous\ vs\ ductal}$ <0.001), whereas the association with number of births ($p_{mucinous\ vs\ ductal}$=0.58) and age at first birth ($p_{mucinous\ vs\ ductal}$=0.22) were similar.

The incidence of medullar, papillary and tubular carcinomas was not significantly related to reproductive history (Table III). The lack of association may, however, be due to low statistical power because of the small number of these types. This is further supported by the fact that the associations were statistically similar to the association between reproductive history and the incidence of ductal carcinomas.

Reproductive history and laterality
DBCG registered 10,241 (95%) cases as unilateral breast cancer. (Table I). Of these 5,153 (50.3%) were left-sided and 5,088 (49,7%) were right-sided, i.e. there was a left-right ratio of 1.01 (0.97-1.05). In patients younger than 45 years of age, the left-right ratio was 1.00 (0.96-1.09) and 1.02 (0.96-1.09) in nulliparous and parous women, respectively. Similar figures for patients aged 45 years or

older were 1.00 (0.94-1.06), and 1.06 (0.90-1.24). Tumour size, receptor status and age at diagnosis were not related to laterality (Table I).

As shown in table IV, the association between parous status on the incidence of left-sided breast cancer was 0.87 (0.80-0.94) and the association with right-sided breast cancer was 0.88 (0.80-0.96). Similarly, there was the same association between the incidence of left and right-sided tumours by number of births (10% decrease in risk per birth) and age at first birth (12% and 9% increase per 5-year, respectively). This pattern was the same when restricting the analysis to women younger or older than 45 years of age, respectively (data not shown).

Reproductive history on location
Patients with a tumour located non-centrally in the breast were on average 44.6 years old at diagnosis, whereas patients with a tumour located centrally in the breast were 45.4 years old at diagnosis (Table I).

The association between reproductive history and the incidence of breast cancer according to the location in the breast is shown in table V. The incidence of tumours in the four non-central parts of the breast (upper lateral, lower lateral, upper medial, lower medial) was statistically similarly related to reproductive history, and the four non-central sites are therefore considered together in the following. The risk of being diagnosed with a tumour in the non-central part of the breast was 10% lower for parous compared with nulliparous women. On average, the risk decreased by 10% per each additional birth and increased by 9% per 5-year postponement of the first birth (Table V). The incidence of tumours located centrally in the breast was 41% lower in parous compared with nulliparous women. There was no significant association with number births. On average, the risk increased by 30% by each 5-year postponement of the first birth. (Table V). Compared with the associations with non-central tumours, the incidence of central tumours was significantly stronger related to nulliparity ($p_{central vs non-central}$=0.003) and age at first birth ($p_{central vs non-central}$=0.02).

Paget's disease in the nipple was registered in 2% of the cases, but in the centrally located tumours the prevalence was 7%. The association between reproductive history and the incidence of centrally located tumours was not altered when excluding cases with Paget's disease in the nipple (67% lower risk in parous compared with nulliparous, 0% risk decrease per additional birth and 30% increased risk per 5-year postponement of the first birth).

Reproductive history and combinations of receptor status, histology and location
Receptor status, histology and location of a tumour are correlated, and the strong associations between a late age at first birth and the incidence of oestrogen receptor positive tumours, lobular and mucinous carcinomas and centrally located tumours may therefore be an expression of the same phenomenon. To investigate this further, we focused on correlated subtypes (e.g. ER+ and lobular

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carcinoma) and analysed the association between age at first birth and the incidence of combinations of these subtypes.

The percentage of oestrogen receptor positive tumours for each of the described subtypes is shown in Table I. Neither centrally located tumours nor mucinous carcinomas were significantly associated with the oestrogen receptor status, whereas lobular carcinomas compared with ductal carcinomas were more frequently oestrogen receptor positive (85% (465/546) versus 68% (3443/5027), p<0.001). We therefore looked at the association between late age at first birth and the incidence of lobular carcinomas according to receptor status. Including only oestrogen receptor negative tumours there was no difference in the association between age at first birth and the incidence of lobular carcinomas (6% increase per 5-year) compared with ductal carcinomas (4% increase per 5-year). In contrast, considering only oestrogen receptor positive tumours, the stronger association with lobular carcinomas (26% per 5-year) compared with ductal carcinomas (10% per 5-year) appeared again. The stronger association between age at first birth and oestrogen receptor positive tumours was seen in both lobular and non-lobular carcinomas.

There was no essential association between neither lobular nor mucinous carcinomas and being diagnosed with a location in the central part of the breast.

We have previously shown late age at first birth to strongly affect especially the incidence of late stage cases as measured by tumour size (Wohlfahrt et al., submitted). As shown in Table I, neither lobular carcinomas nor oestrogen positive tumours were markedly larger at diagnosis compared with ductal carcinomas and oestrogen negative tumours respectively. Tumours located in the central part of the breast, however, were significantly larger at diagnosis compared to non-central tumours (71% (375/525) versus 42% (3822/9052), p<0.001). We therefore looked at the association between age at first birth and the incidence of centrally located tumours according to tumour size. In an analysis including only tumours with a tumour size of 2 cm or less, we found the risk of centrally located tumours to increase by 11% per 5-year postponement of the first birth compared to 5% in non-central tumours. Including only tumours with a tumour size of more than 2 cm in the analysis, we found the risk of centrally located tumours to increase by 44% compared with 15% per 5-year in non-central tumours. In other words: the risk increases per 5-year in central compared with non-central tumours are 2.2 (=11%/5%) and 2.9 (=44%/15%) fold higher in analyses in which tumour size was taken into account compared with the 3.3 (=30%/9%) in the overall analysis, as seen in table V. Thus, less than 1/3 of the difference in effect of late age at first birth according to location can be explained by differences in tumour size.

TABLE I - NUMBER OF CASES ACCORDING TO NUMBER OF BIRTHS, AGE AT FIRST BIRTH, AVERAGE AGE AT DIAGNOSIS OF BREAST CANCER, PERCENTAGE OF OESTROGEN RECEPTOR POSITIVE AND PERCENTAGE OF TUMOURS LARGER THAN 2 CM BY SUBTYPE

	Total	Number of childbirths					Age at first birth					Age at diagn.	ER+	tum. size >2cm
		0	1	2	3	4+	12-19	20-24	25-29	30-34	35+	mean(yr)	pct.	pct.
Total	10,790	1,295	1,910	4,892	2,112	581	1,472	4,437	2,693	710	183	44.6	68%	44%
Oestrogen receptor status														
negative	1,910 (18%)	231	330	908	340	101	273	794	456	132	24	45.0	0%	53%
positive	4,134 (38%)	530	732	1,812	840	220	560	1,652	1,038	264	90	46.5	100%	45%
missing[1]	4,746 (44%)	534	848	2,172	932	260	639	1,991	1,191	314	69	42.9	-	39%
Histology														
ductal	8,669 (80%)	1,039	1,528	3,945	1,700	457	1,211	3,561	2,142	564	152	44.6	69%	44%
lobular	963 (9%)	92	160	458	185	68	108	403	276	68	16	46.1	85%	48%
mucinous	143 (1%)	34	27	52	28	2	10	51	35	10	3	44.8	74%	44%
medullary	294 (2%)	27	64	138	49	16	51	116	75	22	3	42.9	20%	50%
papillary	24 (<1%)	1	8	8	6	1	4	11	6	2	0	42.6	63%	47%
tubular	187 (2%)	22	35	82	42	6	28	78	38	19	2	45.6	83%	11%
other	207 (2%)	31	37	90	33	16	28	94	43	8	3	42.6	38%	52%
missing	303 (3%)	49	51	119	69	15	32	123	78	17	4	45.6	64%	43%
Laterality														
left	5,153 (48%)	612	880	2,374	1010	277	735	2,089	1,310	314	93	44.6	68%	44%
right	5,088 (47%)	597	933	2,276	1001	281	661	2,119	1,264	364	83	44.6	69%	44%
bilateral/missing	549 (5%)	86	97	242	101	23	76	229	119	32	7	45.5	69%	47%
Location														
central	586 (5%)	93	99	236	119	39	72	209	153	42	17	45.4	66%	71%

non-central	9,655 (90%)	1,116	1,714	4,414	1,892	519	1,324	3,999	2,421	636	159	44.6	69%	42%
upper lateral	5,643 (52%)	649	1,002	2,579	1,116	297	786	2,335	1,399	375	99	44.6	68%	45%
lower lateral	1,447 (14%)	165	241	679	283	79	187	617	369	88	21	44.8	71%	38%
upper medialt	1,888 (18%)	222	335	844	385	102	267	772	471	124	32	44.5	69%	39%
lower medialt	677 (6%)	80	136	312	108	41	84	275	182	49	7	44.1	65%	36%
bilateral/missing	549 (5%)	86	97	242	101	23	76	229	119	32	7	45.5	69%	47%

1 The relatively low mean age in the missing category is due to the fact that receptor status was not measured routinely in the earlier programmes of DBCG, i.e. the differences disappear when stratifying by calendar period.

TABLE II - ADJUSTED[1] RELATIVE RISK OF BREAST CANCER BY OESTROGEN RECEPTOR STATUS

Risk factors	ER+ RR (95%-CI)	ER- RR (95%-CI)	Test for: ER+ = ER-
Parous[2]			
no	1	1	p=0.09
yes	0.76 (0.69-0.83)	0.87 (0.76-1.00)	
	p<0.0001	*p*=0.06	
Number of childbirths			
1	1	1	p=0.09
2	0.92 (0.84-1.01)	1.02 (0.89-1.16)	
3	0.89 (0.80-0.99)	0.81 (0.69-0.95)	
4+	0.66 (0.56-0.77)	0.70 (0.55-0.88)	
	p<0.0001	*p*<0.0001	
Risk decrease per birth	*12%*	*10%*	
Age at first birth			
12-19	1.01 (0.92-1.12)	1.02 (0.89-1.18)	p=0.07
20-24	1	1	
25-29	1.23 (1.14-1.34)	1.09 (0.97-1.23)	
30-34	1.25 (1.10-1.43)	1.26 (1.04-1.52)	
35+	1.63 (1.31-2.03)	0.93 (0.62-1.41)	
	p<0.0001	*p*=0.15	
Risk increase per 5-year	*12%*	*4%*	

[1] Adjusted for type specific effects of age, calendar period, parity and age at first birth

[2] Comparing nulliparous with the more homogenous group of uniparous with a childbirth at age 20-24 the relative risks were: ER+: RR=0.77 (0.68-0.87), ER-: RR=0.89 (0.74-1.06).

TABLE III - ADJUSTED[1] RELATIVE RISK OF BREAST CANCER BY HISTOLOGICAL TYPE

Risk factor	Ductal RR (95%-CI)	Lobular RR (95%-CI)	Mucinous RR (95%-CI)	Medullary RR (95%-CI)	Papillary RR (95%-CI)	Tubular RR (95%-CI)
Parous[2]						
no	1	1	1	1	1	1
yes	0.86 (0.80-0.91) p<0.0001	1.03 (0.83-1.28) p=0.80	0.36 (0.24-0.53) p<0.0001	1.32 (0.88-1.97) p=0.17	2.76 (0.37-20.7) p=0.25	0.80 (0.51-1.25) p=0.34
Number of childbirths						
1	1	1	1	1	1	1
2	0.97 (0.91-1.03)	1.07 (0.89-1.29)	0.75 (0.46-1.22)	0.84 (0.62-1.15)	0.34 (0.12-0.95)	0.89 (0.59-1.34)
3+	0.82 (0.76-0.88)[3] p<0.0001	0.94 (0.76-1.17)[4] p=0.26	0.73 (0.42-1.29) p=0.48	0.63 (0.43-0.92) p=0.05	0.42 (0.14-1.27) p=0.13	0.78 (0.48-1.25) p=0.57
Risk decrease per birth	11%	3%	18%	18%	34%	17%
Age at first birth						
12-19	1.00 (0.94-1.07)	0.79 (0.64-0.98)	0.57 (0.29-1.13)	1.32 (0.94-1.84)	1.08 (0.34-3.43)	1.05 (0.68-1.62)
20-24	1	1	1	1	1	1
25-29	1.18 (1.11-1.24)	1.38 (1.18-1.61)	1.34 (0.86-2.08)	1.18 (0.88-1.59)	0.99 (0.36-2.73)	0.96 (0.65-1.42)
30+	1.27 (1.14-1.38)[5] p<0.0001	1.39 (1.09-1.78)[6] p<0.0001	1.51 (0.79-2.89) p=0.06	1.24 (0.79-1.96) p=0.36	0.81 (0.17-3.95) p=0.99	1.64 (0.98-2.74) p=0.29
Risk increase per 5-year	9%	22%	29%	0%	-9%	6%

[1] Adjusted for type specific effects of age, calendar period, parity and age at first birth.
[2] Comparing nulliparous with the more homogenous group of uniparous with a childbirth at age 20-24 the relative risks were: Ductal: RR=0.87 (0.79-0.94), Lobular: RR=0.93 (0.70-1.22), Mucinous: RR=0.43 (0.24-0.77), Medullary: RR=1.44 (0.88-2.34), Papillary: RR=5.48 (0.63-47.3), Tubular: RR=0.88 (0.49-1.58).
[3] 3 births: 0.87 (0.81-0.94), 4+ births: 0.67 (0.60-0.74).
[4] 3 births: 0.92 (0.74-1.16), 4+ births: 1.00 (0.74-1.35).
[5] 30-34 years :1.25 (1.14-1.37), 35+ years: 1.36 (1.15-1.61).
[6] 30-34 years: 1.41 (1.08-1.84), 35+ years: 1.31 (0.78-2.19).

TABLE IV - ADJUSTED[1] RELATIVE RISK OF BREAST CANCER
BY LATERALITY[2]

Risk factor	Left RR (95%-CI)	Right RR (95%-CI)	Test for: Left = Right
Parous[3]			
no	1	1	p=0.85
yes	0.87 (0.80-0.94)	0.88 (0.80-0.96)	
	p=0.001	*p*=0.004	
Number of childbirths			
1	1	1	p=0.32
2	1.01 (0.93-1.10)	0.92 (0.85-0.99)	
3	0.90 (0.82-0.99)	0.85 (0.77-0.94)	
4+	0.70 (0.61-0.81)	0.69 (0.60-0.80)	
	p<0.0001	*p*<0.0001	
Risk decrease per birth	*10%*	*10%*	
Age at first birth			
12-19	1.06 (0.97-1.15)	0.93 (0.85-1.01)	p=0.06
20-24	1	1	
25-29	1.23 (1.15-1.32)	1.17 (1.09-1.25)	
30-34	1.20 (1.06-1.36)	1.35 (1.20-1.51)	
35+	1.46 (1.18-1.81)	1.22 (0.97-1.53)	
	p<0.0001	*p*<0.0001	
Risk increase per 5-year	*12%*	*9%*	

[1] Adjusted for type specific effects of age, calendar period, parity and age at first birth.

[2] Bilateral cases are excluded.

[3] Comparing nulliparous with the more homogenous group of uniparous with a childbirth at age 20-24 the relative risks were: Left: RR=0.83 (0.74-0.93), Right: RR=0.92 (0.83-1.03)).

TABLE V - ADJUSTED[1] RELATIVE RISK OF BREAST CANCER BY LOCATION[2]

	Non-central					Central	Test for: non-centr. = central
Risk factor	Upper lateral RR (95%-CI)	Lower lateral RR (95%-CI)	Upper medial RR (95%-CI)	Lower medial RR (95%-CI)	Total[3] RR (95%-CI)	Total RR(95%-CI)	
Parous[4]							
no	1	1	1	1	1	1	
yes	0.90 (0.83-0.98) *p*=0.02	0.90 (0.76-1.06) *p*=0.19	0.88 (0.76-1.01) *p*=0.08	0.88 (0.70-1.12) *p*=0.32	0.90 (0.84-0.96) *p*=0.001	0.59 (0.47-0.73) *p*<0.0001	*p*=0.003
Number of childbirhts							
1	1	1	1	1	1	1	*p*=0.10
2	0.97 (0.90-1.05)	1.03 (0.88-1.20)	0.95 (0.83-1.08)	0.85 (0.69-1.05)	0.96 (0.91-102)	0.98 (0.76-1.25)	
3	0.88 (0.80-0.96)	0.89 (0.74-1.07)	0.90 (0.77-1.06)	0.63 (0.49-0.83)	0.86 (0.81-0.93)	1.07 (0.80-1.43)	
4+	0.67 (0.58-0.76) *p*<0.001	0.72 (0.55-0.94) *p*=0.01	0.68 (0.54-0.86) *p*=0.007	0.71 (0.49-1.03) *p*=0.006	0.68 (0.61-0.75) *p*<0.0001	1.02 (0.69-1.52) *p*=0.89	
Risk decrease per birth	*10%*	*9%*	*9%*	*16%*	*10%*	*-2%*	
Age at first birth							
12-19	1.01 (0.93-1.09)	1.01 (0.93-1.09)	1.04 (0.90-1.19)	0.91 (0.71-1.16)	0.99 (0.93-1.06)	0.99 (0.75-1.30)	*p*=0.02
20-24	1	1	1	1	1	1	
25-29	1.17 (1.09-1.25)	1.17 (1.03-1.34)	1.20 (1.07-1.35)	1.25 (1.03-1.51)	1.18 (1.12-1.24)	1.51 (1.22-1.87)	
30-34	1.27 (1.14-1.42)	1.14 (0.90-1.44)	1.28 (1.05-1.55)	1.31 (0.95-1.79)	1.25 (1.15-1.37)	1.71 (1.21-2.42)	
35+	1.35 (1.10-1.67) *p*<0.0001	1.11 (0.71-1.74) *p*=0.053	1.35 (0.94-1.94) *p*=0.01	0.73 (0.34-1.56) *p*=0.04	1.27 (1.08-1.49) *p*<0.001	2.78 (1.65-4.67) *p*<0.0001	
Risk increase per 5-year	*9%*	*9%*	*9%*	*11%*	*9%*	*30%*	

[1] Adjusted for type specific effects of age, calendar period, parity and age at first birth.
[2] Bilateral cases are excluded.
[3] The associations between reproductive history and incidence of the four non-central locations were identical (parous status: p=0.99, number of childbirths: p=0.47, age at first birth: p=0.87).
[4] Comparing nulliparous with the more homogenous group of uniparous with a childbirth at age 20-24 the relative risks were: Non-central: RR=0.91 (0.84-0.99), Central: RR=0.49 (0.35-0.68)).

Discussion
In this study we looked at the association between reproductive history and the incidence of subtypes of breast cancer according to receptor status, histology, laterality and location. The study was performed as a prospective analysis on a large population-based cohort and was based on mandatory reported exposure and outcome information, making information bias on exposure and selection bias on cases unlikely. The estimated effects of reproductive history for each subtype were adjusted for subtype specific age and calendar effects, thus taking into account differential age profiles and secular trends in the diagnosis of the subtypes. The large number of cases furthermore allowed us to study the incidence of combinations of subtypes to evaluate whether differences in the associations between reproductive history and subtype were independent.

Reproductive history and receptor status
In previous studies on reproductive risk factors for subtypes of breast cancer, the main focus has been on oestrogen receptor status. Most studies have found nulliparity and late age at first birth to be risk factors for oestrogen receptor positive tumours only, whereas studies on the effect of additional births have revealed less differences (Habel *et al.*, 1993, Stanford *et al.*, 1986, Yoo *et al.*, 1997, Potter *et al.*, 1995). Our finding is in concordance with this, and in particular, we confirm that a late age at first birth only affects the incidence of oestrogen-positive tumours. The pattern was not modified by age and therefore probably not by menopausal status either.

It has been discussed whether oestrogen status reflects different types of breast cancer or rather different stages in the neoplastic process, with oestrogen-positive tumours gradually becoming oestrogen-negative (Habel *et al.*, 1993). Differences in the association with reproductive history would reflect different risk factors for the various subtypes and, in the latter case, different progression factors between the different stages. Our analysis cannot differentiate between - these to interpretations. However, if oestrogen receptor status reflects different types of breast cancer, our finding of a significant association between the incidence of oestrogen-positive tumours and both nulliparity and a late age at first birth (i.e. high risk of being nulliparous at the initiation of a tumour) would be compatible with the hypothesis that the higher level of oestrogen in nulliparous women can stimulate initiation and promotion of breast tumours.

Reproductive history and histological subtypes
Studies on the association between reproductive history and breast cancer according to histological subtype have been limited and with inconsistent results (Mausner *et al.*, 1969, Morrison, 1976, LiVolsi *et al.*,1982, Rosen *et al.*, 1982, Kvåle *et al.*, 1987, Ewertz and Duffy, 1988, Stalsberg *et al.*,1989, Claus *et al.*, 1993). According to three of these studies (Morrison, 1969, LiVolsi *et al.*, 1982, Stalsberg *et al.* 1989), age at first birth had a stronger effect on (or even restricted to) lobular carcinomas compared with ductal carcinomas, but this is not

supported by two other studies (Ewertz and Duffy, 1988, Claus *et al.*, 1993). Our cohort study supported the repeated finding of a significantly stronger effect, but found no evidence of the effect of age at first birth being restricted to lobular carcinomas. Our finding of a stronger association supports the theory according to which additional carcinomas occurring in women with a late age at first birth originate in the lobules rather than ducts by selectively increasing the number of lobular cells at risk (Stalsberg *et al.*, 1989). But also higher hormonal sensitivity in the cells from which lobular carcinomas originate may play a role, as we observed the strong association to be limited to the oestrogen receptor positive lobular carcinomas.

We found the incidence of mucinous carcinomas in parous women to be 36% (24%-53%) of the incidence in nulliparous women. The association is significantly stronger compared with the association with the incidence of ductal carcinomas, and cannot be explained by differences according to receptor status or tumour size. The finding is in line with Stalsberg et al. (Stalsberg *et al.*, 1989) who observed the incidence of mucinous carcinomas in gravi women to be only 30% of the incidence in nulligravi women (p<0.01). We furthermore found a tendency towards a stronger association with age at first birth on the incidence of mucinous carcinomas, which was not reported previously.

It should be noted that the present study only comprises patients in DBCG with available information on reproductive history from the national registries, i.e. women born in 1935 or later. The average age at diagnosis was therefore only 44.6 years. This implies that there is a relatively low proportion of lobular and tubular carcinomas, compared with other settings, as these tumours on average are diagnosed relatively later. For the same reason, there is a relatively higher proportion of medullar carcinomas as they are diagnosed at a relatively early age. However, this introduces no bias as we adjusted for subtype-specific age effects in all analysis.

Reproductive history and laterality
It has become a general belief that the incidence of left-sided breast cancer is higher than that of right-sided breast cancers (Weiss *et al.*, 1996). Two case-studies have found a relation between nulliparity and the left-right ratio. According to the study by Ekbom et al. (Ekbom *et al.*, 1994), nulliparous women under 45 years had a right dominance, whereas Senie et al. (Senie *et al.*, 1980) found left dominance in parous women over 40 years. We found no difference in the association with reproductive history and the incidence of left versus right-sided breast cancer, neither overall nor in women under or over 45 years of age. Therefore, our study does not support the hypothesis that a left-side dominance can be ascribed to reproductive history.

Reproductive history and locality
To our knowledge, no previous reports have focused on the association between reproductive risk factors and the risk of breast cancer according to the localiza-

tion of the tumour in the breast. In our study, parous status and age at first birth were to a much greater extent related to the incidence of centrally located tumours compared with tumours located non-centrally, and the number of additional births was not associated with the incidence of centrally located tumours. These special associations for centrally located tumors were not related to Paget's disease of the nipple or a special proportion of lobular or receptor positive tumours in this area of the breast.

We have in a previous study shown late age at first birth to strongly affect especially the incidence of late stage cases as measured by tumour size (Wohlfahrt *et al.*, submitted). Tumours located in the central part of the breast were significantly larger at diagnosis compared with non-central tumours, probably because they may be more difficult to detect. However, we found that less than 1/3 of the difference in the association with age at first birth according to location could be explained by difference in tumour size.

Women diagnosed with a centrally located tumour were on average relatively older compared with women diagnosed with a non-central tumour, and the same pattern was found in patients with lobular compared with ductal carcinomas and oestrogen-positive compared with oestrogen-negative tumours (Table I). For both non-central tumours, lobular carcinomas and oestrogen-positive tumours, we observed a relatively stronger association with age at first birth (and in the first two types no effect of additional births). A common explanation for these findings could be an effect modification by age or menopausal status, with a stronger association with age at first birth and no association with number of births in older women, and in younger women a smaller association with age at first birth and a strong association with number of births. However, if anything, the literature points in the opposite direction (Velentgas *et al.*, 1994), and in our study we found no effect modification by age, but a tendency in concordance with the literature.

Preterm delivery and risk of breast cancer (Study 6)

Material and methods

Registries
We performed a linkage of data from the Danish Civil Registration System (CRS) with the National Birth Registry, the National Hospital Discharge Registry, the National Registry of Induced Abortions, and the Danish Cancer Registry. since April, 1968, the CRS has assigned a unique identification number to all residents in Denmark which permits accurate linkage of information from different registries. The CRS also keeps updated information on dates of live-births and documents demographic information such as emigration and death.

The National Birth Registry has since 1973 registered all livebirths and stillbirths in Denmark (not including spontaneous and induced abortions). Since 1978, exact (in weeks) gestational age determinations have been included. Gestational age determination is based on information of last menstrual period combined with an early clinical bimanual palpation. In situations of inconsistency between these measures, ultrasound scanning is performed. In the most recent years the use of ultrasound scanning has become widespread and has as such contributed increasingly to the determinations of the gestational age (Sundhedsstyrelsen, 1993). Since 1977, information on spontaneous abortions without specified gestational age has been recorded in the National Hospital Discharge Registry. Information on induced abortions has been recorded in the National Registry of Induced Abortions since reporting became mandatory in 1939. However, information is only available in a computerized format since 1973 (Melbye et al, 1997). The Danish Cancer Registry includes a close to complete registration of cancer diagnoses on all Danish residents back to 1943 (Storm, 1991)).

Subjects
A research database was established from the CRS including all women born in Denmark between April 1, 1935, and March 31, 1978, with information on liveborn children. From the National Birth Registry additional information on stillbirths was added as was gestational age-specific information on all births since 1978. Finally, information on spontaneous (since 1977) and induced abortions (since 1973) was added.

Analyses
The possible impact of gestational age at delivery (stillbirth, preterm, or term delivery) on the risk of breast cancer was investigated among parous women in a log-linear Poisson regression model (Breslow et al, 1987). All women entered the follow-up for breast cancer at the first delivery they had during the period between January 1, 1978, and December 31, 1992, in which gestational age was recorded. Thus, also women with pregnancies before January 1, 1978, were included in the study provided they had a delivery during the study period. The period at risk continued until breast cancer diagnosis, death, emigration, disappearance, or December 31, 1992 (at which time the cancer registration was considered complete), whichever occurred first. Person-years at risk were calculated continuously according to the categorical groups of gestational age of the most recent birth in 1978-92, i.e. women with more than one birth in 1978-92 were in the period between the first and second birth considered at risk according to the gestational age of the first birth; between the second and third birth according to the gestational age of the second birth; and so on. To evaluate the effect of *ever* having a preterm delivery an additional analysis was performed where person-years at risk were calculated continuously in categorical groups according to the birth with the lowest gestational age since 1978.

Adjustments were made for attained age (1-year intervals), calendar period (5-year intervals), age at first birth (12-19,20-24,25-29,30-34,>34 years), and parity

(1,2,3,4,5,6,7+ births; including stillbirths, preterm and term deliveries). In an additional analysis we adjusted for history of spontaneous and induced abortion and whether the birth was a stillbirth or a multiple birth. Note that also information on history of spontaneous and induced abortions, stillbirths, and livebirths prior to January 1, 1978, was used in the adjustment. Estimation of breast cancer incidence rate ratios was performed using the SAS procedure PROC GENMOD (SAS Institute, 1996). These rate ratios were used as a measure of the relative risk. Test for trend was performed with gestational age treated as a continuous variable and the median gestational age used as the value for each group. The linear assumption in the trend test was checked by a likelihood ratio test against the model with gestational age as categorical variable. Effect modification was evaluated as a test for interaction between categorical variables.

To assess the possible effect of misclassification due to unregistered gestational age in births prior to 1978 we estimated the percentage of person-years of follow up and the number of cases in each cell that might be attributed to the "ever a delivery with a gestational age less than 32 weeks"-category instead of the "never"-category, and then performed the analysis with the adjusted figures. The percentage of person-years was calculated on the basis of the age-specific cumulative incidence at the baseline of the study, and the number of cases was calculated as the product of the estimated person-years and the rate in the ever category found in the original analysis. The age-specific cumulative incidence of having a delivery with a gestational age less than 32 weeks was calculated using age specific incidence rates seen in 1983 to 1992.

Results
Overall, 474,156 parous women were included in the cohort study. In the follow-up a total of 740,794 births were recorded and distributed as follows: 254,458 women (53.7%) had one birth, 178,700 women (37.7%) had two, 35,791 women (7.5%) had three, and 5,207 women (1.1%) had four or more births. Among these births, 3,261 were stillbirths (0.4%) and 37,347 (5.0%) were preterm (<37 gestational weeks). Preterm births with a gestational age of 32-36 weeks contributed 4.2%, with a gestational age of 29-31 weeks 0.5%, and with a gestational age of less than 29 weeks 0.3 %. The number of women with a preterm delivery was as follows: 32-36 weeks: 29,488 women; 29-31 weeks: 3,702 women; <29 weeks: 2,181 women. Parous women represented a total of 3.8 million person-years of follow-up and 1,363 of these women developed breast cancer. Table 1 presents a detailed distribution of number of breast cancer diagnoses and person-years of follow-up.

As shown in Table 2, we found a significantly increased relative risk of breast cancer in women with a preterm delivery at <29 gestational weeks of 2.11 (95% confidence intervals (CI): 1.00-4.45) and at 29-31 gestational weeks of 2.08 (1.20-3.60), which subsequently dropped as follows: 32-33 weeks: RR=1.12 (0.62-2.04); 34-35 weeks: RR=1.08 (0.71-1.66); 36-37 weeks: RR= 1.04 (0.83-1.32); 38-39 weeks: RR=1.02 (0.89-1.17), 40 weeks: 1 (reference). The continued

decline in relative risk observed for preterm deliveries was statistically significant (p-trend=0.04). The trend remained significant after adjustment for history of spontaneous abortion, history of induced abortion, and whether the birth was a stillbirth and/or a multiple birth (p-trend=0.04). A stratified analysis which was performed to evaluate whether the increased risk of breast cancer was associated both with preterm births (liveborn) and preterm stillbirths gave the following result with term deliveries as reference: preterm births with gestational age < 32 weeks: RR=1.98 (1.24-3.16); stillbirths with gestational age < 32 weeks: RR=4.62 (0.42-50.9).

The possible effect modification by age of the woman, number of previous births, age at delivery, and history of previous preterm births or stillbirths is evaluated in Table 3. None of these characteristics significantly modified the risk association observed with gestational age. However, the number of cases in some of the stratified subgroups became very small. We evaluated whether possible temporal changes in the validity and completeness of the ascertainment of the gestational age had a measurable effect on the results by testing whether there was a significant effect modification by period of delivery. This was not the case (p=0.62).

Comparing parous women *ever* having a delivery of less than 32 gestational weeks with other parous women we found a significantly increased risk of 1.72 (1.14-2.59). Considering only parous women ever having a delivery less than 32 weeks gestation, but with the most recent delivery being equal to or longer than 32 weeks gestation, we found no increased risk when comparing to parous women who never had had a delivery of less than 32 gestational weeks (RR=0.82; 95% CI: 0.26-2.55). However, this result was based on only three cases of breast cancer in this particular group of women.

Based on the age-specific incidence rates of births with a gestational age less than 32 weeks we estimated that less than 2% will ever experience such a delivery. Taking that into account at the baseline of the analysis the rate ratio between parous women ever having a delivery less than 32 gestational weeks and other women increased from 1.72 to 1.73.

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Table 1. Distribution of number of breast cancer diagnoses and person-years of follow-up according to reproductive history.

	Preterm delivery				Full-term delivery			
	No. cases	(%)	Person-years (x10³)	(%)	No. cases	(%)	Person-years (x10³)	(%)
Age (years)								
<35	16	(20%)	127	(69%)	315	(25%)	2507	(70%)
35-39	31	(38%)	35	(19%)	417	(32%)	714	(20%)
40-44	24	(30%)	16	(9%)	379	(30%)	299	(8%)
45-49	8	(10%)	5	(3%)	147	(11%)	72	(2%)
50+	2	(2%)	1	(0.4%)	24	(2%)	9	(0.2%)
Age at first birth (years)								
<20	9	(11%)	30	(17%)	93	(7%)	464	(13%)
20-24	24	(30%)	82	(45%	432	(34%)	1728	(48%)
25-29	27	(33%)	52	(28%)	501	(39%)	1107	(31%)
30-34	18	(22%)	15	(8%)	191	(25%)	254	(7%)
35+	3	(4%)	4	(2%)	65	(5%)	48	(1%)
Age at latest birth (years)								
<20	0	(0%)	8	(4%)	1	(0.1%)	105	(3%)
20-24	1	(1%)	47	(26%)	54	(4%)	874	(24%)
25-29	23	(28%)	68	(37%)	351	(28%)	1449	(40%)
30-34	29	(36%)	41	(22%)	513	(40%)	872	(24%)
35+	28	(35%)	20	(11%)	363	(28%)	300	(9%)
Number of previous births								
0	23	(28%)	78	(42%)	240	(19%)	1281	(36%)
1	31	(38%)	68	(37%)	611	(48%)	1609	(45%)
2	19	(24%)	27	(15%)	313	(24%)	553	(15%)
3+	8	(10%)	11	(6%)	118	(9%)	157	(4%)
Previous preterm birth or stillbirth·								
Yes	5	(6%)	12	(7%)	17	(1%)	60	(2%)
No	76	(94%)	171	(93%)	1265	(99%)	3540	(98%)
The delivery was a multiple birth								
Yes	9	(11%)	16	(9%)	20	(2%)	35	(1%)
No	72	(89%)	167	(91%)	1262	(98%)	3566	(99%)

· By *previous* means prior to the most recent pregnancy.

Table 2. Adjusted relative risk of breast cancer in 474,156 parous women according to gestational age at delivery.

Gestational* age (weeks)	No. of cases	Person-years (x10³)	RR (95% CI)
<29	7	9	2.11 (1.00-4.45)
29-31	13	17	2.08 (1.20-3.60)
32-33	11	26	1.12 (0.62-2.04)
34-35	22	58	1.08 (0.71-1.66)
36-37	82	214	1.04 (0.83-1.32)
38-39	350	949	1.02 (0.89-1.17)
40	552	1526	1
>40	326	985	1.03 (0.90-1.18)

* Adjusted for age, calendar period, parity, and age at first birth.

Table 3. Adjusted[*] relative risk of breast cancer in parous women according to gestational age at delivery. Stratified by number of previous births, age, and history of previous pre-term births/stillbirths.

	Gestational age					
	≥ 37 weeks		36-32 weeks		<32 weeks	
	No. cases	RR (ref.)	No. cases	RR (95% CI)	No. cases	RR (95% CI)
Age of woman[†]						
<40 years	732	1	37	1.21 (0.87-1.69)	10	2.00 (1.07-3.74)
≥40 years	550	1	24	0.88 (0.58-1.32)	10	2.11 (1.13-3.95)
Number of previous births[§]						
0	240	1	17	1.14 (0.70-1.87)	6	2.41 (1.07-5.42)
1+	1042	1	44	1.03 (0.76-1.39)	14	1.94 (1.14-3.29)
Age at delivery[|]						
<30 years	406	1	20	1.20 (0.77-1.89)	4	1.62 (0.60-4.33)
≥30 years	876	1	41	1.00 (0.73-1.37)	16	2.22 (1.35-3.64)
Previous[‡] pre-term birth[¶] or stillbirth[#]						
No	1265	1	58	1.06 (0.82-1.38)	18	1.97 (1.24-3.14)
Yes	17	1	3	1.02 (0.30-3.49)	2	3.64 (0.84-15.8)

[*] Adjusted for age of the woman, calendar period, parity, and age at first birth., [†] Test for effect modification: p=0.47. A similar lack of effect modification (p=0.73) was found if age of woman was divided by age 50 years., [‡] By *previous* means prior to the most recent pregnancy, [§] Test for effect modification: p=0.86

[|] Test for effect modification: p=0.67, [¶] Pre-term birth: gestational age < 37 weeks., [#] Test for effect modification: p=0.76

Discussion
Based on this large cohort of almost half a million parous women we found as-
suring evidence that a preterm delivery of 32+ weeks gestation does not signifi-
cantly increase the risk of premenopausal breast cancer. Overall, 84% of all pre-
term deliveries are of 32+ weeks gestation. Only for the small group of preterm
deliveries of less than 32 weeks gestation was there a 2-fold increased risk of
breast cancer when comparing with a full-term delivery. This elevated relative
risk was obtained in an analysis in which a woman's person-years at risk were
calculated continuously according to the gestational age of the most recent
birth. In an analysis which instead compared parous women *ever* having a de-
livery of less than 32 gestational weeks with other parous women the risk was
1.7-fold increased. In this last analysis, the preterm birth will not necessarily
have been the most recent birth and we speculate whether the somewhat lower
estimate could indicate that a full-term birth following a preterm birth might
diminish the effect of a preterm birth on breast cancer risk. We found some sup-
port for this assumption in a restricted analysis which estimated the risk in
parous women ever having a delivery of less than 32 weeks gestation but with
the most recent delivery being of 32+ gestational weeks. However, this particu-
lar analysis has very limited power.

The analysis of parous women *ever* having a delivery with a gestational age less
than 32 weeks compared to other women might be subject to some misclassifi-
cation, since many of the included women may have had pre-term births prior to
1978. This misclassification, however, is non-differential, and estimating the ef-
fect, we found that it was ignorable, as only a very small fraction of women
categorised as never having a delivery with a gestational age less than 32
weeks in fact had such a birth prior to 1978.

We used a cohort design for our study based on mandatory reported exposure
and outcome information. However, some limitations of the study should be ac-
knowledged. Our gestational age specific relative risk estimates do not follow a
smooth curve but instead increase rather abruptly below 32 weeks gestation.
This might suggest that the elevated risk of breast cancer among women with a
very preterm delivery was a chance finding. However, another explanation
would be that the small number of cases with very early preterm deliveries
makes it difficult to assess the true magnitude of the effect. In particular, the es-
timate obtained among women with a preterm delivery of less than 29 weeks
was based on only 7 cases of breast cancer and 9,000 person-years of follow-up.
However, it is important to note that this estimate did not stand alone but was
supported by a similarly increased risk for women with a preterm delivery of 29-
31 gestational weeks. We were unable to determine whether the observed risk
was due to the preterm delivery per se or the shorter duration of pregnancy. The
observation that both women with a preterm stillbirth and women with a pre-
term livebirth (<32 weeks) had elevated relative risks of breast cancer would be
in support of the latter but the figures for stillbirths became very small.

The present study allowed us to consider the influence of potentially confound-
ing factors such as age, age at first birth, parity, multiple births, abortion history,

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and history of stillbirths. However, several factors (smoking history, body mass index, age at menarche and menopause, family history, oral contraceptives, postmenopausal hormones) that have been suspected as risk factors for breast cancer could not be accounted for because we lacked the necessary information. The lack of adjustment for such factors would only be important for our results should these factors influence both on the occurrence of breast cancer *and* preterm births. Smoking during pregnancy and high pre-pregnant body weight have been linked to preterm births (Naeye, 1990; Williams et al, 1992). However, there is litle evidence for an association between smoking and breast cancer and the association between body mass and breast cancer remains controversial (Palmer et al, 1993; Hunter et al, 1996). Other factors that have been associated with preterm births are e.g. low social class and low educational level (Pickering et al, 1991). However, breast cancer risk is associated with high social status and thus we would expect the observed relative risks to be underestimated rather than the opposite.

We are not aware of any previous cohort study addressing the risk of breast cancer according to week of gestation at delivery. In a case-control study, Choi et al (Choi et al, 1978) reported an insignificantly 1.4-fold increased risk of breast cancer in premenopausal women who had a terminated pregnancy of more than five gestational months compared to women without such experience. Another case-control study focused on livebirths did not find an increased risk among women with preterm deliveries (Rao et al, 1994) but the total exposure group in that study only counted seven women with a delivery of less than 30 weeks. Stillbirth has not been associated with increased risk of breast cancer, but the available studies have been based on a very limited number of cases and lacked information on gestational length of the pregnancy (Choi et al, 1978; Polednak et al, 1983).

Studies of spontaneous abortion have generally not revealed significantly positive associations (reviewed in Calle et al, 1995). In a recent study by Newcomb et al (Newcomb et al, 1996), a slightly increased risk of breast cancer was recorded but the authors cautioned that the finding might be due to recall bias in their case-control design. Most spontaneous abortions take place early in pregnancy and studies have so far lacked detailed information on gestational week at the time of the abortion. Spontaneous abortion may in certain ways be more like a preterm delivery than an induced abortion but they both represent an interruption of pregnancy (Zang, 1996). The results of case-control studies on induced abortion have been inconsistent with risk estimates ranging from moderately elevated to lowered values (Rosenberg et al, 1994). In a large prospective study we found no overall increased risk of breast cancer after an induced abortion, with the exception of the very small group of women with a late second trimester abortion (Melbye et al, 1997).

In conclusion, a preterm delivery did not significantly increase the woman's risk of contracting premenopausal breast cancer apart from the very small group of women with a preterm delivery of less than 32 weeks gestation. Despite the large size of this study there were only few cases of breast cancer in the subgroups representing the very early deliveries and these results should therefore be considered with due caution.

In the present study we took advantage of the long tradition for mandatory reporting of pregnancy characteristics and cancer diagnoses in Denmark to address in a prospective study whether women with preterm delivery are at increased risk of breast cancer compared to other women.

Maternal risk of breast cancer and birth characteristics of offspring by time since birth (Study 7)

Material and methods
Since April 1, 1968, the Civil Registration System (CRS) in Denmark has assigned a unique registration number to all citizens, thereby facilitating accurate linkage of registries. Information on dates and gender of live births, emigration and vital status was obtained from the CRS. From the National Birth Registry we obtained information on dates and gender of stillbirths and information on gestational age (in weeks) and birth weight (in groups of 250 g) on all births since 1973. To identify multiple pregnancies we looked for children (live or stillbirths) born to the same mother within two days.

Invasive primary breast cancers were identified in the Danish Breast Cancer Group's registry (DBCG). [9-10] This registry has since 1978 collected detailed information on the breast cancer diagnosis including the size of the tumor, number of positive nodes, receptor status, histology, localization and laterality. Through a linkage between the DBCG's registry and the Danish Cancer Registry, the DBCG's registry was found to contain information on 94% of all breast cancer patients reported to the Danish Cancer Registry. The Danish Cancer Registry is considered close to complete regarding incident cases of malignant neoplasms diagnosed in Denmark since 1943. [11]

A research parity database was established from the CRS including all women born between April 1, 1935, and March 31, 1978 as earlier described. [12-13] Based on the person-identifiable CRS number a linkage was performed with the DBCG giving information on registered invasive primary breast cancers in the period from January 1, 1978, to September 30, 1994.

We investigated the possible impact of the plurality, birth weight and gender of the latest offspring on the subsequent incidence of the maternal breast cancer risk using a follow-up study, with analysis by log-linear Poisson regression models. [14] All parous women entered the follow-up for breast cancer on January 1, 1978, or on the date of their first childbirth, whichever came last. The period at risk continued until breast cancer, death, emigration, or September 30, 1994, whichever occurred first. Adjustment was made for attained age (=25,26,27,......,56,57,58), calendar period (1978-1982,1983-1988,1989-1992,1993-1994), age at first birth (<20,20-24,25-29,30-34,=35), number of births (1,2,3,4,5,6,7+). As we have previously shown mothers with an extremely preterm birth as the latest birth to have an increased risk of breast cancer[15], we therefore furthermore adjusted for extremely preterm birth (<32 weeks, = 32 weeks, unknown). Due to lower number of cases in the tumor size-specific

analysis number of births were categorized (1,2,3,4+) and age adjustment was performed by quadratic splines (with knots: 30,35,40,45,50,55). [16] All variables were treated as time-dependent variables. Using year of birth instead of calendar period had no effect on the results in Tables 1 and 2. No residual confounding was revealed by adjustment with a main effect of time since latest birth categorized more than "<5 years" and "=5 years". The numbers of person-years at risk for birth characteristic groups were calculated according to birth characteristics of the latest birth, as the focus was the effect in the first years after delivery. Women with more than one birth were, in the period between the first and the second birth, considered at risk according to the characteristics of the first birth; between the second and the third birth they were considered at risk according to the characteristics of the second birth; and so on. In the analysis of gender and birth weight of offspring, the observation periods with the latest birth as a multiple birth were excluded from follow-up.

Results

During the 12.8 million person-years of follow-up 9,495 cases of breast cancer aged 22 to 58 years were identified.

In table 1 the association is shown between birth characteristics of a woman's latest birth and her risk of breast cancer according to the time interval since the birth. In the first 5 years following a multiple versus a singleton birth, the risk of breast cancer was higher (RR=1.8 (1.1-2.8)). The higher risk was seen in both uniparous (RR=1.9 (0.8-4.6) and multiparous (RR=1.7 (1.0-3.0) mothers. After 5 years there was no appreciably increased risk (RR=1.1 (0.9-1.3)). Mothers delivering a heavy-weighted child subsequently had a higher risk of breast cancer compared with mothers delivering a small child. The risk increased by 10% per 1 kilogram increase in birth weight (RR_{trend}=1.1 (1.0-1.2) per kg) (Table 1). In the first 5 years following a birth the risk of breast cancer increased by 20% per kg (RR_{trend}=1.2 (1.0-1.5) per kg). The trends were RR_{trend}=1.1 (0.8-1.5) per kg and RR_{trend}=1.2 (1.0-1.5) per kg in uniparous and multiparous, respectively. After the 5-year period the relative increase per kg was 10% (RR_{trend} =1.1 (1.0-1.2) per kg). According to additional analysis, mothers delivering a child with a birth weight from 3.75 up to 4 kg and larger than 4 kg, respectively, both had a 10 % overall higher risk ($RR_{3.75kg-4kg}$ =1.1 (1.0-1.2), $RR_{>4kg}$ =1.1 (1.0-1.3)) compared with mothers with a newborn of 3 kg or less. There was no difference in the breast cancer incidence according to gender of the child (Table 1).

Additional information on the characteristics of the breast cancer at diagnosis and the large number of cases in each birth weight category allowed us to estimate the risk according to birth weight of latest offspring by tumor size (Table 2). The overall increase in risk during the first 5 years following a birth in mothers delivering a heavy-weighted child was primarily due to an increase in larger tumors (>2 cm) (RR_{trend}= 1.5 (1.1-2.1) per kg). The effect on small tumors (=2 cm) was smaller (RR_{trend}=1.2 (0.8-1.6) per kg). The effect of birth weight of offspring in the first 5 years after the birth was seen primarily on the incidence on estrogen negative (RR_{trend} =1.3 (0.8-2.1) per kg) compared with estrogen positive tumors (RR_{trend} =0.9 (0.6-1.3) per kg).

TABLE 1. Adjusted* effect of birth characteristics of latest offspring on the maternal risk of breast cancer overall and according to time since latest birth.

| Birth characteristics of the latest offspring | Person years | Rate Ratio overall | | Rate Ratio according to time since latest birth | | | |
| | | | | <5 years | | =5 years | |
		no.	RR(95%-CI)	no.	RR (95%-CI)	no.	RR (95%-CI)
Multiple birth							
no	$12,592 \times 10^3$	9,327	1	663	1	8,664	1
yes	185×10^3	168	1.1 (1.0-1.3)	18	1.8 (1.1-2.8)	150	1.1 (0.9-1.3)
Birth weight[†,‡]							
= 3 kg	$1,617 \times 10^3$	739	1	115	1	624	1
3-3.25 kg	$1,241 \times 10^3$	560	1.0 (0.9-1.1)	78	0.9 (0.7-1.2)	482	1.0 (0.9-1.1)
3.25-3.5 kg	$1,610 \times 10^3$	758	1.0 (0.9-1.1)	130	1.1 (0.9-1.5)	628	1.0 (0.9-1.1)
3.5-3.75 kg	$1,388 \times 10^3$	666	1.0 (0.9-1.1)	116	1.1 (0.9-1.4)	550	1.0 (0.9-1.1)
>3.75 kg	$2,063 \times 10^3$	1151	1.1 (1.0-1.2)	198	1.2 (0.9-1.5)	953	1.1 (1.0-1.2)
Increase in risk per kg[§]			1.1 (1.0-1.2)		1.2 (1.0-1.5)		1.1 (1.0-1.2)
Gender[†]							
boy	$6,422 \times 10^3$	4,786	1	331	1	4,455	1
girl	$6,170 \times 10^3$	4,541	1.0 (1.0-1.0)	332	1.0 (0.9-1.2)	4,209	1.0 (0.9-1.0)

* Adjustment was made for attained age, calendar period, age at first birth, number of births and extremely preterm birth.
† Only singleton births are included.
‡ Only mothers with a birth from 1973 and onwards are considered in these analyses.
§ Birth weight was treated as a continuous variable, with the median for each category as the category value.

TABLE 2. Adjusted* effects of birth weight of latest offspring on the maternal risk of breast cancer according to time since latest birth and tumor size at diagnosis.

Birth weight of the latest offspring	Rate ratio according to time since latest birth and tumor size at diagnosis							
	< 5 years				= 5 years			
	=2 cm		>2 cm		=2 cm		>2 cm	
	no.	RR (95%-CI)	no.	RR (95%-CI)	no.	RR (95%-CI)	no.	RR (95%-CI)
Birth weight†								
= 3 kg	51	1	46	1	346	1	240	1
3-3.25 kg	40	1.1 (0.7-1.6)	27	0.8 (0.5-1.3)	261	1.0 (0.8-1.2)	194	1.1 (0.9-1.3)
3.25-3.5 kg	61	1.2 (0.8-1.8)	55	1.2 (0.8-1.8)	328	0.9 (0.8-1.1)	252	1.0 (0.9-1.2)
3.5-3.75 kg	49	1.1 (0.7-1.6)	52	1.2 (0.8-1.8)	280	0.9 (0.8-1.1)	236	1.1 (0.9-1.3)
>3.75 kg	91	1.2 (0.9-1.7)	93	1.4 (0.9-1.9)	495	1.0 (0.9-1.2)	409	1.2 (1.0-1.4)
Increase in risk per kg‡		1.2 (0.8-1.6)		1.5 (1.1-2.1)		1.0 (0.9-1.2)		1.2 (1.0-1.4)

*Adjustment was made for attained age, calendar period, age at first birth, number of births and extremely preterm birth.

†Only singleton births are considered and only mothers with a birth from 1973 and onwards are included in these analyses.

‡Birth weight was treated as a continuous variable, with the median for each category as the category value.

Discussion

The present study was motivated by the hypothesis that hormone-associated birth characteristics of offspring are related to the maternal risk of breast cancer in the first years following a birth. Our population-based cohort study supported this hypothesis, as we found an increased risk of breast cancer in mothers with multiple births or heavy-weighted newborn children in the first 5 years following the birth, whereas the associations diminished in subsequent years. Due to our prospective study design it is unlikely that these results are subject to selection bias or differential misclassification.

Mothers with a multiple birth or a heavy-weighted newborn child are likely to have higher estrogen concentrations (oestradiol, oestriol and unconjugated oestriol) during pregnancy. [2-4] The increased risk in these mothers during the first five years after birth is therefore compatible with the idea that estrogen is involved in the etiology of breast cancer, and the increased incidence of large tumors in mothers with a heavy-weighted newborn child furthermore supports the idea that also the progression of occult tumors may be affected. We note that the effect on breast cancer risk of a multiple birth is larger compared with a delivery of a relatively heavy child. This finding could be due to a larger difference in hormonal levels in mothers having a multiple versus singleton birth compared with a heavy-weighted versus light-weighted child.

Women with diabetes may have an increased risk of breast cancer[17,18] and their offspring have a higher average birth weight due to the higher concentrations of different growth factors in these women. Part of the increased risk in mothers with heavy-weighted newborn children could therefore also be attributed to a high proportion of diabetics among these mothers.

A few studies of mothers with multiple births have previously reported an increased risk of breast cancer in the first years following a multiple birth. [19-21] However, these studies have compared the incidence to all other mothers irrespective of the time factor, meaning time since latest birth. Thus previously published effects cannot be separated from the overall short-term increased risk of breast cancer after a birth reported by Lambe and Albrektsen. [22-23] By analyzing the short-term effect of birth characteristics as an effect modification of the overall effect of time since latest birth, we avoided this problem, and found that indeed there is a higher short-term risk in mothers with a multiple birth or a heavy-weighted newborn child compared with others.

Women with high body mass index (BMI) have an increased risk of breast cancer. [24] Mothers that deliver a heavy-weighted child on average have a higher BMI themselves, which may explain the overall enhanced risk in these mothers. Furthermore, part of the increased incidence of large tumors might be due to difficulties for early detection in these women because of more breast tissue. However, it cannot explain why the effect is largest in the first 5 years following a birth. Furthermore, most studies indicate that the negative effect of high BMI is restricted to post-menopausal women, whereas in this study most women are in a pre-menopausal age group in the first five years following a birth.

In conclusion, we found support for the hypothesis that hormone-associated birth characteristics influence the maternal risk of breast cancer in the first five years following a birth. This is compatible with the idea that hormonal changes during pregnancy influence the subsequent short-term risk of breast cancer.

Multivariate competing risks in a poisson regression model: An application with two correlated characteristics of breast cancer (Study 8)

<u>Material and methods</u>

A MOTIVATING EXAMPLE

The concept of multivariate competing risks was developed in the course of analysing a follow-up study of breast cancer. The study was based on information on breast cancer cases from the Danish Breast Cancer Cooperative Group[3] and a population-based cohort of Danish women with information on vital status and reproductive factors[4,5]. In the cohort of 1.5 mill women (22.3 mill person-years) we identified 10,790 women with breast cancer.

The purpose of the following analysis was to investigate whether a woman's number of (live)births, besides being an important risk factor for breast cancer as such[6], was predictive for the severity of the disease at diagnosis, in order to select women for a targeted breast cancer screening. The analysis was performed as a competing risks analysis comparing the effect of number of births on the incidence of breast cancer according to two measures of severity at diagnosis: tumour size (=20mm, 21-50 mm, >50 mm) and number of positive nodes (no positive nodes, 1-3 positive nodes, and 4 or more positive nodes).

Both tumour size and nodal status reflect different stages rather than different subtypes. A competing risks analysis might therefore not seem to be the obvious approach because a breast cancer with a tumour size larger than 50 mm at diagnosis must have been 10 mm previously, i.e. the 'types' do not seem to compete. However, competing risks models are applicable in this setting because the two classifications are measures of severity *at diagnosis* and a case can only have a single level of severity at diagnosis according to a given classification scheme. Nevertheless such an approach does not allow for differentiation between differences in progression and detection rate, i.e. an aetiologically more relevant explanation of why differences may exist. The following analysis is, therefore, primarily meant as an illustration on the use of multivariate competing risks rather than a definitive aetiological analysis of the data at hand.

The competing risks analysis (described in detail in section 4.1) revealed that number of births had a stronger effect on the incidence of small tumours compared to the effect on the incidence of larger tumours. Similarly, the effect on the incidence of node-negative breast cancers was stronger than the effect on the incidence of node-positive cases. As small tumours tend to be node-negative it is natural to speculate whether the two findings reflect the same phenomenon. An intuitive way to evaluate this hypothesis is to look at the effect of number of births on the incidence of different combinations of tumour size and nodal status, and then see whether the relatively stronger effect on the incidence of small tumours can be found in both node-negative and node-positive cases. The

concept of multivariate competing risks analysis formalises this intuitive idea, and we will now describe the method in detail.

MULTIVARIATE COMPETING RISKS MODELS

3.1 Multivariate competing risks models using Cox regression
If the purpose of a study is to evaluate the effect of an exposure on the rates of a specific type of outcome (e.g. breast cancer), the rate for individual i is commonly modelled in a log-additive model as $l_i(t) = l_0(t)\exp(bx_i)$, with t representing age and x_i being a coded variable representing the exposure for women i. Extension to several exposures and adjustment for confounders is well known. To ease notation, the index i will be dropped in the following.

If instead of only one type there are J subtypes of outcome, one can apply a competing risks model, with the cause specific rates modelled as: $l_j(t) = l_{0j}(t)\exp(b_jx)$, with t being age and $j=1,...,J$ outcome subtype. In this model the effect of the exposure is different for each subtype outcome, and the likelihood function factorizes corresponding to j completely separate models. The model with the same effect of the exposure for all outcome subtypes can be stated as $l_j(t) = l_{0j}(t)\exp(bx)$. In this model the likelihood function does no longer correspond to j completely separate models, however, the model can still be analysed using standard Cox regression techniques as described in Andersen et al[1] p. 493ff.

In order to introduce the multivariate competing risks model we will now describe the situation where two subtype classifications ($j=1,...,J$ and $k=1,...,K$) of outcome are being studied simultaneously. As a straightforward extension of the previous model one can consider the cross-product of the two subtype classifications letting $l_{jk}(t)$ be the rate of having subtypes j and k simultaneously and t representing age. These rates could be modelled as $l_{jk}(t) = l_{0jk}(t)\exp(b_{jk}x)$, i.e. a model with different baseline hazards and different effects of exposure for all combinations of subtypes. This would be a standard competing risks model. However, a more parsimonious log-additive model would be $l_{jk}(t) = l_{0jk}(t)\exp(b^0x + b^1_jx + b^2_kx)$, where the effect of the exposure is log-additive on both subtype classifications. This model offers a natural means for testing for no differences in effects according to one subtype classification when adjusting for differences according to the other subtype classification, i.e. testing the models: $l_{jk}(t) = l_{0jk}(t)\exp(b^0x + b^1_jx)$ or $l_{jk}(t) = l_{0jk}(t)\exp(b^0x + b^2_kx)$. These models for $l_{jk}(t)$ are what we will propose to call *multivariate competing risks models* as they can be applied for analysing two or more sets of competing risks, making it possible to test hypotheses about the multivariate effect of risk factors on these sets of competing risks. The models can be analysed using the same techniques as for standard 'univariate' competing risks models, with cause specific rates for every combination of subtypes.

3.2 Multivariate competing risks models using Poisson regression
Under the assumption of piecewise constant baseline rates the Cox regression model is identical to a Poisson regression model. Poisson regression often provides a more feasible approach in large studies since one may work with abbre-

viated tables of cases and person-years at risk rather than with the individual data records[7].

Competing risks analysis using Poisson regression can be performed if an extra dimension in the cross-classification of cases according to the type of disease is created as described for linear models by Pierce and Preston[2] and for log-linear models by Larson[8]. Person-years at risk are independent of this factor. Test for the same effect of a risk factor is then simply a test for no interaction between the risk factor and this new factor.

Multivariate competing risks models can be analysed using Poisson regression following the same arguments and techniques as for 'univaritate' competing risks models, i.e. by creating an extra dimension according to each of the $J \cdot K$ combinations of subtypes. However, in order to facilitate the new parsimonious additive models this extra dimension should be further classified into two new dimensions according to each of the two classifications (i.e., with J and K levels, respectively). Tests for hypotheses of identical effects of the risk factor according to classification number one can be performed as a test for no interaction between the risk factor and the factor according to classification number one while including an interaction term between classification number two and the risk factor.

THE EXAMPLE REVISITED
We will now return to the example introduced in section 2. We will shortly describe the 'univariate' competing risks analyses and thereafter illustrate multivariate competing risks models.

4.1 The 'univariate' competing risks analysis
Due to the large number of observations the breast cancer rates were analysed using log-linear Poisson regression models, i.e. assuming piecewise constant baseline rates. The effects of number of births according to number of positive nodes were estimated in three independent models of the form:

$$l_j(t) = l_{0j}(t) \exp[a_{period,j} + b_{age\ 1.\ birth,j} + d_{no.\ of\ births,j}],$$

with j being the number of positive nodes (0,1-3,4+) and $a_{period,j}$, $b_{age\ 1.\ birth,j}$ and $d_{no.\ of\ births,j}$ being the node-specific effects according to levels of calendar period, age at first birth and number of births. A significant effect of number of births was found for breast cancers with no positive nodes ($p<0.0001$) or one, two or three positive nodes ($p=0.0006$), whereas there was no effect of number of births on the risk of breast cancer cases with four or more positive nodes ($p=0.42$) (Table 1). Whether these differences in effect could be due to chance can be answered within the framework of competing risks, i.e. by testing whether $d_{no.\ of\ births,j} = d_{no.\ of\ births}$. Doing so, we found a significant difference between the effects of number of births on the incidence of breast cancer according to the number of positive nodes, i.e. a significant interaction between number of births and the dummy variable created according to the number of positive nodes in the breast cancer cases
(Likelihood ratio test: $-2\log Q=33.07$, d.f.$=6$, $p<0.0001$) (Table 1).

Similarly, a significant effect of number of births was found for breast cancers with size $=20$ mm ($p<0.0001$) or 21-50 mm ($p=0.012$), whereas there was no effect of number of births on the incidence of large tumours ($p=0.98$) (Table 1). As for number of positive nodes, the three effects of number of births were significantly different although the differences were less pronounced (Likelihood ratio test: $-2\log Q=13.41$, d.f.$=6$, $p=0.04$).

4.2 The multivariate competing risks analysis

Number of positive nodes and tumour size are highly correlated and it is therefore natural to speculate whether the latter finding simply reflects differences according to number of nodes. The effects of number of births for each combination of tumour size and number of positive nodes are presented in Table 2. The differences in the effect of number of births according to number of positive nodes that were significant in the 'univariate' competing risks analysis remained significant within constant levels of tumour size ($=20$ mm: $p=0.02$, 21-50 mm: $p=0.02$, >50 mm: $p=0.38$). However, the data disclosed a tendency to a uniform effect of number of births according to tumour size within a constant level of number of positive nodes (0 nodes: $p=0.11$, 1-3 nodes: $p=0.95$, 4+ nodes: $p=0.62$). Application of a multivariate competing risks model makes it possible to make a formal test of whether there is a uniform effect of number of births according to tumour size adjusted for differences according to number of positive nodes.

In this multivariate competing risks model we initially checked whether the differences in effects in Table 2 could be described as a log-additive effect of differences according to tumour size and differences according to number of nodes, i.e. a test of

$$l_{jk}(t)= l_{0jk}(t)\exp[a^1_{period,j}+a^2_{period,k}+b^1_{age\ 1.\ birth,j}+ b^2_{age\ 1.birth,\ k}+ d^1_{no.\ of\ births,j}+ d^2_{no.\ of\ births,k}]$$

against

$$l_{jk}(t) = l_{0jk}(t)\exp[a^1_{period,j}+a^2_{period,k}+b^1_{age\ 1.\ birth,j}+b^2_{age\ 1.\ birth,k}+ d_{no.\ of\ births,jk}\qquad]$$

with j being number of positive nodes and k the tumour size. This was accepted (Likelihood ratio test: $-2\log Q=15.11$, d.f.$=12$, $p=0.24$). The underlying assumptions of log-additivity for the effects of calendar period and age at first birth were checked using the same types of test (data not shown).

Finally, we tested whether there were differences in the effect of number of births according to tumour size or number of positive nodes, i.e. the hypothesis $d^1_{no.\ of\ births,j} = d^1_{no.\ of\ births}$ and $d^2_{no.\ of\ births,k} = d^2_{no.\ of\ births}$. The estimates based on this multivariate competing risks model clearly demonstrated that the differences according to tumour size can be ascribed to differences according to number of nodes (Likelihood ratio test:
$-2\log Q=2.29$, d.f.$=6$, $p=0.89$). While there were no differences relative to the reference effect for tumour size, there were still noticeable differences for number of positive nodes when adjusting for tumour size (Likelihood ratio test: $-2\log Q=22.37$ d.f.$=6$, $p=0.001$).

Table 1 The adjusted effect of number of births on the breast cancer incidence according to number of positive nodes and tumour size.

Breast cancer characteristics Likelihood ratio test (d.f.=6)	1 child	2 children RR (95%-CI)	3 children RR (95%-CI)	4 or more children RR (95%-CI)	Likelihood ratio test for effect (d.f.=3)	for interaction
Positive nodes* p<0.0001						
0 nodes	1 (ref.)	0.97 (0.90-1.05)	0.82 (0.75-0.90)	0.59 (0.51-0.68)	p<0.0001	
1-3 nodes	1	0.98 (0.88-1.09)	0.89 (0.79-1.01)	0.71 (0.59-0.85)	p=0.0006	-2logQ=33.07
4+ nodes	1	0.98 (0.85-1.13)	1.08 (0.91-1.28)	1.11 (0.89-1.39)		p=0.42
Tumour size† 2logQ=13.41 p=0.04						
=20mm	1	0.95 (0.89-1.03)	0.83 (0.76-0.91)	0.61 (0.53-0.70)	p<0.0001	
21-50 mm	1	0.98 (0.89-1.08)	0.90 (0.80-1.01)	0.80 (0.68-0.94)	p=0.012	-
>50 mm	1	1.00 (0.80-1.25)	0.99 (0.76-1.29)	1.06 (0.74-1.52)	p=0.98	

* Adjustment made for node-specific effects of age, calendar period and age at first birth.
† Adjustment made for size-specific effects of age, calendar period and age at first birth.

Table 2 The adjusted* effect of number of births on the breast cancer incidence according to combinations of number of positive nodes and tumour size.

Number of nodes	Number of births	Tumour size			Likelihood ratio test for interaction (d.f=6)
		=20 mm RR (95%-CI)	21-50mm RR (95%-CI)	>50mm RR (95%-CI)	
0 positive nodes	1 child	1	1	1	*p*=0.11
	2 child.	0.95 (0.86-1.04)	1.00 (0.87-1.16)	0.74 (0.43-1.27)	-2logQ=10.28
	3 child.	0.78 (0.69-0.87)	0.86 (0.72-1.02)	1.08 (0.60-1.95)	
	4+ child.	0.56 (0.47-0.67)	0.61 (0.47-0.79)	1.30 (0.62-2.75)	
1-3 positive nodes	1 child	1	1	1	*p*=0.95
	2 child.	0.97 (0.83-1.12)	0.97 (0.82-1.15)	0.99 (0.65-1.50)	-2logQ=1.60
	3 child.	0.87 (0.73-1.04)	0.87 (0.71-1.06)	0.78 (0.47-1.31)	
	4+ child.	0.65 (0.50-0.85)	0.80 (0.60-1.06)	0.55 (0.24-1.26)	
4+ positive nodes	1 child	1	1	1	*p*=0.62
	2 child.	0.99 (0.74-1.33)	0.91 (0.74-1.10)	1.07 (0.79-1.45)	-2logQ=4.42
	3 child.	1.24 (0.90-1.71)	0.98 (0.78-1.23)	1.04 (0.73-1.49)	
	4+ child.	0.98 (0.62-1.55)	1.11 (0.82-1.51)	1.10 (0.68-1.78)	
Likelihood ratio test for interaction (d.f.=6)		*p*=0.02 -2logQ=14.98	*p*=0.02 -2logQ=14.95	*p*=0.38 -2logQ=6.41	

*Adjustment made for size and node-specific effects of calendar period and age at first birth and for the effect of age for each of the nine combinations of size and nodal status.

Discussion
We have with the above example illustrated the use of the concept of multivariate competing risks introduced in section 3. Using a competing risks model we showed that a woman's number of births is predictive of the severity at diagnosis of breast cancer, measured as tumour size or nodal status. We speculated whether these two findings reflected one phenomenon, and with the use of the multivariate competing risks analysis we were able to formally confirm this hypothesis.

As noted, the example chosen for illustrative purposes does not evaluate an aetiological hypothesis as one cannot distinguish between differences in progression and detection rates. An example of a multivariate competing risks analysis of a aetiological hypothesis within breast cancer research would be to compare risk factors for receptor negative versus receptor positive breast tumours. Many have found that reproductive risk factors might be stronger for oestrogen receptor positive than for oestrogen receptor negative tumours and some have found the same relation using the progesterone receptor status[9]. Progesterone receptor status and oestrogen receptor status are highly correlated. It has, therefore, been speculated, whether these two results reflect the same phenomenon[9]. Multivariate competing risks models offer a natural way to analyse this hypothesis with follow-up data.

Furthermore, it has been speculated that certain combinations of the oestrogen and progesterone receptor status might be more related to reproductive history than others[10]. This opens for yet another use of the multivariate competing risks model, because this can be studied as a goodness-of-fit test for the additive model. We have performed these analyses in our dataset, however, the multivariate competing risks analyses turned out less useful in this case as we found no strong relation between progesterone receptor status and reproductive history in the 'univariate' competing risks analysis.
In the models described above we have used a multiplicative modelling of competing risks. However, it could be argued that competing risks are intrinsically additive, and that the effects of the two classifications should not be mutually multiplicatively adjusted. An alternative model could, therefore, be to adjust them additively in a more complicated model like

$$l_{jk}(t) = l_{0jk}(t)\exp[a^1_{period,j}+a^2_{period,k}+b^1_{age\ 1.\ birth,j}+b^2_{age\ 1.birth,k}]$$
$$\cdot(\exp(d^1_{no.\ of\ births,j}) +\exp(d^2_{no.\ of\ births,k}))$$

This will no longer be a standard log-linear model of the rates but it could be analysed as a Poisson regression model using Epicure[11].

In conclusion, we have here introduced a new type of competing risks models which we think, may prove relevant in practical situations.

Reproductive history and stage of breast cancer (Study 9)

Subjects and methods

Population Registries

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Since April 1, 1968, the Civil Registration System in Denmark has assigned an individually unique, national registration number to all citizens. This number permits accurate linkage of information from different registries. The Civil Registration System also keeps updated information on dates of live births, emigration and vital status.

The Danish Breast Cancer Cooperative Group started a series of national prospective studies in 1978 to systematically evaluate breast cancer treatment programs. A detailed description of this registry has been given elsewhere (2, 3). The Danish Breast Cancer Cooperative Group collects detailed information on the breast cancer at diagnosis including the size of the tumor, number of positive nodes, and histology. During a limited time period (1977-81), the Danish Breast Cancer Cooperative Group collected additional information such as whether the tumor was discovered by the woman herself, the date the woman experienced the first symptom(s) of her disease, and the date of her first consultation with a medical doctor (4).

Through a linkage between the Danish Breast Cancer Cooperative Group and the Danish Cancer Registry, the Danish Breast Cancer Cooperative Group was found to contain information on 94% of all breast cancer patients reported to the Danish Cancer Registry. The Danish Cancer Registry is considered close to complete regarding incident cases of malignant neoplasms diagnosed in Denmark since 1943 (5) with world standardized breast cancer rates in the periods 1978-82, 1983-87 and 1988+ being 64.8, 69.5 and 74.6, respectively.

Study cohort
A research parity database was established from the Civil Registration System including all women born between April 1, 1935, and March 31, 1978, as earlier described (6, 7). Based on the person identifiable number from the Civil Registration System a linkage was performed with the Danish Breast Cancer Cooperative Group giving information on registered invasive primary breast cancers in the period from January 1, 1978, to September 30, 1994.

Statistical analyses
The possible impact of reproductive history on the incidence of breast cancer of a specific size or a particular nodal status was investigated in a follow-up study analyzed by log-linear Poisson regression models (8). Each stage-specific subtype of breast cancer was analyzed separately. All women entered the follow-up for each of the stage specific breast cancer diagnoses on January 1, 1978, or on their 12-year birthday, whichever came last. The period at risk continued until first diagnosis of breast cancer (whatever stage), death, emigration, or September 30, 1994, whichever occurred first. Pregnancies after a diagnosis of breast cancer were not included in the study. Incidence rate ratios are referred to as relative risks. Adjustment was made for attained age (12-24 years, 25-29, 30-34 ,....., 50-54, >54), calendar period (1978-82, 1983-87, 1988-92, 1993-1994), parity (0,1,2,3,4+ live births) and age at first live birth (nulliparous, 12-19, 20-24, 25-29, 30-34, >34). All variables were treated as time-dependent variables. The effects of the confounders were allowed to be different according to stage, making it possible to take into account that temporal trends and other effects could differ by size and nodal status. Test for effect modification by attained age was performed with age categorized as <45 years, ≥45 years. Analyses were performed using the SAS procedure PROC GENMOD (9).

The associations between reproductive history and factors connected with tumor-detection such as whether the woman discovered the tumor herself (yes/no), the time-interval from first symptom to first visit at the doctor in days (patient delay), and the time from first visit at the doctor to time of operation in days (doctor delay), were evaluated by Mann-Whitney and Chi-squared tests.

<u>Results</u>

Incidence
In total, 1,529,512 women were included in the cohort. Of these, 1,000,276 (65.3%) had 2,071,415 births before end of follow-up: 254,694 (25.5 %) women had one birth, 494,697 (49.5%) two, 193,250 (19.3%) three, and 57,635 (5.6%) four or more births. A total of 10,790 primary invasive breast cancers below 60 years of age were detected in this cohort during 22.3 million person-years of follow-up. Table 1 gives the distribution of cases and person-years by age, calendar-period, parity, and age at first birth.

Overall, we documented a significantly lower incidence of breast cancer among ever parous compared with never parous women (Relativ Risk=0.87; 95 percent confidence interval: 0.82-0.92). Among parous women, we found a significantly increasing incidence of breast cancer with increasing age at first birth (p<0.0001) and decreasing parity (p<0.0001) (Table 2).

Table 2 also shows the association between these reproductive factors and the risk for breast cancer according to the tumor size. Ever parous women had a significantly lower incidence of larger tumors than nulliparous women; for tumors less or equal to 20 mm in diameter, we found no such association. In other terms, nulliparous women had a significantly increased risk of being diagnosed with a large tumor compared to parous women (Relative Risk=1.69; 95 percent confidence interval: 1.37-2.04). Among ever parous women, age at first birth was largely unrelated to the incidence of breast cancers less than or equal to 20 mm. In contrast, increasing age at first birth was positively associated with risk for larger tumors. Risk increased monotonously and the gradient was largest, about 2.5-fold, for tumors larger than 50 mm. The protective effect of multiparity was significantly stronger for small tumors (=20 mm) than for larger tumors. Indeed, we found no association between the number of births and the risk for breast tumors above 50 mm (Table 2). Similar associations with reproductive history were found when breast cancer cases were classified by nodal status instead of by tumor size (without positive nodes, 1-3 positive nodes, 4+ positive nodes, data no shown). To evaluate whether our results were modified by age and in particular by pre- or postmenopausal status, we performed a test for interaction with age categorized as <45 years and ≥45 years. Our analysis did not show any effect modification by attained age.

The associations shown in table 2 are further illustrated in figures 1 and 2. Here the predicted breast cancer *rates* (based on the model from table 2) were calculated by tumor size at diagnosis for women aged 50-54 years in the period 1993-1994, according to their reproductive history. In figure 1 the tumor size specific rates in nulliparous women are compared with the rates in uniparous women according to their age at the birth. Having the first birth at a young age slightly increases the woman's risk of being diagnosed with a small tumor, whereas the risk of a medium and large tumor is reduced after the first birth. The reduction in medium and large tumors becomes smaller the older the women is at time of childbirth. For women 35+ years at the first birth there is even a small increase in the risk. The incidence of tumors less than 21 mm at diagnosis is only slightly increased the older the women is at her first birth. Therefore, much of the overall increase in risk with increasing age at first birth can be attributed to the fact that the reduction in the incidence of tumors with a diameter between 20 and 50 mm after a birth is smaller the older the women is at her first birth. Although the *relative* increase in risk with increasing age at first birth is highest in the group of tumors larger than 50 mm (as shown in table 2) the *absolute* contribution to the overall risk is small.

Figure 2 illustrates the effect of having additional births beyond the first. The rates are calculated for women who were between 20 to 24 years of age at their first birth. This restriction only affects the level of rates not the shape of the figure. Figure 2 shows that additional births beyond the first in general do not affect the incidence of large tumors and only slightly reduce the incidence of medium sized tumors. The overall reduction in breast cancer risk with additional births is therefore attributable to a reduction in the incidence of small tumors.

Diagnostic delay

For women diagnosed in the period 1978-82 additional information had been obtained about whether the woman discovered the tumor herself, about the time interval between the first symptoms observed by the woman and her first visit to her doctor (patient's delay), and finally about the time interval between the first visit to her doctor and the time of the definitive surgery or biopsy (doctor's delay) (4). Overall, 93.3 % of the women discovered their tumor themselves and among these women the median patient's delay was 9 days. The median doctor's delay was 29 days. A more detailed presentation of the figures is given in table 3. We evaluated the association between the three tumor detection related variables and the reproductive variables presented in Table 2. There was no significant association in any of the 9 tests (Table 3).

Discussion

This study showed that parity and age at first birth are associated not only with the incidence rate of breast cancer, but also with the stage of the disease at diagnosis. Whereas nulliparous compared to parous women and women with a late compared to early age at first childbirth were at a similar risk of being diagnosed at an early stage (small tumor, no metastatic spread), nulliparous women and women with a late first childbirth were at significantly increased risk of being diagnosed with advanced breast cancer (large tumors, extensive metastatic spread to regional lymph nodes). In contrast, multiparity was protective against being diagnosed with a small tumor but not with a large tumor. These results can be ascribed to differences in tumor progression rates and/or to differences in detection rates. Obviously, a large tumor must at some point have been small. Under the assumption that certain tumors grow more rapidly than others, they will stay in the category of small tumors for a shorter time before they move on to become medium and eventually large tumors. Thus, according to one interpretation, nulliparous women and women with a late age at first childbirth who were at particularly high risk of being diagnosed with large tumors may have tumors with a rapid growth potential.

The rival explanation would be that associations exist between reproductive factors and the probability of early tumor detection. For example, differences in detection rates might arise if breast self examination is more difficult in nulliparous compared to parous women or in women with a late compared to early age at first childbirth. The breast tissue of a nulliparous woman is more firm and homogenous than the breast tissue of a parous women which might make detection of a tumor more difficult. However, it is equally conceivable that the nodularity present in a parous woman's breast would make it difficult to distinguish glandular tissue from tumor tissue. Thus, the extent and direction in which reproductive factors may influence detection of tumors is difficult to predict. Differential use of mammography according to reproductive history could also cause differences in time of detection. The vast majority of women in our study were, however, below age 50. In Denmark, mammography is offered only for women aged 50 years or older and even today only in few parts of the country.

Finally, behavioural differences according to reproductive history could cause differences in time of detection. For example parous women and those considering pregnancy may be more frequently in contact with the medical care system, leading to shorter delays in

detection compared to nulliparous or older women. However, the difference in the effects of reproductive history were the same regardless of the age of the women. Furthermore, based on detailed referral information on a subset of the women included in this study we found no evidence of an association between delay in referral or delay in diagnosis and the reproductive factors in question. Therefore, the most likely explanation for our findings is that a woman's reproductive status influences both the risk for tumor development and the biologic features of the tumor, notably its growth potential.

Our prospective analysis was performed on a large population-based cohort which made selection and information bias very unlikely. A potential limitation of our study was the lack of data on other reproductive factors such as age at menarche and age at menopause. The confounding introduced by lack of adjustment for these variables should, however, be limited (10). Temporal trends in breast cancer incidence might differ by tumor characteristics. This was taken into account by allowing for different effects of calendar period in the different stage specific analyses. The cohort included only women younger than 60 years at the end of follow-up. Therefore, our results are primarely obtained among premenopausal women. However, it should be stressed that the effects of reproductive history were the same regardless of age indicating that the effects may be applicable to both pre- and postmenopausal women.

It is well-established that advanced breast cancer at time of diagnosis (large tumor, lymphatic spread) is associated with a particular poor prognosis. Thus, the association with more advanced disease observed for nulliparous women and women with a late age at first childbirth also give them a higher risk of lethal disease. Based on a large cohort of women who had undergone breast cancer treatment, we have previously investigated whether the prognostic effect of parity and age at first childbirth also had an independent effect on these women's survival. We found in that study a significant independent negative prognostic effect of a late age at first birth, but no prognostic effect of number of births. To evaluate the independent effect on the *prognosis* of breast cancer in that study we adjusted for the differences in tumor size and nodal status at the time of diagnosis (in addition to age, histological grading, treatment regimens and others) (11). Taken together, the two studies illustrate how reproductive risk factors have a further negative effect on the progression rate besides those seen as differences in tumor size and nodal status at diagnosis.

In conclusion, we provide novel knowledge that a woman's reproductive status may also influence the stage of breast cancer at diagnosis and thereby her long-term disease-specific survival. In particular, nulliparous women and women who give birth to a first child at a late age are at increased risk of being diagnosed with large tumors with extensive metastatic growth and a poor prognosis. Regardless of the underlying biologic mechanism, these results motivate initiatives to achieve earlier detection of breast cancer perhaps through a combination of increased awareness and more frequent mammography in a subset of women who tend to develop more lethal breast cancer.

TABLE 1. Number of Cases of Breast Cancer and Person-Years of Follow-Up by Age, Calendar Period and Reproductive History, Denmark, 1978-1994

	No. Cases (%)	Person-years
Age		
12-29	158 (1.5%)	10,399,000
30-39	2,054 (19.0%)	5,973,000
40-49	6,072 (56.3%)	4,665,000
50+	2,506 (23.2%)	1,234,000
Calendar Period		
1978-82	1,390 (12.9%)	5,850,000
1983-87	2,734 (25.4%)	6,656,000
1988-92	4,656 (43.2%)	7,244,000
1993-94	2,010 (18.6%)	2,519,000
Parous Status		
nulliparous	1,295 (12.0%)	9,501,000
parous	9,495 (88.0%)	12,770,000
Age at First Birth		
12-19	1,472 (15.5%)	2,362,000
20-24	4,437 (46.7%)	6,480,000
25-29	2,693 (28.4%)	3,164,000
30-34	710 (7.5%)	648,000
35+	183 (1.9%)	116,000
Number of Births		
1	1,910 (20.1%)	3,469,000
2	4,892 (51.5%)	6,188,000
3	2,112 (22.5%)	2,390,000
4+	581 (6.1%)	723,000

TABLE 3.   Percentage of self-discovered tumours and median patient's and doctor's delay according to reproductive history in women diagnosed with breast cancer 1978-82, Denmark

	Self-discovered tumour*	Median patient's delay in days [†]	Median doctor's delay in days[‡]
Parous Status			
nulliparous	94%	15	34
parous	93%	9	28
test for difference	p=0.78	p=0.09	p=0.14
Age at First Birth			
12-19	93%	10	28
20-24	93%	8	29
25-29	93%	9	27
30-34	93%	8	27
35+	92%	10	25
test for difference	p=0.99	p=0.98	p=0.88
Number of Births			
1	92%	7	28
2	93%	7	28
3	93%	13	29
4+	97%	13	31
test for difference	p=0.62	p=0.27	p=0.75

*87%=1215/1390 had non-missing information.
[†] 76%=1055/1390 had non-missing information.
[‡] 87%=1203/1390 had non-missing information.

Gender of offspring and maternal breast cancer risk. (study 12)

Materials and methods

Since April 1, 1968, the Civil Registration System (CRS) in Denmark has assigned an individually unique national registration number to all citizens. This number permits accurate linkage of information from different registries. The Civil Registration System also keeps updated information on gender and dates of live births, emigration and vital status. Information on stillbirths was available from the National Birth Register. To identify multiple deliveries we looked for children born to the same mother within two days. A research parity database was established from the CRS including all women born between April 1, 1935, and March 31, 1978, as earlier described (Melbye et al, 1997; Westergaard et al, 1997). Based on the person-identifiable CRS number, a linkage was performed with the Danish Breast Cancer Group's registry (DBCG) (Andersen and Mouridsen, 1988; Kroman et al, 1997) giving information on registered invasive breast cancers in the period from January 1, 1978, to September 30, 1994. The DBCG's registry was found to contain information on 94% of all breast cancer patients reported to the Danish Cancer Registry, which has nearly complete registration of all incident cases of malignant neoplasms diagnosed in Denmark since 1943 (Storm, 1991). The impact of gender on the incidence of breast cancer was investigated in a follow-up study analysed by log-linear Poisson regression models (Breslow and Day, 1987). All women entered the follow-up for breast cancer diagnoses on January 1, 1978,

or on the date of their first childbirth, whichever came last. The period at risk continued until breast cancer, death, emigration or September 30, 1994, whichever occurred first. Incidence rate ratios are referred to as relative risks. In all analyses adjustment was made for age (<30, 31, 32, ..., 57, 58), calendar period (1978-82,1983-87,1988-92,1993-94) and age at first birth (<20,20-24,25-29,30-34,35+). The gender of n. birth was categorised as (n-1. parous or less, boy, girl). The first parameter in the gender variables were redundant when the gender variables for all births were included and these parameters were therefore set to zero.

Methods
In all we observed 9,495 cases of breast cancer during 12.8 million years of follow-up. All the following analyses are restricted to 5 or more years after latest birth in mothers with a history of only singleton births in order to focus on long-term effects of singleton births. With this restriction we observed 8,607 cases during 8.7 million years of follow-up. Using 10 years instead of 5 years gave similar results. The mother's risk of breast cancer decreased significantly with number of births: 1 birth: 1 (reference), 2 births: 1.0 (0.9-1.0), 3 births: 0.9 (0.8-0.9), 4 births: 0.7 (0.6-0.8), 5+ births: 0.5 (0.4-0.7). Table 1 shows the risk of breast cancer according to the gender distribution of the mother's offspring. We observed that women with many compared to few boys and women with many compared to few girls had a lower breast cancer risk. However, the effects are similar and can be described more simply by the total number of births. This can be seen by the very similar estimates within the diagonals from left-bottom to right-top, i.e. within strata of similar parity. Within the parity-specific strata the distribution of boys and girls does not modify the risk. The pattern was the same in women younger than 45 years of age and in women aged 45 years or older. In an alternative approach we estimated the gender difference in the long-term effects of the 1st to 6th birth (Table 2). The effect of 1st to 6th birth was not modified by the gender of the offspring.

Discussion
Our study shows that gender of offspring does not modify the effect of a childbirth on the breast cancer risk. This is true for both the short-term increase and the long-term decrease of breast cancer risk after a childbirth. The gender modification of the long-term effect was investigated by studying breast cancer risk 5 or more years after the latest birth according to the gender distribution of offspring as well as the effects of each birth. The long-term decrease in risk following a childbirth is believed to originate from permanent changes in the susceptibility of the stem cells, changes that perhaps partly are determined by the hormonal level during pregnancy (Adami et al, 1998). We therefore used these approaches as it is most plausible that a potential gender induced modification of the long-term effect would be an effect of the gender of all previous births some years after the latest birth, i.e. after the most marked effects of these transient negative effects of the births.

The short-term effect of a childbirth is, on the other hand, believed to be due to hormonally induced growth of pre-malignant and malignant tumours. A study of the gender modification of the short-term effect should therefore ei-

ther focus on the latest birth in a short time-interval after the latest birth or the short term effects of each of the births separately. Studying the gender modification of the short term effect according to gender distribution of all births disregarding the order of appearance and only restricting to young women, as in the study by Hsieh et al of the gender effect in "childbearing ages" (Hsieh et al, 1999), is therefore most likely going to obscure the true short-term effect. As argued above, such an approach is more appropriate in the study of long-term effects. We have recently looked at the effect of gender of the most recent birth within the first 5 years following birth (Wohlfahrt and Melbye, 1999) and observed no modifying effect of gender of offspring. Based on these findings in a large population-based cohort study we conclude that gender of offspring neither modifies the short nor the long-term effect of breast risk following a childbirth. Our findings do no necessarily imply that the hormones related to gender are of no importance in the etiology of breast cancer, but probably illustrate that the gender differences in hormonal level during pregnancy are small compared with the hormonal changes induced by a pregnancy irrespective of gender.

Table 1 Relative maternal risk[a] of breast cancer 5 or more years after latest birth according to gender distribution of offspring

Number of girls	Number of boys					
	0	1	2	3	4+	All
0	-	1.0 (0.9-1.1)	1.0 (0.9-1.1)	0.9 (0.8-1.1)	0.6 (0.4-0.8)	1.2 (1.1-1.2)
1	1 (ref.)	0.9 (0.9-1.0)	0.9 (0.8-1.0)	0.7 (0.6-0.9)	0.5 (0.3-0.9)	1 (ref.)
2	1.0 (0.9-1.1)	0.9 (0.8-0.9)	0.8 (0.6-0.9)	0.5 (0.4-0.8)	0.2 (0.1-0.7)	0.9 (0.8-1.0)
3	0.8 (0.6-0.9)	0.7 (0.6-0.9)	0.7 (0.4-1.0)	0.2 (0.1-0.9)	0.9 (0.3-2.8)	0.7 (0.7-0.8)
4+	0.7 (0.4-0.1)	0.4 (0.2-0.9)	1.3 (0.7-2.4)	0.4 (0.1-2.7)	- (no cases)	0.7 (0.5-0.9)
All	1.1 (1.1-1.2)	1 (ref.)	1.0 (0.9-1.0)	0.9 (0.8-1.0)	0.6 (0.4-0.7)	

[a] All relative risks are adjusted for attained age, calendar period and age at first birth and with 95% confidence interval. The effects of number of boys and number of girls are furthermore mutually adjusted.

Age at any birth is equally important for breast cancer risk (Study 13)

Methods

POPULATION REGISTRIES

Since April 1, 1968, the Civil Registration System in Denmark has assigned an individually unique national registration number to all citizens. This number permits accurate linkage of information from different registries. The Civil Registration System also keeps updated information on dates of live births, emigration and vital status.

The Danish Cancer Registry is considered close to complete regarding incident cases of malignant neoplasms diagnosed in Denmark since 1943.[4]

STUDY COHORT

A research parity database was established from the Civil Registration System including all women born between April 1, 1935, and March 31, 1978, as earlier described[5,6]. Based on the person identifiable number from the Civil Registration System, a linkage was performed with the Danish Cancer Registry giving information on registered invasive primary breast cancers in the period from April 1, 1968, to September 30, 1994.

STATISTICAL ANALYSES

The impact of age at birth on the incidence of breast cancer was investigated in a follow-up study analyzed by log-linear Poisson regression models.[7] All women entered the follow-up for breast cancer diagnoses on April 1, 1968, or on their 12-year birthday, whichever came last. The period at risk continued until breast cancer, death, emigration, a seventh birth, or September 30, 1994 (end of follow-up), whichever occurred first. The effect of age at birth were analyzed using two different approaches. In one approach we performed a parity stratified analysis including age at the most recent and previous births categorized as (<25,25-29, 30-34, =35), age (quadratic splines with knots: 30,35,40,45,50,55)[8] and calendar period. In a second approach, we used models including information from all parity strata. In these analyses, adjustment was made for age categorized in one-year categories and calendar period (1968-72,1973-77,1978-82,1983-87,1988-92,1993-94). In the following models these categorical variables and an intercept are represented by the term $c_1(age,period)$. All variables were treated as time-dependent variables. To estimate the change in risk after 1st to 4th birth we used the following model for the logarithm of the incidence rate (l):

$$(1) \qquad \log(\lambda) = c_1(age, period) + \sum\nolimits_{k=1}^{4} \alpha_k x_k$$

with x_k equal to 1 if parity= k and equal to 0 otherwise. a_k represents the change in risk (on the log-scale) after kth birth. In other words, the effect of reproductive history in e.g. biparous women is in this model represented by a_1+a_2, where a_1 represents the change in risk after 1st birth and a_2 the change in risk after 2nd birth. To see how risk changes after 1st to 4th birth varied with age at 1st to 4th birth we used the following model:

$$(2) \qquad \log(\lambda) = c_1(age, period) + \sum\nolimits_{k=1}^{4} \sum\nolimits_{l=1}^{4} \beta_{kl} y_{kl} + c_2(5th\ and\ 6th\ birth)$$

l=1 to 4 represents the categories (<25, 25-29, 30-34, =35) and y_{kl} equals 1 if parity is k or larger and age at kth birth is in lth "age at birth"-category, y_{kl} is 0 otherwise. The term c_2(5th and 6th birth) represents the effects of 5th and 6th birth and is explained below. b_{kl} represents the change in risk after a kth birth occuring in lth "age at birth"-category. The effect of reproductive history in e.g. biparous women with a first birth before 25 years of age and a second after 35 years age is in this model represented by $b_{11}+b_{24}$, where b_{11} represents the change in risk after a relatively early 1st birth and b_{24} the change in risk after a relatively late 2nd birth. As a parsimony alternative we estimated the increase in risk per increase in age at birth by:

(3)

$$\log(\lambda) = c_1(age, period) + \sum_{k=1}^{4}\sum_{l=1}^{4}(\varepsilon_k x_k + \chi_k z_{kl}) + c_2(5th\ and\ 6th\ birth)$$

with z_{kl} equal to the lth age-level if parity is k or lager and age at kth birth is in lth "age at birth"-category, z_{kl} is otherwise 0. The categories <25, 25-29,30-34,=35 were assigned the levels 22.5, 27.5, 32.5 and 37.5. $5c_k$ represents the increase in risk per 5 years increase in age at kth birth. Due to small numbers we used this expression for 5th and 6th birth in all analyses (except model (1)), i.e. the effect of age at 5th and 6th birth were repre-

sented by trends using $c_2(5th\ and\ 6th\ birth) = \sum_{k=5}^{6}\sum_{l=1}^{4}(\varepsilon_k x_k + \chi_k z_{kl})$. To es-

timate the general increase per 5 years in age at 1st to 4th birth we substituted c_k with d:
(4)

$$\log(\lambda) = c_1(age, period) + \sum_{k=1}^{4}\sum_{l=1}^{4}(\varepsilon_k x_k + \delta z_{kl}) + c_2(5th\ and\ 6th\ birth)$$

with d representing the general effect for 1st to 4th birth. In more elaborate models we investigated how the changes after 1st to 4th birth are modified by attained age and time since kth birth by the use of interaction terms. Estimation of the effect of time since latest birth with adjustment for age and age at first birth when including uniparous has been discussed by Heuch et al. [9] Finally, we estimated the effect of age at latest birth extending model(2) to:
(5)

$$\log(\lambda) = c_1(age, period) + \sum_{k=1}^{4}\sum_{l=1}^{4}(\beta_{kl} y_{kl} + \beta_l^{latest} y_{kl}^{latest}) + c_2(5th\ and\ 6th\ birth)$$

with y_{kl}^{latest} equal to 1 if parity is precisely k and age at kth birth is in lth "age at birth"-category, y_{kl}^{latest} is equal to 0 otherwise. y_{kl} and y_{kl}^{latest} are identical for k-parous women. However, as the variables differ in other strata, it is possible to perform meaningful estimations of b_{kl} and b_l^{latest} simultaneously, assuming that they represent two distinct aetiological effects.

Results

Overall, 1,529,414 women were observed during 31.2 million person-years of follow-up (mean follow-up 20.4 years; range: <1 year to 26.5 years). Of these, 13,049 women were diagnosed with breast cancer before the end of the study with the following parity status: 1,599 were nulliparous, 2,350

were uniparous, 5,869 were biparous, 2,516 were triparous, 583 had four children, and 132 had 5 or 6 children. At the seventh birth women were excluded from follow-up. Age during follow-up ranged from 12 to 59 years. Among cases, 10,281 women were younger than 50 years at diagnosis and 2,768 were 50 years or older.

Table 1 shows the estimated effect of age at 1st birth in uniparous women, the estimated effect of age at 1st and 2nd birth in biparous, the estimated effect of age at 1st, 2nd and 3rd birth in triparous and the estimated effect of age at 1st, 2nd, 3rd and 4th birth in 4-parous women. These stratified analyses show that the woman's age at 1st to 4th birth is associated with breast cancer risk and that the associations are also observed after subsequent births.

The overall relative risk after the 1st, 2nd, 3rd and 4th birth is: 0.98 (0.92-1.05), 0.90 (0.86-0.95), 0.86 (0.83-0.91) and 0.81 (0.75-0.88), adjusted for age and calendar period (model 1). Table 2 illustrates how these effects are affected by age at birth; i.e, it shows the effect of the 1st to 4th birth on the maternal risk of breast cancer according to age at birth. Compared with nulliparous women, a 1st birth induced a decreased risk of breast cancer if the woman was less than 25 years at the time of giving birth. A 2nd, 3rd and 4th birth induced a reduced breast cancer risk among woman less than 30 years at the time of giving birth compared with uniparous, biparous and triparous, respectively (model 2).

To evaluate whether age at any birth was equally important for breast cancer risk, we furthermore compared the increase in risk according to increase in maternal age at 1st to 4th birth. The risk of breast cancer increased by 9% per 5 years increase in age at first birth and 7%, 5% and 14% per 5 years increase in age at 2nd, 3rd and 4th birth, respectively (model 3). The general increase per 5 years in age at 1st, 2nd, 3rd and 4th birth was 8% (model 4). The general increase per 5 years in age at 5th and 6th birth was 5%(-12%-26%). Including only age at first birth and adjusting for number of births, the trend for age at first birth was 13% per 5 years. The associations with age at 2nd, 3rd and 4th birth were not due to residual confounding introduced by the categorization of age at first birth in four groups. Adjusting for age at first birth in one-year categories the increase per 5 years in age at 2nd, 3rd and 4th birth was 7%, 4%, 14% (Table 2, modified model 3). We found no interaction between the effects of age at different births. Among women with a first birth below 25 years of age the increase in risk after 2nd, 3rd, and 4th birth was 6%, 4% and 14%.

The effects of age at birth differed according to time since birth (Table 2), i.e. the effect of age at nth birth differed according to time since nth birth. The first 10 years after birth there was only a minor effect of age at birth (2% risk increase per 5 years), whereas 10 or more years after birth the effect of birth was modified by the age at birth (7% risk increase per 5 years) (model 5). The ratio between the trends was 1.07/1.02=1.05 (1.01.-1.10). Comparing the overall trends according to attained age we found only a minor difference; <50 years: 8% risk increase per 5 years; and =50 years: 6%

risk increase per 5 years (Table 2, model 5). The ratio between the trends was 1.08/1.06=1.02 (0.99-1.05).

In additional analyses we examined whether effects of each childbirth could explain a possible effect of age at latest birth (model 6), i.e. whether there was an additional effect of age at latest birth beside the effects of each birth. Adjusting only for age at first birth we found a strong effect of age at latest birth (<25 years: 1 (reference), 25-29 years: 1.02 (0.96-1.07), 30-34 years: 1.12 (1.05-1.21), =35 years: 1.25 (1.13-1.38); increase per 5 years: 8% (4%-11%). However, after additional adjustment for the effect of age at subsequent births we found no effect of age at latest birth (<25 years: 1 (reference), 25-29 years: 0.98 (0.91-1.06), 30-34 years: 1.02 (0.92-1.14), =35 years: 0.99 (0.79-1.24); increase per 5 years: 0% (-5%-4%)).

We only had complete information on livebirths, but information on stillbirths occurring from 1973 to 1993 were available. Including these births in the analyses gave similar results (e.g. general increase per 5 year: 8%(6%-9%)).

Discussion
Women with low age at first birth are at reduced risk of breast cancer, i.e. women with a short nulliparous period (defined as the period between menarche and first birth) have a low risk of breast cancer later in life.[1] This observation has led to the hypothesis that the nulliparous period represents a critical time window in a woman's life where breast cancer may be initiated.[10] While subsequent births are known to reduce breast cancer risk, it is not well understood whether the timing of these childbirths during reproductive life are of importance. Our data show that the timing of all childbirths, and not only the first, affects a woman's breast cancer risk. We found that all childbirths result in a long-term reduction in maternal breast cancer risk if the woman delivers at an early age, whereas childbirths occurring later in a woman's life evidently induce no reduction in risk. This effect was observed irrespective of parity and attained age. These findings suggest that the early reproductive years, rather than just the initial nulliparous period, are the critical time-window and that the risk of negative long-term effects initiated during this time window is reduced by any early childbirth, with age at first birth not being more important than age at subsequent births.

The lower risk in women with a young age at birth was not due to their relatively long time since birth. Thus, after stratification for time since birth we still found an effect of age at birth. We did find, however, that the effect of age at birth was modified by time since birth. There was essentially no effect of age at birth the first 10 years after birth, perhaps owing to a time delay in the effect of the birth. Thus, our data suggest that the effect of the age at birth primarily is a long-term effect.

Few previous studies have focused on the independent effect of age at births subsequent to the first. The results have been conflicting: some have found effects of age at second birth [11,12,13,14] while others observed no effect of subsequent births.[15,16] These studies, however, have included fewer

observations, and analysing the independent effect of each birth requires the inclusion of many cases in the study to achieve sufficient precision. Lack of precision might explain why some of these smaller studies reported an effect of age at first birth and age at second birth but not for other births. Recently a large study found effect of any birth, although with a stronger effect of age at first birth compared with subsequent births.[17] This study and most previous studies, however, have examined differences in risk increase per increase in maternal age, i.e. trend differences. In contrast, our study included more than 13,000 cases, which allowed us to study not only the trend differences but also to use a statistical approach that could identify the age-at-birth categories associated with a reduced risk, taking into account latency period and short-term effects following a birth. This latter approach was essential in order to identify a potential critical period in a woman's life.

An alternative interpretation of our data is that early age at first birth is important combined with shortness of interbirths intervals, i.e. that early timing of subsequent births are important due to short interbirth time intervals. Nevertheless, the fact that we do not find any interaction between the effects of age at different births does not support this interpretation. Regardless of interpretation, however, it seems evident that the timing of any birth is important.

Although our study emphasizes the effects of childbirths subsequent to the first, our observations give no support to the idea that age at *latest* birth has any special importance. Studies in Norway and Brazil have previously found an effect of age at latest birth.[3,18,19] Neither of these studies, however, took into account the ages at intermediate births in their analyses, as pointed out by Hsieh et al.[20]. We initially observed a strong effect of age at latest birth when only adjusting for age at first birth, but after adjustment for the effects age at first *and* subsequent births, there was no independent effect of age at latest birth. Therefore, in addition to the lack of a biological rational for an effect of age at latest birth, we think that these findings are artifacts representing the effects of age at first and subsequent births observed in the present study.

Our findings are not likely to be due to information or selection bias as the study was performed as a prospective analysis on a large population-based cohort and was based on mandatorily reported information on reproductive history and breast cancer. A limitation of the study, however, was the lack of possible confounder information such as oral contraceptives and infertility (and the associated treatment). We had no information on menopausal status, which might modify the long-term effect of age at birth. Nevertheless, we observed almost the same pattern in women while below and while over 50 years of age, suggesting that the hormonal changes during menopause do not affect the long-term risk reduction conferred by early reproduction.

In conclusion, our study shows that we should modify the traditional view that age at first birth and number of births are the main reproductive long-term risk factors for breast cancer. Our data suggest that the fundamental factor behind the association between a woman's reproductive

history and breast cancer risk is simpler, i.e. that early timing of *any* additional birth induces an additional long-term reduction of maternal risk of breast cancer. Thus, the effect of an early 1st birth is no stronger than that of subsequent births.

Table 1: Relative risk* (RR) of breast cancer according to age at 1st to 4th birth stratified by parity

	Uniparous women		Biparous women		Triparous women	
	no.	RR (95%-CI)	no.	RR (95%-CI)	no.	RR (95%-CI)
Age at 1st birth						
<25	944	1 (reference)	3,605	1 (reference)	1,978	1 (reference)
25-29	808	1.17 (1.06-1.28)	1,846	1.10 (1.03-1.18)	486	1.08 (0.96-1.23)
30-34	432	1.24 (1.11-1.39)	377	1.14 (1.00-1.30)	49	1.15 (0.82-1.60)
=35	166	1.29 (1.10-1.53)	41	0.96 (0.68-1.33)	3	1.57 (0.46-5.31)
Age at 2nd birth						
<25			1,304	1 (reference)	1,144	1 (reference)
25-29			2,685	1.04 (0.97-1.11)	1,080	1.06 (0.96-1.17)
30-34			1,493	1.14 (1.04-1.25)	267	1.25 (1.04-1.50)
=35			387	1.29 (1.12-1.49)	25	1.28 (0.80-2.04)
Age at 3rd birth						
<25					218	1 (reference)
25-29					929	1.09 (0.94-1.27)
30-34					1,016	1.18 (1.00-1.39)
=35					353	1.14 (0.93-1.39)
Age at 4th birth						
<25						
25-29						
30-34						
=35						

* Estimated within strata of parity with adjustment for age, calendar period and other effects presented in the column.

† Furthermore adjusted for the effects of age at 5th and 6th birth.

TABLE 2: Relative risk* (RR) of breast cancer following 1st to 4th birth according to age at birth

	no. of cases	Overall† RR (95% CI)	According to time since birth†		According to attained age†	
			<10 yr RR (95% CI)	=10 yr RR (95% CI)	<50 yr RR (95%CI)	=50 yr RR (95% CI)
Age at 1st birth						
<25	7,158	0.91 (0.85-0.98)	0.96 (0.82-1.12)	0.91 (0.84-0.97)	0.93 (0.86-1.00)	0.87 (0.74-1.00)
25-29	3,216	1.01 (0.94-1.08)	1.10 (0.97-1.23)	0.98 (0.91-1.06)	1.02 (0.94-1.11)	0.95 (0.81-1.11)
30-34	866	1.08 (0.99-1.18)	1.11 (0.97-1.26)	1.05 (0.95-1.17)	1.12 (1.02-1.24)	0.93 (0.77-1.14)
=35	210	1.10 (0.95-1.27)	1.00 (0.82-1.21)	1.19 (0.96-1.46)	1.05 (0.88-1.25)	1.23 (0.93-1.62)
nulliparous	1,599	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
increase per 5 yr	11,450	9%(5%-12%)	2% (-5%-10%)	9% (5%-13%)	9% (5%-12%)	9% (2%-16%)
Age at 2nd birth						
<25	2,912	0.88 (0.82-0.94)	1.09 (0.90-1.31)	0.86 (0.80-0.93)	0.88 (0.82-0.95)	0.87 (0.75-1.02)
25-29	3,995	0.92 (0.87-0.97)	1.05 (0.95-1.17)	0.89 (0.84-0.95)	0.93 (0.87-0.99)	0.88 (0.77-1.00)
30-34	1,779	1.00 (0.94-1.07)	1.06 (0.96-1.16)	0.97 (0.90-1.04)	1.01 (0.94-1.09)	0.96 (0.83-1.10)
=35	414	1.09 (0.97-1.21)	1.05 (0.92-1.21)	1.10 (0.94-1.29)	1.15 (1.02-1.30)	0.88 (0.69-1.11)
uniparous	2,350	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
increase per 5 yr	9,100	7%(4%-11%)	0% (-6%-7%)	6% (2%-10%)	9% (5%-13%)	2% (-4%-10%)
Age at 3rd birth						
<25	432	0.84 (0.74-0.94)	0.99 (0.63-1.56)	0.83 (0.73-0.94)	0.86 (0.75-0.98)	0.76 (0.58-0.99)
25-29	1,274	0.89 (0.83-0.95)	0.96 (0.81-1.13)	0.88 (0.81-0.95)	0.89 (0.82-0.97)	0.86 (0.74-1.00)
30-34	1,140	0.94 (0.88-1.00)	1.01 (0.91-1.13)	0.92 (0.85-0.99)	0.97 (0.90-1.05)	0.85 (0.74-0.98)
=35	385	0.97 (0.87-1.07)	0.95 (0.83-1.09)	1.00 (0.86-1.17)	0.94 (0.84-1.07)	1.04 (0.85-1.27)
biparous	5,869	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
increase per 5 yr	3,231	5% (0%-10%)	-1% (-10%-9%)	5% (-1%-11%)	4% (-1%-10%)	8% (-1%-19%)
Age at 4th birth						
<25	46	0.76 (0.54-1.07)	0.37 (0.05-2.73)	0.78 (0.55-1.11)	0.72 (0.49-1.07)	0.89 (0.45-1.75)
25-29	208	0.77 (0.65-0.91)	0.77 (0.50-1.19)	0.78 (0.65-0.92)	0.76 (0.63-0.92)	0.79 (0.58-1.09)
30-34	285	0.92 (0.81-1.05)	0.84 (0.66-1.07)	0.96 (0.83-1.12)	0.93 (0.80-1.09)	0.90 (0.70-1.15)
=35	176	1.03 (0.89-1.21)	1.01 (0.82-1.24)	1.09 (0.87-1.37)	1.03 (0.86-1.23)	1.06 (0.80-1.42)
triparous	2,516	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
increase per 5 yr	715	14%(4%-26%)	18% (-4%-46%)	15% (3%-30%)	15% (3%-29%)	11% (-7%-34%)
General increase per 5 yr per birth		8% (6%-9%)‡	2%(-2%-5%)	7% (6%-9%)	8% (6%-10%)	6% (3%-9%)

* Adjusted for age, calendar period and age at 1. to 6. birth. The age at birth specific estimates are estimated using formula (2) in the method section, parity specific risk increase estimates using formula (3) and general risk increase estimates using formula (4). Overall increase per 5 years for age at 5th birth and 6th birth were included for in all analyses and estimated to be 9% (-13%-38%) (n=104) and -9%(-45%-51%) (n=28), respectively. The general increase per 5 yr for 5th and 6th birth was 5%(-12%,26%). †Estimating the general effect for 1st to 6th birth (instead of 1st to 4th birth) gave the same result.

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Alpha-fetoprotein levels during pregnancy and maternal breast cancer incidence. (study 14)

<u>Material and methods</u>
For this investigation we linked data from the National Civil Registration System (CRS), the National Birth Registry, and the Danish Breast Cancer Cooperative Group with data from a population-based screening of MS-AFP. This study was approved by the Scientific Ethics Committee and the National Data Protection Board of Denmark.

Population Registries
Since April 1, 1968, the CRS has assigned a unique identification number to all residents of Denmark, which permits information from different registries to be linked. The CRS registers dates of any live births (which allows reconstruction of reproductive history for each woman) and dates of emigration and deaths *(18)*. Information on stillbirths were available from the National Birth Registry.

AFP Assessment
MS-AFP testing has been offered to all women attending antenatal care in three Danish counties since 1978 and a screening program was introduced in 1980. Serum samples used for MS-AFP screening were taken during the second trimester of each pregnancy before any amniocentesis was performed. Gestational age was recorded in completed weeks estimated from ultrasound examination or from the date of the last menstrual period. MS-AFP levels were standardized to gestational age by dividing the absolute value by the median value across all singleton live births obtained for each gestational week for each calendar year (multiples of the median = MoM). For women with more than one birth, repeated measures of AFP-levels were available. Prepregnancy weight was available only on a subset of these women.

Breast Cancer Cases
Women who developed breast cancer were identified by linkage with the Danish Breast Cancer Cooperative Group (DBCG) as this register was complete until September 1, 1998. The DBCG collects detailed information on the breast cancer diagnosis, including tumor size, nodal status, and receptor status *(19,20)*.In a linkage between the DBCG registry and the Danish Cancer Registry, the DBCG registry was found to contain information on 94% of all breast cancer patients reported to the Danish Cancer Registry *(3)*. Reporting of cancer to the cancer registry is mandatory in Denmark since 1943 *(21)*.

Design and Statistical Analysis
A cohort of Danish women was retrospectively established to include all women born in Denmark between April 1, 1935, and March 31, 1978 (since information on reproductive history could be obtained for these women by linkage with the CRS), who gave their first birth in 1978 or thereafter, and for whom an AFP-measurement was available for at least the first birth.

Women contributed person-time from the date of their first MS-AFP screening to a diagnosis of invasive breast cancer, death, emigration, or the end of follow-up on September 1, 1998 (at which date the DBCG was considered complete), whichever occurred first. Relative risk estimates were obtained by modeling breast cancer incidence rates using a log-linear Poisson regression model.

Since the relation between MS-AFP levels and breast cancer incidence appeared to be non-linear, MS-AFP levels were categorized into four categories using quartiles of the MoM based on the person-years distribution as cut points. For women who gave birth more than once and for whom MS-AFP levels were available for more than one pregnancy, information was updated at the time the new measurement was taken. The study variable representing a woman's AFP level is therefore the AFP level relating to her latest birth and the variable is a time-dependent variable that can change after each childbirth. Complete MS-AFP values for all pregnancies were available for 50% of the cohort. If MS-AFP levels were missing for a pregnancy subsequent to the first, the latest value was carried forward until the next pregnancy with an available MS-AFP level. Compared to mothers with singleton births, mothers with a multiple birth have a 2-fold higher level of MS-AFP during pregnancy and probably also a different breast cancer risk. This potential confounding effect of multiple births was avoided by excluding person-time from the analysis if the latest birth was a multiple birth. The person-time after a singleton birth in mothers with a previous multiple birth, however, was included in the analysis.

Analyses were adjusted for attained age in 5-year intervals, calendar period (<1993, >=1993), age at first birth (<=24, 25-29, 30-34, 35+), and parity (1, 2+). Attained age means age at any time during follow-up; for cases this is age at the time of diagnosis of breast cancer. All variables were treated as time-dependent variables in the analyses. To evaluate potential effect modification of the AFP-breast cancer association, analyses stratified by age at first birth, age at birth at which MS-AFP was measured, and number of births were also conducted. For the stratified analyses, MS-AFP levels of median value or above were compared to levels below the median.

Tests for trend of the relative risk of pregnancies with AFP>=1 MoM compared to AFP<1 MoM across the levels of the stratification variables were performed by including an interaction term between AFP and the stratification variable. The stratification variable was used as a trend variable using the median value of the categories.

The association between level of MS-AFP in the latest pregnancy and survival after a breast cancer diagnosis was analyzed by Cox's proportional hazards method with adjustment for tumor size, number of positive nodes, age at diagnosis, and protocol allocation. Vital status was followed up from date of diagnosis to October 1, 1998.

Results
The 42,057 women who fulfilled the inclusion criteria for this study contributed 379,287 person-years and 79,531 pregnancies for 70% of which MS-AFP values were available. The median age at first birth in this population was 26 years (this is representative for the Danish female population during the respective time period). Of these women, 117 developed invasive breast cancer during follow-up. About 96% of cancer cases were 50 years of age or younger at diagnosis.

Table 1 shows the distribution of factors characterizing the population at risk including number of cases of breast cancer and person-years of follow-up. In Table 2 we present the association between MS-AFP levels and breast cancer incidence.

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Women who contributed person-time to the highest two MS-AFP quartiles had a significantly lower incidence of breast cancer than women who contributed time to the lowest two MS-AFP quartiles. Women in the two highest MS-AFP quartiles had about half the risk of breast cancer compared to women with MS-AFP levels just below the median after adjusting for age, calendar period, parity, and age at first birth. Adjusting only for age and calendar period produced virtually identical results. Adjustment for pre-pregnancy weight did not alter the results and results are therefore presented unadjusted for this variable. When we restricted the cohort to women for whom we had complete information on MS-AFP for each of their births during follow-up the relative risk estimates relative to the second lowest quartile was 0.91 (95% CI 0.51-1.64) for the lowest, 0.56 (0.29-1.08) for the second highest, and 0.46 (0.25-0.86) for the highest quartile, respectively.

In Table 3 we present the association between AFP levels and breast cancer incidence stratified by age, age at first birth, age at birth at which MS-AFP was measured, number of births, and time since latest birth. An MS-AFP level of median value or above was associated with a 41% decreased risk of breast cancer compared to a level below the median. The association between MS-AFP and breast cancer incidence was even stronger among younger women, and if the pregnancy occurred at a young age, whereas it was the same for the first and subsequent births.

Table 4 presents the association between MS-AFP and breast cancer incidence stratified by certain tumor characteristics. The difference with respect to estrogen receptor status was rather modest. However, the reduction in breast cancer incidence in women with high MS-AFP levels was stronger for tumors with positive nodal status (relative risk (RR)=0.48 (0.30-0.79)) than negative nodal status (RR=0.70 (0.39-1.25)). Similarly, the reduction was also more pronounced for large tumors (RR=0.24 (0.11-0.50)) than for small tumors (RR=0.83 (0.2-1.33)). Of the 117 women who developed breast cancer, 22 had died before October 1, 1998. Comparing the prognosis according to the level of MS-AFP we found that the adjusted relative risk of dying in patients with a MS-AFP level of median value or above was 0.70 (0.22-2.24) compared to patient with a MS-AFP level below the median.

Discussion
In a large population of more than 42,000 Danish women we found a high level of MS-AFP during second trimester pregnancy to be associated with a significantly reduced incidence of breast cancer. This finding confirmed our expectation which was mainly based on AFP's antiestrogenic properties. The association between MS-AFP levels and breast cancer was particularly strong among women with high MS-AFP levels in pregnancies at an early age.

To date, the current study is the only one in which repeated measures of MS-AFP levels are available for consecutive pregnancies. To our knowledge, the only previous epidemiologic study in which the association between MS-AFP and breast cancer has been considered was a case-control study nested in the Californian Child Health and Development Studies (CHDS) (22). In this study including 573 women, third trimester blood samples taken between 1959 and 1966 and subsequently frozen were later used to determine MS-AFP levels. Blood levels had only been taken during one pregnancy, thus MS-AFP levels were only available for one birth (at arbitrary birth order) for each woman included. No overall asso-

ciation between MS-AFP levels obtained during one pregnancy and breast cancer risk was found in this study. The authors did, however, report a reduced breast cancer risk for women with high MS-AFP levels during the one index pregnancy provided they had an early age at first birth. We also found the lowest relative risk of breast cancer among women with the youngest age at first birth. In contrast to the previous authors, however, we did not find an increased risk of breast cancer associated with high MS-AFP levels among women with age at first birth above 26 years.

We and others have previously shown that the effect of pregnancy on breast cancer risk approximates to a short term increase in risk followed by longer term protection (23, Wohlfahrt, unpublished data). In the present study we found the lower risk associated with high AFP to be greatest for diagnosis of breast cancer at a young age, when the effect of a recent pregnancy is to increase risk above that of nulliparous women. Therefore, in theory high AFP might be associated not with a reduction in risk but with a smaller increase in risk. The present study did for obvious reasons not include nulliparous women. However, as shown in Table 3 we found no difference in the association with high AFP according to years since childbirth. The short-term effect is therefore not likely related to the AFP level during pregnancy.

There are a number of possible explanations for the difference in the overall association reported by the CHDS group and our results. Firstly, AFP levels from one pregnancy only was available from the CHDS cohort and for each woman the index pregnancy could represent a different order pregnancy. In contrast, we had repeated measures on MS-AFP; we measured MS-AFP levels for all first pregnancies and as well for the majority of the remaining pregnancies. If there are important differences among MS-AFP levels of different pregnancies we would capture their influence more effectively with our updated analyses. Furthermore, MS-AFP was measured during the third trimester in the CHDS cohort, our measures were taken during the second trimester. As the authors of the previous study acknowledge as being a potential limitation of their cross sectional approach, the consistency of third trimester MS-AFP levels between subsequent pregnancies has not been studied (24). Also, the majority of breast cancer cases in the CHDS study were postmenopausal at diagnosis while most of our cases were diagnosed premenopausally. Our conclusions of a positive association between high MS-AFP level during pregnancy and a lower breast cancer incidence cannot be extrapolated to postmenopausal women on the basis of our data. Finally, although both studies had a large number of cases overall, the number of cases in the stratified analyses were relatively small leaving room for some variability between results.

Our cancer registry included detailed information on tumor characteristics at time of diagnosis which allowed a more detailed analysis of the association with MS-AFP level. There was a clear tendency among women with high levels of MS-AFP to have a particularly low incidence of breast tumors with aggressive characteristics at time of diagnosis, such as large tumor size and positive lymph node status. Most striking was the finding that women with high AFP levels reduced their incidence of large tumors (above 2 cm) to ¼ of that seen for women with low levels of MS-AFP. In line with these results women with high levels of MS-AFP also appeared to have a better overall survival compared to those with low levels even after adjusting for important characteristics influencing survival. This finding was,

however, based on a limited number of deaths and should be taken with due care.

The nature of this prospective cohort design limited the potential for biases related to differential misclassification and selection. Thus, all covariate information was obtained independently of the exposure and not dependent on recall. We were not able to adjust in our analysis for a number of known risk factors for breast cancer such as family history, height, body mass index (BMI), age at menarche, and age at menopause. Since the majority of breast cancer cases were diagnosed before age 50 confounding by menopausal status is unlikely. In line with what was reported by the CHDS group, we found no confounding effect of pre-pregnancy weight. Thus, it is unlikely that adjustment for height or BMI would alter the results. The Danish population is very homogeneous with the vast majority representing white Caucasians. The present investigation only included women born in Denmark and the study population therefore almost exclusively represents white Caucasians. Thus, confounding by race is not an issue.

In conclusion, we found a high AFP level in maternal serum during the second trimester of pregnancy to reduce subsequent breast cancer incidence overall, and advanced disease in particular among primarily premenopausal women. This association appeared strongest if the pregnancy occurred at a young age. The present findings are potentially important. Firstly they may offer a complementary explanation as to why the risk of breast cancer is lower in parous compared to nulliparous women. Moreover, our results indicate that even time-limited exposure to high levels of AFP can lower the risk of breast cancer overall and of advanced breast cancer in particular. If confirmed in future studies, and given the availability of recombinant AFP, these findings may open up new venues for the prevention of breast cancer. However, any practical implications awaits a better understanding of as to what extent the observed association with high levels of AFP reflects a direct effect of AFP on tumor carcinogenesis or the effect of another substance closely interacting with AFP.

Table 1. Distribution of factors characterizing the population at risk, including number of cases of breast cancer and person-years of follow-up.

	Number of cases	Person-years of follow-up (x 1000)
Attained age[A]		
≤34	23	263.7
35-39	45	73.8
40-44	17	30.2
≥45	32	11.6
Age at first birth		
≤24	14	139.5
25-29	34	153.7
30-34	34	59.2
≥35	35	26.7
Age at birth at which AFP was measured[$]		
≤29	24	236.4
30-34	40	98.9
35-37	34	28.2
≥38	19	15.7
Number of births		
1	63	209.0
≥2	54	170.2
Time since latest birth		
<2	18	135.4
2-4	39	135.8
5-9	40	87.8
≥10	20	20.3

[A] Age at any time during follow-up; for cases this is age at the time of diagnosis of breast cancer.
[$] Time-dependent variable.

Table 2. Relative risk of breast cancer according to serum levels of alpha-feto protein during the second trimester of pregnancy

Alpha-fetoprotein		Number of cases	Relative risk (95% CI)*
<0.8 MoM	(1. quartile)	32	0.74 (0.46-1.20)
0.8-0.99 MoM	(2. quartile)	34	1
1-1.29 MoM	(3. quartile)	28	0.51 (0.31-0.85)
≥1.3 MoM	(4. quartile)	23	0.49 (0.29-0.83)

* Relative risk and 95% confidence interval adjusted for age, calendar period, parity and age at first birth

MoM: Multiples of the median

Table 3. Serum levels of alpha-fetoprotein (AFP) and relative risk of breast cancer stratified by age and reproductive variables

Stratification variable	Number of cases		Relative Risk for AFP≥1 MoM vs. AFP<1 MoM (95% CI)*	P for Trend
	AFP<1 MoM	AFP≥1 MoM		
Overall	66	51	0.59 (0.41-0.85)	
Attained age[†]				
≤34	19	4	0.17 (0.06-0.51)	
35-39	25	20	0.65 (0.36-1.16)	
40-44	8	9	0.85 (0.33-2.19)	
≥45	14	18	0.86 (0.43-1.73)	p=0.01
Age at first birth				
≤24	11	3	0.22 (0.06-0.80)	
25-29	21	13	0.51 (0.25-1.01)	
30-34	17	17	0.80 (0.41-1.57)	
≥35	17	18	0.70 (0.36-1.35)	p=0.11
Age at birth at which AFP was measured[$]				
≤29	19	5	0.21 (0.08-0.56)	
30-34	23	17	0.61 (0.32-1.14)	
35-37	15	19	0.96 (0.49-1.89)	
≥38	9	10	0.71 (0.29-1.75)	p=0.02
Number of births				
1	36	27	0.53 (0.32-0.88)	
≥2	30	24	0.66 (0.39-1.13)	p=0.57
Time since latest birth				
<2	10	8	0.65 (0.26-1.65)	
2-4	25	14	0.45 (0.23-0.87)	
5-9	23	17	0.54 (0.29-1.01)	
≥10	8	12	1.06 (0.43-2.58)	p=0.39

* Relative risk and 95% confidence interval adjusted for age, calendar period, parity, and age at first birth (unless the variable was stratified on).
[†] Age at any time during follow-up; for cases this is age at the time of diagnosis of breast cancer
[$] Time-dependent variable

Table 4. Serum levels of alpha-fetoprotein (AFP) and relative risk of breast cancer stratified by estrogen receptor status, nodal status, and tumor size

Stratification variable	Number of cases		Relative Risk for AFP≥1 MoM vs. AFP<1 MoM (95% CI)*
	AFP<1 MoM	AFP≥1 MoM	
Estrogen receptor status			
negative	21	19	0.69 (0.37-1.28)
positive	32	24	0.57 (0.34-0.97)
Nodal status			
negative	24	22	0.70 (0.39-1.25)
positive	41	26	0.48 (0.30-0.79)
Tumor size			
≤ 2 cm	33	36	0.83 (0.52-1.33)
> 2 cm	29	9	0.24 (0.11-0.50)

* Relative risk and 95% confidence interval adjusted for age, calendar period, parity and age at first birth

Risk of late stage breast cancer following a childbirth. (Study 16)

Study cohort

Since April 1, 1968, the Civil Registration System (CRS) in Denmark has assigned an individually unique national registration number to all citizens. Based on this number the CRS keeps updated information on dates of live births, emigration and vital status. The CRS-number also permits accurate individual-based linkage of information from other registries. We used the CRS-registry to establish a national parity database including all women born between April 1, 1935, and March 31, 1978 as earlier described (6,7). To be able to study breast cancer rates during pregnancy, we added information on induced and spontaneous abortions and gestational age of births from the National Registry of Induced Abortions, the National Discharge Registry and the Danish National Birth Registry.

Detailed information on registered invasive primary breast cancers in the period January 1, 1978 to September 30, 1994 including the size of the tumor, number of positive nodes and histological grading was obtained from the Danish Breast Cancer Cooperative Group (DBCG) registry. DBCG initiated a series of national prospective studies in 1977 to systematically evaluate breast cancer treatment programs. A detailed description of this registry has been given elsewhere (8,9). During a limited time period (1977-81), the DBCG collected additional information such as the date at which the woman experienced the first

symptom(s) of her disease, and the date of her first consultation with a medical doctor (10). Through a linkage between the DBCG registry and the Danish Cancer Registry, the DBCG registry was found to contain information on 94 percent of all breast cancer patients reported to the Danish Cancer Registry. The Danish Cancer Registry is considered close to complete regarding incident cases of malignant neoplasms diagnosed in Denmark since 1943 (11).

Statistical analyses

The impact of time since birth on the incidence of breast cancer with a specific tumor characteristic was investigated in a follow-up study analyzed using log-linear Poisson regression models (12). Each stage specific diagnosis of breast cancer was analyzed separately. Both tumor size, nodal involvement and histologic grading are used as indicator of stage. All women entered follow-up for each of the stage specific breast cancer diagnoses on January 1, 1978 or on their 12-year birthday whichever came last. The period at risk continued until breast cancer (whatever stage), death, emigration, or September 30, 1994 whichever occurred first. Incidence rate ratios are referred to as relative risks. All variables were treated as time-dependent variables. Calculations were performed using the SAS procedure PROC GENMOD (13). Adjustment was made for age (quadratic splines with knots: 30,35,40,45,50,55) (14) and calendar period (1978-82,1983-87,1988-92,1993-94). Using age in 1-year categories in the overall analysis had no impact on the conclusions. In the analysis of time since latest birth we furthermore adjusted for age at first birth (nulliparous, 12-19, 20-24, 25-29, 30-34, >34) and parity (nulliparous,1,2,3,4+). Estimation of the effect of time since latest birth with adjustment for age and age at first birth when including uniparous women has been discussed by Heuch et al (15). Test for effect modification by parity (1,2,3+) was performed as a test for interaction between categorical variables.

In an alternative approach we compared the mother's risk with what would have been her risk had she not delivered a child. This was done according to time since each delivery categorized as: <2 years, 2-3 years, 4-5 years, 6-7 years, 8-9 years, 10+ years, i.e. four time-dependent variables representing time since 1st ,2nd, 3rd and 4th birth were included in the model. In these analyses women were followed until a possible fifth birth. In addition to age and calendar period we also adjusted for age at 1st, 2nd, 3rd and 4th birth. As we only found minor insignificant effects of age at 1st to 4th birth the first 10 years after birth, we only added the effects of age at 1st to 4th birth in the model 10 years after birth. This was done by further categorizing the category "10+ years" in each of the four "time since birth"-variables according to age at birth (12-24 years, 25-29 years, 30-34 years, 35+ years). In other words, the "short-term" effect of a birth (<10 years) was categorized according to time since birth, and the "long-term" effect (=10 years) according to age at birth.

In an additional analysis we estimated the rate of breast cancer during pregnancy using a similar approach including additional information on interrupted pregnancies and gestational age at delivery. A woman was considered pregnant from the time of conception (estimated by gestational age) until birth or time of interruption of the pregnancy. Her parous status during pregnancy was the number of births prior to the pregnancy.

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To evaluate whether an increased risk after childbirth could be ascribed to delayed diagnosis we estimated the cumulative difference between the observed number of incident breast cancer cases in newly pregnant nulliparous and uniparous women in the cohort according to time since latest birth and the predicted number of cases had they not had the latest birth. The prediction was based on a model including age, calendar period, parity and age at first birth. The deficit of cases in these women during pregnancy was estimated using the distribution of person-years in uni- and biparous women with less than one year since latest birth (multiplied by 9/12) assuming that they were one year younger and have had one childbirth less.

Results

Overall, 1,529,512 women were included in the cohort. A total of 10,790 primary invasive breast cancers were observed during 22.3 million person-years of follow-up. Of these 1,295 women were nulliparous at time of diagnosis.

The association between time since latest birth and the incidence of breast cancer is shown in Table 1. Overall, there was a small but significant association between time since latest birth and the breast cancer rate ($p=0.0002$). After adjustment for the differences in age and other confounders the risk was highest (1.16-fold) 2-3 years after delivery compared to 10-14 years after. Table 1 furthermore shows the association between the time interval since latest birth and the risk of breast cancer by tumor size at diagnosis. The rate of large tumors was significantly associated with the time interval since latest birth ($p=0.002$), for instance, women with 2-3 years since latest birth had a 2.27-fold (95 percent confidence interval 1.49-3.44) higher risk of breast cancer compared to women with 10 to 14 years since latest birth. Overall the risk of being diagnosed with a tumor with a diameter larger than 5 cm was 53 percent higher the first 10 years after birth compared to later. There was no association between time since latest birth and the rate of small tumors (<21mm) ($p=0.17$). The rate of medium sized tumors (21-50 mm) was only slightly associated with the time since latest birth ($p=0.06$). The association between time since latest birth and large breast cancer was not modified by parity ($p=0.56$). We found similar patterns of an increased risk of tumors with adverse features when the cases were divided according to nodal status or histological grading (Table 2).

In an alternative approach we compared a mother's risk with what would have been her risk had she not delivered a child. In the first 10 years after the first and second birth the breast cancer risk was increased by ratios of 1.07 (0.97-1.19) and 1.07 (0.98-1.15) compared to nulliparous and uniparous women, respectively. Overall, the increase the first 10 years after first and second birth was 1.07 (1.01-1.13) and according to time since birth: <2 years: 0.97 (0.82-1.15), 2-3 years: 1.13 (0.99-1.29), 4-5 years: 1.08 (0.96-1.21), 6-7 years: 1.11 (1.00-1.22), 8-9 years: 1.06 (0.97-1.15). In the first 10 years after the third and fourth birth there was no increased risk (RR=0.99 (0.90-1.08) and 0.89 (0.74-1.07), respectively). As illustrated in Figure 1A and 1B we performed the same analyses according to tumor size at diagnosis. During the first 10 years after the second and third birth, mothers had up to a 2-fold higher risk of being diagnosed with a tumor larger than 50 mm. The relative risk the first 10 years after fourth birth compared with triparous women was 1.34 (0.74-2.43) for being diagnosed with tumors larger than 5 cm and 0.88 (0.72-1.08) for being diagnosed with tumors 5 cm or smaller. The same type of analysis was not informative for uniparous women because there was an

overall higher rate of late stage tumors in nulliparous women as mentioned in the discussion.

Based on 20 cases of breast cancer detected in *pregnant* women during 706,234 years of follow-up, we estimated that the rate of breast cancer in pregnant women was 72 percent (95 percent confidence interval from 51 percent to 84 percent) lower than expected. To evaluate whether this lower rate of breast cancer during pregnancy could explain the increased risk of breast cancer in the first years after first and second birth we estimated the cumulative difference between the observed number of incident breast cancer cases in the newly pregnant nulliparous and uniparous women in the cohort and the expected number of cases had they not had the latest birth. During pregnancy, we estimated that there was a total deficit of around 31 breast cancer cases compared with non-pregnant women. However, in the first 10 years after birth the women who had been pregnant experienced an excess of 88 cases. Assuming that the deficit of cases during pregnancy was exclusively due to postponed diagnosis it could only account for the excess during 4 to 5 years after birth.

For a subgroup of women diagnosed in the period from 1978 to 1982 we had information about the time interval between the first symptoms observed by the woman and her first visit to her doctor (patient's delay), and between the first visit to her doctor and the time of definitive surgery or biopsy (doctor's delay) (10). There was no significant relationship between the two measures of diagnostic delay and the time since latest birth when compared by a Mann-Whitney test. Within five years after a birth the median patient's and doctor's delay was 12 and 29 days, respectively. During 5 to 9 years after a childbirth the similar figures were 11 days and 30 days, respectively, and in the subsequent years the figures were 7 days and 28 days, respectively.

TABLE 1. Adjusted* relative risk of breast cancer overall and according to time since latest birth by tumor size at diagnosis†, Denmark, 1978-1994.

| Years since latest birth | Overall† | | | Tumor size at diagnosis‡ | | | | | | | |
| | Person-years (10³) | No. | RR (95% CI) | <21mm | | 21-50 mm | | >50 mm | | | |
				No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)		
<2	1,794	211	1.02 (0.87-1.19)§	97	0.91 (0.73-1.14)	77	1.16 (0.89-1.50)	14	1.14 (0.63-2.07)
2-3	1,479	303	1.16 (1.02-1.32)	139	1.04 (0.86-1.26)	102	1.21 (0.97-1.52)	35	2.27 (1.49-3.44)
4-5	1,191	369	1.13 (1.00-1.27)	170	1.03 (0.86-1.22)	122	1.16 (0.94-1.42)	35	1.80 (1.21-2.69)
6-7	1,080	486	1.14 (1.03-1.27)	243	1.13 (0.98-1.31)	149	1.09 (0.90-1.30)	39	1.53 (1.05-3.23)
8-9	1,022	591	1.08 (0.99-1.19)	290	1.04 (0.91-1.19)	202	1.14 (0.97-1.34)	40	1.22 (0.85-1.76)
10-14	2,414	1993	1 (reference)	1038	1 (reference)	659	1 (reference)	118	1 (reference)
15+	3,791	5542	0.92 (0.86-0.98)	3042	0.93 (0.85-1.01)	1876	0.90 (0.81-1.00)	304	0.95 (0.74-1.21)
Homogenity test			p=0.0002		p=0.17		p=0.06		p=0.002

* Adjusted for overall and tumor size specific age, calendar period, parity and age at first birth.

† When excluding uniparous women from the analysis we found a similar association: <2: 1.05 (0.88-1.26), 2-3: 1.15 (0.99-1.33), 4-5: 1.16 (1.03-1.32), 6-7: 1.13 (1.01-1.27), 8-9: 1.07 (0.97-1.19), 10-14: 1 (reference), 15+: 0.91 (0.85-0.97).

‡ Number of cases by tumor size do not add up to number of cases overall due to missing information on tumor size for some cases (7%).

§ 0 yr: 0.92 (0.74-1.15), 1 yr: 1.10 (0.90-1.33).

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TABLE 2. Adjusted* relative risk of breast cancer according to time since latest birth by nodal status and histological grading at diagnosis, Denmark, 1978-1994.

Years since latest birth	Nodal status at diagnosis[†]				Histological grading at diagnosis[‡]			
	Node negative		Node positive		I		II+III	
	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)
<2	88	0.85 (0.67-1.08)	109	1.01 (0.75-1.36)	30	0.77 (0.52-1.14)	137	1.13 (0.93-1.38)
2-3	140	1.06 (0.88-1.29)	139	1.28 (1.00-1.63)	55	1.07 (0.79-1.44)	178	1.19 (1.00-1.41)
4-5	163	0.99 (0.83-1.17)	184	1.34 (1.08-1.65)	74	1.09 (0.84-1.41)	213	1.17 (1.00-1.36)
6-7	260	1.21 (1.05-1.39)	202	1.11 (0.91-1.36)	95	1.02 (0.82-1.28)	287	1.24 (1.08-1.42)
8-9	281	1.01 (0.88-1.16)	266	1.27 (1.08-1.51)	134	1.07 (0.88-1.30)	321	1.11 (0.98-1.26)
10-14	1020	1 (reference)	862	1 (reference)	490	1 (reference)	1014	1 (reference)
15+	2972	0.96 (0.89-1.05)	2364	0.89 (0.79-1.00)	1500	0.91 (0.80-1.02)	2770	0.98 (0.90-1.07)
Homogenity test		$p=0.06$		$p=0.0002$		$p=0.33$		$p=0.03$

* Adjusted for nodal and grading specific effects of age, calendar period, parity and age at first birth.
[†] Number of cases according to nodal status do not add up to number of cases overall (Table 1) due to missing information on nodal status (4%).
[‡] Histological grading is only registered for ductal carcinomas. Number of ductal cases with missing information on histological grading were 4%.

DISCUSSION

The present study documented that a mother's age-adjusted risk of breast cancer is highest the first 10 years following the latest birth and in particular that their risk of late stage tumors is significantly elevated. That the overall breast cancer risk is increased after a childbirth has been observed before (2,3,4,5), but that in particular the risk of late stage breast cancer is elevated is a novel observation that may give further insight to the mechanisms behind the increased risk.

The high rate of late stage breast cancer in the first years following a birth could be due to delayed diagnosis/surgery of breast cancer during pregnancy. Either because of difficulties in detecting the tumor during pregnancy or because breast surgery was postponed to after the delivery. A delayed diagnosis/surgery due to pregnancy would result in larger tumors after the delivery, but the breast cancer rate during pregnancy should also be correspondingly low. In concordance with three previous studies (4,16,17) we observed a 72 percent lower risk of breast cancer during pregnancy. Some of this lower rate might very well be explained by a "healthy women" effect, but, even if we assumed that the lower rate during pregnancy should exclusively be explained by delayed diagnosis, we found that such a diagnostic delay only could account for an excess of cases equivalent to e.g. the observed increased breast cancer rate in the first four or five years after first and second delivery. Thus, delayed diagnosis/surgery due to pregnancy did not appear to explain the entire excess of cases in the years following pregnancy.

We furthermore investigated whether the higher rate of late stage breast cancers after the first years could be due to delayed detection because of woman's primary attention being devoted to childcare during the first years after delivery. However, based on detailed referral information on a subset of the women in this study we found no elevated diagnostic delay in women diagnosed in the these years after a childbirth compared to later years. Altogether, delayed diagnosis during pregnancy and delayed detection in the first years following birth appeared unable to explain the significantly elevated age-adjusted risk of late stage breast cancer in the first 10 years after a birth.

Part of the higher rate of late stage breast cancer in the first 10 years after a birth is probably explained by cases diagnosed in the first 10 years being initiated before the birth, whereas the malignant process in cases diagnosed after the first 10 years more likely are initiated after the birth where the rate is reduced by the protective effect of an additional birth. However, if this was the only ex-planation for the higher rate of breast cancer after a delivery we would anticipate that uniparous mothers in the first years after childbirth had the same *overall* risk as nulliparous women (and likewise when comparing biparous with uniparous women) or maybe even a lower risk in the first years due to a "healthy women" effect. Nevertheless, in additional analyses we observed that uniparous and bipa-rous women had a slightly increased overall breast cancer risk in the first 10 years after the latest birth when compared with women with one birth less. Such analy-ses suggest that a mother transiently has a higher risk compared to what would have been her risk had she not delivered a child and therefore directly support the idea that pregnancy related factors, e.g. the elevated hormonal level during pregnancy, transiently increase a mother's overall risk of breast cancer by stimu-

lating high growth rate in already malignant cells and/or inducing a new tumor growth.

An enhanced tumor growth following a birth might just mean that relatively indolent tumors are accelerated and therefore discovered sooner, but at the same stage as without a growth rate change. However, performing the same kind of analysis on the rate of late stage tumors we observed an even more dramatic transient increase of the rate of late stage breast cancer after a second and third delivery. During the first years following the second or third birth we observed a more than 2-fold higher risk of late stage cancer when comparing with women everything else equal but the latest birth. This novel observation should not alarm the average pregnant women as the rate of late stage breast cancer is very small. In other words, the absolute effect is small and therefore has no direct implications for primary prevention, but the finding is of etiologic interest because it supports the hypothesis that the transient increased risk of breast cancer after birth is due to an increased growth rate in malignant and premalignant cells that to some extent leads to discovery at a later stage compared to what would have been the case had the women not delivered a child.

The stage-specific analysis should be considered with due caution. We have previously shown that the rate of late stage tumors in general is much higher in nulliparous compared with parous women, which can either be because a woman's reproductive history influences the time of detection or it affects the progression rate of the tumor (18). The lack of a transient increased risk of late stage breast cancer after the first birth is most likely due to this generally lower risk of late stage breast cancer in uniparous compared to nulliparous women. Because of this phenomenon we have in this paper also focused on comparisons between mothers with the same number of births, thereby excluding differences in the rate of late stage breast cancer attributable to parity per se. Using this approach we observed the same association between the time since latest birth and the rate of late stage breast cancer irrespective of parity, which suggests that regardless of number of prior births, a recent pregnancy transiently increases the number of late stage cases of breast cancer.

We have used two different analytic approaches with different features. In one approach we compare the risk in mothers at a given time-interval after the birth compared to what would have been her risk had she not delivered a child. With such an approach one is able to estimate the transient increase, which is of etiological interest. However, as one estimates the combined effect of the birth and the time interval one cannot determine whether differences in the effects according to stage is related to the birth per se, the time interval or both. In the other approach we avoid this problem by comparing the risk according to time since latest birth between women with the same number of births. This approach can, however, not be used to estimate the transient increase (19) and it has furthermore been argued that one cannot estimate the effect of time since latest birth in uniparous women while adjusting for age at first birth and age (5). However, estimation in uniparous is possible when including nulliparous and assuming a common age effect for all women (15). A recent paper reveals that this approach is reasonable (20). Nevertheless we have in Table 1 provided results where uniparous are excluded.

The study was performed as a prospective analysis on a large population-based cohort and was based on mandatory reported exposure and outcome information making information bias on exposure and selection bias on cases unlikely. As noted by Hsieh et al (3) the cohort follow-up design is more powerful than a case-control design when studying a time-dependent variable as time since latest birth, since all births are included in the study and not just the last birth. A limitation of the study was the lack of data on other reproductive breast cancer risk factors such as age at menarche, age at menopause, family history and use of exogenous hormones.

Breast cancer risk after a childbirth in young women with a family history. (Study 18)

Material and Methods
Study population and ascertainment of cases
A research parity database was established from the Civil Registration System (CRS). It includes all women born in Denmark between April 1, 1935, and March 31, 1978, as earlier described (Melbye 1997, Westergaard 1997). Based on the personal identification number from the Civil Registration System, we linked data with the Danish Cancer Registry, which has information on invasive primary breast cancers since 1943. As earlier described were 2860 women born 1935 or later diagnosed with breast cancer in the period 1943 to 1990 before the age of 40 years (Olsen et al, 1999). The research parity database includes 2770 of these cases (i.e. excluding cases diagnosed Jan 1, 1935 to Marts 31, 1935 and cases born outside Denmark).

Identification of mothers
The method for identification of mothers of the women in the cohort differed according to mother's birth cohort. For women with a mother born in April 1, 1935 or later the mother could be identified in the CRS. For women with a mother born before April 1, 1935 the identity of the mother was not necessarily available from the CRS. For cases among these women the mother was identified from parish registries as described in Olsen et al (Olsen et al, 1999). The identity of the mothers was found in 94% of the cases (Olsen et al, 1999). Breast cancer cases among mothers were identified in the Cancer Registry with follow-up to end of 1993.

Determination of person-years of follow-up
For women with a mother born in April 1, 1935 or later the mothers identity was known for all women, and it was therefore possible to directly calculate person-years of follow-up in strata according to both FHBC and other factors. For women with a mother born before April 1, 1935 the identity of the mother was only known for cases. The distribution of person-years of follow-up in these women was therefore estimated on the basis of the distribution among women with a mother born April 1, 1935 or later. This was done by estimating, for each strata according to other factors, the proportion of persons-years of follow-up contributed by women with FHBC using logistic regression (with adjustment for age, parity and age at first birth) based on the person-years distribution in women with a mother born April 1, 1935 or later. To test the robustness of these imputations of person-years of follow-up we alternatively scaled the estimated proportions by a factor 5 and as a second alternative used the average proportion regardless of age and reproductive history. Using these two alternative approaches did not change the conclusion, e.g. the general relative increase in risk the first 5 years after a

birth in women with compared to without FHBC was found to be 1.30 using the logistic regression approach, and 1.30 and 1.27 in the two alternatives. The main effect of FHBC might be modified by mothers birth cohort (due to differences in mothers mean age). Therefore, as we used a birth-cohort dependent imputation procedure, we did not estimate the main effect of FHBC on a woman's breast cancer risk. However, it is much less likely that this should affect the estimation of the interaction between FHBC and reproductive history which is the focus of this paper.

Statistical methods
We investigated the impact of 1st, 2nd and 3rd birth on the incidence of breast cancer according to FHBC in a follow-up study using log-linear Poisson regression models (Breslow and Day, 1987). The impact of 1st (2nd and 3rd) birth was modeled as a comparison between uniparous and nulliparous (biparous versus uniparous, triparous versus biparous) according to time since and age at 1st (2nd and 3rd) birth. A more formal statistical description of the model is given elsewhere (Wohlfahrt and Melbye, 2001). As described in that paper the effect of age at birth only affect the breast cancer risk more than 10 years after birth, the risk in the first 10 years after birth is therefore not stratified according to age at birth (Wohlfahrt and Melbye, 2001). FHBC, i.e. family history of breast cancer, was a constant variable defined as having a mother with breast cancer diagnosed before the end of 1993. All women entered the follow-up for breast cancer diagnoses on April 1, 1968, or on their 12th birthday, whichever came latest. The period at risk continued until breast cancer, 40th birthday, death, emigration, or December 31, 1990 (end of follow-up), whichever occurred first. Adjustment was made for age (one year categories), calendar period (5 years categories), an interaction between having a fourth birth (yes/no) and FHBC, and an interaction between mother's birth cohort (<1935,=1935) and FHBC. When adjusting for the interaction between FHBC and age, the age factor was modeled by quadratic splines with knots (age=30,35) (Greenland 1995). Common effects for 1st, 2nd and 3rd, for example the effect in the first 5 years after birth, were estimated by substituting the three related indicator variables (0/1) in the model by their sum.

Results
In all 2,770 cases of breast cancer were observed during 22.7 mill person-years of follow-up. Among the cases 276 (10%) had a mother with breast cancer. Table 1 shows the distribution of number of cases and distribution of person-years of follow-up in women with and without a family history of breast cancer (FHBC) according to age and number of births.

In table 2 is shown the effect of 1st, 2nd and 3rd birth on breast cancer risk according to family history of breast cancer. The relative risk of breast cancer in the first 5 years after the first birth compared to nulliparous is 1.5 in women with FHBC and 1.1 in women without FHBC, i.e. the relative risk is 1.4-fold higher in women with FHBC. The same figure for the 2nd and 3rd birth is 1.2 and 1.2, and the general estimate for 1st, 2nd and 3rd birth is 1.30 (95-confidence interval: 1.03-1.64). In other words the increased risk the first 5 years after birth is 30% higher in women with compared to without FHBC. Performing the same analysis of the relative risk of breast cancer 5 to 9 years after birth, compared with women with a birth less, the general effect is 1.02 (0.84-1.23). Including an interaction between age and FHBC the two estimates were 1.30 and 1.01.

More than 10 years after birth the relative risk is categorized according to age at birth. The relative risk of breast cancer more than 10 years after birth in women that were 25 to 29 years at first birth compared to nulliparous was 0.7 in women with FHBC and 1.0 in women without FHBC, i.e. the relative risk in woman with FHBC was 0.7-fold that of women without FHBC. The same figure for 2nd and 3rd birth was 0.9 and 1.4. The general estimate for 1st, 2nd and 3rd birth was 0.89 (0.67-1.19). When performing the same analysis for women younger than 25 at childbirth the general estimate obtained was 0.93 (0.78-1.12). Including an interaction between age and FHBC the two estimates were 0.87 and 0.93.

Table 1. Number of cases and distribution of person-years of follow-up in women with and without family history of breast cancer (FHBC) according to attained age and parity.

	with FHBC			without FHBC		
	no.	%cases	%pyrs	no.	%cases	%pyrs
Attained age						
12-20	1	0.4%	25.9 %	5	0.2%	30.1 %
20-24	2	0.7%	17.8%	23	0.9%	19.3 %
25-29	27	9.8%	20.3%	211	8.5%	18.8 %
30-34	81	29.4%	20.1%	706	28.3%	17.6%
35-39	165	59.8%	16.0%	1,549	62.1%	14.1 %
Parity						
0	42	15.2%	50.2%	353	14.3%	54.0 %
1	61	22.1%	17.3%	492	19.7%	16.1 %
2	124	44.9%	23.1%	1165	46.6%	21.3%
3	44	15.9%	7.5%	397	15.9%	6.8%
4+	5	1.8%	1.9%	87	3.5%	1.7%

Table 2. Relative risk* (RR) of breast cancer after 1st, 2nd and 3rd birth compared to women with one birth less according to time since and age at birth by family history of breast cancer (FHBC).**

	with FHBC		without FHBC	
	no.	RR (95%-CI)	no.	RR (95%-CI)
Time since and age at 1st birth				
Nulliparous	42	1 (ref.)	353	1 (ref.)
<5 yr after 1st birth	38	1.5 (0.9-2.4)	218	1.1 (0.9-1.3)
5-9 yr after 1st birth	62	1.0 (0.6-1.6)	529	1.1 (0.9-1.3)
=10 yr after 1st birth, 12-24 yr at 1st birth	114	0.9 (0.6-1.5)	1,138	1.0 (0.8-1.1)
=10 yr after 1st birth, 25-29 yr at 1st birth	20	0.7 (0.4-1.3)	256	1.0 (0.9-1.3)
Time since and age at 2nd birth				
Uniparous	103	1 (ref.)	845	1 (ref.)
<5 yr after 2nd birth	49	1.3 (0.9-2.0)	342	1.1 (0.9-1.2)
5-9 yr after 2nd birth	60	1.1 (0.7-1.6)	610	1.1 (0.9-1.2)
=10 yr after 2nd birth,12-24 yr at 2nd birth	35	0.7 (0.4-1.3)	410	0.9 (0.7-1.0)
=10 yr after 2nd birth,25-29 yr at 2nd birth	29	0.9 (0.6-1.6)	287	1.0 (0.8-1.2)
Time since and age at 3rd birth				
Biparous	227	1 (ref.)	2,010	1 (ref.)
<5 yr after 3rd birth	19	1.1 (0.7-1.8)	153	0.9 (0.7-1.0)
5-9 yr after 3rd birth	21	1.2 (0.7-1.9)	206	1.0 (0.8-1.2)
=10 yr after 3rd birth,12-24 yr at 3rd birth	2	0.4 (0.1-1.8)	63	0.9 (0.7-1.3)
=10 yr after 3rd birth,25-29 yr at 3rd birth	7	1.1 (0.5-2.4)	62	0.8 (0.6-1.0)

* Adjusted for age, calendar period and subsequent births.

** -2logL in the model is 4352.4. In the model with common effect for 1st, 2nd and 3rd birth -2logL is 4367.3 (with 12 parameters less).

Discussion
In a comprehensive review of 74 studies from the period from 1935 to 1995 on the association between family history of breast cancer (FHBC) and breast cancer the authors found based on meta-analysis that the strongest association with family history was among young women (Pharoah et al, 1997). Factors interacting with FHBC in the older ages might therefore be different from the factors acting in younger women and studies in post-menopausal can not necessarily be used to predict the effect modifications in pre-menopausal women. The situation is further complicated by the fact that the effect of reproductive history might be modified by age (Andrieu et al, 2000). Our study concentrates on pre-menopausal women and is by far the largest study among women 40 years or younger.

The focus in previous studies on the interaction between FHBC and reproductive history has been on number of births and age at first birth. Some studies have found an interaction with age at first birth (Dupont and Page, 1987; Negri et al, 1988; Byrne et al, 1991; Sellers et al, 1992; Colditz et al, 1993; 1996) or parity (Negri et al, 1988; Parazzini et al, 1992; Colditz et al, 1996), some found no interaction with age at first birth (Brinton et al, 1982; Parazzini et al, 1992; McCredie et al, 1997; Magnusson et al, 1998; Andriu et al, 1998) or parity (Bain et al,1980; Colditz et al,1993; Sellers et al, 1992;1993; McCredie et al, 1997; Andrieu et al 1998). However, a dominant reproductive risk factor in the young years is the short-term increase in risk following a childbirth, and we have therefore in our study focused on the negative short-term effect of a childbirth.

We found that the increase in risk the first 5 years after a childbirth was stronger in young women with compared to without FHBC. More than five years after a childbirth the protective effect was equal to the effect seen in other young women, or if anything even larger. Our finding is in correspondence with the 50% larger transient increased effect seen in women with FHBC in the Nurses' Health Study (Colditz et al, 1996) . Furthermore, a small Swedish study found an increased risk of pregnancy related breast cancer among carriers of BRAC1 and BRAC2 compared with a references population (Johansson et al, 1998). In other words, although FHBC can reflect genetic factors, shared environmental factors or both, and although only a small fraction of women with FHBC are BRAC1 or BRAC2 carriers, our results might be applicable to this particular group.

One interpretation is related to the hypothesis that the short-term increase after childbirth is due to a childbirth induced increase in growth potential in occult tumors. Young women with family history have a higher risk of having occult breast tumors during the reproductive years (due to their higher risk in the young years) and the impact of the growth inducing effect might therefore be stronger in women with FHBC. However, although the relative risk the first 5 years after birth is high, the absolute rate in the young years is relative low and the actual excess number of cases the first five years is therefore small. The importance of our study is therefore primarily related to the etiologic interpretation of breast cancer in relation to the mechanism of the short-term effect of a childbirth.

The study of the effect of FHBC is related to several technical issues. Firstly, family history of breast cancer when assessed by questionnaire can be subject to recall bias (Floderus and Mack, 1990). This type of information bias is

avoided in our study by using register data. Secondly, there might be some non-differential misclassification due to the fact that a mother can develop breast cancer after follow-up. However, it is unlikely that this should affect the interaction with reproductive history. A limitation of the study was the lack of detailed confounder information in the registries. One potential confounder could be the lower average age at diagnose in women with FHBC, that would give them higher likelihood of being diagnosed few years after a birth. However, we found the same result when allowing for different age effects according to FHBC. Our findings are not likely to be due to selection bias as the study was performed as a prospective analysis on a large population-based cohort and was based on mandatorily reported information on reproductive history and breast cancer.

III. Factors influencing the prognosis of breast cancer

Time since childbirth and prognosis in primary breast cancer (study 2)

Material and methods
The Danish Breast Cancer Cooperative Group, DBCG, started its national prospective studies in 1977. Up till now three treatment programs have been in function, DBCG 77 (patient accrual from 1978-1982), DBCG 82 (patient accrual from 1983-1989), and DBCG 89 (ongoing accrual started 1990). The Danish Cancer Registry contains information on close to all incident cases of malignant neoplasms diagnosed in Denmark since 1943 [8]. DBCG has information on 93 percent of all breast cancer patients aged less than 45 years at diagnosis reported to The Danish Cancer Registry.

The primary surgical treatment of the patients included total mastectomy plus axillary sampling (90 percent of the population), or lumpectomy with axillary sampling. Patients were hereafter classified as either low-risk or high-risk according to histopathological criteria. Low-risk patients had tumours ≤ 5 centimetres in diameter without axillary lymph node metastases and without invasion into the skin or the deep resection line (DBCG 77 and DBCG 82). In the DBCG 89 program, premenopausal node negative patients in addition were required to have tumours classified as histologic grade I. High-risk patients were those with a primary tumour > 5 centimetres or with lymph node metastases in the axilla or with tumour growth into the skin or the deep resection line (DBCG 77 and DBCG 82). In the DBCG 89 program premenopausal patients with grade II and III of anaplasia were classified as high-risk patients. Patients with bilateral breast cancer, distant metastases, inflammatory cancer, with contraindication to the planned postoperative therapy, or patients who were not treated according to the surgical guidelines were not allocated to treatment protocols (miscellaneous group). The miscellaneous patient group could be separated into a group with a favourable prognosis and a group with a bad prognosis. The patients who were not treated according to surgical guidelines had an overall good prognosis compared with patients excluded for other reasons. In all three programs low-risk patients were given no systemic treatment after surgery. In the DBCG 77 program, high-risk patients were allocated to either postoperative radiotherapy or radiotherapy and systemic therapy as it has been described elsewhere [9]. In the DBCG 82 program, high-risk patients were allocated to systemic therapy and radiotherapy or to systemic therapy alone [9]. The target for radiotherapy following mastectomy included the chest wall and regional lymph nodes (axillary, supra-/infra clavicular, and parasternal nodes). In the

DBCG 89 programme, high-risk patients were given systemic therapy according to the steroid hormone receptor status. Radiotherapy including the chest wall was given if the tumour invaded the deep resection line. All tumourectomized patients were given radiotherapy to the residual breast tissue.

Since 1968, the Civil Registration System (CRS) has assigned a unique 10-digit identification number to all residents in Denmark that permits accurate linkage of information from different registries. The CRS-registry also keeps updated files on dates of childbirths and vital status. Information about stillbirths was added from the National Birth Registry.

Permission was obtained in advance from the National Scientific Ethics Committee and the Data Protection Board to link information on patients in the DBCG-registry with the CRS-registry. Women born before 1935 have no systematic link to all their children in the CRS- registry. Therefore, to obtain complete reproductive history of the women we restricted our study group to those born since 1st. April 1935. Because our objective was to study the influence of time since birth on breast cancer survival and we furthermore wanted to limit the analysis to premenopausal women, we only included women aged 45 years or less at the time of their breast cancer diagnosis. All women diagnosed before 1st. October 1994, were included and followed until 1st. October 1995, with respect to vital status.

The associations between the study variables and survival were investigated using the Cox Proportional Hazards method [10]. Multivariate analyses included tumour characteristics, time between diagnosis and most recent previous childbirth, parity at diagnosis, age at diagnosis, year of treatment, and protocol allocation. Parity was eliminated from the final multivariate model as it was not significant. Based on the finding of a rather constant survival for the age categories representing six and more years after childbirth we defined a reference category for the variable "time since birth" as six+ years to be used in the multivariate analyses (Table 2). The adequacy of the proportional hazard assumptions for the included variables was checked by log(-log)plots from stratified multivariate analyses. The Cox-regression was performed in four strata (information on tumour size and lymph node status available, only tumour size missing, only lymph node status missing, both missing). Estimation was done using the SAS procedure PROC PHREG [11].

Results

Overall, 5,752 women aged 45 years or less were identified for this particular study. The influence of pregnancy subsequent to treatment of breast cancer is unknown [12], and hence 100 patients were excluded due to delivery after the time of their diagnosis, leaving 5,652 patients for further analyses. The follow-up time ranged from 13 months to 17 years representing a total of 34,130 person-years of follow-up. Overall, 4,957 women (87.7 percent) were parous and 695 women (12.3 percent) were nulliparous. The distribution of patient age, tumour characteristics, and risk group allocation according to time since last birth is given in Table 1.

Figure 1 illustrates the overall 5 and 10-year survival for women according to time since birth. Women diagnosed less than two years after having given birth had a crude 5-year survival of 58.7 percent and a 10-year survival of 46.1 percent, compared with 78.4 percent (5-year) and 66.0 percent (10-

year), respectively, for women who had their last delivery more than two years prior to their cancer diagnosis. Recent pregnancy conferred a negative effect both on patients who received adjuvant treatment and those who did not. Women with a recent birth (< 2 years) who were classified with low-risk breast cancer and as such did not receive adjuvant systemic treatment, had a crude survival of 75.0 percent (5-year) and 55.6 percent (10-year), respectively, compared with 88.5 percent (5-year) and 77.8 percent (10-year) for women whose last child birth was more than two years prior to their diagnosis. Women classified with high-risk disease, receiving adjuvant treatment, had a crude survival of 53.2 percent (5-year) and 41.2 percent (10-year), respectively, compared with 72.0 percent (5-year) and 58.2 percent (10-year) for women whose last child birth was more than two years prior to their diagnosis.

The effect of time since birth was further evaluated for parous women in a multivariate analysis that considered the influence of age at diagnosis, tumour size at diagnosis, numbers of positive axillary lymph nodes, grade of anaplasia, protocol allocation, year of treatment, and number of full-term pregnancies. As shown in Table 2, the prognosis remained significantly worse for women who gave birth to a child within the past two years (relative risk: 1.58 (95 percent confidence interval: 1.24-2.02) compared with women who had given birth six or more years ago (p=0.0002). The increased risk associated with a recent birth was found to be 2.1-fold in the first year and 1.3-fold in the second year.

In order to investigate whether the negative effect of a recent birth was modified by age at diagnosis, stage of disease (measured by number of positive axillary lymph nodes), or tumour size, we performed a stratified analysis that adjusted for all other considered factors as given above (Table 3). Neither age at diagnosis, nodal status, nor tumour size had any significant modifying effect on the poor survival for the group of women with a history of a recent birth (< 2 years).

Table 1. Distribution of 5,652 breast cancer patients 45 years or less at diagnosis according to tumour characteristics, age, risk group allocation, and time since birth.

	Time since birth n (%)				
	Nulliparous	< 2 years	2-3 years	4-5 years	6 years
Total No	695	201	280	349	4127
Age					
<30 years	46 (6.6)	33 (16.4)	24 (8.6)	16 (4.6)	4 (0.1)
30-39 years	261 (37.6)	144 (71.6)	211(75.4)	224 (64.2)	1157 (28.0)
40-45 years	388 (55.8)	24 (11.9)	45 (16.1)	109 (31.2)	2966 (71.9)
Tumour size					
2 cm	299 (43.0)	94 (46.8)	134 (47.9)	167 (47.9)	2240 (54.3)
>2. 5 cm	260 (37.4)	74 (36.8)	94 (33.6)	115 (33.0)	1308 (31.7)
> 5 cm	72 (10.4)	14 (7.0)	33 (11.8)	33 (9.5)	266 (6.5)
No information	64 (9.2)	19 (9.5)	19 (6.8)	34 (9.7)	313 (7.6)
Positive nodes					
0	328 (47.2)	81 (40.3)	129 (46.1)	153 (43.8)	2180 (52.8)
1-3	200 (28.8)	56 (27.9)	86 (30.7)	115 (33.0)	1134 (27.5)
4-9	85 (12.2)	34 (16.9)	33 (11.8)	44 (12.6)	449 (10.9)
10	24 (3.5)	18 (9.0)	10 (3.6)	17 (4.9)	135 (3.3)
No information	58 (8.4)	12 (6.0)	22 (7.9)	20 (5.7)	229 (5.6)
Histologic grading					
I	146 (21.0)	30 (14.9)	52 (18.6)	71 (20.3)	994 (24.1)
II + III	394 (56.7)	132 (65.7)	166 (59.3)	205 (58.7)	2219 (53.8)
ND*	155 (22.3)	39 (19.4)	62 (22.1)	73 (20.9)	914 (22.2)
Protocol allocation					
Yes	523 (75.3)	156 (77.6)	228 (81.4)	289 (82.8)	3442 (83.4)
No Not treated according to surgical guidelines	100 (14.4)	35 (17.4)	42 (15.0)	44 (12.6)	521 (12.6)
Not allocated due to other reasons†	72 (10.4)	10 (5.0)	10 (3.6)	16 (4.6)	164 (4.0)

*Including patients with non-ductal carcinomas and patients without information on histologic grading

†Medical contraindications, bilateral breast cancer, distant metastasis, or inflamatory cancer.

Table 2. Adjusted relative risk (aRR) of dying according to prognostic factors, age at diagnosis, and time since birth among 4,957 parous breast cancer patients 45 years or less.

Variables	aRR (95% CI)[*]
Age at diagnosis	
<30 years	1.0 ref.
30-39 years	0.88 (0.62-1.27)
40-45 years	0.80 (0.55-1.16)
Tumour size	
2 cm	1.0 ref.
>2. 5 cm	1.67 (1.48-1.89)
> 5 cm	2.44 (2.03-2.92)
Positive nodes	
0	1.0 ref.
1-3	1.58 (1.39-1.81)
4-9	3.04 (2.61-3.54)
10	3.90 (3.12-4.87)
Histologic grading	
I	1.0 ref.
II + III	2.27 (1.93-2.67)
ND†	1.26 (1.04-1.54)
Time since birth	
< 2 years	1.64 (1.28-2.09)
2-3 years	1.00 (0.80-1.26)
4-5 years	1.20 (0.98-1.46)
6 years	1.0 ref.

[*]Adjusted relative risk (95% confidence intervals) adjusted for the other characteristics listed   above and overall parity, age at first birth, year of treatment, and protocol allocation.

†Patients with non-ductal carcinomas.

Table 3. Adjusted relative risk, aRR (95% confidence interval) of dying according to age at diagnosis, nodal status, tumour size, and time since birth among 4,957 parous breast cancer patients 45 years or less.

| | Time since birth | | | |
| | < 2 years | 2-3 years | 4-5 years | 6 years |
	aRR	aRR	aRR	aRR
Age at diagnosis†	1.6 (1.2-2.3)*	1.1 (0.8-1.6)	1.2 (0.8-1.9)	1.0 ref.
33 years	1.7 (1.2-2.3)*	0.9 (0.7-1.2)	1.2 (1.0-1.5)	1.0 ref.
> 34 years				
Tumour size				
2 cm	1.7 (1.1-2.4)*	1.4 (1.0-2.0)	1.4 (1.0-1.9)	1.0 ref.
> 2 cm	1.5 (1.1-2.0)*	0.8 (0.6-1.1)	1.0 (0.8-1.4)	1.0 ref.
Nodal status				
Negative	1.6 (1.0-2.4)*	1.1 (0.8-1.7)	1.0 (0.7-1.4)	1.0 ref.
Positive	1.5 (1.1-2.0)*	1.0 (0.7-1.3)	1.3 (1.1-1.7)*	1.0 ref.

*p<0.05

†Patients separated into two groups according to median age among patients with child birth less than two years before diagnosis.

Appendix

Number of primary breast cancer patients under 45 years of age (N=5,652).
Five and ten-year survival according to time since birth. Denmark 1977 -
1994.

Time since birth	N	5-year survival	10-year survival
Nulliparous	695	73.1 (69.5-76.7)	57.7 (53.0-62.5)
< 2 year	201	58.7 (51.2-66.1)	46.1 (37.5-54.6)
2-3 years	280	78.8 (73.7-83.9)	60.8 (53.8-67.8)
4-5 years	349	72.8 (67.8-77.7)	61.1 (55.3-66.9)
6-7 years	448	75.8 (71.5-80.0)	62.6 (57.3-67.8)
8-9 years	526	77.0 (73.2-80.8)	66.9 (62.3-71.4)
10 years	3,153	79.6 (78.0-81.1)	67.3 (65.2-69.3)

Discussion

We documented a particularly poor survival for women who were diagnosed
with a breast cancer within two years after having given birth. This finding
was obtained using a large and very complete population-based database
with detailed information on tumour characteristics, treatment regimes,
reproductive factors, and vital status. The adverse effect on the prognosis
was observed irrespective of the woman s age, the size of the tumour, and
the stage of the disease. In a small multicenter study involving nine centres
and a total of 152 young mothers (<30 years) with breast cancer, Guinee et
al. [6] found an increased mortality in women who gave birth up to four years
prior to their diagnosis. Other studies indicate that breast cancer diagnosed
during lactation is associated with poor survival [13,14]. However, a recent
study by von Schoultz and colleagues [7] failed to support such an association.
A limitation in all these studies has been their sample size. Furthermore,
they have generally been unable to adequately adjust for confounders such
as other reproductive history, tumour size, axillary lymph node status, and
histological grading.

To diagnose a breast cancer among young women in general and in
pregnant women and lactating women in particular are difficult due to the
density of the mammary glands. This is reflected in a significant diagnostic
delay among these patients [12,15]. In the present study there was a tendency
for recently pregnant women to be classified with more advanced disease
that, at least to some extent, could be caused by delayed diagnosing.
However, our detailed information on each woman s tumour characteristics
allowed us to adjust for this phenomenon thoroughly. Thus, independent of
the influence caused by delayed diagnosis, women with a recent birth prior
to their diagnosis conferred an increased risk of dying of about 60 percent
compared to other women with breast cancer.

Breastfeeding was earlier considered to influence the risk of breast cancer development but most recent evidence suggests that there be no important overall association [19]. Whether breastfeeding should influence the prognosis of the disease is unknown but the lack of effect on the risk of disease does not necessarily strengthen a possible effect on its prognosis. In our study, we did not have information on breast feeding. Lactating women entails a very different hormonal enviroment to that of non-lactating post partum women, which makes the group of women with recent pregnancy heterogenous. However, we note that a poor survival was observed also in the second year after birth, at which time most women have stopped breastfeeding.

Experimental data support that pregnancy may confer a growth-enhancing effect on tumour cells [20]. However, a simple growth-enhancing effect would tend to increase the volume of the tumour at time of diagnosis shortly after pregnancy. We find that the negative effect of a recent birth remains present also after having taken into account factors that reflect the volume of the tumour, i. e. tumour size and nodal status (Table 3). Therefore, we suggest that the most likely explanation for our finding is that the pregnancy changes the course of the disease by increasing the risk of a highly malignant growth-pattern of already existing tumour cells.

It has long been known that early age at first full-term pregnancy is associated with a low risk of breast cancer *development,* whereas women aged 35 years or more at first child birth are at a particularly high risk [1]. In our study, neither tumour size, nodal status, nor age modified the specific *prognostic* effect of recent last delivery. Because breast cancer is rare before the age of 30 years [21], the likelihood of giving birth close to the development of a breast cancer diagnosis is significantly larger for women who have their children at an advanced age. Therefore, the adverse influence of pregnancy on breast cancer survival will naturally have the greatest impact in modern societies where women are postponing the time of childbearing to higher ages.

The negative effect of recent pregnancy was pronounced both in the group of women who did not receive adjuvant treatment (low risk group) as well as among patients who all received adjuvant therapy (high risk group). However, it is unknown whether more intensive adjuvant treatment will change the course of the disease in this group of patients. These new findings need be considered while counselling such patients and furthermore be taken into account when the decision of adjuvant treatment is made. We therefore recommend that pregnancy history be recorded at admission of premenopausal breast cancer patients. Furthermore we recommend that such information be recorded in future prospective clinical trials in order for response to adjuvant treatment according to time since last childbirth to be assessed.

Parity, age at first childbirth and the prognosis of breast cancer (Study 3)

Materials and methods
We used the DBCG register as described in detail in study 2.

Information on reproductive history was obtained by linkage with the Civil Registration System (CRS). The CRS was established on 1 April 1968 where all residents in Denmark were registered and assigned a unique identification number that permits identity secure linkage of information between registries. Parents were recorded with a link to most of their children born in the beginning of the 1950´s or later and alive in 1968. Since then, the CRS registry has kept updated files on dates on all live-births and residents in Denmark including updated files on vital status. A more detailed description of the reproductive information included in this registry is given elsewhere (Melbye *et al.*, 1997). Information on stillbirths was available during the period 1978-1993 from the National Birth Registry.

Permission was obtained in advance from the National Scientific Ethics Committee and the Data Protection Board to link information on patients in the DBCG registry with the CRS registry, and the National Birth Registry. Women born before 1935 have no systematic link to all their children in the CRS registry. Therefore, we restricted our study group to women born since 1th. April 1935. All women with a diagnosis of breast cancer before 1th. October 1994, were included and followed until 1th. October 1995, with respect to vital status.

The associations between the study variables and survival were investigated using the Cox Proportional Hazards method (Cox, 1972). Multivariate analyses included tumour size (2 cm, >2 and up till 5 cm, >5 cm), positive lymphnodes (0,1-3,4-9, 10), histological grading (I, II-III, non-ductal patients and those without information on histological grading), age at first birth (nulliparous, <20, 20-24,25-29, 30 years), parity at diagnosis (0,1,2,3, 4), age at diagnosis (<35, 35-39,40-44,45-49, 50 years), year of diagnosis (1977-81,82-87,88-94), and protocol allocation (see table 1). The adequacy of the proportional hazard assumptions for the included variables was checked by log(-logS) plots from stratified multivariate analyses. For both tumour size and lymph node status the hazard rate of the heterogeneous category of missing information was not proportional to the hazard rates of the other categories. Therefore, the Cox-regression was performed in four strata (information on tumour size and lymph node status available, only tumour size missing, only lymph node status missing, both missing). The estimates were only slightly changed if women with missing tumour size or nodal status were excluded from the analysis. Tests for effect modification were performed as tests for interaction between categorized variables. In an exploratory analysis we categorized year of treatment in one-year intervals, but this did not affect the results - a finding that argues against residual confounding. All analyses were performed using likelihood ratio tests by means of the SAS procedure PROC PHREG (SAS Institute Inc., 1992).

Results
By the first of October 1994, 10,803 women with primary breast cancer born after April 1, 1935, were registered in the DBCG. One hundred patients were excluded due to delivery after the time of diagnosis. 1,260 patients (11.8%) were nulliparous, and 9,443 patients (88.2%) were parous. The follow-up time ranged from 13 months to 17 years representing a total of 60,322 person-years of follow-up. Distribution of patients according to age at diagnosis, tumour characteristics, protocol allocation, parity, and age at first birth is given in table 1. The influence of these factors on breast cancer prognosis were evaluated in a multivariate analysis. The relative risk of dying according to tumour characteristics and status as nulliparous or parous is

given in table 2. Table 3 presents the relative risk of dying according to parity and age at first childbirth in parous women. Parous women were found to have a minor insignificantly reduced risk of dying compared with nulliparous women (relative risk: 0.95; 95% confidence interval: 0.86-1.06). The prognosis was unaffected by the number of children in the group of parous women (p=0.78, table 3).

The adjusted relative risk of dying varied significantly according to age at first birth as shown in table 3 (p=0.005). Women having their first child at the age of 25 - 29 years had the best prognosis. The relative risk of dying was significantly reduced for women having there first child between the age of 20 years to 24 years (RR: 0.88, 95% CI: 0.78-0.99) and women with primary childbirth between the age of 25 years to 29 years (RR: 0.80, 95% CI: 0.70-0.91) compared with women having primary childbirth below the age of 20 years (reference group). To investigate whether the prognostic effect of age at first birth was modified by age at diagnosis, extend of disease (measured by number of positive axillary lymph nodes), or tumour size, we tested for effect modification with adjustment for all other considered factors as given above (Table 4). Neither tumour size (p=0.63) nor nodal status (p=0.74) had a significantly modifying effect on the prognostic influence of age at first birth. There was a trend towards the prognostic effect of age at first childbirth being more pronounced among women diagnosed between the age of 40 to 50 years. However, this finding was not significant (p=0.27).
Oestrogen receptor (ER) status was available on 6,016 patients. Sixty-nine percent were classified as ER positive and 31% were classified as ER negative. The negative prognostic effect of age at first childbirth was not affected by ER status.

Table 1. Distribution of 10,703 women with primary breast cancer born after April 1, 1935, diagnosed during 1978-1994 according to age at diagnosis, tumour characteristics, protocol allocation, parity, and age at first childbirth.

		Age at first birth n (%)			
	Nulliparous	< 20 years	20-24 years	25-29 years	30 years
Total No	1,260	1,468	4,416	2,670	889
Age at diagnosis					
< 35 years	138 (11.0)	71 (4.8)	225 (5.1)	184 (6.9)	31 (3.5)
35-39 years	169 (13.4)	211 (14.4)	595 (13.5)	374 (14.0)	122 (13.7)
40-44 years	318 (25.2)	434 (29.6)	1,128 (25.5)	701 (26.3)	258 (29.0)
45-49 years	337 (26.8)	452 (30.8)	1,392 (31.5)	781 (29.3)	273 (30.7)
50 years	298 (23.7)	300 (20.4)	1,076 (24.4)	630 (23.6)	205 (23.1)
Tumour size					
2 cm	576 (45.7)	837 (57.0)	2,446 (55.4)	1,429 (53.5)	461 (51.9)
>2, 5 cm	480 (38.1)	457 (31.1)	1,477 (33.5)	936 (35.1)	300 (33.7)
> 5 cm	119 (9.4)	87 (5.9)	261 (5.9)	158 (5.9)	76 (8.5)
No information	85 (6.8)	87 (5.9)	232 (5.3)	147 (5.5)	52 (5.8)
Positive nodes					
0	600 (47.6)	784 (53.4)	2,301 (52.1)	1,359 (50.9)	448 (50.4)
1-3	374 (29.7)	401 (27.3)	1,204 (27.3)	777 (29.1)	237 (26.7)
4-9	152 (12.1)	160 (10.9)	538 (12.2)	307 (11.5)	127 (14.3)
10	49 (3.9)	48 (3.3)	165 (3.7)	110 (4.1)	39 (4.4)
No information	85 (6.8)	75 (5.1)	208 (4.7)	117 (4.4)	38 (4.3)
Histologic grading					
I	302 (24.0)	362 (24.7)	1,135 (25.7)	668 (25.0)	210 (23.6)
II + III	664 (52.7)	802 (54.6)	2,268 (51.4)	1,353 (50.7)	471 (53.0)
ND[a]	294 (23.3)	304 (20.7)	1,013 (22.9)	649 (24.3)	208 (23.4)
Protocol allocation					
Yes	980 (77.8)	1,234 (84.1)	3,748 (84.9)	2,245 (84.1)	740 (83.2)
No Not treated according to surgical guidelines	158 (12.5)	168 (11.4)	457 (10.4)	291 (10.9)	101 (11.4)
Not allocated due to other reasons[b]	122 (9.7)	66 (4.5)	211 (4.8)	134 (5.0)	48 (5.4)
Parity	-				
1		157 (10.7)	586 (13.3)	648 (24.3)	489 (55.0)
2		639 (43.5)	2,325 (52.7)	1,555 (58.2)	350 (39.4)
3		471 (32.1)	1,199 (27.2)	399 (14.9)	42 (4.7)
4		201 (13.7)	306 (6.9)	68 (2.6)	8 (0.9)

[a] Including patients with non-ductal carcinomas (n=2089, 84.6%) and patients without information on histologic grading (n=379, 15.4%). [b] Medical contraindications, bilateral breast cancer, distant metastasis, or inflamatory cancer.

Table 2. Adjusted relative risk (aRR) of dying according to prognostic factors, protocol allocation, and parity, in 10,703 breast cancer patients born after April 1, 1935, and diagnosed 1978-1994.

Variables	aRR (95% CI)[a]
Tumour size	
2 cm	1 ref.
>2, 5 cm	1.63 (1.49-1.78)[b]
> 5 cm	2.17 (1.90-2.49)[b]
Positive nodes	
0	1 ref.
1-3	1.71 (1.53-1.91)[b]
4-9	3.32 (2.97-3.72)[b]
10	4.72 (4.02-5.52)[b]
Histologic grading	
I	1 ref.
II + III	2.33 (2.07-2.62)[b]
ND[c]	1.18 (1.02-1.36)[b]
Protocol allocation	
Allocated patients	1 ref
Not treated according to guidelines	1.04 (0.91-1.17)
Not allocated due to other reasons[d]	2.76 (2.43-3.13)[b]
Parity	
Nulliparous	1 ref.
Parous	0.95 (0.85-1.06)

[a]Adjusted relative risk (95% confidence intervals) adjusted for all characteristics listed above and age at diagnosis and year of diagnosis.[b]$p<0.05$. [c]Patients with non-ductal carcinomas and patients without information on histologic grading. [d]Medical contraindications, bilateral breast cancer, distant metastasis, or inflamatory cancer.

Table 3. Adjusted relative risk (aRR) of dying according to number of full-term pregnancies, and age at first childbirth in 9,443 parous breast cancer patients born after April 1, 1935, and diagnosed 1978-1994.

Variables	aRR (95% CI)[a]	aRR (95% CI)[b]
Parity		
Nulliparous	1.04 (0.90-1.19)	
1	1 ref.	1 ref.
2	0.96 (0.86-1.07)	0.97 (0.86-1.08)
3	0.99 (0.88-1.12)	0.98 (0.85-1.11)
4	1.07 (0.90-1.28)	1.04 (0.87-1.25)
Age at first birth		
Nulliparous	0.92 (0.80-1.06)	
< 20 years	1 ref.	1 ref.
20-24 years	0.87 (0.78-0.98)[c]	0.88 (0.78-0.99)[c]
25-29 years	0.79 (0.70-0.90)[c]	0.80 (0.70-0.91)[c]
30 years	0.94 (0.80-1.11)	0.94 (0.79-1.12)

[a] Adjusted relative risk (95% confidence intervals) adjusted for age at diagnosis, tumour size, nodal status histologic grading, protocol allocation, and year of diagnosis. [b] Adjusted relative risk further adjusted for parity factors listed above [c] $p < 0.05$

Table 4. Stratified analysis of risk of dying according to age at diagnosis, nodal status, tumour size, and age at first childbirth among 9,443 parous breast cancer patients.

| | Age at first birth | | | |
| | < 20 years | 20-24 years | 25-29 years | 30 years |
	aRR[a]	aRR[a]	aRR[a]	aRR[a]
Age at diagnosis				
<35 years	1 ref.	1.6 (0.99-2.5)	1.2 (0.8-2.0)	2.0 (0.96-4.1)
35-39 years	1 ref.	0.9 (0.7-1.1)	0.9 (0.7-1.2)	1.1 (0.8-1.6)
40-44 years	1 ref.	0.7 (0.6-0.9)[b]	0.7 (0.6-0.9)[b]	0.8 (0.6-1.0)
45-49 years	1 ref.	0.8 (0.6-1.0)	0.7 (0.6-0.9)[b]	0.9 (0.7-1.2)
50 years	1 ref.	1.1 (0.8-1.5)	0.9 (0.6-1.3)	1.0 (0.6-1.5)
Tumour size				
2 cm	1 ref.	0.8 (0.6-0.9)[b]	0.8 (0.6-0.9)[b]	0.9 (0.7-1.2)
> 2 cm	1 ref.	0.9 (0.7-1.0)	0.8 (0.7-0.9)[b]	0.9 (0.7-1.1)
Nodal status				
Negative	1 ref.	0.8 (0.7-1.0)	0.8 (0.6-0.97)[b]	1.0 (0.7-1.3)
Positive	1 ref.	0.9 (0.8-1.0)	0.8 (0.7-0.9)[b]	0.9 (0.8-1.1)

[a]Adjusted relative risk (95% confidence intervals) adjusted for age at diagnosis, tumour size, nodal status histologic grading, protocol allocation, and year of diagnosis. [b]p<0.05

Discussion

We found strong evidence that young age at first birth is associated with poor survival of breast cancer, despite its protective effect on breast cancer development. Although some studies have not supported this observation (Ewertz et al., 1991; Lees et al., 1989; Mohle Boetani et al., 1988), there is accumulating evidence that supports it (Schouten et al., 1997; Kogevinas, 1990; Greenberg et al., 1985). A limitation of previous studies has been their small sample sizes (range 582-1,744 subjects) compared with the present study. Furthermore, these studies have primarily been based on retrospectively collected information obtained among cases and controls through interviews. The present population-based study was based on prospectively collected data, with detailed exposure and outcome information that limits possibilities for recall bias.

Previous reports have shown the risk of developing breast cancer to be reduced among women who have their first child at an early age (MacMahon

et al., 1970; Ewertz *et al.*, 1990). Based on a large cohort of 1.5 million women and including more than 10,000 breast cancer cases we have similarly found a strongly increasing risk of breast cancer with increasing age at first childbirth (Wohlfahrt et al., unpublished). Thus, one could argue that some women who avoided breast cancer because of a delivery at an early age would have developed breast cancer if they had their primary childbirth late or if they had remained nulliparous. These avoided breast cancers might be those with the most favourable course. Following this argument the observed reduced survival in breast cancer patients with early first childbirth might reflect a selection of more aggressive cases rather than a direct biologic effect of the early pregnancy on the carcinogenic process. We acknowledge that women with an early first childbirth did not have a poorer profile of the available prognostic factors. However, these prognostic factors do not necessarily offer a complete picture of the biological behaviour of the tumours.

There was an indication, although not being significant, that early first childbirth primarily served as a negative prognostic indicator of breast cancer in older premenopausal women aged 40 to 49 years. The assumption that the negative effect of early first childbirth is a consequence of a selection is supported by epidemiologic data showing that the protective effect of early first childbirth on breast cancer development is most pronounced in older premenopausal women (Ewertz *et al.*, 1990). In the western world the median age of first childbirth has increased over the past decades. It is generally accepted that this postponement of motherhood has contributed to the rising incidence of breast cancer. Our study suggests that the postponement of motherhood might have a beneficial effect on overall breast cancer prognosis.

Studies on overall parity as a prognostic factor have been contradictory (von Schoultz *et al.*, 1995; Palmer *et al.*, 1982; Guinee *et al.*, 1994; Mason *et al.*, 1990; Lees *et al.*, 1989; Lehrer *et al.*, 1992; Wang *et al.*, 1985; Orr and Fraher, 1995; Mohle Boetani *et al.*, 1988; Korzeniowski and Dyba, 1994; Black *et al.*, 1983; Papatestas *et al.*, 1980). We have previously found that pregnancy within two years before a diagnosis of breast cancer was associated with reduced survival (Kroman *et al.*, 1997). This combined with the present observation of early first childbirth being a negative prognostic factor could explain the finding reported by some researchers of an association between high parity and poor prognoses (Wang *et al.*, 1985; Lees *et al.*, 1989; Korzeniowski and Dyba, 1994). Women with high parity would be expected to have their first child early and have their last child late. Therefore, women with high parity would be overrepresentated in the two high-risk groups defined by us. In the present study high parity alone did not serve as an independent prognostic factor.

The observation that breast cancer may be a high social status disease has been related to differences in childbirth patterns (Kelsey and Horn Ross, 1993). In contrast, several studies have shown that low social class is associated with reduced survival (Gordon *et al.*, 1992; Karjalainen and Pukkala, 1990; Kogevinas *et al.*, 1991). It may be of relevance for the latter finding that poorly educated women tend to have their first child earlier than women with higher education level (Knudsen, 1993).

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Should women be advised against pregnancy after breast-cancer treatment? (Study 4)

<u>Material and methods</u>
We used information from the DBCG register as described in detail in study 2.

Patients with bilateral breast cancer, distant metastases, inflammatory cancer, with contraindication to the planned postoperative therapy, or patients who were not treated according to the surgical guidelines were not allocated to treatment protocols (miscellaneous group). The miscellaneous patient group could be separated into a group with favourable prognosis and a group with poor prognosis. Patients who were not treated according to surgical guidelines had an overall good prognosis compared with patients excluded for other reasons.

The Danish Civil Registration System (CRS) was established in 1968 and since then a unique identification number has been assigned to all residents in Denmark. Individual information is kept under the personal identification number in all national registers permitting accurate linkage of information between these registries. The CRS registry keeps updated files on vital status and dates of childbirths with a systematic link to the children of women born after April 1, 1935. A detailed description of the information included in this registry is given elsewhere [20,21]. Information on stillbirths after 1977 and induced abortions after 1973, including gestational age of the foetus, was available from the National Birth Registry and the National Induced Abortion registry.

Permission to perform the study was obtained in advance from the National Scientific Ethics Committee and the Data Protection Board. Information on patients in the DBCG registry was linked with the other national registries to obtain information on pregnancy history and vital status. As women born before 1935 have no systematic link to all their children in the CRS registry, we restricted our study group to women born since April 1, 1935. Since the aim was to identify women with pregnancies, we further restricted the study group to women aged 45 years or less at the time of diagnosis. All women diagnosed before October 1, 1994, were included and followed until October 1, 1995, with respect to vital status.

The associations between the study variables and survival were investigated using the Cox Proportional Hazards method. Multivariate analyses included tumour characteristics, time between diagnosis and most recent previous childbirth (with nulliparous in a separate category), age at diagnosis, year of treatment, protocol allocation, full-term pregnancy after diagnosis, induced abortion after diagnosis, and spontaneous abortion after diagnosis. The three last variables were included in the analysis as time-dependent variables. The adequacy of the proportional hazard assumptions for the included variables was checked by log(-log)S-plots from stratified multivariate analyses. For both tumour size and lymph node status, the hazard rate of the heterogeneous category of missing information was not proportional to the hazard rates of the other categories. Therefore, the Cox regression was performed in four strata (information on tumour size and lymph node status available, only tumour size missing, only lymph node status missing, both missing). Estimation was done using the SAS procedure PROC PHREG.

Results
Overall, 5,752 women aged 45 years or less with primary breast cancer were identified. Since the specific aim of the study was to evaluate the prognostic effect of having a pregnancy subsequent to breast cancer treatment, we excluded 27 women who might have been pregnant at the time of diagnosis, i. e. women who had a childbirth less than 10 months after the breast cancer diagnosis, or women who had an abortion with a gestational age indicating that they might have been pregnant at the time of diagnosis of breast cancer. This left 5,725 patients with a total of 35,067 person-years of follow-up for further study. Among these, 173 women (3.0%) experienced a total of 211 pregnancies (97 full-term pregnancies, 22 spontaneous abortions, and 92 induced abortions). Thirty-two women had more than one pregnancy after breast cancer diagnosis. The median time between breast cancer diagnosis and time of birth or abortion was as follows: a) birth 32 months (range 11-147 months), b) spontaneous abortion 23 months (range 6-50 months), and c) induced abortion 22 months (range 3-89 months). Distribution of patients according to histopathological tumour criteria, protocol allocation, and reproductive status after diagnosis of breast cancer is shown in Table 1. These factors plus the year of treatment and time since last previous childbirth, known to be of prognostic influence, were introduced in a multivariate analysis. The adjusted relative risk of dying according to reproductive history after treatment of breast cancer, age at diagnosis, and tumour characteristics is given in Table 2. Women with a full-term pregnancy after treatment of breast cancer had an insignificantly reduced risk of dying (RR: 0.55; 95% CI: 0.28-1.06, p=0.08) compared to other women with breast cancer. Women having induced abortion or spontaneous abortion experienced no significant risk alteration. Information on recurrence was available in the group of protocol-allocated patients (n=4,695 (82%)). If for this subgroup recurrence was introduced in the multivariate model, the relative risk estimate for women with full-term pregnancy was unchanged (RR: 0.79; 95% CI: 0.39-1.61).

Further analysis showed that the effect of subsequent pregnancy was not significantly modified by age at diagnosis, tumour size, nodal status, status as parous/nulliparous before diagnosis, time since most recent previous pregnancy before breast cancer diagnosis, age at subsequent pregnancy, or time to subsequent pregnancy (data not shown).

We subsequently performed a restricted analysis including only women who were classified as having a low-risk tumour (n=2,110). Also in this group of breast cancer patients, the survival was favourable for women with a full-term pregnancy subsequent to breast cancer treatment (RR: 0.61; 95% CI: 0.19-1.91) compared to other women with low-risk breast cancer. Calculated on the basis of the age-standardized incidence rates of childbirths in Danish women, the expected number of full-term pregnancies in the entire cohort was 285 compared with the observed 97.

Table 1. Distribution of 5,725 breast cancer patients, diagnosed 1978-95, according to age at diagnosis, tumour characteristics, protocol allocation, and reproductive status subsequent to their diagnosis. Danish women born after April 1, 1935 and less than 45 years of age at diagnosis.

	Reproductive status after diagnosis of breast cancer n (%)			
	Full-term pregnancy*	Induced abortion†	Spontaneous abortion	No pregnancy
Total No	84	77	12	5,552
Age at diagnosis				
<35 years	62 (74%)	35 (45%)	6 (50%)	603 (11%)
35-39 years	17 (20%)	29 (38%)	3 (25%)	1,436 (26%)
40-45 years	5 (6%)	13 (17%)	3 (25%)	3,513 (63%)
Tumour size				
2 cm	47 (56%)	42 (55%)	6 (50%)	2,876 (52%)
>2, 5 cm	17 (20%)	23 (30%)	4 (33%)	1,823 (33%)
> 5 cm	5 (6%)	4 (5%)	0 (0%)	414 (7%)
No information	15 (18%)	8 (10%)	2 (17%)	439 (8%)
Positive nodes				
0	49 (58%)	46 (60%)	7 (58%)	2,812 (51%)
1-3	19 (23%)	20 (26%)	4 (33%)	1,563 (28%)
4-9	6 (7%)	5 (6%)	0 (0%)	640 (12%)
10	0 (0%)	2 (3%)	0 (0%)	202 (4%)
No information	10 (12%)	4 (5%)	1 (8%)	335 (6%)
Histologic grading				
I	15 (18%)	16 (21%)	4 (33%)	1,270 (23%)
II + III	35 (42%)	45 (58%)	7 (58%)	3,058 (55%)
ND‡	34 (40%)	16 (21%)	1 (8%)	1,224 (22%)
Protocol allocation				
Yes	55 (65%)	65 (84%)	9 (75%)	4,556 (82%)
No				
Not treated according to surgical guidelines	21 (25%)	11 (14%)	3 (25%)	726 (13%)
Not allocated due to other reasons§	8 (10%)	1 (1%)	0 (0%)	270 (5%)

* Including 8 women with both induced abortion and full-term pregnancy, 5 women with spontaneous abortion and full-term pregnancy, and 1 woman with both induced abortion, spontaneous abortion and full-term pregnancy. †Including 1 woman with both induced abortion and spontaneous abortion. ‡Including patients with non-ductal carcinomas and patients without information on histologic grading. §Medical contraindications, bilateral breast cancer, distant metastasis, or inflammatory cancer.

Table 2. Adjusted relative risk (aRR) of dying according to reproductive status after diagnosis of breast cancer, age at diagnosis, and prognostic factors among 5,725 women.

Variables		aRR (95% CI)[*]
Reproductive status after diagnosis of breast cancer		
Full-term pregnancy	No	1 ref.
	Yes	0.55 (0.28-1.06)
Induced abortion	No	1 ref.
	Yes	1.00 (0.67-1.50)
Spontaneous abortion	No	1 ref.
	Yes	0.36 (0.09-1.45)
Age at diagnosis		
<35 years		1 ref.
35-39 years		1.01 (0.88-1.17)
40-45 years		0.93 (0.82-1.06)
Tumour size		
2 cm		1 ref.
>2. 5 cm		1.74 (1.54-1.96)†
> 5 cm		2.46 (2.06-2.93)†
Positive nodes		
0		1 ref.
1-3		1.60 (1.41-1.82)†
4-9		3.02 (2.60-3.50)†
10		4.06 (3.26-5.06)†
Histologic grading		
I		1 ref.
II + III		2.25 (1.92-2.64)†
ND‡		1.13 (0.93-1.37)

[*]Adjusted relative risk (95% confidence intervals) adjusted for the other characteristics listed   above, year of treatment, protocol allocation, and time since last previous childbirth.
†p<0.05
‡Patients with non-ductal carcinomas.

Discussion
The present study documented that a pregnancy subsequent to treatment of breast cancer conferred no negative effect on the prognosis. Because women with a poor prognosis are believed to avoid pregnancies, there is a potential problem of the exposed group being selected. This problem is not easy to overcome and has been the main concern regarding the interpretation of results from previous studies on this subject [7-16]. The present investigation

took advantage of the clinical population-based DBCG database that over many years has recorded detailed information on breast tumour characteristics. Also, in the present study the group of women with subsequent pregnancy tended to have smaller tumours and a slightly lower risk of nodal involvement. However, we were able to perform a detailed adjustment for the influence of such important prognostic factors and thus to minimize selection bias. Furthermore, the use of time-dependent variables in a cohort design enabled us to adequately adjust for the important influence of time from breast cancer diagnosis to time of birth or abortion. Thus, the length of the relapse free period is believed to significantly influence the woman´s decision regarding pregnancy. Women with known recurrence are not believed to get pregnant deliberately which might introduce a selection bias. However, the estimated relative risk of dying was not significantly influenced by the introduction of recurrence in the multivariate model.

The proportion of protocol-allocated patients was lower in the group of women who subsequently gave birth compared to other groups. This may partly be explained by some of these women choosing breast conserving therapy at a time when this treatment was not established as equal to mastectomy (before 1989). In those circumstances they have not been included in treatment protocols because they fell outside the surgical guidelines. However, we adjusted for this discrepancy by introducing protocol allocation in the multivariate analysis.

We acknowledge that despite these efforts, there are likely to be other selection mechanisms for which we were unable to adequately adjust with the available prognostic factors. This may explain why women with a full-term pregnancy subsequent to breast cancer treatment, even after adjustment for established prognostic factors, tended to have a better outcome than women without a subsequent pregnancy. Although we may not have completely adjusted for all factors, it seems implausible that we should have overlooked a negative prognostic effect of a pregnancy after breast cancer treatment. Thus, in a restricted analysis which included only women allocated to the low-risk group of breast cancer patients, we found women with a subsequent pregnancy to also have a favourable prognosis. The group of women allocated to the low-risk protocol constitutes a homogeneous population with localized disease unlikely to give symptoms that might influence a woman's decision regarding pregnancy. The risk of selection bias should therefore be particularly small in this group.

Certain reproductive factors such as age at first birth and time since last childbirth have been shown to have prognostic effect [5,6,22-24]. We were able to adjust for these factors in the analysis and furthermore to show that none of the reproductive factors modified the prognostic influence of pregnancy subsequent to treatment of breast cancer. The fertility rate, calculated on the basis of full-term pregnancies, was reduced to one third of the expected level in the group of treated breast cancer patients. This is due to an overall lower number of pregnancies as well as an increased incidence of induced abortions in this group of women. In Denmark, the number of induced abortions based on figures from the mid of our study period constituted 36% of the number of full-term pregnancies [25]. In our material breast cancer patients chose induced abortion almost as often as fulfilling pregnancy, whereas the number of spontaneous abortions was as expected. Un-

planned pregnancy when a woman is seriously ill most likely leads to a higher rate of induced abortions. It is obvious that many women have avoided getting pregnant after their breast cancer diagnosis. Furthermore, it is possible that some women have chosen induced abortion due to lack of knowledge of the influence a pregnancy might have on the course of their treated breast cancer. However, women with a history of induced abortion after breast cancer treatment did not have a different profile of prognostic factors than other women, which suggests that induced abortion was not chosen primarily among patients with a poor prognosis. This finding further supports the credibility of the overall result.

Factors influencing the effect of age on prognosis in breast cancer: population based study (Study 10)

Materials and methods

Population data-base
In 1977, nation-wide prospective studies on breast cancer treatment and survival were initiated by the Danish Breast Cancer Cooperative Group. Up till today, three programs have been launched including the DBCG 77 (patient accrual from 1977-1982), DBCG 82 (patient accrual from 1982-1989), and DBCG 89 (patient accrual since 1989). Primary clinical and histopathological data and data concerning postoperative therapy and status at follow-up visits have all been registered by the Danish Breast Cancer Cooperative Group based on specific forms submitted by departments of surgery, pathology and oncology in Denmark. A linkage between the Danish Breast Cancer Cooperative Group register and the Danish Cancer Registry, which is considered close to complete regarding reporting of breast cancer diagnoses among residents in Denmark [22], has revealed a 94 percent concordance (authors' unpublished result).

Patient records in the Danish Breast Cancer Cooperative Group registry were linked with the Danish Civil Registration System registry to obtain complete information on vital status. Since 1968, the Civil Registration System registry has assigned a unique identification number to all residents in Denmark. Individual information is kept under this personal identification number in all national registries permitting accurate linkage of information between different registries. The Civil Registration System registry keeps updated files on dates of childbirth's and vital status. A detailed description of the information included in this registry is given elsewhere [23].

Recent studies have shown that in particular age at first birth and short interval to last birth prior to breast cancer diagnosis may influence the prognosis of breast cancer [24,25]. Information on childbirth history was available among women born since April 1, 1935.

Treatments
Patients were classified as either low-risk or high-risk according to histopathological criteria. Detailed information on risk-group allocation is given elsewhere [24]. For all three programmes, the primary surgical treatment of patients allocated in treatment protocols included total mastectomy plus axillary dissection (90% of the population), or lumpectomy with axillary dissection. Standard adjuvant cytotoxic chemotherapy was used in all three programmes [20,21]. Overview of the adjuvant treatment is given in Table 1.

Patients with bilateral breast cancer or inflammatory cancer, distant metastases, with contraindication to the planned postoperative therapy, or patients who were not treated according to the surgical guidelines were not allocated to any of the protocols.

Statistical analysis

Women diagnosed with breast cancer between January 1978 and July 1, 1996, were included and followed up to ten years after diagnosis or until July 1, 1996, whichever came first, with respect to vital status. The study was restricted to pre-menopausal women less than 50 years of age at time of diagnosis. The overall death rate was modelled by a sum of two terms. The first term was the age and calendar-specific expected mortality rate as a known time-dependent offset. Expected mortality rates were obtained from life tables according to five-year age groups, and five-year calendar periods for the total female population in Denmark supplied by the Danish Central Bureau of Statistics [26]. The second term in the overall model was the exponential function of a linear expression including the categorical variables: age at diagnosis (5-year groups), tumour size (<=2 cm, >2-5 cm, >5 cm), number of positive nodes (0,1-3,4-9,10+), histological grading (I, II+III, non-ductal carcinomas), protocol allocation (allocated, not treated according to surgical guidelines, not allocated due to other reasons) and year of diagnosis (1977-81, 1982-87, 1988-96). This model can be viewed as a log-linear model of the observed death rate minus the expected death rate, i.e. a log-linear model of the excess death rate. Expected number of deaths due to breast cancer only amounts to a small proportion of all expected deaths [26]. Therefore, the adjusted relative risks were interpreted as relative risks of death due to breast cancer. Poisson regression was chosen instead of Cox regression in order to facilitate the additive adjustment for the expected mortality rates. In additional analyses we performed multivariate analyses without adjustment for the expected mortality. In this situation we could perform both Poisson regression and Cox regression. The two approaches gave identical estimates of the relative risk. All tests in the Poisson regression analyses were performed as likelihood ratio tests using Epicure [27]. Tests for difference in the age effect in low risk patients compared to high risk patients receiving cytotoxic treatment were performed by including an interaction term between age and risk group. Association between age at diagnosis and tumour characteristics was analysed by chi-squared tests.

Results

By July 1, 1996, 10,356 pre-menopausal women less than 50 years of age with primary breast cancer were registered by the Danish Breast Cancer Cooperative Group. Our cohort represented a total of 52,432 person-years of follow-up. Distribution of patients according to tumour characteristics, protocol allocation, and age at diagnosis is given in Table 2. Compared with older patients, patients less than 35 years of age at diagnosis were at higher risk of being node positive (51%=404/795 vs 46%=4,061/8,854, p=0.02). The proportion of patients with histological grading I was significant lower in patients less than 35 years of age compared with patients 35 years or more (18%=122/668 vs 32%=2,321/7,303, p<0.001).

To evaluate the independent effect of age at diagnosis on breast cancer specific survival, we performed a multivariate analysis that included age at diagnosis, tumour size, axillary nodal status, histologic grading, year of treat-

ment, protocol allocation, and expected mortality (Table 3). Women aged 45-49 years were chosen as reference category because they constituted the largest group around the time of menopause. Compared with this group, women in the two age groups less than 40 years at diagnosis were at significantly increased risk of dying (Table 3). Women below 35 years of age had the worst prognosis with a 1.46-fold increased risk of dying of their disease. Performing the multivariate analysis with adjustment for oestrogen receptor status in the subgroup of patients with available oestrogen receptor status did not change the results (data not shown).

In order to evaluate the effect of adjuvant cytotoxic therapy in relation to age at diagnosis, we allowed for an interaction between age at diagnosis and low-risk patients (all receiving no adjuvant treatment, n=4,329), versus high-risk patients receiving adjuvant cytotoxic treatment (n=2,824) (Figure 2). Among patients not receiving adjuvant cytotoxic treatment, there was a highly significant increasing risk of dying with decreasing age (adjusted relative risk: 45-49 years: 1 (reference); 40-44 years: 1.12 (0.89-1.40); 35-39 years: 1.40 (1.10-1.78); <35 years: 2.18 (95% CI: 1.64-2.89). A similar trend was not observed in young patients receiving adjuvant cytotoxic therapy (high risk disease) (Figure 1). The negative effect of young age among women without adjuvant cytotoxic treatment was significantly more pronounced than that observed in the group of treated patients (test for effect modification: p=0.02).

In further analyses we looked explicitly at the effect of treatment among node negative women (Table 4). In line with findings above, young women were only at increased risk in the group receiving no treatment whereas no increased effect was observed among those receiving adjuvant cytotoxic treatment. A similar pattern was observed when looking only at women diagnosed with small tumours (< 2 cm) or women only diagnosed with large tumours (2 cm or more) (Table 4).

We have previously shown that age at first childbirth and time since last previous childbirth are independent prognostic factors [24,25]. Complete information regarding reproductive history was available on 3,373 low-risk patients (77.9 %). Adjusting for age at first childbirth or time since last previous childbirth had only insignificant influence on the estimated prognostic effect of age at diagnosis (data not shown).

Discussion
In agreement with previous studies, we found that breast cancer in young women has a particularly poor prognosis [1,4-19]. These patients are at high risk of having axillary lymph node involvement and having tumours with high histopathological grading, and oestrogen receptor negative status [1-3].

Part of the explanation for being diagnosed with more advanced and aggressive disease as a young woman has been sought in the potentials for having a delayed diagnosis [17,28]. Thus, detecting tumours in the breast of young women is difficult because of the density of the mammary glands. This problem is particularly pronounced among pregnant and lactating women [29]. Our detailed information on tumour characteristics at diagnosis enabled us to adjust for the effect of factors such as tumour size, nodal status and histologic grading, and thereby more clearly judge the independent ef-

fect of age. Furthermore, on a subset of the women we had complete reproductive history and could therefore include the previously reported negative prognostic effect of a recent childbirth in our multivariate analyses. However, none of these adjustments changed the overall result that young age at time of diagnosis is associated with a particularly poor prognosis. This argues in favour of a tendency towards breast cancers among young women being biologically more aggressive than those diagnosed in older women but does not indicate how these cancers respond to adjuvant cytotoxic chemotherapy. However, other results suggest that tumours in young women respond adequately to chemotherapy. A metaanalysis of 133 randomised trials involving 75,000 women with high-risk breast cancer found the relative benefit of adjuvant cytotoxic chemotherapy to be larger in patients less than 50 years compared with patients above 50 years of age [30].

Henderson and Patek [31] have argued against accepting young age alone as a criterion for adjuvant treatment. The International Consensus Panel on the treatment of primary breast cancer came to a similar conclusion in their 1995 report [32] but has in their most recent report changed their recommendation to include young age below 35 years without presenting scientific evidence to back this decision [33]. In order to evaluate the role of postoperative adjuvant cytotoxic treatment in relation to age at diagnosis we allowed for an interaction between age at diagnosis and low-risk patients all receiving no adjuvant treatment versus high-risk patients receiving adjuvant cytotoxic treatment. Our analysis documented that the negative effect of young age is almost exclusively seen in the group of patients classified as having low-risk disease, whereas the negative prognostic effect of young age was nonsignificant in high-risk patients receiving cytotoxic adjuvant treatment. We found the same difference as above when comparing women receiving no treatment with those receiving adjuvant cytotoxic treatment within strata of node negative patients and patients with the same tumor size. This raises the question of whether the negative effect of young age seen in low risk patients is directly due to lack of adjuvant cytotoxic therapy. We acknowledge that the results cannot be taken as direct evidence that young patients classified today, as having low-risk disease will benefit from adjuvant cytotoxic treatment in line with young patients diagnosed with high-risk disease. However, Fischer et al. [34] recently showed that women classified with low risk disease do indeed benefit when given adjuvant cytotoxic treatment, and that the most pronounced positive effect is seen in premenopausal women. Therefore, we feel confident that the low risk tumours associated with a poor prognosis in young women will respond to adjuvant cytotoxic treatment leading to a better prognosis for this group of women.
The relative risk of dying was adjusted for expected mortality that includes death due to breast cancer. In some age categories, particularly among young women, this leads to an underestimation of the disease-specific risk because death from breast cancer accounts for up to 15% of the total mortality in this age category [26]. Thus, the prognosis for young compared with middle-aged women is most likely worse than we estimated in the present study. However, this approach did not introduce an age-differential bias when comparing the age specific effects in women receiving no treatment with those receiving adjuvant treatment.

In conclusion, we found young age at diagnosis of breast cancer, and in particular an age below 35 years, to be associated with the worst prognosis of

all age groups. Whereas the age effect was only marginally present in the group of women receiving adjuvant cytotoxic treatment, there was a highly significant age effect in the group of low-risk patients receiving no adjuvant treatment. In this group of patients, the adjusted risk of dying was more than 2-fold increased compared with the reference group of 45-49-year-olds. These results suggest that young women with breast cancer on the basis of age alone, should be regarded as high risk patients and be offered adjuvant cytotoxic therapy.

Table 1. Overview of postoperative adjuvant treatment given 1977-1996 to Danish premenopausal high-risk breast cancer patients

Treatment protocol		Treatment randomization
DBCG 77		Radiotherapy or
		Radiotherapy + levamisol or
		Radiotherapy + cyclofosfamide or
		Radiotherapy + CMF
DBCG 82		CMF or
		CMF + radiotherapy or
		CMF + tamoxifen
DBCG 89	ER positive	CMF or
		Castration
	ER negative	CMF or
		CEF or
		CMF + pamidronate or
		CEF + pamidronate

CMF=Cyclofosfamide + Methotrexate + Fluorouracil

CEF=Cyclofosfamide + Epirubicin + Fluorouracil

Table 2. Distribution of 10,356 pre-menopausal women with primary breast cancer operated in Denmark 1977-1996 according to tumour characteristics, risk group allocation, and age at diagnosis.

	Age at diagnosis n (%)							
	<35 years		35-39 years		40-44 years		45-49 years	
Total No	867		1,733		3,354		4,402	
Tumor size								
≤ 2 cm	431	(49.7)	948	(54.7)	1,769	(52.7)	2,322	(52.8)
> 2, ≤ 5 cm	330	(38.0)	595	(34.3)	1,169	(34.9)	1,652	(37.5)
> 5 cm	69	(8.0)	133	(7.7)	278	(8.3)	291	(6.6)
No information	37	(4.3)	57	(3.3)	138	(4.1)	137	(3.1)
Positive nodes								
0	391	(45.1)	886	(51.1)	1,691	(50.4)	2,216	(50.3)
1-3	259	(29.8)	478	(27.6)	910	(27.1)	1,258	(28.6)
4-9	114	(13.2)	174	(10.0)	397	(11.8)	497	(11.3)
≥ 10	31	(3.6)	76	(4.4)	127	(3.8)	144	(3.3)
No information	72	(8.3)	119	(6.9)	229	(6.8)	287	(6.5)
Histologic grading								
I	122	(14.7)	351	(20.3)	812	(24.2)	1,158	(26.3)
II + III	546	(63.0)	1,017	(58.7)	1,785	(53.2)	2,180	(49.5)
ND*	199	(23.0)	365	(21.1)	757	(22.6)	1,064	(24.2)
Oestrogen receptor status†								
Positive	198	(51.2)	469	(57.8)	1,086	(65.9)	1,634	(71.0)
Negative	189	(48.8)	342	(42.2)	561	(34.1)	667	(29.0)
Risk group								
Low	315	(36.3)	733	(42.3)	1,423	(42.4)	1,920	(43.6)
High	349	(40.3)	677	(39.1)	1,319	(39.3)	1,715	(38.9)
Not treated according to guidelines‡	143	(16.5)	231	(13.3)	443	(13.2)	496	(11.3)
Not allocated due to other reasons§	60	(6.9)	92	(5.3)	169	(5.0)	271	(6.2)

*Patients with non ductal carcinomas and patients without available histologic grading. †Oestrogen receptor status among 5,146 patients (49.7%) with available information. ‡ Patients not allocated because surgical treatment did not follow guidelines. §Patients not allocated due to medical contraindications, bilateral or inflamatory breast cancer, or distant metastasis.

Table 3. Adjusted relative risk of dying after diagnosis of primary breast cancer according to age at diagnosis, tumour characteristics and protocol allocation in 9,541 breast cancer patients* diagnosed 1978-1996.

Variables	Adjusted Relative Risk (95% CI)†
Age at diagnosis	
<35 years	1.46 (1.27-1.70)
35-39 years	1.26 (1.12-1.42)
40-44 years	1.07 (0.97-1.19)
45-49 years	1 ref.
Tumour size	
2 cm	1 ref.
>2, 5 cm	1.78 (1.61-1.97)
> 5 cm	2.31 (2.00-2.67)
Positive nodes	
0	1 ref.
1-3	1.80 (1.62-2.01)
4-9	3.44 (3.05-3.89)
10	4.71 (3.96-5.59)
Histologic grading	
I	1 ref.
II + III	2.44 (2.12-2.81)
ND ‡	1.12 (1.00-1.43)
Protocol allocation	
Allocated patients	1 ref.
Not treated according to surgical guidelines	1.11 (0.95-1.28)
Not allocated due to other reasons §	2.61 (2.26-3.01)

* 815 patients (7.9%) excluded due to missing information on tumour size or nodal status. † Adjusted relative risk (95% confidence intervals) adjusted for all characteristics listed above, year of diagnosis, and expected mortality. ‡ Patients with non-ductal carcinomas and patients without information on histologic grading. § Medical contraindications, bilateral or inflamatory breast cancer, or distant metastasis.

Influence of tumor location on axillary nodal status and breast cancer prognosis (Study 17)

Material and methods

Registries
In 1977, the Danish Breast Cancer Cooperative Group (DBCG) started nationwide prospective studies on treatment of breast cancer (9). The primary surgical treatment of patients allocated in treatment protocols included total mastectomy plus axillary clearance (90% of the population), or lumpectomy with axillary dissection. Patients were classified as having either low-risk disease or high-risk disease according to histopathological criteria. Low-risk patients were observed without further adjuvant treatment apart from radiotherapy to the residual breast of women who had breast conserving surgery. High-risk patients were allocated to adjuvant chemotherapy and/or radiotherapy. Guidelines for risk group allocation and treatment have been described in detail elsewhere (9-12).

Primary clinical and histopathological data and data concerning postoperative therapy and status at follow-up visits are all registered by the DBCG based on specific forms submitted by the participating departments of surgery, pathology and oncology. Location of the tumor was determined based on an indication made by the surgeon on a figure (Figure 1). When a tumor was located in the borderline between two areas, it was assigned to one of the two areas by randomization according to date of birth.

The Danish Civil Registration System (CRS) was established in 1968 and since then a unique identification number has been assigned to all residents in Denmark. Individual information is kept under the personal identification number in all national registers permitting accurate linkage of information between different registries. The CRS registry keeps updated files on vital status including dates of death and emigration. A detailed description of the information included in this registry is given elsewhere (13).

Subjects
Permission to perform the study was obtained in advance from the National Scientific Ethics
Committee and the Data Protection Board. Information on patients in the DBCG-registry was linked with the CRS-registry to obtain information on vital status. The study was restricted to women less than 70 years at diagnosis, because the DBCG in the DBCG 82 program restricted the data collection to this group of women. Women included in the DBCG-program since 1977 and diagnosed with breast cancer before September 1, 1998, were followed from time of diagnosis until date of death, emigration, or October 1, 1998, whichever occurred first.

Statistical analysis
Associations between tumor characteristics and location were evaluated by chi-square statistics. The association between location and survival was investigated using Cox proportional hazard regression with adjustment for axillary nodal status (0, 1-3, 4-9, ≥10 positive nodes), tumor size (≤2cm, >2 cm and up to 5 cm, >5 cm) histologic grading (I, II-III, non-ductal carcinomas, and patients without information on histologic grading), year of diagnosis (1977-1981, 1982-1988, 1989-1998) and protocol allocation (allocated, not treated according to surgical guidelines, not allocated for other reasons). Test for effect modification was performed as test for interaction between categorized variables. All analyses were performed with the use of SAS (14).

Results
By September 1, 1998, 35,319 women with primary breast cancer less than 70 years of age were registered in the DBCG. The cohort represented a total of 237,364 person-years of follow-up. Distribution of patients according to tumor characteristics and tumor site is given in Table 1. Compared with laterally located tumors, tumors located medially tended to be smaller (p<0.001) and the chance of nodal involvement was significantly reduced (p<0.001). Tumors with central location were found to be larger (p<0.001), associated with higher risk of nodal involvement (p<0.001), and with lower chance of having histologic grading I (p<0.001) compared to laterally located tumors.

In order to further analyze tumor characteristics according to the tumor location in the four quadrants, women with central tumors and women without information on tumor location or nodal status were excluded, leaving 27,234 women for further analysis. Nodal status according to tumor site is given in Figure 2, and further details on tumor site, tumor size and nodal status is given in Table 2. The chance of being axillary node negative was significantly greater for women with medial tumors compared with lateral tumors in the subgroup with tumors ≤ 2 cm (p<0.001) and women with tumors being > 2 cm and ≤ 5 cm (p<0.001). The same trend was seen for the group of women with large tumors (> 5 cm), but the differences did not reach significance (p=0.38).

The independent prognostic effect of tumor location was analyzed by performing a multivariate analysis including tumor size, nodal status, histologic grading, age at diagnosis, protocol allocation, year of treatment, and tumor site. Compared to women presenting with a tumor in the upper lateral quadrant, women with other tumor locations had significantly impaired prognosis (Table 3). Axillary nodal status did not modify the negative prognostic effect among women with lower lateral and lower medial tumors. However, the negative effect of tumor location in the upper medial quadrant was almost exclusively restricted to women classified as axillary nodal negative (upper medial node negative RR=1.30, 95 percent confidence interval, 1.20 to 1.40; upper medial node positive RR=1.08, 95 percent confidence interval, 0.996 to 1.16). The differences in prognosis according to tumor location were not modified by tumor size (p=0.77, data not shown).

Discussion

The present study shows that the prognosis in breast cancer patients differs significantly according to tumor location. Compared to women with tumors in the other three quadrants, women with tumors located in the upper lateral quadrant clearly had the best survival. They were, however, also the group of women most likely to be diagnosed with metastatic spread to the axillary lymph nodes. In contrast, women with tumors in the upper medial quadrant had the worst prognosis but were the least likely to be diagnosed with axillary node positive tumors. An explanation for these seemingly contradictory associations is that treatment allocation according to axillary lymph node spread is insufficient. Thus, a proportion of women with tumors in the upper medial quadrant and with no spread to axillary nodes most likely had lymphatic dissemination of their disease to lymph nodes outside the axilla, and thus should have been allocated to a more aggressive treatment program than the one given to them. Support for this view is given by our finding that women with upper medial and lateral tumor locations had similar survival when restricting the analysis to those with positive axillary nodes whereas survival was 30 percent worse among women with upper medial compared to upper lateral tumors among those classified as axillary node negative. The internal mammary lymphnodes have been found the most important destination of lymph drainage outside the axilla (15). It seems likely that more accurate diagnosis and surgical treatment of the internal mammary nodes could lead to improved prognosis for patients with tumors located in the upper medial quadrant of the breast. The impact on survival after treatment of the internal mammary nodes in women with medially located tumors is the subject of an ongoing EORCT trial (16).

Compared to tumors in the upper lateral quadrant we also found an impaired survival for women with lower medial and lower lateral tumors. However, for these tumor locations survival was independent of axillary nodal status. This observation indicates that other factors than nodal misclassification and consequently wrong allocation to existing treatment protocols should be considered.

It is documented that a proper axillary dissection is important not only regarding staging of the disease but also with respect to the local tumor control (1,17). Hence, women with tumors in the upper lateral quadrant are likely to have the most complete surgical management of the tumor burden when mastectomy/lumpectomy and axillary dissection is the standard treatment. Compared with these patients, women with other tumor locations must be expected to have a higher risk of having residual tumor tissue after surgical treatment. Thus, incomplete removal of tumor tissue among women with tumors located away from the axilla may explain why survival disadvantage is observed also among certain groups of axillary node positive patients who receive adjuvant treatment.

Some centers have evaluated whether more extended operations including internal mammary chain dissection can improve survival of the patients (18-22). Based on these studies between 6% and 9% (some old studies even up to 19%) of the patients have been found to have metastases in the internal mammary chain without axillary nodal involvement. Although some authors

found a beneficial effect of the extended operation for women with medial tumors, the overall conclusion was that due to increased morbidity of the intensive procedure, it was not found recommendable.

Recent studies on sentinel node procedures have revealed that about three percent of breast cancer patients without positive axillary lymph nodes have metastatic nodes outside the axilla (3,15). The present study underlines that axillary nodal staging is insufficient in a proportion of women with breast cancer. The sentinel node technique may offer an attractive opportunity to identify women with primary lymph drainage to lymph nodes outside the axilla and thus lead to changed treatment procedures for some women. However, based on the present results such altered procedures may primarily be beneficial to women with upper medially located tumors. Unfortunately, a better classification of nodal status does not appear to remove the differential survival for all tumors in the breast. The differences in survival according to tumor location are substantial and suggest that other factors of prognostic importance need be considered. It is unlikely that the biology of the tumors should differ based on tumor location in the breast. Rather, factors such as differences in the surgical efficacy of removing metastatic tissue might show important for the differential survival according to tumor location observed in the present study.

KEY RESEARCH ACCOMPLISHMENTS

I. Abortion and breast cancer

- We found no overall effect of induced abortion on breast cancer risk.

II. Reproductive history and breast cancer

- Early reproductive years rather than just the nulliparous years constitute the critical period. In other words, the risk reduction of having the first birth at an early age is no greater than the additional risk reduction following a second birth, as long as the second birth also occurs at an early age. Having all births at a very young age gives the highest risk reduction.

- Nulliparous women were more likely than parous women to be diagnosed with a large tumour and with spread to regional lymph nodes.
- Women with a late compared to an early first birth were at significantly increased risk of being diagnosed with a large tumour and with spread to the regional lymph nodes.

- The incidence of breast cancer in parous women increased by 10% by each 5-year postponement of the first birth.

- On average, there was a 10% decrease in breast cancer risk by each additional birth.

- Lopular and mucinous carcinomas and centrally located tumours may have risk effect profiles which differ from other types of breast cancer.

- After a child birth, morthers experience a transient increased risk of breast cancer, and in particular a relatively high risk of late stage disease.

- Women with family history had a stronger adverse short-term effect of a child birth compared to others.

- Mothers having a multiple birth compared to singleton mothers had an increased risk of breast cancer in the first 5 years after birth.

- Mothers having a heavy weighted child compared with a lighter weighted child had an increased risk of breast cancer in the first 5 years after birth.

- Gender of offspring did not influence the mother's breast cancer risk.

- A high alpha-fetoprotein level during any pregnancy is associated with a low overall breast cancer incidence, and in particular with a low incidence of advanced disease. This association appears particularly strong if the pregnancy occurs at a young age.

III. Factors influencing the prognosis of breast cancer

- A diagnosis of breast cancer less than 2 years after having given birth is associated with a particularly poor survival.

- Low age at first birth, but not parity, is associated with a poor prognosis of breast cancer.

- A pregnancy subsequent to breast cancer treatment did not aggravate the prognosis.

- The well-known negative prognostic effect of young age was almost exclusively seen in women diagnosed with low-risk disease not receiving adjuvant cytotoxic therapy, whereas young women who received adjuvant cytotoxic therapy had the same prognosis as middle-aged women.

- We found a highly significant 20-30% difference in chance of survival after breast cancer diagnosis according to in what quadrant of the breast the primary tumour was first diagnosed.

REPORTABLE OUTCOMES

Papers published or accepted for publication

1. Melbye M, Wohlfahrt J, Olsen JH, Frisch M, Westergaard T, Larsen KH, Andersen PK. Induced abortion and the risk of breast cancer. **N Eng J Med 1997; 336: 81-85.**

2. Kroman N, Wohlfahrt J, Andersen KW, Mouridsen HT, Westergaard T, Melbye M. Time since birth is a prognostic factor in primary breast cancer. **BMJ 1997; 315: 851-55.**

3. Kroman N, Wohlfahrt J, Andersen KW, Mouridsen HT, Westergaard T, Melbye M. Parity, age at first childbirth and the prognosis of primary breast cancer. **Br J Cancer 1998; 78: 1529-33.**

4. Kroman N, Jensen M, Melbye M, Wohlfahrt J, Mouridsen HT. Should women be advised against pregnancy subsequent to breast cancer treatment? **Lancet 1997; 350: 319-22.**

5. Wohlfahrt J, Mouridsen HT, Andersen PK and Melbye M. Reproductive risk factors for breast cancer by receptor status, histology, laterality and location. **Int J Cancer 1999; 81: 49-55.**

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7. Wohlfahrt J and Melbye M. Maternal risk of breast cancer and birth characteristics of offspring by time since birth. **Epidemiology 1999: 10: 441-44.**
8. Wohlfahrt J, Andersen PK and Melbye M. Multivariate competing risks in a poisson regression model: An application with two correlated characteristics of breast cancer. **Stat Med 1999; 18: 1023-30.**

9. Wohlfahrt J, Andersen PK, Mouridsen HT, Adami HO and Melbye M. Reproductive history and stage of breast cancer. **Am J Epidemiol 1999;150:1325-1330.**

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12. Wohlfahrt J and Melbye M. Gender of offspring and maternal breast cancer risk. **Br J Cancer 2000;82:1070-1072.**

13. Wohlfahrt J and Melbye M. Age at any birth is equally important for breast cancer risk. **Epidemiology 2000 (in press).**

14. Melbye M, Wohlfahrt J, Lei U, Nørgaard-Pedersen B, Lambe M and Michels KB. Alpha-fetoprotein levels during pregnancy and maternal breast cancer incidence. **JNCI 2000; 92: 1001-05.**

15. Melbye M, Wohlfahrt J and Andersen PK. Induced abortion and risk of breast cancer (letter) **Epidemiology 2000;11:235.**

16. Wohlfahrt J, Andersen PK, Mouridsen HT, Melbye M. Risk of late stage breast cancer following a childbirth. **Am J Epidemiol 2000 (accepted for publication).**

Papers submitted for publication

17. Kroman N, Wohlfahrt J, Mouridsen HT, Melbye M. Influence of tumor location on axillary nodal status and breast cancer prognosis. Submitted 2000.

18. Wohlfahrt J, Olsen JH, Melbye M. Breast cancer risk after a childbirth in young women with family history. Submitted 2000.

Degrees

Lei U. Alfaføtoprotein under graviditeten og risiko for brystkræft – Et populationsbaseret studie. In Danish [Alphafetoprotein and risk of breast cancer]. University Diploma (Osval II). Degree obtained 1998

Kielgast U. Har aetiologien bag brystkraeft en immunologisk komponent? [Does the etiology of breast cancer have an immunologic component?]. University Diploma (Osvald II). Degree obtained 2000.

Kroman N. Prognostic influence of age and reproductive factors in premenopausal breast cancer patients. DMSc thesis 2000 (submitted).

List of personnel receiving pay from the research effort

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CONCLUSIONS

The following conclusions were reached regarding topics covered under category:

I. Abortion and breast cancer

There is no overall effect of induced abortions on breast cancer risk. Our finding of a significantly increased risk in the special group of second trimester abortions was based on a limited number of cancer events and should be considered with caution. (study 1,11,15)

II. Reproductive history and breast cancer

Overall, the incidence in parous women increased by 10% by each 5-year postponement of their first birth. For the incidence of lobular carcinomas this increase was significantly stronger and for mucinous carcinomas it tended to be stronger than for ductal carcinomas. For the incidence of centrally located tumours the increase was stronger than for non-centrally located tumours. On average, there was a 10% decrease in breast cancer risk by each additional birth. This decrease was seen in most subtypes, but not for lobu-

lar carcinomas and centrally localised tumours. According to our findings, lobular and mucinous carcinomas and centrally located tumours may have risk factor profiles which differ from other types of breast cancer. (study 5)

A preterm delivery did not significantly increase a woman's risk of contracting pre-menopausal breast cancer apart from a very small group of women with a preterm delivery of less than 32 weeks' gestation. Despite the large size of the this study, there were only few cases of breast cancer in the subgroups representing the very early deliveries, and these results should therefore be considered with due caution. (study 6)

Mothers having a multiple birth compared to singleton mothers had an increased risk of breast cancer in the first five years after a birth (RR=1.8; (95% CI 1.1-2.8). Mothers having a heavy-weighted child compared with a lighter-weighted child were also at increased risk (RR_{trend}= 1.2 (1.0-1.5) per kg). This latter effect was primarily due to an increased incidence of tumors larger than 2 cm at diagnosis (RR_{trend}=1.5 (1.1-2.1) per kg). Our findings are compatible with the hypothesis that the hormonal level during pregnancy influences the risk of breast cancer in the early years after delivery. (study 7)

Competing risks models can be used to compare the effect of risk factors for different causes of death or subtypes of a disease. However, sometimes more than one outcome classification is available and if two such classifications are correlated, one may speculate whether differences in the effect of a risk factor according to one classification simply may be an effect of differences according to the other correlated classification. We introduce in this paper the new concept of *multivariate competing risks* to formally test such a hypothesis. (study 8)

Nulliparous compared to parous women and women with a late compared to an early age at first childbirth were at significantly increased risk of being diagnosed with a large tumor and with spread to regional lymph nodes. However, such an association was not seen for women diagnosed with a small tumor and a tumor without spread to regional lymph nodes. Reproductive history did not appear to influence patient's and doctor's delay before diagnosis of the tumor. In conclusion, reproductive history is associated both with the incidence of breast cancer and with the stage of the disease at diagnosis, indicating possible influences on progression and growth rate of the tumor. Intensified awareness is warranted to achieve earlier diagnosis in nulliparous women and women with late age at first childbirth, with the hope of improving their prognosis. (study 9)

Gender of offspring is related to maternal hormonal level during pregnancy. The hormonal level might influence the subsequent maternal breast cancer risk. However, analysing national birth and cancer registrations in a cohort of 998,499 women, we found no association between gender of offspring and subsequent breast cancer risk. (study 12)

According to our results, a woman's breast cancer risk is related to her age at any of her births. The risk increase per 5 year's increase in maternal age at 1st, 2nd, 3rd and 4th birth was 9%, 7%, 5% and 14%. For 5th and 6th birth it was 5%. We observed a risk reduction after any birth occurring before 30 years of age (in uniparous women before 25 years of age). These effects were strongest more than 10 years after birth. In conclusion, our study shows that early timing of any additional birth induces an additional long-term reduction in maternal risk of breast cancer, i.e. that early reproductive years, rather than just the nulliparous years, constitute the critical period. (study 13)

Women with median or higher AFP-levels during pregnancy had a 41% lower risk of breast cancer than women with AFP levels below the median (Relative Risk=0.59; 95% CI 0.41-0.85). The association between maternal AFP levels and breast cancer incidence was strongest among women with high AFP levels during pregnancy at young ages. Stratifying by age at birth gave the following results: $\leq$29 years RR=0.21, 30-34 years RR=0.61, 35-37 years RR=0.96, $\geq$38 years RR=0.71 (p trend = 0.02). Further analyses documented that high AFP levels in particular reduced the incidence of aggressive disease. A high AFP level during any pregnancy is associated with a low overall breast cancer incidence and in particular with a low incidence of advanced disease. This association appears particularly strong if the pregnancy occurs at a young age. (study 14)

In conclusion, after a childbirth mothers experience a transient increased risk of breast cancer and in particular a relatively high risk of late stage disease. This finding suggests that pregnancy related factors transiently induce a high growth rate in cells that are already malignant and induce new tumor growth. (study 16)

Women with family history had a stronger adverse short-term effect of a childbirth compared to others, which corresponds with the hypothesis that a childbirth induce growth potential in occult tumors. (study 18)

III. Factors influencing the prognosis of breast cancer

A diagnosis of breast cancer less than two years after having given birth is associated with a particularly poor survival irrespective of the stage of disease at debut. Therefore, a recent pregnancy should be regarded as a negative prognostic factor, and as such be considered in the counselling of these patients and in the decisions regarding adjuvant treatment regimens. (study 2)

Low age at first childbirth, but not parity, was associated with a poor prognosis of breast cancer. We speculate whether women who develop breast cancer despite an early first full-term pregnancy might represent a selected group with a particular malignant disease. (study 3)

We found no evidence that a pregnancy subsequent to breast cancer treatment should aggravate the prognosis. (study 4)

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Overall, young patients with low risk disease who did not receive adjuvant treatment had a significantly increasing risk of dying with decreasing age at diagnosis (adjusted relative risk: 45-49 years: 1 (reference); 40-44 years: 1.12 (0.89-1.40); 35-39 years: 1.40 (1.10-1.78); <35 years: 2.18 (1.64-2.89). However, a similar trend was not seen in young patients who received adjuvant cytotoxic therapy. We found the same difference as above when comparing women receiving no treatment with those receiving adjuvant cytotoxic therapy within strata of node negative patients and patients with the same tumor size. The negative prognostic effect of young age is almost exclusively seen in women diagnosed with low risk disease not receiving adjuvant cytotoxic therapy, whereas young women who receive adjuvant cytotoxic therapy have the same prognosis as middle-aged women. These results suggest that young women with breast cancer, on the basis of age alone, should be regarded as high risk patients and be given adjuvant cytotoxic therapy. (study 10)

Survival is significantly better for women with a tumor in the upper lateral quadrant than tumors located elsewhere in the breast. Better staging of the tumor and extensive surgery to dissect lymph nodes for staging purposes out into the axilla are likely explanations for the superior survival of women with such tumor location. This suggests that a more aggressive treatment of tumors in other locations might increase these women's chance of survival. (study 17)

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INDUCED ABORTION AND THE RISK OF BREAST CANCER

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ABSTRACT

Background It has been hypothesized that an interrupted pregnancy might increase a woman's risk of breast cancer because breast cells could proliferate without the later protective effect of differentiation.

Methods We established a population-based cohort with information on parity and vital status consisting of all Danish women born from April 1, 1935, through March 31, 1978. Through linkage with the National Registry of Induced Abortions, information on the number and dates of induced abortions among those women was combined with information on the gestational age of each aborted fetus. All new cases of breast cancer were identified through linkage with the Danish Cancer Registry.

Results In the cohort of 1.5 million women (28.5 million person-years), we identified 370,715 induced abortions among 280,965 women (2.7 million person-years) and 10,246 women with breast cancer. After adjustment for known risk factors, induced abortion was not associated with an increased risk of breast cancer (relative risk, 1.00; 95 percent confidence interval, 0.94 to 1.06). No increases in risk were found in subgroups defined according to age at abortion, parity, time since abortion, or age at diagnosis of breast cancer. The relative risk of breast cancer increased with increasing gestational age of the fetus at the time of the most recent induced abortion: <7 weeks, 0.81 (95 percent confidence interval, 0.58 to 1.13); >12 weeks, 1.38 (1.00 to 1.90) (reference category, 9 to 10 weeks).

Conclusions Induced abortions have no overall effect on the risk of breast cancer. (N Engl J Med 1997; 336:81-5.)

A FULL-TERM pregnancy increases a woman's short-term risk of breast cancer, possibly as a result of the growth-enhancing properties of pregnancy-induced estrogen secretion. By contrast, such a pregnancy decreases the long-term risk of breast cancer, perhaps by inducing terminal differentiation of the susceptible mammary cells.[1-5] Studies in animals suggest that the potential for terminal differentiation of breast cells is lower for a pregnancy terminated by abortion than for a full-term pregnancy. On this basis Russo and Russo[3] have proposed that a full-term pregnancy allows complete differentiation of breast cells, thereby protecting against cancer, whereas an abortion forestalls the late protective effect of differentiation, thereby increasing the risk of breast cancer.

Epidemiologic studies of the association between abortion and the subsequent risk of breast cancer have yielded inconsistent results, with estimates of risk ranging from moderately elevated to significantly lowered.[6-24] In a recent case–control study, Daling et al. found evidence of an elevated risk in women who had an induced abortion between 9 and 12 weeks' gestation, but this finding was based on a very limited number of women.[7] In the present study, we took advantage of Denmark's mandatory reporting of all induced abortions, together with the week of gestation, to evaluate the hypothesis of Russo and Russo.[3]

METHODS

Population Registries

Before initiating this study, we obtained permission from Denmark's National Scientific Ethics Committee and Data Protection

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Board. For this investigation we linked data from the Civil Registration System (CRS) with data from the National Registry for Induced Abortions and the Danish Cancer Registry. Since April 1, 1968, the CRS has assigned a unique identification number to all Danish residents, which permits information from different registries to be linked. The CRS also keeps updated files on the dates of live births and documents demographic variables such as emigration and deaths.

The reporting of induced abortions to the National Board of Health has been mandatory since 1939. In 1973, the legal right to an induced abortion through 12 weeks' gestation was established for women with residence in Denmark. Induced abortions after week 12 were permitted under medical or other circumstances, such as rape, that could greatly interfere with the proper care of the newborn child. Since 1973, information on all induced abortions, including the date of the procedure and the week of gestation at the time, has been computerized in the national registry of induced abortions.[25] The induced abortions included in this analysis (those occurring between 1973 and 1992) were performed almost exclusively by surgical removal.

The Danish Cancer Registry contains information on all cases of cancer diagnosed in the country since 1943. It receives reports from clinicians, pathologists, clinics, radiotherapy units, and hospitals.[26]

Subjects

A research data base comprising all Danish women born between April 1, 1935, and March 31, 1978, and including information on any live-born children, was established on the basis of information from the CRS. The individually identifiable CRS numbers were used to form a link with the national registry of induced abortions, which supplied information on the date of any induced abortion and the gestational age of the aborted fetus. Subjects' CRS numbers were subsequently linked with the Danish Cancer Registry to identify the subjects with a diagnosis of invasive breast cancer.

Statistical Analysis

Follow-up for breast cancer for all the women began on April 1, 1968, or on their 12th birthday, whichever came later. The period at risk continued until a diagnosis of breast cancer, death, emigration, loss to follow-up, or December 31, 1992 (at which date the cancer registry was considered complete) — whichever occurred first. The possible effect of the duration of the pregnancies that ultimately ended in induced abortions was investigated in a log-linear Poisson regression model.[27] The numbers of person-years at risk were calculated for groups defined according to the week of gestation for induced abortions that took place at <7, 7 to 8, 9 to 10, 11 to 12, 13 to 14, 15 to 18, and >18 weeks' gestation. Women with more than one induced abortion were, in the period between the first and second abortion, considered at risk according to the week of gestation at the time of the first induced abortion; between the second and third abortions they were considered at risk according to the week of gestation at the time of the second induced abortion; and so on.

Adjustment was made for attained age in one-year intervals and for the calendar period in which the abortion occurred (1968–1972, 1973–1977, 1978–1982, 1983–1987, and 1988–1992), parity (0, 1, 2, 3, 4, 5, 6, and ≥7), and age at delivery of a first child (12 to 19, 20 to 24, 25 to 29, 30 to 34, and >34 years). In an exploratory analysis we also categorized the women according to calendar period and age at first delivery in one-year intervals, but this had no effect on the results — a finding that argues against residual confounding. For simplicity, the attained age of a woman is denoted as her "age at the time of diagnosis of breast cancer." "Calendar period" and "calendar period at time of diagnosis of breast cancer" are used synonymously. Tests for trend were performed with gestational age treated as a continuous variable and the mean gestational age used as the value for each group. Rate ratios for the incidence of breast cancer were estimat-

ed with the use of the SAS procedures software package PROC GENMOD.[28] These rate ratios are referred to as relative risks in this article.

RESULTS

Overall, 1,529,512 women were included in the cohort. Of these, 280,965 (18.4 percent) had a total of 370,715 induced abortions, distributed as follows: 215,902 women (76.8 percent) each had one induced abortion; 47,906 women (17.1 percent) each had two; and 17,157 women (6.1 percent) each had three or more. The distribution of the number of induced abortions according to gestational age was as follows: <7 weeks, 3.1 percent; 7 to 8 weeks, 37.1 percent; 9 to 10 weeks, 41.8 percent; 11 to 12 weeks, 15.7 percent; >12 weeks, 2.3 percent. Women without a history of induced abortion accounted for 25,850,000 person-years of follow-up. In this group, there were 8908 cases of breast cancer. In comparison, among women with a history of induced abortion, accounting for 2,697,000 person-years of follow-up, there were 1338 cases of breast cancer.

Overall, the risk of breast cancer in women with a history of induced abortion was not different from that in women without such a history, after potential confounding by age, parity, age at delivery of a first child, and calendar period was taken into account (relative risk, 1.00; 95 percent confidence interval, 0.94 to 1.06).

Table 1 presents the association between variables related to abortion history and the risk of breast cancer. We calculated both the relative risk adjusted for age, parity, calendar period, and age at first delivery and the further adjusted multivariate relative risk (adjusted also for the other variables shown in the table). The adjustment had little or no effect on any of the risk estimates. Age at the time of the induced abortion did not significantly influence the overall risk, but there was a tendency toward a higher risk of breast cancer among women in the lowest age category — between 12 and 19 years of age (relative risk, 1.29; 95 percent confidence interval, 0.80 to 2.08). Neither the number of induced abortions nor whether or not the woman had given birth to a live infant (i.e., whether the induced abortion occurred in a nulliparous woman or either before or after a live birth) significantly influenced the risk of breast cancer. We also examined the time interval between the induced abortion and the diagnosis of breast cancer but found no indication of a differential effect (<1 year, relative risk = 0.97; 1 to 4 years, relative risk = 0.99; ≥5 years, relative risk = 1 [reference category]) (Table 1).

There was no effect of induced abortion on the risk of breast cancer after adjustment for the ages of the women at the time of the diagnosis of breast cancer (12 to 34 years, relative risk = 0.95 [95 percent confidence interval, 0.78 to 1.14]; 35 to 39 years,

TABLE 1. ADJUSTED RELATIVE RISK OF BREAST CANCER IN WOMEN WITH A HISTORY OF INDUCED ABORTION.

ABORTION HISTORY	NO. OF CANCERS	PERSON-YEARS (THOUSANDS)	RELATIVE RISK (95% CI)*	MULTIVARIATE RELATIVE RISK (95% CI)†
Wk of gestation				
<7	36	82	0.81 (0.58–1.13)	0.81 (0.58–1.13)
7–8	526	1012	1.01 (0.89–1.14)	1.01 (0.89–1.14)
9–10‡	534	1118	1	1
11–12	205	422	1.12 (0.95–1.31)	1.12 (0.95–1.31)
13–14	6	14	1.13 (0.50–2.52)	1.13 (0.51–2.53)
15–18	17	35	1.24 (0.76–2.01)	1.23 (0.76–2.00)
>18	14	14	1.92 (1.13–3.26)	1.89 (1.11–3.22)
Age at induced abortion (yr)				
12–19	23	458	1.32 (0.82–2.12)	1.29 (0.80–2.08)
20–24‡	68	617	1	1
25–29	161	552	0.91 (0.68–1.20)	0.93 (0.69–1.25)
30–34	366	529	0.99 (0.76–1.29)	1.03 (0.77–1.38)
≥35	720	541	1.04 (0.81–1.34)	1.07 (0.80–1.43)
No. of induced abortions				
1‡	1105	2220	1	1
2	191	376	1.08 (0.92–1.26)	1.09 (0.94–1.28)
≥3	42	101	0.99 (0.73–1.35)	1.02 (0.75–1.40)
Time since induced abortion (yr)				
<1	63	339	0.97 (0.75–1.25)	0.97 (0.75–1.25)
1–4	315	1048	0.99 (0.87–1.12)	0.99 (0.87–1.13)
≥5‡	960	1310	1	1
Time of induced abortion and live-birth history				
Nulliparous women	95	694	1.04 (0.83–1.29)	1.04 (0.83–1.31)
Parous women				
Induced abortion before 1st live birth	77	350	1.08 (0.85–1.36)	1.08 (0.82–1.44)
Induced abortion after 1st live birth‡	1154	1582	1	1
Other§	12	71	0.76 (0.43–1.34)	0.74 (0.41–1.33)

*The relative risks were calculated separately for each of the five variables, with adjustment for women's age, calendar period, parity, and age at delivery of a first child. CI denotes confidence interval.

†Values were adjusted for women's age, calendar period, parity, age at delivery of a first child, and the other variables shown in the table.

‡The women with this characteristic served as the reference group.

§"Other" denotes induced abortion occurring after delivery of a first child in women who also had induced abortion before delivery of a first child.

relative risk = 0.99 [0.87 to 1.14]; 40 to 44 years, relative risk = 1.01 [0.91 to 1.12]; 45 to 49 years, relative risk = 1 [reference category]; ≥50 years, relative risk = 1.03 [0.88 to 1.21]; P for trend = 0.97). Also, neither the calendar period at the time of diagnosis of breast cancer (P = 0.17) nor the calendar period at the time of induced abortion (P = 0.83) modified the relation between induced abortion and the risk of breast cancer.

With each one-week increase in the gestational age of the fetus, however, there was a 3 percent increase in the risk of breast cancer. The relative risk increased from 0.81 (95 percent confidence interval, 0.58 to 1.13) among women whose most recent induced abortion was at less than 7 weeks of gestation to 1.38 (95 percent confidence interval, 1.00 to 1.90) among women whose most recent abortion was at more than 12 weeks of gestation. We acknowledge

the small number of cases in the group with abortions later than 12 weeks, but we evaluated this period further and found the following relative risks: weeks 13 to 14, 1.13 (95 percent confidence interval, 0.51 to 2.53); weeks 15 to 18, 1.23 (0.76 to 2.00); weeks >18, 1.89 (1.11 to 3.22) (P for trend = 0.016, Table 1).

DISCUSSION

Our study of a population-based cohort uncovered no overall increased risk of breast cancer among women with a history of induced abortion. This result is very much in line with the results of previous retrospective cohort studies,[9,10,15,16] two of which actually suggested a decreased risk.[10,15] However, all previously published retrospective cohort studies lack detailed information on the week of gestation at the time of abortion. The results of case–control

studies have been inconsistent,[6-8,11-14,17-24] but several groups have reported an increased risk of breast cancer among women with a history of induced abortion.[7,8,13,21-24]

A recent meta-analysis found an overall increased risk of breast cancer among women with a history of induced abortion of 1.3 (95 percent confidence interval, 1.2 to 1.4).[24] The authors concluded that "such a broad base of statistical agreement rules out any reasonable possibility that the association is the result of bias or any other confounding variable." However, since almost all 23 studies included in the analysis were case–control studies, it is not unreasonable to assume that many of them were inherently biased, making the pooled conclusions biased as well. Furthermore, the authors based their results on a crude analysis of published odds ratios and relative risks with no attempt to incorporate the original raw data into a more sophisticated statistical analysis.

Almost inevitably, case–control studies arouse concern about the potential problem of differential misclassification. Even after its legalization, abortion remains a sensitive issue. It is possible that women with breast cancer might be more willing to report induced abortions than healthy women. A Swedish study that compared registry information with interview data regarding induced abortion attributed an increase in the risk of breast cancer of between 16 and 50 percent to differential misclassification in interview data.[7,29] The problem of misclassification based on reporting led Newcomb et al. to conclude that studies that do not rely on interviews with case and control subjects are necessary to resolve whether there is a link between induced abortion and breast cancer.[8] In our study, all the information on dates and the number of induced abortions, reproductive history, and cancer diagnosis was obtained from national registries, which are compiled through a system of mandatory reporting for the entire population. Follow-up included complete information on death and emigration and was performed through computerized linkage of registry information by means of individually identifiable registration numbers. These measures, we believe, allowed us to avoid some of the major methodologic problems of previous studies.

A limitation of our research data base was that information on induced abortions has been computerized only since 1973. Therefore, we might have obtained an incomplete history of induced abortions for some of the oldest women in the cohort. However, we found that the risk of breast cancer among women with a history of induced abortion was no different from that among women without such a history, nor did we find that the number of induced abortions influenced the risk of breast cancer. Therefore, it is unlikely that missing information about abortions before 1973 affected the results of our analysis.

Induced abortion had no overall effect on the risk of breast cancer, but we found a statistically significant increase in risk among women with a history of second-trimester abortion. The fact that such an increase did not affect the overall result clearly indicates that it is based on small numbers and therefore requires cautious interpretation. The increased risk among women who had had second-trimester abortions finds biologic support in experiments in rats and is in line with the hypothesis of Russo and Russo.[3]

We were concerned that women whose breast cancer was diagnosed during pregnancy might have been advised to have induced abortions, a situation that would not be equally distributed according to the week of gestation at the time of the abortion. Since the time at risk was calculated only up to the diagnosis of breast cancer, only late abortions that were misclassified as occurring before the diagnosis of cancer could represent a problem. However, a stratified analysis of the risk of breast cancer according to the length of time since an induced abortion showed no differential risk and, in particular, no increased risk within the first year after abortion. Abortions induced at gestational ages of more than 12 weeks were performed primarily for medical or social reasons. The women who had such abortions could have had a relatively high risk of breast cancer, but we could not identify any medical condition associated with both a high risk and late induced abortion. Women with drinking problems might delay the interruption of their unwanted pregnancies, but the association between alcohol and breast cancer is weak and inconsistent.[30]

We cannot explain why a very early induced abortion was associated with a slight, although insignificant, decrease in risk. Nulliparous women with a history of induced abortion did not differ from parous women in their risk of breast cancer. Among nulliparous women, the possible effects of lactation and later births are irrelevant. We are therefore confident that neither of these variables had any confounding effect on our overall result.

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Time since childbirth and prognosis in primary breast cancer: population based study

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Abstract

Objective: To investigate whether time since birth of last child was of prognostic importance in women with primary breast cancer.

Design: Retrospective cohort study based on a population based database of breast cancer diagnoses with detailed information on tumour characteristics, treatment regimens, reproductive factors, and vital status.

Setting: Denmark.

Subjects: 5652 women with primary breast cancer aged 45 years or less at the time of diagnosis.

Main outcome measures: 5 and 10 year survival; relative risk of dying.

Results: Women diagnosed in the first 2 years after last childbirth had a crude 5 year survival of 58.7% and 10 year survival of 46.1% compared with 78.4% and 66.0% for women whose last childbirth was more than 2 years before their diagnosis. After adjustment for age, reproductive factors, and stage of disease (tumour size, axillary nodal status, and histological grading), a diagnosis sooner than 2 years since last childbirth was significantly associated with a poor survival (relative risk 1.58, 95% confidence interval 1.24 to 2.02) compared with women who gave birth more than 5 years previously. Further analyses showed that the effect was not modified by age at diagnosis, tumour size, and nodal status.

Conclusion: A diagnosis of breast cancer less than 2 years after having given birth is associated with a particularly poor survival irrespective of the stage of disease at debut. Therefore, a recent pregnancy should be regarded as a negative prognostic factor and should be considered in counselling these patients and in the decisions regarding adjuvant treatment.

Introduction

An early first delivery and a large number of childbirths are among the best established factors conferring a low risk of breast cancer.[1] Recent studies have described a dual effect of full term pregnancy on the risk of breast cancer, with a transiently increased risk immediately after childbirth followed by a long term reduction in the risk.[2-4]

Although these findings relate to the risk of developing breast cancer, they could also have implications for the prognosis of this disease. A breast cancer that is established before or during pregnancy might accelerate its growth under the influence of high

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concentrations of pregnancy hormones, primarily oestrogens. However, reports on this point are conflicting because of problems with small study sizes or the lack of adjustment for relevant tumour characteristics and reproductive history.[5-7]

We used three nationwide Danish registries, one containing detailed information on tumour characteristics, treatment regimens, and clinical outcome and two others containing complete information on parity, to evaluate the influence of reproductive history on breast cancer survival.

Methods

Population registries

The Danish Breast Cancer Cooperative Group, DBCG, started its national prospective studies in 1977. Three treatment programmes have been run, DBCG 77 (patient accrual from 1978 to 1982), DBCG 82 (patient accrual from 1983 to 1989), and DBCG 89 (ongoing accrual started in 1990). The Danish cancer registry contains information on almost all cases of malignant neoplasms diagnosed in Denmark since 1943.[8] The Danish Breast Cancer Cooperative Group has information on 93% of all breast cancer patients aged under 45 at diagnosis reported to the registry.

Since 1968, the civil registration system has assigned a unique 10 digit identification number to all residents in Denmark, which permits accurate linkage of information from different registries. The system's registry also keeps updated files on dates of childbirths

and vital status. Information about stillbirths was added from the national birth registry.

Subjects

Permission was obtained in advance from the national scientific ethics committee and the data protection board to link information on patients in the Danish Breast Cancer Cooperative Group's registry with the civil registration system registry. This registry does not systematically link women born before 1935 to all their children; therefore, to obtain the complete reproductive histories of the women we restricted our study group to women born since 1 April 1935. Because our objective was to study the influence of time since birth on breast cancer survival and because we also wanted to limit the analysis to premenopausal women, we included only women aged 45 or less at the time their breast cancer was diagnosed. All women diagnosed before 1 October 1994 were included and followed until 1 October 1995 with respect to vital status.

Treatment

Primary surgical treatment was total mastectomy plus axillary sampling (90% of the population) or lumpectomy with axillary sampling, after which patients were classified as low risk or high risk according to histopathological criteria. Low risk patients had tumours <5 cm in diameter without axillary lymph node metastases and without invasion into the skin or the deep resection line (DBCG 77 and DBCG 82); in the DBCG 89 programme, premenopausal node negative patients had tumours classified as histological grade I. High risk patients were those with a primary tumour >5 cm or with lymph node metastases in the axilla or with tumour growth into the skin or the deep resection line (DBCG 77 and DBCG 82). In the DBCG 89 programme, premenopausal patients with grade II and III anaplasia were classified as high risk patients. Patients with bilateral breast cancer, distant metastases, or inflammatory cancer or with contraindication to the planned postoperative treatment or who were not treated according to the surgical guidelines were not allocated to treatment protocols.

In all three programmes, low risk patients were given no systemic treatment after surgery. In the DBCG 77 programme, high risk patients were allocated to either postoperative radiotherapy or radiotherapy and systemic treatment, as described elsewhere.[9] In the DBCG 82 programme, high risk patients were allocated to systemic treatment and radiotherapy or to systemic treatment alone.[9] The target for radiotherapy after mastectomy included the chest wall and regional lymph nodes (axillary, supraclavicular, infraclavicular, and parasternal nodes). In the DBCG 89 programme, high risk patients were given systemic treatment according to steroid hormone receptor status. Radiotherapy including the chest wall was given if the tumour invaded the deep resection line. All patients who had lumpectomy were given radiotherapy to the residual breast tissue.

Statistical analysis

The associations between the study variables and survival were investigated using the Cox proportional hazards method.[10] Multivariate analyses included tumour characteristics, time between diagnosis and

Table 1 Distribution of 5652 breast cancer patients aged 45 or less at diagnosis according to tumour characteristics, age, risk group allocation, and time since birth. Values are numbers (percentages)

| Variable | Time since last childbirth | | | | |
	Nulliparous (n=695)	< 2 years (n=201)	2-3 years (n=280)	4-5 years (n=349)	≥6 years (n=4127)
Age (years):					
<30	46 (6.6)	33 (16.4)	24 (8.6)	16 (4.6)	4 (0.1)
30-34	92 (13.2)	84 (41.8)	93 (33.2)	77 (22.1)	180 (4.4)
35-39	169 (24.2)	60 (29.9)	188 (42.1)	147 (42.1)	977 (23.7)
40-45	388 (55.8)	24 (11.9)	45 (16.1)	109 (31.2)	2966 (71.9)
Tumour size (cm):					
≤2	299 (43.0)	94 (46.8)	134 (47.9)	167 (47.9)	2240 (54.3)
>2 ≤5	260 (37.4)	74 (36.8)	94 (33.6)	115 (33.0)	1308 (31.7)
>5	72 (10.4)	14 (7.0)	33 (11.8)	33 (9.5)	266 (6.5)
No information	64 (9.2)	19 (9.5)	19 (6.8)	34 (9.7)	313 (7.6)
No of positive nodes:					
0	328 (47.2)	81 (40.3)	129 (46.1)	153 (43.8)	2180 (52.8)
1-3	200 (28.8)	56 (27.9)	86 (30.7)	115 (33.0)	1134 (27.5)
4-9	85 (12.2)	34 (16.9)	33 (11.8)	44 (12.6)	449 (10.9)
≥10	24 (3.5)	18 (9.0)	10 (3.6)	17 (4.9)	135 (3.3)
No information	58 (8.4)	12 (6.0)	22 (7.9)	20 (5.7)	229 (5.6)
Histological grading:					
I	146 (21.0)	30 (14.9)	52 (18.6)	71 (20.3)	994 (24.1)
II+III	394 (56.7)	132 (65.7)	166 (59.3)	205 (58.7)	2219 (53.8)
Other*	155 (22.3)	39 (19.4)	62 (22.1)	73 (20.9)	914 (22.2)
Protocol allocation:					
Yes	523 (75.3)	156 (77.6)	228 (81.4)	289 (82.8)	3442 (83.4)
No					
Not treated according to surgical guidelines	100 (14.4)	35 (17.4)	42 (15.0)	44 (12.6)	521 (12.6)
Not allocated for other reasons†	72 (10.4)	10 (5.0)	10 (3.6)	16 (4.6)	164 (4.0)

*Including patients with non-ductal carcinomas and patients without information on histologic grading.
†Medical contraindications, bilateral breast cancer, distant metastasis, or inflammatory cancer.
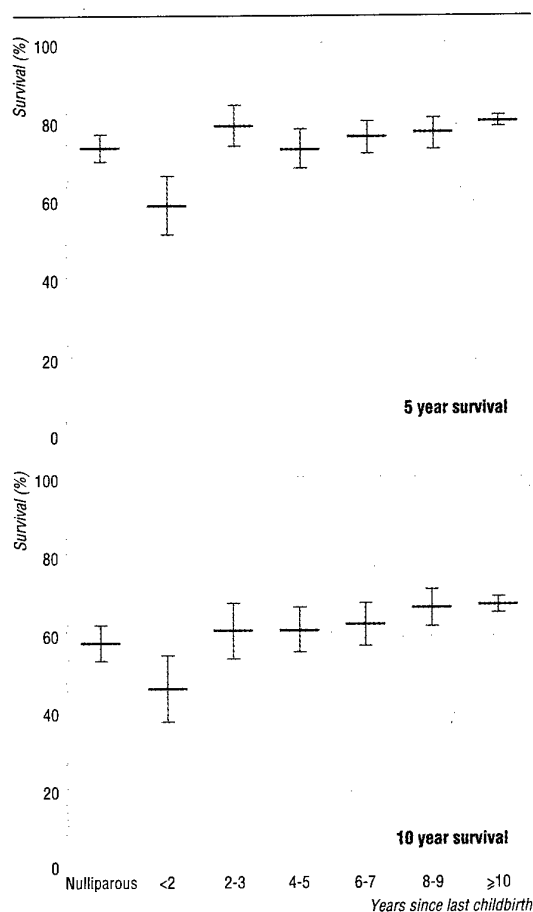
Five year survival (top) and 10 year survival (bottom) according to time since last childbirth in 5652 women with primary breast cancer. Bars indicate 95% confidence intervals

most recent childbirth, age at diagnosis, year of treatment, and protocol allocation. Parity was eliminated from the final multivariate model as it was not significant. Because survival for the age categories representing six and more years after childbirth was similar, we defined a reference category for the variable "time since birth" as ≥6 years to be used in the multivariate analyses. The adequacy of the proportional hazard assumptions for the included variables was checked by log(−log) plots from stratified multivariate analyses. The Cox regression was performed in four strata (information on tumour size and lymph node status available, only tumour size missing, only lymph node status missing, both missing). Estimation was done using the SAS procedure PROC PHREG.[11]

Results

Overall, 5752 women aged 45 years or less were identified for our study. The influence of pregnancy subsequent to treatment of breast cancer has been debated,[12] and hence 100 patients were excluded due to delivery after the time of their diagnosis, leaving 5652 patients for further analyses. Follow up ranged from 13 months to 17 years, representing a total of 34 130 person years of follow-up. Overall, 4957 women (87.7%) were parous and 695 (12.3%) were nulliparous. Table 1 shows the distribution of patient

age, tumour characteristics, and risk group allocation according to time since last birth.

The figure shows the overall 5 year and 10 year survival for women according to time since birth. Women whose breast cancer was diagnosed less than 2 years after they gave birth had a crude 5 year survival of 58.7% and a 10 year survival of 46.1%, compared with 78.4% and 66.0% for women who had their last delivery more than 2 years before their cancer diagnosis. Recent pregnancy had a negative effect in patients who received adjuvant treatment and those who did not. Women with a recent birth (<2 years) who were classified with low risk breast cancer and thus did not receive adjuvant systemic treatment had a crude survival of 75.0% (5 year) and 55.6% (10 year) compared with 88.5% and 77.8% for women whose last childbirth was more than 2 years before their diagnosis. Women classified with high risk disease, who received adjuvant treatment, had a crude survival of 53.2% (5 year) and 41.2% (10 year) compared with 72.0% and 58.2% for women whose last childbirth was more than 2 years before their diagnosis.

The effect of time since last childbirth was further evaluated for parous women in a multivariate analysis that considered the influence of age at diagnosis, tumour size at diagnosis, numbers of positive axillary lymph nodes, grade of anaplasia, protocol allocation, and year of treatment. The prognosis remained significantly worse for women who gave birth to a child within the past 2 years (relative risk 1.58 (95% confidence interval 1.24 to 2.02)) than for women who had given birth six or more years ago (P = 0.0002) (table 2). The risk associated with a recent birth was increased 2.1-fold in the first year and 1.3-fold in the second year.

To investigate whether the negative effect of a recent birth was modified by age at diagnosis, stage of

Table 2 Adjusted relative risk of dying according to prognostic factors, age at diagnosis, and time since birth among 4957 parous women with breast cancer

Variable	Adjusted relative risk (95% CI)*
Age at diagnosis (years):	
<30	1
30-34	0.88 (0.61 to 1.28)
35-39	0.88 (0.61 to 1.27)
40-45	0.79 (0.58 to 1.15)
Tumour size (cm):	
≤2	1
>2 ≤5	1.67 (1.48 to 1.89)
>5	2.46 (2.06 to 2.95)
No of positive nodes:	
0	1
1-3	1.56 (1.37 to 1.78)
4-9	3.01 (2.58 to 3.50)
≥10	3.87 (3.09 to 4.83)
Histological grade:	
I	1
II + III	2.28 (1.93 to 2.68)
Non-ductal carcinoma	1.26 (1.04 to 1.54)
Years since last childbirth:	
<2	1.58 (1.24 to 2.02)
2-3	0.96 (0.77 to 1.21)
4-5	1.15 (0.95 to 1.40)
≥6	1

*Adjusted for characteristics listed and for year of treatment, and protocol allocation.

Table 3 Adjusted relative risk (95% confidence interval) of dying according to age at diagnosis, nodal status, tumour size, and time since birth among 4957 parous woen with breast cancer aged 45 or less

	Time since last childbirth			
	< 2 years	2-3 years	4-5 years	≥6 years
Age at diagnosis (years):†				
≤33	1.6 (1.1 to 2.3)*	1.1 (0.8 to 1.6)	1.2 (0.8 to 1.9)	1
>34	1.6 (1.2 to 2.3)*	0.9 (0.7 to 1.2)	1.1 (0.9 to 1.4)	1
Tumour size (cm):				
≤2	1.6 (1.1 to 2.3)*	1.3 (0.9 to 1.9)	1.3 (1.0 to 1.8)	1
>2	1.4 (1.0 to 2.0)*	0.8 (0.6 to 1.1)	1.0 (0.8 to 1.3)	1
Nodal status:				
Negative	1.5 (1.0 to 2.4)*	1.1 (0.7 to 1.6)	1.0 (0.7 to 1.4)	1
Positive	1.4 (1.1 to 2.0)*	1.0 (0.7 to 1.3)	1.3 (1.0 to 1.6)*	1

Relative risk adjusted for age at diagnosis, tumor size, moal status, histological grade, years of treatment, and protocol allocation.
*P<0.05.
†Patients separated into two groups according to median age among patients with childbirth <2 years before diagnosis.

disease (measured by number of positive axillary lymph nodes), or tumour size, we performed tests for effect modification with adjustment as given above (table 3). Neither age at diagnosis, nodal status, nor tumour size had any significant modifying effect on the poor survival for the group of women who had recently (<2 years) given birth.

Discussion

Using a large and complete population based database with detailed information on tumour characteristics, treatment regimens, reproductive factors, and vital status, we documented a particularly poor survival for women who were diagnosed with breast cancer within 2 years after giving birth. The adverse effect on the prognosis was seen irrespective of the woman's age, the size of the tumour, and the stage of the disease. In a small multicentre study involving nine centres and a total of 152 young mothers (<30 years) with breast cancer, Guinee et al found an increased mortality in women who had given birth up to four years before their diagnosis.[6] Other studies indicate that breast cancer diagnosed during lactation is associated with poor survival,[13 14] though one recent study failed to support such an association [7]. A limitation in all these studies has been their sample size. Furthermore, they have generally been unable to adequately adjust for confounders such as other reproductive history, tumour size, axillary lymph node status, and histological grading.

Delayed diagnosis

The difficulty of diagnosing breast cancer in young women in general and pregnant and lactating women in particular, because of the density of the mammary glands, is reflected in a significant diagnostic delay among these patients.[12 15] In our study the tendency for recently pregnant women to have more advanced disease could, at least to some extent, be caused by delayed diagnosing. However, our detailed information on each woman's tumour characteristics allowed us to adjust for this. Thus, independent of the influence caused by delayed diagnosis, a recent birth before the diagnosis of breast cancer conferred an increased risk of dying of about 60% in comparison to other women with breast cancer.

Influence of breast feeding

Breast feeding was earlier considered to influence the risk of developing breast cancer, but most recent evidence suggests that there is no important overall association.[19] Whether breast feeding influences the prognosis of the disease is unknown, but the lack of effect on the risk of disease does not necessarily strengthen a possible effect on its prognosis. In our study, we did not have information on breast feeding. Lactation entails a different hormonal environment to that in non-lactating women after delivery, which makes the group of women with recent pregnancy heterogeneous. However, poor survival was observed when breast cancer was diagnosed not only in the first but also in the second year after birth, at which time most women have stopped breast feeding.

Influence of pregnancy

In 1988, Mohle-Boetani and colleagues observed an insignificantly increased risk of relapse among women with a recent delivery and suggested that the special hormonal and immunological conditions associated with pregnancy might lower the survival of breast cancer patients.[5] Although immunological changes occur during pregnancy, it is no longer widely accepted that pregnancy results in a state of immunodeficiency.[16 17] Even if some kind of immunosuppression should occur during pregnancy, this would not necessarily be expected to have a negative influence on the course of breast cancer.[18]

In vitro experiments show that pregnancy may confer a growth enhancing effect on tumour cells.[20] However, a simple growth enhancing effect would tend to increase the volume of the tumour at time of diagnosis shortly after pregnancy. The negative effect of a recent birth remains present after factors that reflect the volume of the tumour (tumour size and nodal status) are taken into account (table 3). Therefore, the most likely explanation for our finding is that the pregnancy changes the course of the disease by increasing the risk of a highly malignant growth pattern of already existing tumour cells.

It has long been known that early age at first full term pregnancy is associated with a low risk of developing breast cancer, whereas women aged 35 years or more at first childbirth are at a particularly high risk.[1] In our study, neither tumour size, nodal status, nor age modified the specific prognostic effect of recent last delivery. Because breast cancer is rare before

Key messages

- A childbirth close to subsequent diagnosis of breast cancer has a negative effect on the woman's cancer prognosis

- The negative effect of recent childbirth is not affected by age at diagnosis, nodal status, and tumour size

- The negative effect is found both in patients who receive adjuvant treatment and those who do not

- Childbirth history should be taken into account when counselling young women with breast cancer

the age of 30,[21] the likelihood of giving birth near the time of a breast cancer diagnosis is significantly greater for women who have their children at an advanced age. Therefore, the adverse influence of pregnancy on breast cancer survival will be greatest when women postpone childbearing to older ages.

The negative effect of recent pregnancy was pronounced in women who did not receive adjuvant treatment (low risk group) as well as among those who did (high risk group). Therefore, it is not known whether more intensive adjuvant treatment will change the course of the disease in these patients. These findings need be considered in counselling such patients and in deciding on adjuvant treatment. Pregnancy history should be recorded for premenopausal breast cancer patients and in prospective clinical trials so that response to adjuvant treatment according to time since last childbirth can be assessed.

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Parity, age at first childbirth and the prognosis of primary breast cancer

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Summary Reproductive factors are known to be aetiologically important in breast cancer, but less is known regarding their effect on breast cancer prognosis. We have investigated the prognostic effect of age at first birth and total parity using data from the Danish Breast Cancer Cooperative Group that, since 1977, has collected population-based information on tumour characteristics, treatment regimes and follow-up status on Danish women with breast cancer. Details of pregnancy history were added from the Danish Civil Registration System and the National Birth Registry. Included in the study were 10 703 women with primary breast cancer. After adjusting for age and stage of disease (tumour size, axillary nodal status and histological grading), the number of full-term pregnancies was found without prognostic value. However, women with primary childbirth between 20 and 29 years experienced a significantly reduced risk of death compared with women with primary childbirth below the age of 20 years [20–24 years: relative risk (RR) = 0.88, 95% confidence interval (CI) 0.78–0.99; 25–29 years: RR = 0.80, 95% CI 0.70–0.91]. Further adjustment for oestrogen receptor status did not influence these results. The effect was not modified by age at diagnosis, tumour size or nodal status. In conclusion, low age at first childbirth, but not parity, was associated with a poor prognosis of breast cancer. We speculate whether women who develop breast cancer despite an early first full-term pregnancy might represent a selected group with a more malignant disease.

Keywords: breast cancer; reproductive factors; survival; prognostic factors; oestrogen receptor

It is well-established that reproductive factors influence the risk of breast cancer development (McPherson et al, 1994). Based on animal studies, it has been hypothesized that pregnancy induces differentiation and maturation of the breast cells and that the cells subsequently become less vulnerable to carcinogenic stimuli (Russo et al, 1990). Parous women and in particular multiparous women are known to be at a lower risk of breast cancer than nulliparous women. Women having their first childbirth at a young age seem to experience a particular reduction in risk (MacMahon et al, 1970; Ewertz et al, 1990).

Factors influencing the development of breast cancer might also affect its course, but studies of the prognostic influence of reproductive factors have been contradictory (Papatestas et al, 1980; Palmer et al, 1982; Black et al, 1983; Wang et al, 1985; Mohle Boetani et al, 1988; Lees et al, 1989; Mason et al, 1990; Lehrer et al, 1992; Guinee et al, 1994; Korzeniowski and Dyba, 1994; Orr and Fraher, 1995; von Schoultz et al, 1995; Schouten et al, 1997). We took advantage of the population-based registration of breast cancer patients established by the Danish Breast Cancer Cooperative Group (DBCG) and a database containing complete information on parity to evaluate the possible importance of childbirth history and age at first birth as prognostic factors in primary breast cancer.

MATERIALS AND METHODS

Population registries

Primary clinical and histopathological data together with data concerning post-operative therapy and follow-up have been registered by the DBCG since 1977 (Andersen and Mouridsen, 1988). The Danish Cancer Registry contains information on nearly all incident cases of malignant neoplasms diagnosed in Denmark since 1943 (Storm, 1991). The DBCG has information on 93% of all breast cancer patients born after 1 April 1935, reported to The Danish Cancer Registry.

The primary surgical treatment of the patients included total mastectomy plus axillary sampling (90% of cases), or lumpectomy with axillary sampling. Patients were hereafter classified as either low risk or high risk according to histopathological criteria. Treatment guidelines, strategy for risk group allocation and post-operative treatment have previously been described in detail (Andersen and Mouridsen, 1988; Kroman et al, 1994).

Patients with bilateral breast cancer, distant metastases, inflammatory cancer, with contraindication to the planned post-operative therapy, or patients who were not treated according to the surgical guidelines, were not allocated to treatment protocols (miscellaneous group). The miscellaneous patient group could be separated into a group with a favourable prognosis and a group with a bad prognosis. Patients who were excluded from protocol allocation because of a surgical treatment that did not follow the guidelines had a better prognosis than patients excluded for other reasons.

Information on reproductive history was obtained by linkage with the Civil Registration System (CRS). The CRS was established on 1 April 1968 when all residents in Denmark were registered and assigned a unique identification number that permits

Table 1 Distribution of 10 703 women with primary breast cancer born after 1 April 1935, diagnosed during 1978–94 according to age at diagnosis, tumour characteristics, protocol allocation, parity, and age at first childbirth

	Age at first birth No. (%)				
	Nulliparous	**<20 years**	**20–24 years**	**25–29 years**	**≥30 years**
Total no.	1260	1468	4416	2670	889
Age at diagnosis					
<35 years	138 (11.0)	71 (4.8)	225 (5.1)	184 (6.9)	31 (3.5)
35–39 years	169 (13.4)	211 (14.4)	595 (13.5)	374 (14.0)	122 (13.7)
40–44 years	318 (25.2)	434 (29.6)	1128 (25.5)	701 (26.3)	258 (29.0)
45–49 years	337 (26.8)	452 (30.8)	1392 (31.5)	781 (29.3)	273 (30.7)
≥50 years	298 (23.7)	300 (20.4)	1076 (24.4)	630 (23.6)	205 (23.1)
Tumour size					
≤2 cm	576 (45.7)	837 (57.0)	2446 (55.4)	1429 (53.5)	461 (51.9)
>2, ≤5 cm	480 (38.1)	457 (31.1)	1477 (33.5)	936 (35.1)	300 (33.7)
>5 cm	119 (9.4)	87 (5.9)	261 (5.9)	158 (5.9)	76 (8.5)
No information	85 (6.8)	87 (5.9)	232 (5.3)	147 (5.5)	52 (5.8)
Positive nodes					
0	600 (47.6)	784 (53.4)	2301 (52.1)	1359 (50.9)	448 (50.4)
1–3	374 (29.7)	401 (27.3)	1204 (27.3)	777 (29.1)	237 (26.7)
4–9	152 (12.1)	160 (10.9)	538 (12.2)	307 (11.5)	127 (14.3)
≥10	49 (3.9)	48 (3.3)	165 (3.7)	110 (4.1)	39 (4.4)
No information	85 (6.8)	75 (5.1)	208 (4.7)	117 (4.4)	38 (4.3)
Histological grading					
I	302 (24.0)	362 (24.7)	1135 (25.7)	668 (25.0)	210 (23.6)
II + III	664 (52.7)	802 (54.6)	2268 (51.4)	1353 (50.7)	471 (53.0)
ND[a]	294 (23.3)	304 (20.7)	1013 (22.9)	649 (24.3)	208 (23.4)
Protocol allocation					
Yes	980 (77.8)	1234 (84.1)	3748 (84.9)	2245 (84.1)	740 (83.2)
No					
Not treated according to surgical guidelines	158 (12.5)	168 (11.4)	457 (10.4)	291 (10.9)	101 (11.4)
Not allocated because of other reasons[b]	122 (9.7)	66 (4.5)	211 (4.8)	134 (5.0)	48 (5.4)
Parity					
1		157 (10.7)	586 (13.3)	648 (24.3)	489 (55.0)
2	–	639 (43.5)	2325 (52.7)	1555 (58.2)	350 (39.4)
3		471 (32.1)	1199 (27.2)	399 (14.9)	42 (4.7)
≥4		201 (13.7)	306 (6.9)	68 (2.6)	8 (0.9)

[a]Including patients with non-ductal carcinomas (n = 2089, 84.6%) and patients without information on histological grading (n = 379, 15.4%). [b]Medical contraindications, bilateral breast cancer, distant metastasis, or inflamatory cancer.

identity secure linkage of information between registries. Parents were recorded with a link to most of their children born in the beginning of the 1950s or later and alive in 1968. Since then, the CRS registry has kept updated files on dates on all live births and residents in Denmark including updated files on vital status. A more detailed description of the reproductive information included in this registry is given elsewhere (Melbye et al, 1997). Information on stillbirths was available during the period 1978–93 from the National Birth Registry.

Subjects

Permission was obtained in advance from the National Scientific Ethics Committee and the Data Protection Board to link information on patients in the DBCG registry with the CRS registry and the National Birth Registry. Women born before 1935 have no systematic link to all their children in the CRS registry. Therefore, we restricted our study group to women born since 1 April 1935.

All women with a diagnosis of breast cancer before 1 October 1994 were included and followed until 1 October 1995, with respect to vital status.

Statistical analysis

The associations between the study variables and survival were investigated using the Cox Proportional Hazards method (Cox, 1972). Multivariate analyses included tumour size (≤2 cm, >2 and up to 5 cm, >5 cm), positive lymph nodes (0, 1–3, 4–9, ≥10), histological grading (I, II and III, non-ductal patients and those without information on histological grading), age at first birth (nulliparous, <20, 20–24, 25–29, ≥30 years), parity at diagnosis (0, 1, 2, 3, ≥4), age at diagnosis (<35, 35–39, 40–44, 45–49, ≥50 years), year of diagnosis, and protocol allocation (see Table 1). The adequacy of the proportional hazard assumptions for the included variables was checked by log(– logS) plots from stratified multivariate analyses. For both tumour size and lymph node

Table 2 Adjusted relative risk (aRR) of dying according to prognostic factors, protocol allocation and parity in 10 703 breast cancer patients born after 1 April 1935 and diagnosed 1978–94

Variables	aRR (95% CI)[a]
Tumour size	
≥2 cm	1 (reference)
>2, ≤5 cm	1.63 (1.49–1.78)[b]
> 5 cm	2.17 (1.90–2.49)[b]
Positive nodes	
0	1 (reference)
1–3	1.71 (1.53–1.91)[b]
4–9	3.32 (2.97–3.72)[b]
≥10	4.72 (4.02–5.52)[b]
Histological grading	
I	1 (reference)
II + III	2.33 (2.07–2.62)[b]
ND[c]	1.18 (1.02–1.36)[b]
Protocol allocation	
Allocated patients	1 (reference)
Not treated according to guidelines	1.04 (0.91–1.17)
Not allocated because of other reasons[d]	2.76 (2.43–3.13)[b]
Parity	
Nulliparous	1 (reference)
Parous	0.95 (0.85–1.06)

[a]Adjusted relative risk (95% confidence intervals) adjusted for all characteristics listed above and age at diagnosis and year of diagnosis. [b]$P<0.05$. [c]Patients with non-ductal carcinomas and patients without information on histological grading. [d]Medical contraindications, bilateral breast cancer, distant metastasis, or inflamatory cancer.

Table 3 Adjusted relative risk (aRR) of dying according to number of full-term pregnancies, and age at first childbirth in 9443 parous breast cancer patients born after 1 April 1935 and diagnosed 1978–94

Variables	aRR (95% CI)[a]	aRR (95% CI)[b]
Parity		
Nulliparous	1.04 (0.90–1.19)	
1	1 (reference)	1 (reference)
2	0.96 (0.86–1.07)	0.97 (0.86–1.08)
3	0.99 (0.88–1.12)	0.98 (0.85–1.11)
≥4	1.07 (0.90–1.28)	1.04 (0.87–1.25)
Age at first birth		
Nulliparous	0.92 (0.80–1.06)	
<20 years	1 (reference)	1 (reference)
20–24 years	0.87 (0.78–0.98)[c]	0.88 (0.78–0.99)[c]
25–29 years	0.79 (0.70–0.90)[c]	0.80 (0.70–0.91)[c]
≥30 years	0.94 (0.80–1.11)	0.94 (0.79–1.12)

[a]Adjusted relative risk (95% confidence intervals) adjusted for age at diagnosis, tumour size, nodal status histological grading, protocol allocation, and year of diagnosis. [b]Adjusted relative risk further adjusted for parity factors listed above. [c]$P < 0.05$.

Table 4 Stratified analysis of risk of dying according to age at diagnosis, nodal status, tumour size, and age at first childbirth among 9443 parous breast cancer patients

	Age at first birth			
	<20 years aRR[a]	20–24 years aRR[a]	25–29 years aRR[a]	≥30 years aRR[a]
Age at diagnosis				
<35 years	1 (reference)	1.6 (0.99–2.5)	1.2 (0.8–2.0)	2.0 (0.96–4.1)
35–39 years	1 (reference)	0.9 (0.7–1.1)	0.9 (0.7–1.2)	1.1 (0.8–1.6)
40–44 years	1 (reference)	0.7 (0.6–0.9)[b]	0.7 (0.6–0.9)[b]	0.8 (0.6–1.0)
45–49 years	1 (reference)	0.8 (0.6–1.0)	0.7 (0.6–0.9)[b]	0.9 (0.7–1.2)
≥50 years	1 (reference)	1.1 (0.8–1.5)	0.9 (0.6–1.3)	1.0 (0.6–1.5)
Tumour size				
≤2 cm	1 (reference)	0.8 (0.6–0.9)[b]	0.8 (0.6–0.9)[b]	0.9 (0.7–1.2)
>2 cm	1 (reference)	0.9 (0.7–1.0)	0.8 (0.7–0.9)[b]	0.9 (0.7–1.1)
Nodal status				
Negative	1 (reference)	0.8 (0.7–1.0)	0.8 (0.6–0.97)[b]	1.0 (0.7–1.3)
Positive	1 (reference)	0.9 (0.8–1.0)	0.8 (0.7–0.9)[b]	0.9 (0.8–1.1)

[a]Adjusted relative risk (95% confidence intervals) adjusted for age at diagnosis, tumour size, nodal status, histological grading, protocol allocation, and year of diagnosis. [b]$P < 0.05$.

status, the hazard rate of the heterogeneous category of missing information was not proportional to the hazard rates of the other categories. Therefore, the Cox regression was performed in four strata (information on tumour size and lymph node status available, only tumour size missing, only lymph node status missing, both missing). The estimates were only slightly changed if women with missing tumour size or nodal status were excluded from the analysis. Tests for effect modification were performed as tests for interaction between categorized variables. In an exploratory analysis, we categorized year of treatment in 1-year intervals, but this did not affect the results – a finding that argues against residual confounding. All analyses were performed using likelihood ratio tests by means of the SAS procedure PROC PHREG (SAS Institute, 1992).

RESULTS

By 1 October 1994, 10 803 women with primary breast cancer born after 1 April 1935 were registered in the DBCG. One hundred patients were excluded because of delivery after diagnosis. Of the remaining 10 703 patients, 1260 (11.8%) were nulliparous and 9443 patients (88.2%) were parous. The follow-up time ranged from 13 months to 17 years representing a total of 60 322 person–years of follow-up. Distribution of patients according to age at diagnosis, tumour characteristics, protocol allocation, parity and age at first birth is given in Table 1.

The influence of these factors on breast cancer prognosis was evaluated in a multivariate analysis. The relative risk of dying according to tumour characteristics and status as nulliparous or parous is given in Table 2. Table 3 shows the relative risk of dying

according to parity and age at first childbirth in parous women. Parous women were found to have a minor insignificantly reduced risk of dying compared with nulliparous women [relative risk (RR) 0.95; 95% confidence interval (CI) 0.86–1.06]. The prognosis was unaffected by the number of children in the group of parous women ($P = 0.78$, Table 3).

The adjusted relative risk of dying varied significantly according to age at first birth as shown in Table 3 ($P = 0.005$). Women having their first child at the age of 25–29 years had the best prognosis. The relative risk of dying was significantly reduced for women having their first child between the ages of 20 and 24 years (RR 0.88; 95% CI 0.78–0.99) and women with primary childbirth between the ages of 25 and 29 years (RR 0.80; 95% CI 0.70–0.91) compared with women having primary childbirth below the age of 20 years (reference group).

To investigate whether the prognostic effect of age at first birth was modified by age at diagnosis, extent of disease (measured by number of positive axillary lymph nodes) or tumour size, we tested for effect modification with adjustment for all other considered factors as given above (Table 4). Neither tumour size ($P = 0.63$) nor nodal status ($P = 0.74$) had a significantly modifying effect on the prognostic influence of age at first birth. There was a trend towards the prognostic effect of age at first childbirth being more pronounced among women diagnosed between the ages of 40 and 50 years. However, this finding was not significant ($P = 0.27$).

Oestrogen receptor (ER) status was available on 6016 patients. Sixty-nine per cent were classified as ER positive and 31% were classified as ER negative. The negative prognostic effect of age at first childbirth was not affected by ER status.

DISCUSSION

We found strong evidence that young age of the mother at first birth is associated with poor survival of breast cancer, despite its protective effect on breast cancer development. Although some studies have not supported this observation (Mohle Boetani et al, 1988; Lees et al, 1989; Ewertz et al, 1991), there is accumulating evidence that does support it (Greenberg et al, 1985; Kogevinas, 1990; Schouten et al, 1997). A limitation of previous studies has been their small sample sizes (range 582–1744 subjects) compared with the present study. Furthermore, these studies have primarily been based on retrospectively collected information obtained among cases and controls through interviews. The present population-based study was based on prospectively collected data, with detailed exposure and outcome information that limits possibilities for recall bias.

Previous reports have shown the risk of developing breast cancer to be reduced among women who have their first child at an early age (MacMahon et al, 1970; Ewertz et al, 1990). Based on a large cohort of 1.5 million women and including more than 10 000 breast cancer cases, we have similarly found a strongly increasing risk of breast cancer with increasing age at first childbirth (J Wohlfahrt, PK Anderson, HT Mouridsen, HO Adami and M Melbye personal communication). Thus, one could argue that some women who avoided breast cancer because of a delivery at an early age would have developed breast cancer if they had had their first childbirth late or if they had remained nulliparous. These women who avoided breast cancers might be those with the most favourable course. Following this argument, the observed reduced survival in breast cancer patients with early first childbirth might reflect a selection of more aggressive cases rather than a direct biological effect of the early pregnancy on the carcinogenic process. We acknowledge that women with an early first childbirth did not have a poorer profile of the available prognostic factors. However, these prognostic factors do not necessarily offer a complete picture of the biological behaviour of the tumours.

There was a suggestion, although not statistically significant, that early first childbirth is a negative prognostic factor of breast cancer in older premenopausal women aged 40–49 years. The assumption that the negative effect of early first childbirth is a consequence of a selection is supported by epidemiological data showing that the protective effect of early first childbirth on breast cancer development is most pronounced in older premenopausal women (Ewertz et al, 1990).

In the Western world, the median age of first childbirth has increased over the past decades. It is generally accepted that this postponement of motherhood has contributed to the rising incidence of breast cancer. Our study suggests that the postponement of motherhood might have a beneficial effect on overall breast cancer prognosis.

Studies on overall parity as a prognostic factor have been contradictory (von Papatestas et al, 1980; Palmer et al, 1982; Black et al, 1983; Wang et al, 1985; Mohle-Boetani et al, 1988; Lees et al, 1989; Mason et al, 1990; Lehrer et al, 1992; Guinee et al, 1994; Korzeniowski and Dyba, 1994; Orr and Fraher, 1995; Schoultz et al, 1995). We have previously found that pregnancy within 2 years before a diagnosis of breast cancer was associated with reduced survival (Kroman et al, 1997). This, combined with the present observation of early first childbirth being a negative prognostic factor, could explain the finding reported by some researchers of an association between high parity and poor prognoses (Wang et al, 1985; Lees et al, 1989; Korzeniowski and Dyba, 1994). Women with high parity would be expected to have their first child early and have their last child late. Therefore, women with high parity would be over-represented in the two high-risk groups defined by us. In the present study, high parity alone did not serve as an independent prognostic factor.

The observation that breast cancer may be a high social status disease has been related to differences in childbirth patterns (Kelsey and Horn Ross, 1993). In contrast, several studies have shown that low social class is associated with reduced survival (Karjalainen and Pukkala, 1990; Kogevinas et al, 1991; Gordon et al, 1992). It may be of relevance for the latter finding that poorly educated women tend to have their first child earlier than women with higher education level (Knudsen, 1993).

In conclusion, we found that age at first birth is a prognostic factor in breast cancer, whereas parity did not affect the survival. These findings may provide further insight into breast tumour pathogenesis and should be considered in future evaluations of other prognostic factors of importance for this disease.

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Benign anal lesions, inflammatory bowel disease and risk for high-risk human papillomavirus-positive and -negative anal carcinoma

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Summary A central role in anal carcinogenesis of high-risk types of human papillomaviruses (hrHPV) was recently established, but the possible role of benign anal lesions has not been addressed in hrHPV-positive and -negative anal cancers. As part of a population-based case–control study in Denmark and Sweden, we interviewed 417 case patients (93 men and 324 women) diagnosed during the period 1991–94 with invasive or in situ anal cancer, 534 patients with adenocarcinoma of the rectum and 554 population controls. Anal cancer specimens (n = 388) were tested for HPV by the polymerase chain reaction. Excluding the 5 years immediately before diagnosis, men, but not women, with anal cancer reported a history of haemorrhoids [multivariate odds ratio (OR) 1.8; 95% confidence interval (CI) 1.04–3.2] and unspecific anal irritation (OR 4.5; CI 2.3–8.7) significantly more often than controls. Women with anal cancer did not report a history of benign anal lesions other than anal abscess to any greater extent than controls, but they had used anal suppositories more often (OR 1.5; CI 1.1–2.0). Patients with hrHPV in anal cancer tissue (84%) and those without (16%) reported similar histories of most benign anal lesions, but anal fissure or fistula was more common among hrHPV-positive cases. Ulcerative colitis and Crohn's disease, reported by <1% of study participants, were not associated with anal cancer risk. The higher proportion of hrHPV-positive anal cancers among case patients with anal fissure or fistula suggests that such mucosal lesions may provide direct viral access to basal epithelial layers. Since risk associations with benign anal lesions in men may be confounded by unreported sexual behaviour, and since risk associations in women were generally negative, it seems unlikely that benign anal lesions act as promoters in hrHPV-associated anal carcinogenesis. Moreover, benign anal lesions appear not to be linked to an alternative, hrHPV-unassociated causal pathway to anal cancer. Ulcerative colitis and Crohn's disease were not supported as causal factors for anal cancer.

Keywords: anus neoplasms; risk factors; haemorrhoids; anal fistula; anal fissure; inflammatory bowel diseases; ulcerative colitis; Crohn's disease

The incidence of epidermoid anal cancer, a rare neoplasm of the anal canal and perianal skin, has increased considerably during the past decades (Goldman et al, 1989; Frisch et al, 1993; Melbye et al, 1994). It has been shown that anal cancer has a sexually transmitted aetiology (Daling et al, 1987; Holly et al, 1989; Frisch et al, 1997). Substantial evidence now points to the causal involvement of certain high-risk types of human papillomaviruses (hrHPV), notably HPV type 16, in the majority of anal cancers (Holm et al, 1994; Frisch et al, 1997). One case–control study (Holly et al, 1989) provided data that were interpreted as supportive of the old belief that anal inflammation predisposes to anal cancer (Brofeldt, 1927; Buckwalter and Jurayj, 1957). However, another case–control study (Holmes et al, 1988) and two subsequent cohort studies did not accord with this view (Frisch et al, 1994; Lin et al, 1995). Based on data from a nationwide case–control study

in Denmark and Sweden, we attempted to re-evaluate the association between benign anal lesions and the risk for hrHPV-positive and -negative anal cancer.

MATERIALS AND METHODS

We identified all incident cases of histologically verified invasive and in situ anal and rectal epidermoid carcinoma (hereafter referred to as anal cancer) in Denmark and Sweden for the period 1991–94 (and five cases from 1995) as described in detail elsewhere (Frisch et al, 1997). Two control groups were included: one consisting of patients with adenocarcinoma of the rectum (cancer controls) and another consisting of population controls drawn from national population registers. Each control group was frequency matched within each country on sex and age (±5 years) and for cancer controls, on the year of diagnosis.

Data collection

Participants were interviewed by telephone using a structured questionnaire covering a large number of possible risk factors for anal cancer. A separate report gives a detailed analysis of sexual

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Summary

Background Oestrogen is an established growth factor in breast cancer. There has, therefore, been much discussion about whether women should be advised against becoming pregnant after breast-cancer treatment because of a possible negative prognostic effect from the high oestrogen concentrations associated with pregnancy.

Methods We studied 5725 women with primary breast cancer. Information on these women was obtained from the Danish Breast Cancer Cooperative Group. Since 1977 this group has collected population-based data on tumour characteristics, treatment regimens, and follow-up status of Danish women with breast cancer. Details of reproductive history were obtained from The Danish Civil Registration System, the National Birth Registry, and the National Induced Abortion registry. We estimated the relative risk of death among women who became pregnant after breast-cancer treatment compared with women who had not become pregnant.

Findings 5725 women with primary breast cancer aged 45 years or younger at the time of diagnosis were followed up for 35 067 patient-years. Among these, 173 women became pregnant after treatment of breast cancer. Women who had a full-term pregnancy after breast-cancer treatment had a non-significantly reduced risk of death (relative risk 0·55 [95% CI 0·28–1·06]) compared with women who had had no full-term pregnancy after adjustment for age at diagnosis, stage of disease (tumour size, axillary nodal status, and histological grading), and reproductive history before diagnosis. The effect was also not significantly modified by age at diagnosis, tumour size, nodal status, or reproductive history before diagnosis of breast cancer. Neither miscarriages nor induced abortions after breast-cancer treatment influenced the prognosis.

Interpretation We found no evidence that a pregnancy after breast-cancer treatment increased the risk of a poor outcome.

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Introduction

Much attention has been given to the importance of endocrine factors in breast-cancer development and prognosis since Beatson[1] first reported 100 years ago on the positive effect of oophorectomy in women with breast cancer. A woman's reproductive history strongly influences her risk of developing breast cancer; one of the best-known associations is the protective effect of having many children, preferably at a young age.[2,3] Although childbearing may reduce the risk of breast cancer overall, evidence suggests that childbearing, at least in some circumstances, may have a negative effect on the prognosis—studies suggest that breast cancer diagnosed in the first few years after childbearing is associated with a poor prognostic outcome.[4-6]

An outstanding question is whether pregnancy after breast-cancer treatment may worsen the prognosis. Reports on this topic seem to indicate that, contrary to expectations, pregancy has no negative effect after treatment of breast cancer. However, the evidence is weak and based on small studies that have generally not been able to adjust adequately for important confounders.[7-16] Another important obstacle in the study of this question has been that the group of women who decide to have a child after breast-cancer diagnosis is thought to be highly selected.[4]

In the western world, the median age at first childbirth has increased. Since motherhood generally starts later, more patients are seeking medical advice about pregnancy after treatment of breast cancer. In this study we investigated the prognostic influence of pregnancy after breast-cancer treatment based on a linkage analysis between the population-based Danish Breast Cancer Cooperative Group (DBCG) registry and other national registries. Detailed information on stage of disease allowed us to address specifically the potential problem of selection bias.

Methods

The DBCG started its national prospective studies in 1977 and has since recorded primary clinical and histopathological data together with data on postoperative therapy and follow-up status among women with breast cancer in Denmark. The DBCG has information on 93% of all breast-cancer patients born after April 1, 1935, reported to the Danish Cancer Registry, which contains information on almost all incident cases of malignant neoplasms diagnosed in Denmark.[17]

The primary surgical treatment of the patients assigned treatment protocols included total mastectomy plus axillary sampling (90% of the population), or lumpectomy with axillary sampling. We classified patients low risk or high risk, according to histopathological criteria. Further information about treatment guidelines, risk-groups, and postoperative treatment has been published.[18,19]

Patients with bilateral breast cancer, distant metastases, inflammatory cancer, or contraindications to the planned postoperative therapy, or patients who were not treated according to the surgical guidelines were not assigned treatment

	Reproductive status after diagnosis of breast cancer			
	Full-term pregnancy* (n=84)	Induced abortion† (n=77)	Miscarriage (n=12)	No pregnancy (n=5552)
Age at diagnosis				
<35 years	62 (74%)	35 (45%)	6 (50%)	603 (11%)
35-39 years	17 (20%)	29 (38%)	3 (25%)	1436 (26%)
40-45 years	5 (6%)	13 (17%)	3 (25%)	3513 (63%)
Tumour size				
≤2 cm	47 (56%)	42 (55%)	6 (50%)	2876 (52%)
>2 to ≤5 cm	17 (20%)	23 (30%)	4 (33%)	1823 (33%)
>5 cm	5 (6%)	4 (5%)	0	414 (7%)
No information	15 (18%)	8 (10%)	2 (17%)	439 (8%)
Positive nodes				
0	49 (58%)	46 (60%)	7 (58%)	2812 (51%)
1-3	19 (23%)	20 (26%)	4 (33%)	1563 (28%)
4-9	6 (7%)	5 (6%)	0	640 (12%)
≥10	0	2 (3%)	0	202 (4%)
No information	10 (12%)	4 (5%)	1 (8%)	335 (6%)
Histological grading				
I	15 (18%)	16 (21%)	4 (33%)	1270 (23%)
II + III	35 (42%)	45 (58%)	7 (58%)	3058 (55%)
Non-ductal‡	34 (40%)	16 (21%)	1 (8%)	1224 (22%)
Protocol allocation				
Yes	55 (65%)	65 (84%)	9 (75%)	4556 (82%)
No				
Not treated according to surgical guidelines	21 (25%)	11 (14%)	3 (25%)	726 (13%)
Not assigned for other reasons§	8 (10%)	1 (1%)	0	270 (5%)

*Including eight women with induced abortion and full-term pregnancy, five women with miscarriage and full-term pregnancy, and one woman with induced abortion and full-term pregnancy.
†Including one woman with both induced abortion and miscarriage.
‡Including patients without information on histological grading.
§Medical contraindications, bilateral breast cancer, distant metastasis, or inflammatory cancer.

Table 1: **Distribution of patients diagnosed between 1978 and 1995 according to age at diagnosis, tumour characteristics, protocol assignment, and reproductive status after diagnosis**

protocols, and we classified these women as the miscellaneous group. We separated the miscellaneous group into subgroups of favourable and poor prognosis.

The Danish Civil Registration System (CRS) was established in 1968 and, since then, a unique identification number has been assigned to all residents of Denmark. Individual information is kept under the personal identification number in all national registers, which permits accurate linkage of information between these registries. The CRS resgistry keeps updated files on vital status and dates of childbirths with a systematic link to the children of women born after April 1, 1935. A detailed description of the information included in this registry is given elsewhere.[20,21] Information on stillbirths after 1977 and induced abortions after 1973, including gestational age of the fetus, was available from the National Birth Registry and the National Induced Abortion registry.

Permission to do the study was obtained in advance from the National Scientific Ethics Committee and the Data Protection Board of Denmark. Information on patients in the DBCG registry was linked with the other national registries to obtain information on pregnancy history and vital status. Since women born before 1935 have no systematic link to all their children in the CRS resgistry, we restricted our study to women born after April 1, 1935. We aimed to identify women who might become pregnant after breast-cancer diagnosis, so we further restricted the study group to women aged 45 years or younger at the time of diagnosis. All women diagnosed before Oct 1, 1994, were included and vital status was followed up until Oct 1, 1995.

The associations between the study variables and survival were analysed by Cox's proportional hazards method. Variables in the multivariate analyses included tumour characteristics, time between diagnosis and latest birth (with nulliparous in a separate category), age at diagnosis, year of treatment, protocol assignment, full-term pregnancy after diagnosis, induced abortion after diagnosis, and miscarriage after diagnosis. The three latter variables were included in the analysis as time-dependent variables. The adequacy of the proportional-hazard assumptions for these variables was checked by log(-log)S-plots from stratified multivariate analyses. For both tumour size and

lymph-node status, the hazard rate of the heterogeneous category of missing information was not proportional to the hazard rates of the other categories. Therefore, Cox regression was done in four strata (information on tumour size and lymph-node status available; only tumour size missing; only lymph-node status missing; both missing). Estimation was done by the SAS procedure PROC PHREG (release 6.11).

Results

We identified 5752 women with primary breast cancer aged 45 years or younger. Since the specific aim of the study was to assess the prognostic effect of pregnancy after breast-cancer treatment, we excluded 27 women who might have been pregnant at the time of diagnosis (ie, women who gave birth less than 10 months after diagnosis, or women who had abortion at stages of gestation that indicated possible pregnancy at the time of diagnosis). 5725 patients were followed up for a total of 35 067 patient-years. Of these, 173 (3·0%) women had 211 pregnancies (97 full-term pregnancies, 22 miscarriages, and 92 induced abortions). 32 women had more than one pregnancy after breast-cancer diagnosis. The median times between diagnosis and time of end of pregnancy were: birth, 32 months (range 11–147); miscarriage, 23 months (6–50); and induced abortion, 22 months (3–89). Distribution of patients according to histopathological tumour criteria, protocol assignment, and reproductive status after diagnosis of breast cancer is shown in table 1. These factors, plus the year of treatment and time since latest birth, which are known to influence prognosis, were introduced in a multivariate analysis. The adjusted relative risks of death according to reproductive history after treatment of breast cancer, age at diagnosis, and tumour characteristics are given in table 2. Women who had a full-term pregnancy after treatment had a non-significantly reduced risk of dying (relative risk 0·55 [95%

Variables	Adjusted relative risk* (95% CI)
Reproductive status after diagnosis of breast cancer†	
Full-term pregnancy	0·55 (0·28–1·06)
Induced abortion	1·00 (0·67–1·50)
Miscarriage	0·36 (0·09–1·45)
Age at diagnosis	
<35 years	1·00
35–39 years	1·01 (0·88–1·17)
40–45 years	0·93 (0·82–1·06)
Tumour size	
≤2 cm	1·00
>2 to ≤5 cm	1·74 (1·54–1·96)‡
>5 cm	2·46 (2·06–2·93)‡
Positive nodes	
0	1·00
1–3	1·60 (1·41–1·82)‡
4–9	3·02 (2·60–3·50)‡
≥10	4·06 (3·26–5·06)‡
Histological grading	
I	1·00
II + III	2·25 (1·92–2·64)‡
Non-ductal	1·13 (0·93–1·37)

*Adjusted for the other variables in the table and for year of treatment, protocol assignment, and time since last childbirth.

†Reference group for full-term pregnancy was women without full-term pregnancy; for women with induced abortions it was women without induced abortion; for women with miscarriage it was women without miscarriage.

‡p<0·05.

Table 2: **Adjusted relative risk of death according to reproductive status after diagnosis of breast cancer, age at diagnosis, and prognostic factors**

CI 0·28–1·06] p=0·08) compared with other women with no full-term pregnancy. Women who had induced abortion or miscarriage had no significant risk alteration. Information on recurrence was available in the group of protocol-assigned patients (n=4695 [82%]). For this subgroup introduction of recurrence to the multivariate model did not change the relative risk estimate for full-term pregnancy (0·79 [0·39–1·61]).

Further analysis showed that the effect of pregnancy was not significantly modified by age at diagnosis, tumour size, nodal status, whether the woman was parous or nulliparous before diagnosis, time since last pregnancy before breast-cancer diagnosis, age at pregnancy after diagnosis, or time to pregnancy after diagnosis (data not shown).

We did a restricted analysis, including only women who were classified as having a low-risk tumour (n=2110). In this subgroup, the survival was better for women who had had a full-term pregnancy after breast-cancer treatment (0·61 [0·19–1·91]) than for women who had had no full-term pregnancy. Patients who were not treated according to surgical guidelines had overall better prognosis than patients excluded for other reasons.

From age-standardised rates of childbirth among Danish women, we expected the number of full-term pregnancies in the entire cohort to be 285, compared with the observed 97.

Discussion

Breast cancer diagnosed during pregnancy or in the first few years afterwards has been associated with a poor outcome.[4-6] Furthermore, several hormones that are present in high concentrations during pregnancy are known to induce breast-tissue growth. Such findings have led to much discussion on the possible negative prognostic effect of pregnancy after breast-cancer treatment.

However, we found that pregnancy after breast cancer had no negative effect on prognosis. Because women with a poor outlook are believed to avoid pregnancy there is a potential problem of selection bias in the exposed group. This problem is not easy to overcome and has been the main concern about the interpretation of results in previous studies.[7-16]

We took advantage of the clinical population-based DBCG database, which includes detailed information on breast tumours from many years. Also, the group of women who became pregnant tended to have smaller tumours and a slightly lower risk of nodal involvement. However, we were able to adjust for the influence of such prognostic factors and, therefore, to keep potential problems with selection bias to a minimum. Furthermore, the use of time-dependent variables in a cohort design enabled us to adjust adequately for the influence of time from breast-cancer diagnosis to time of birth or abortion. This is important because we believe that the length of the relapse-free period significantly influences the woman's decision about pregnancy. Women with known recurrence are not thought to become pregnant deliberately which might introduce a selection bias. However, the introduction of recurrence in the multivariate model did not significantly alter the relative risk of death.

The proportion of protocol-assigned patients was lower in the group of women who gave birth than other groups. This difference may be explained partly by some of these women having chosen breast-conserving therapy at a time when this treatment was not established as equal to mastectomy (before 1989). Under those circumstances treatment protocols would not have been offered because the women did not meet the surgical guidelines. However, we adjusted for this discrepancy by introducing protocol assignment to the multivariate analysis.

We acknowledge that, despite these efforts, there are likely to be other selection mechanisms for which we were unable to adjust adequately with the available prognostic factors. Inadequate adjustment may explain why women with full-term pregnancies after breast-cancer treatment, even after adjustment for established prognostic factors, tended to have a better outcome that women who did not become pregnant. Although we may not have completely adjusted for all factors, the possibility that we missed a negative prognostic effect of a pregnancy after breast-cancer treatment seems unlikely. In a restricted analysis of only low-risk breast-cancer patients, we found women who became pregnant after diagnosis also had a favourable outlook. The group of women assigned the low-risk protocol constitutes a homogeneous population in which localised disease is unlikely to give symptoms that might influence a woman's decision about pregnancy. The risk of selection bias should, therefore, be particularly small in this group.

Certain reproductive factors, such as age at first birth and time since latest birth, have been shown to have prognostic effect.[5,6,22,23] We were able to adjust for these factors in the analysis and also to show that none of the reproductive factors modified the prognostic influence of pregnancy after treatment of breast cancer.

The fertility rate, calculated as the number of full-term pregnancies, was about a third of the expected rate in the group of treated breast-cancer patients. This difference is due to an overall lower number of pregnancies as well as an increase in induced abortions in this group of women.

In Denmark, the number of induced abortions, based on figures from the middle of our study period, was 36% of the number of full-term of pregnancies.[24] In our study, breast-cancer patients chose induced abortion almost as often as full-term pregnancy, whereas the number of miscarriages was as expected. Unplanned pregnancy when a woman is seriously ill is likely to lead to a higher rate of induced abortions. Many of these women avoided becoming pregnant after diagnosis of breast cancer. Furthermore, some women may have chosen induced abortion because of lack of knowledge about the influence a pregnancy might have on the course of their treated breast cancer. However, women with a history of induced abortion after breast-cancer treatment had a similar profile of prognostic factors to other women, which suggests that induced abortion was not chosen primarily by patients with a poor prognosis. This finding further supports the credibility of the overall result.

We conclude that pregnancy subsequent to breast-cancer treatment does not have a negative effect on the woman's survival.

Contributors
Niels Kroman created the idea for the study, obtained the necessary permissions, and contributed to the planning and execution. Mads Melbye participated in the planning, execution, and analysis. Maj-Britt Jensen did the statistical analyses. Jan Wohlfahrt participated in the planning, and statistical execution of the analyses. Henning Mouridsen created the idea for the study, took part in the design, and was essential to the establishment of the DBCG register. All the authors contributed to writing of the paper.

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Maternal Risk of Breast Cancer and Birth Characteristics of Offspring by Time Since Birth

Jan Wohlfahrt and Mads Melbye

We examined the association between birth characteristics of offspring and the subsequent maternal risk of breast cancer in a population-based cohort of 998,499 women, 13 to 48 years of age at entry. There were 9,495 incident cases of breast cancer during 12.8 million person-years of follow-up among these women. Compared with mothers of singleton infants, mothers having a multiple birth had an increased risk of breast cancer in the first 5 years after a birth (relative risk (RR) = 1.8; 95% confidence interval (CI) = 1.1–2.8). The risk for mothers having a heavy-weighted child (>3.75 kg), as compared with a child of light weight (≤3 kg), was also slightly increased (RR = 1.2; 95% CI = 0.9–1.5). This latter effect was primarily due to an increased incidence of tumors larger than 2 cm at diagnosis (RR = 1.4; 95% CI = 0.9–1.9). Our findings are compatible with the hypothesis that the hormonal level during pregnancy influences the risk of breast cancer in the early years after delivery. (Epidemiology 1999;10:441–444)

Keywords: breast neoplasm, birth weight, multiple births, gender of offspring, population-based, Denmark, incidence.

Hormonal levels during pregnancy may influence the maternal risk of breast cancer.[1] We investigated this hypothesis by studying the association between certain birth characteristics of the latest offspring and the subsequent maternal risk of breast cancer. The birth characteristics studied (birth weight, gender of offspring, and multiple births) are related to the hormonal level during pregnancy.[2-8]

Materials and Methods

Since April 1, 1968, the Civil Registration System (CRS) in Denmark has assigned a unique registration number to all citizens, thereby facilitating accurate linkage of registries. We obtained information on dates and gender of live births, emigration, and vital status from the CRS. We also obtained, from the National Birth Registry, information on the dates and genders of stillbirths and on the gestational age (in weeks) and birth weight (in groups of 250 g) for all births since 1973. To identify multiple pregnancies, we looked for children (live or stillbirths) born to the same mother within 2 days.

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We identified invasive primary breast cancers through the registry of the Danish Breast Cancer Cooperative Group (DBCG).[9,10] Since 1978, this registry has collected detailed information on breast cancer diagnoses including the size of the tumor, number of positive nodes, receptor status, histology, localization, and laterality. The DBCG's registry was found to contain information on 94% of all breast cancer patients reported to the Danish Cancer Registry, which has nearly complete registration of all incident cases of malignant neoplasms diagnosed in Denmark since 1943.[11]

We established a research parity database from the CRS that included all women born between April 1, 1935 and March 31, 1978.[12,13] We then linked this database with the DBCG to obtain information on registered invasive primary breast cancers in the period from January 1, 1978 to September 30, 1994.

We investigated the possible impact of the plurality, birth weight, and gender of the latest offspring on the subsequent incidence of maternal breast cancer using a follow-up study. Data was analyzed by log-linear Poisson regression models.[14] All parous women entered the follow-up for breast cancer on January 1, 1978, or on the date of their first childbirth, whichever came later. The period at risk continued until whichever occurred first: breast cancer, death, emigration, or the date of September 30, 1994. Adjustment was made for attained age (≤25, 26, 27,......, 56, 57, 58), calendar period (1978–1982, 1983–1988, 1989–1992, 1993–1994), age at first birth (<20, 20–24, 25–29, 30–34, ≥35), and number of births (1, 2, 3, 4, 5, 6, 7+). We have previously shown that mothers with an extremely preterm birth as the latest birth have an increased risk of breast cancer.[15] We therefore adjusted for extreme preterm

birth (<32 weeks, ≥32 weeks, unknown). In tumor-sized specific analyses we categorized number of births (1, 2, 3, 4+) and adjusted for age by quadratic splines (with knots: 30, 35, 40, 45, 50, 55).[16] All variables were treated as time-dependent variables. We calculated numbers of person-years at risk for birth characteristic groups according to birth characteristics of the latest birth, as the focus was the effect in the early years after delivery. Women with more than one birth were, in the period between the first and the second birth, considered at risk according to the characteristics of the first birth; between the second and the third birth, they were considered at risk according to the characteristics of the second birth; and so on. In the analysis of gender and birth weight of offspring, we excluded from follow-up the observation periods when the latest birth was a multiple one.

Results

There were 9,495 incident cases of breast cancer among 998,499 women, 13 to 48 years of age at entry, during the 12.8 million person-years of follow-up.

Table 1 presents the association between birth characteristics of a woman's latest birth and her risk of breast cancer according to the time interval since the birth. The risk of breast cancer was higher in the first 5 years after a multiple, vs a singleton, birth (RR = 1.8; 95% CI = 1.1–2.8). The higher risk was seen in both uniparous (RR = 1.9; 95% CI = 0.8–4.6) and multiparous (RR = 1.7; 95% CI = 1.0–3.0) mothers. After 5 years there was no appreciably increased risk (RR = 1.1; 95% CI = 0.9–1.3). Mothers delivering a heavy-weighted child (>3.75 kg) subsequently had a higher risk of breast cancer compared with mothers delivering a small child (≤3 kg) (RR = 1.1; 95% CI = 1.0–1.2). The higher risk of breast cancer in these women was primarily seen the first 5 years after a birth (RR = 1.2; 95% CI = 0.9–1.5). The relative risks were 0.9 (95% CI = 0.6–1.5) and 1.3 (95% CI = 1.0–1.7) in uniparous and multiparous,

respectively. After the 5-year period, there was a smaller increased risk in mothers delivering a heavy-weighted child (RR = 1.1; 95% CI = 1.0–1.2). Mothers delivering a child with a birth weight from 3.75 up to 4 kg and >4 kg, respectively, had a 10% overall higher risk compared with mothers with a newborn of 3 kg or less (data not shown). There was no difference in the breast cancer incidence according to gender of the child (Table 1).

Additional information on the characteristics of the breast cancer at diagnosis and the large number of cases in each birth weight category allowed us to estimate the risk, according to birth weight of latest offspring, by tumor size (Table 2). The overall increase in risk during the first 5 years after a birth in mothers delivering a heavy-weighted child (>3.75 kg) was primarily due to an increase in larger tumors (>2 cm) (RR = 1.4; 95% CI = 0.9–1.9). The effect on small tumors (≤2 cm) was less pronounced (RR = 1.2; 95% CI = 0.9–1.7). The effect of birth weight of offspring in the first 5 years after the birth was seen primarily on the incidence on estrogen negative (RR = 1.6; 95% CI = 0.9–2.8) compared with estrogen positive tumors (RR = 0.8; 95% CI = 0.5–1.3).

Using year of birth instead of calendar period had no effect on the results in Tables 1 and 2. No residual confounding was revealed by adjustment with a main effect of time since latest birth categorized more than "<5 years" and "≥5 years".

Discussion

The present study examined whether hormone-associated birth characteristics of offspring are related to the maternal risk of breast cancer in the early years after a birth. We found an increased risk of breast cancer in mothers with multiple births or heavy-weighted newborn children in the first 5 years after the birth, whereas the associations diminished in subsequent years. Owing to our prospective study design, it is unlikely that these results are subject to selection bias or differential misclassification.

TABLE 1. Adjusted* Effect of Birth Characteristics of Latest Offspring on the Maternal Risk of Breast Cancer Overall and According to Time Since Latest Birth

Birth Characteristics of the Latest Offspring	Person-years	Rate Ratio Overall		Rate Ratio According to Time Since Latest Birth			
				<5 years		≥5 years	
		No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)
Multiple birth							
No†	12,592 × 10³	9,327	1	663	1	8,664	1
Yes	185 × 10³	168	1.1 (1.0–1.3)	18	1.8 (1.1–2.8)	150	1.1 (0.9–1.3)
Birth weight‡,§							
≤3 kg†	1,617 × 10³	739	1	115	1	624	1
3–3.25 kg	1,241 × 10³	560	1.0 (0.9–1.1)	78	0.9 (0.7–1.2)	482	1.0 (0.9–1.1)
3.25–3.5 kg	1,610 × 10³	758	1.0 (0.9–1.1)	130	1.1 (0.9–1.5)	628	1.0 (0.9–1.1)
3.5–3.75 kg	1,388 × 10³	666	1.0 (0.9–1.1)	116	1.1 (0.9–1.4)	550	1.0 (0.9–1.1)
>3.75 kg	2,063 × 10³	1151	1.1 (1.0–1.2)	198	1.2 (0.9–1.5)	953	1.1 (1.0–1.2)
Gender‡							
Boy†	6,422 × 10³	4,786	1	331	1	4,455	1
Girl	6,170 × 10³	4,541	1.0 (1.0–1.0)	332	1.0 (0.9–1.2)	4,209	1.0 (0.9–1.0)

* Adjustment was made for attained age, calendar period, age at first birth, number of births, and extremely preterm birth.
† Referent category.
‡ Only singleton births are included.
§ Only mothers with a birth from 1973 and onward are considered in these analyses.

REPRODUCTIVE RISK FACTORS FOR BREAST CANCER BY RECEPTOR STATUS, HISTOLOGY, LATERALITY AND LOCATION

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It is well established that a woman's reproductive history influences her risk of breast cancer. We examined whether the effect of reproductive history was similar for different sub-types of breast cancer. The study was based on a population-based cohort of 1.5 million Danish women born between 1935 and 1978, with individual information on births. Between 1978 and 1994, 10,790 incident cases of breast cancer were identified in a nationwide cancer registry, including detailed information on receptor status, histology, laterality and location of the tumour. Overall, the incidence of breast cancer was 13% lower in parous compared with nulliparous women. This reduction was significantly stronger for mucinous than for ductal carcinomas and for tumours located centrally than for those non-central in the breast. Overall, the incidence in parous women increased by 10% by each 5-year postponement of their first birth. For the incidence of lobular carcinomas this increase was significantly stronger, and for mucinous carcinomas it tended to be stronger than for ductal carcinomas. For the incidence of centrally located tumours the increase was stronger than for non-centrally located tumours. On average, there was a 10% decrease in breast-cancer risk by each additional birth. This decrease was seen in most sub-types, but not for lobular carcinomas of for centrally located tumours. According to our findings, lobular and mucinous carcinomas and centrally located tumours may have risk-factor profiles that differ from other types of breast cancer. *Int. J. Cancer* 81:49–55, 1999.
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It is well established that a woman's reproductive history influences her risk of breast cancer (Kelsey *et al.,* 1993), but the mechanisms behind are unknown. Hormonal changes induced by pregnancy could play a role, and, since cells in the breast may respond differently to hormone stimuli, it has been suggested that the effect of reproductive history on the incidence of breast cancer varies by subtypes of breast cancer.

Hitherto, investigations have pursued this idea by examining whether there are differences in the effect of reproductive factors according to oestrogen-receptor (ER) status. The majority have found nulliparity and late age at first birth only to influence the development of ER-positive tumours, but not ER-negative tumours (Habel and Stanford, 1993; Stanford *et al.,* 1986; Yoo *et al.,* 1997; Potter *et al.,* 1995).

We extended this line of pursuit, investigating in more detail the importance not only of ER status, but also of histology, laterality and location of the tumour, using a large population-based cohort of Danish women which was linked to a tumour registry with detailed information on breast-tumour characteristics.

MATERIAL AND METHODS

Population registries

Since April 1, 1968, the Civil Registration System (CRS) in Denmark has assigned an individually unique national registration number to all citizens. This number permits accurate linkage of information from different registries. The Civil Registration System also keeps updated information on dates of live births, emigration and vital status.

In 1977, the Danish Breast-Cancer Cooperative Group (DBCG) started a series of national prospective studies to systematically evaluate breast-cancer treatment programmes. A detailed descrip-

tion of this registry has been given elsewhere (Andersen *et al.,* 1988; Kroman *et al.,* 1997). The DBCG collects detailed information on the breast-cancer diagnosis, including tumor size, nodal status, receptor status, histology, laterality and location. The histological sub-types were categorized according to the WHO classification. The location of a tumour was determined on the basis of an indication, received from the surgical departments, of the location of the tumour on a figure of the four quadrants and the central part of the right and left breasts respectively. When a tumour was located in the borderline between 2 areas, it was assigned to one of the 2 adjacent areas by randomization.

The presence of oestrogen receptors in breast-cancer tissue was determined by quantitative methods (Thorpe, 1988; Thorpe *et al.,* 1986) or by a semi-quantitative method (Andersen *et al.,* 1990). Positive receptor status was defined by a level of receptor ≥ 10 fmol/mg cytosol protein for the quantitative assays and/or by staining of $\geq 10\%$ cells in the semi-quantitative method. Cases considered ER-positive by at least one of the assays were considered as receptor-positive (ER$^+$).

Through a linkage between the DBCG and the Danish Cancer Registry, the DBCG was found to contain information on 94% of all breast-cancer patients reported to the Danish Cancer Registry. The Danish Cancer Registry is considered close to complete regarding incident cases of malignant neoplasms diagnosed in Denmark since 1943 (Storm, 1991).

Study cohort

A research parity database was established from the CRS including all women born between April 1, 1935, and March 31, 1978, as earlier described (Westergaard *et al.,* 1997; Melbye *et al.,* 1997). Based on the person-identifiable CRS number, a linkage was performed with the DBCG giving information on registered invasive primary breast cancers in the period from January 1, 1978, to September 30, 1994.

Statistical analyses

The possible impact of reproductive history on the incidence of different types of breast cancer was investigated in a follow-up study analyzed by log-linear Poisson regression models (Breslow and Day, 1987). All women entered the follow-up for each type of breast cancer on January 1, 1978, or on their 12th birthday, whichever came last. The period at risk continued until a first-time diagnosis of breast cancer (regardless of type), death, emigration, or September 30, 1994, whichever occurred first. Incidence-rate ratios are referred to as relative risks. Adjustment was made for age using quadratic splines (with knots: 30, 35, 40, 45, 50, 55) (Greenland, 1995), calendar period (1978–1982, 1983–1988, 1989–

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1992, 1993–1994), age at first birth (nulliparous, 12–19, 20–24, 25–29, 30–34, ≥35) and parity (nulliparous, 1, 2, 3, 4+). Splines were used in age adjustment in order to reduce the number of parameters in the type-specific analysis. If there was a relatively small number of cases of a specific sub-type, fewer knots were used. All variables were treated as time-dependent. Differences in the association between reproductive history and the incidence of different sub-types were evaluated by competing risks analysis adjusting for type-specific effects of confounders. p values for these tests have indices indicating the sub-types compared. For some of the sub-types, the associations with number of births and age at first birth could not be statistically modelled solely by a log-linear trend. All tests and confidence intervals are therefore based on categorized variables. However, in order to describe the overall trends and ease comparison of sub-types, we have also chosen to give the average risk increase, but without confidence intervals. The average risk increases were estimated with the categorized continuous variables included in the model as continuous variables using the median value within each category as the category score. Traditionally, the risk of breast cancer in nulliparous women is compared with the risk in parous women, disregarding that the parous women have a non-homogeneous risk profile, depending on their reproductive history. To facilitate comparison with other studies, we followed this tradition, but in the notes of the tables we compare the risk in nulliparous women with the more homogeneous group of parous women with only one birth at the age of 20 to 24 years. All calculations were performed using the SAS procedure PROC GENMOD (SAS, 1996).

RESULTS

In total, 1,529,512 women were included in the cohort. Of these, 1,000,276 (65.3%) women had 2,071,415 births before follow-up as follows: 254,694 (25.5%) had one birth, 494,697 (49.5%), two, 193,250 (19.3%) three, and 57,635 (5.6%) four or more births. A total of 10,790 primary invasive breast cancers were detected in this cohort during 22.3 million person-years of follow-up. Number

of cases according to reproductive history, average age at diagnosis, percentage ER-positive and percentage of tumours that were larger than 2 cm are shown for each type of breast cancer in Table I.

Reproductive history and the risk of breast cancer

Compared with nulliparous women, parous women had a 13% (8%–18%) lower risk of breast cancer. In parous women, the risk of breast cancer increased by 10% by each 5-year postponement of the first birth [age (years) at first birth: 12–19: 0.99 (0.93–1.05), 20–24: 1 (ref), 25–29: 1.19 (1.13–1.24), 30–34 1.27 (1.17–1.37), 35+: 1.33 (1.14–1.55)], and there was a 10% decrease in risk by each additional birth: 1 childbirth: 1 (ref), 2 childbirths: 0.97 (0.92–1.02), 3 childbirths: 0.88 (0.82–0.94), 4+ childbirths: 0.70 (0.63–0.77). The association with reproductive history was not significantly modified by age. In women under 45 years of age, parous women had a significant 10% reduced risk compared with nulliparous, on average an 8% decreased risk per each additional birth, and an 11% increased risk per each 5-year postponement of the first birth. In women 45 years of age or more, parous women had an 18% reduced risk compared with nulliparous, on average a 12% decreased risk per each additional birth and a 9% increased risk per 5-year postponement of the first birth.

Reproductive history and receptor status

ER status was available on 6,044 (56%) cases. Of these, 68% were ER-positive (ER+), with an average age of 46.5 years at time of diagnosis, whereas patients with ER-negative (ER−) tumours were on average 45.0 years at diagnosis (Table I).

Table II shows the association between reproductive history and the incidence of ER− and ER+ tumours. Parous women had a 13% (0%–24%) lower risk of an ER− tumour compared with nulliparous women and on average a 10% decreased risk by each additional birth. The woman's age at first birth was not significantly associated with her risk of developing ER− tumours.

Compared with nulliparous women, parous women had a 24% (17%–31%) lower risk of developing an ER+ tumour. The risk

TABLE I – NUMBER OF CASES ACCORDING TO NUMBER OF BIRTHS, AGE AT FIRST BIRTH, AVERAGE AGE AT DIAGNOSIS OF BREAST CANCER, PERCENTAGE OF OESTROGEN-RECEPTOR POSITIVE ER+ AND PERCENTAGE OF TUMOURS LARGER THAN 2 CM BY SUB-TYPE

	Total	Number of childbirths					Age at first birth					Age at diagnosis Mean (years)	ER+ (%)	Tumor size >2 cm (%)
		0	1	2	3	4+	12–19	20–24	25–29	30–34	35+			
Total	10,790	1,295	1,910	4,892	2,112	581	1,472	4,437	2,693	710	183	44.6	68	44
ER status														
negative	1,910 (18%)	231	330	908	340	101	273	794	456	132	24	45.0	0	53
positive	4,134 (38%)	530	732	1,812	840	220	560	1,652	1,038	264	90	46.5	100	45
missing[1]	4,746 (44%)	534	848	2,172	932	260	639	1,991	1,191	314	69	42.9	—	39
Histology														
ductal	8,669 (80%)	1,039	1,528	3,945	1,700	457	1,211	3,561	2,142	564	152	44.6	69	44
lobular	963 (9%)	92	160	458	185	68	108	403	276	68	16	46.1	85	48
mucinous	143 (1%)	34	27	52	28	2	10	51	35	10	3	44.8	74	44
medullary	294 (2%)	27	64	138	49	16	51	116	75	22	3	42.9	20	50
papillary	24 (<1%)	1	8	8	6	1	4	11	6	2	0	42.6	63	47
tubular	187 (2%)	22	35	82	42	6	28	78	38	19	2	45.6	83	11
other	207 (2%)	31	37	90	33	16	28	94	43	8	3	42.6	38	52
missing	303 (3%)	49	51	119	69	15	32	123	78	17	4	45.6	64	43
Laterality														
left	5,153 (48%)	612	880	2,374	1010	277	735	2,089	1,310	314	93	44.6	68	44
right	5,088 (47%)	597	933	2,276	1001	281	661	2,119	1,264	364	83	44.6	69	44
bilateral/missing	549 (5%)	86	97	242	101	23	76	229	119	32	7	45.5	69	47
Location														
central	586 (5%)	93	99	236	119	39	72	209	153	42	17	45.4	66	71
non-central	9,655 (90%)	1,116	1,714	4,414	1,892	519	1,324	3,999	2,421	636	159	44.6	69	42
upper lateral	5,643 (52%)	649	1,002	2,579	1,116	297	786	2,335	1,399	375	99	44.6	68	45
lower lateral	1,447 (14%)	165	241	679	283	79	187	617	369	88	21	44.8	71	38
upper medial	1,888 (18%)	222	335	844	385	102	267	772	471	124	32	44.5	69	39
lower medial	677 (6%)	80	136	312	108	41	84	275	182	49	7	44.1	65	36
bilateral/missing	549 (5%)	86	97	242	101	23	76	229	119	32	7	45.5	69	47

[1]The relatively low mean age in the missing category is due to the fact that receptor status was not measured routinely in the earlier programmes of DBCG, *i.e.*, the differences disappear when stratifying by calendar period.

Risk factors	ER$^+$ RR (95% CI)	ER$^-$ RR (95% CI)	Test for: ER$^+$ = ER$^-$
Parous[2]			
no	1	1	$p = 0.09$
yes	0.76 (0.69–0.83) $p < 0.0001$	0.87 (0.76–1.00) $p = 0.06$	
Number of childbirths			
1	1	1	$p = 0.09$
2	0.92 (0.84–1.01)	1.02 (0.89–1.16)	
3	0.89 (0.80–0.99)	0.81 (0.69–0.95)	
4+	0.66 (0.56–0.77) $p < 0.0001$	0.70 (0.55–0.88) $p < 0.0001$	
Risk decrease per birth	12%	10%	
Age at first birth			
12–19	1.01 (0.92–1.12)	1.02 (0.89–1.18)	$p = 0.07$
20–24	1	1	
25–29	1.23 (1.14–1.34)	1.09 (0.97–1.23)	
30–34	1.25 (1.10–1.43)	1.26 (1.04–1.52)	
35+	1.63 (1.31–2.03) $p < 0.0001$	0.93 (0.62–1.41) $p = 0.15$	
Risk increase per 5 years	12%	4%	

[1]Adjusted for type-specific effects of age, calendar period, parity and age at first birth.–[2]RR for uniparous with a childbirth at age 20–24 *vs.* nulliparous: ER$^+$, 0.77 (0.68–0.87); ER$^-$, 0.89 (0.74–1.06).–95% CI, 95% confidence interval.

decreased on average by 12% by each additional birth, but was 12% higher by each 5-year increase in age of the woman at her first birth. The association between reproductive history and the incidence of ER$^+$ tumours was not statistically different from the association with the incidence of ER$^-$ tumours, although, especially, late age at first birth tended to be more strongly related to the risk of ER$^+$ tumours (12% increase compared with 4%, $p_{ER^+ \, vs. \, ER^-} = 0.07$).

The pattern was the same when restricted to women under 45 years of age and women aged 45 years or more. In women under 45 years of age, the risk of ER$^+$ and ER$^-$ tumours decreased by 6% and 5%, respectively, by each additional birth ($p_{ER^+ \, vs. \, ER^-} = 0.81$), but was 17% and 8% higher by each 5-year increase in age of the woman at her first birth ($p_{ER^+ \, vs. \, ER^-} = 0.17$). In women aged 45 years or more, the risk of ER$^+$ and ER$^-$ tumors decreased by 11% and 17% by each additional birth ($p_{ER^+ \, vs. \, ER^-} = 0.17$), but was 10% and 2% higher by each 5-year increase in age of the woman at her first birth ($p_{ER^+ \, vs. \, ER^-} = 0.11$).

Reproductive history and histological sub-type

Patients diagnosed with ductal carcinomas averaged 44.6 years at diagnosis, compared with 46.1 years in patients diagnosed with lobular carcinomas (Table I).

Table III shows the association between parous status, number of births, age at first birth and the incidence of 6 histological sub-types. Since more than 80% of the tumours were ductal carcinomas, the association between reproductive history and the incidence of this sub-type was, as expected, almost identical to the association with the overall incidence of breast cancer. The incidence was 14% lower in parous than in nulliparous women; the risk decreased on average by 11% by each additional birth, and increased by 9% by each 5-year postponement of the first birth (Table III).

The incidence of lobular carcinomas followed a different pattern (Table III). There was no association with parous status or number of births. However, each 5-year post-ponement of the first birth increased the risk on average by 22%. The association between parous status ($p_{lobular \, vs. \, ductal} = 0.10$), number of births ($p_{lobular \, vs. \, ductal} = 0.09$) and the risk of lobular carcinoma was not significantly

different from the association with the incidence of ductal carcinomas, but age at first birth was found to have a significantly stronger association with the incidence of lobular carcinomas compared with ductal carcinomas ($p_{lobular \, vs. \, ductal} = 0.01$).

The risk of developing a mucinous carcinoma was 64% (47%–76%) lower in parous than in nulliparous women. There was no significant association with number of births, but a tendency towards an association with late age at first birth, with a 29% increased risk by each 5-year postponement of first birth ($p = 0.06$). As compared with the association with incidence of ductal carcinomas, the association with parous status was significantly stronger ($p_{mucinous \, vs. \, ductal} < 0.001$), whereas the association with number of births ($p_{mucinous \, vs. \, ductal} = 0.58$) and age at first birth ($p_{mucinous \, vs. \, ductal} = 0.22$) were similar.

The incidence of medullar, papillary and tubular carcinomas was not significantly related to reproductive history (Table III). The lack of association may, however, be due to low statistical power due to the small number of these types. This is further supported by the fact that the associations were statistically similar to the association between reproductive history and the incidence of ductal carcinomas.

Reproductive history and laterality

The DBCG registered 10,241 (95%) cases as unilateral breast cancer (Table I). Of these 5,153 (50.3%) were left-sided and 5,088 (49.7%) were right-sided, *i.e.*, there was a left-to-right ratio of 1.01 (0.97–1.05). In patients younger than 45 years of age, the left-to-right ratio was 1.00 (0.96–1.09) and 1.02 (0.96–1.09) in nulliparous and parous women respectively. Similar figures for patients aged 45 years or older were 1.00 (0.94–1.06), and 1.06 (0.90–1.24). Tumour size, ER status and age at diagnosis were not related to laterality (Table I).

As shown in Table IV, the association between parous status and the incidence of left-sided breast cancer was 0.87 (0.80–0.94), and that of right-sided breast cancer, 0.88 (0.80–0.96). Similarly, there was the same association between the incidence of left- and right-sided tumours by number of births (10% decrease in risk per birth) and age at first birth (12% and 9% increase per 5-year respectively). This pattern was the same when analysis was restricted to women younger or older than 45 years of age respectively (data not shown).

Reproductive history and location

Patients with a tumour located non-centrally in the breast were on average 44.6 years old at diagnosis, whereas patients with a tumour located centrally in the breast were 45.4 years old at diagnosis (Table I).

The association between reproductive history and the incidence of breast cancer according to location in the breast is shown in Table V. The incidence of tumours in the 4 non-central parts of the breast (upper lateral, lower lateral, upper medial, lower medial) was statistically similarly related to reproductive history, and the 4 non-central sites are therefore considered together in the following. The risk of a tumour in the non-central part of the breast was 10% lower for parous than for nulliparous women. On average, the risk decreased by 10% per each additional birth and increased by 9% per 5-year postponement of the first birth (Table V). The incidence of tumours located centrally in the breast was 41% lower in parous than in nulliparous women. There was no significant association with number of births. On average, the risk increased by 30% by each 5-year postponement of the first birth. (Table V). In comparison with associations with non-central tumours, the incidence of central tumours was significantly more strongly associated with nulliparity ($p_{central \, vs. \, non-central} = 0.003$) and age at first birth ($p_{central \, vs. \, non-central} = 0.02$).

Paget's disease in the nipple was registered in 2% of the cases, but in centrally located tumours the prevalence was 7%. The association between reproductive history and the incidence of centrally located tumours was not altered when cases with Paget's

TABLE III – ADJUSTED[1] RELATIVE RISK (RR) OF BREAST CANCER BY HISTOLOGICAL TYPE

Risk factor	Ductal RR (95% CI)	Lobular RR (95% CI)	Mucinous RR (95% CI)	Medullary RR (95% CI)	Papillary RR (95% CI)	Tubular RR (95% CI)
Parous[2]						
no	1	1	1	1	1	1
yes	0.86 (0.80–0.91)	1.03 (0.83–1.28)	0.36 (0.24–0.53)	1.32 (0.88–1.97)	2.76 (0.37–20.7)	0.80 (0.51–1.25)
	$p < 0.0001$	$p = 0.80$	$p < 0.0001$	$p = 0.17$	$p = 0.25$	$p = 0.34$
Number of childbirths						
1	1	1	1	1	1	1
2	0.97 (0.91–1.03)	1.07 (0.89–1.29)	0.75 (0.46–1.22)	0.84 (0.62–1.15)	0.34 (0.12–0.95)	0.89 (0.59–1.34)
3+	0.82 (0.76–0.88)[3]	0.94 (0.76–1.17)[4]	0.73 (0.42–1.29)	0.63 (0.43–0.92)	0.42 (0.14–1.27)	0.78 (0.48–1.25)
	$p < 0.0001$	$p = 0.26$	$p = 0.48$	$p = 0.05$	$p = 0.13$	$p = 0.57$
Risk decrease per birth	11%	3%	18%	18%	34%	17%
Age at first birth						
12–19	1.00 (0.94–1.07)	0.79 (0.64–0.98)	0.57 (0.29–1.13)	1.32 (0.94–1.84)	1.08 (0.34–3.43)	1.05 (0.68–1.62)
20–24	1	1	1	1	1	1
25–29	1.18 (1.11–1.24)	1.38 (1.18–1.61)	1.34 (0.86–2.08)	1.18 (0.88–1.59)	0.99 (0.36–2.73)	0.96 (0.65–1.42)
30+	1.27 (1.14–1.38)[5]	1.39 (1.09–1.78)[6]	1.51 (0.79–2.89)	1.24 (0.79–1.96)	0.81 (0.17–3.95)	1.64 (0.98–2.74)
	$p < 0.0001$	$p < 0.0001$	$p = 0.06$	$p = 0.36$	$p = 0.99$	$p = 0.29$
Risk increase per 5 year	9%	22%	29%	0%	−9%	6%

[1]Adjusted for type-specific effects of age, calendar period, parity and age at first birth.–[2]RR for uniparous with a childbirth at age 20–24 vs. nulliparous: ductal, 0.87 (0.79–0.94), lobular, 0.93 (0.70–1.22); mucinous, 0.43 (0.24–0.77); medullary, 1.44 (0.88–2.34); papillary, 5.48 (0.63–47.3); tubular, 0.88 (0.49–1.58).–[3]3 births: 0.87 (0.81–0.94), 4+ births: 0.67 (0.60–0.74).–[4]3 births: 0.92 (0.74–1.16), 4+ births: 1.00 (0.74–1.35).–[5]30–34 years: 1.25 (1.14–1.37), 35+ years: 1.36 (1.15–1.61).–[6]30–34 years: 1.41 (1.08–1.84), 35+ years: 1.31 (0.78–2.19).–95% CI, 95% confidence interval.

TABLE IV – ADJUSTED[1] RELATIVE RISK (RR) OF BREAST CANCER BY LATERALITY[2]

Risk factor	Left side RR (95% CI)	Right side RR (95% CI)	Test for left = right
Parous[3]			
no	1	1	$p = 0.85$
yes	0.87 (0.80–0.94)	0.88 (0.80–0.96)	
	$p = 0.001$	$p = 0.004$	
Number of childbirths			
1	1	1	$p = 0.32$
2	1.01 (0.93–1.10)	0.92 (0.85–0.99)	
3	0.90 (0.82–0.99)	0.85 (0.77–0.94)	
4+	0.70 (0.61–0.81)	0.69 (0.60–0.80)	
	$p < 0.0001$	$p < 0.0001$	
Risk decrease per birth	10%	10%	
Age at first birth			
12–19	1.06 (0.97–1.15)	0.93 (0.85–1.01)	$p = 0.06$
20–24	1	1	
25–29	1.23 (1.15–1.32)	1.17 (1.09–1.25)	
30–34	1.20 (1.06–1.36)	1.35 (1.20–1.51)	
35+	1.46 (1.18–1.81)	1.22 (0.97–1.53)	
	$p < 0.0001$	$p < 0.0001$	
Risk increase per 5 years	12%	9%	

[1]Adjusted for type-specific effects of age, calendar period, parity and age at first birth.–[2]Bilateral cases excluded.–[3]RR for uniparous with a childbirth at age 20–24 vs. nulliparous: left, 0.83 (0.74–0.93); right, 0.92 (0.83–1.03). 95% CI, 95% confidence interval.

disease in the nipple were excluded (67% lower risk in parous than in nulliparous, 0% risk decrease per additional birth, 30% increased risk per 5-year post-ponement of the first birth).

Reproductive history and combinations of ER status, histology and location

ER status, histology and location of a tumour are correlated, and the strong associations between late age at first birth and the incidence of ER+ tumours, lobular and mucinous carcinomas and centrally located tumours may therefore be an expression of the same phenomenon. To investigate this further, we focused on correlated sub-types (e.g., ER+ and lobular carcinoma) and ana-lyzed the association between age at first birth and the incidence of combinations of these sub-types.

The percentage of ER+ tumours for each of the described sub-types is shown in Table I. Neither centrally located tumours nor mucinous carcinomas were significantly associated with ER status, whereas lobular carcinomas were more frequently ER+ than ductal

carcinomas [85% (465/546) vs. 68% (3,443/5,027), $p < 0.001$]. We therefore looked at the association between late age at first birth and the incidence of lobular carcinomas according to ER status. When only ER− tumours were included, there was no difference in the association between age at first birth and the incidence of lobular carcinomas (6% increase per 5 years) compared with ductal carcinomas (4% increase per 5 years). In contrast, when only ER+ tumours were considered, the stronger association with lobular carcinomas (26% per 5 years) as compared with ductal carcinomas (10% per 5-year) appeared again. The stronger association between age at first birth and ER+ tumours was seen both in lobular and in non-lobular carcinomas.

There was no essential association between lobular or mucinous carcinoma and location in the central part of the breast.

We found that late age at first birth strongly affected, especially, the incidence of late-stage cases as measured by tumour size (data not shown). As shown in Table I, neither lobular carcinomas nor ER+ tumours were markedly larger at diagnosis, as compared with

TABLE V – ADJUSTED[1] RELATIVE RISK (RR) OF BREAST CANCER BY LOCATION[2]

| Risk factor | Non-central | | | | | Central | Test for: |
	Upper lateral RR (95% CI)	Lower lateral RR (95% CI)	Upper medial RR (95% CI)	Lower medial RR (95% CI)	Total[3] RR (95% CI)	Total RR (95% CI)	non-central = central
Parous[4]							
no	1	1	1	1	1	1	$p = 0.003$
yes	0.90 (0.83–0.98)	0.90 (0.76–1.06)	0.88 (0.76–1.01)	0.88 (0.70–1.12)	0.90 (0.84–0.96)	0.59 (0.47–0.73)	
	$p = 0.02$	$p = 0.19$	$p = 0.08$	$p = 0.32$	$p = 0.001$	$p < 0.0001$	
Number of child-births							
1	1	1	1	1	1	1	$p = 0.10$
2	0.97 (0.90–1.05)	1.03 (0.88–1.20)	0.95 (0.83–1.08)	0.85 (0.69–1.05)	0.96 (0.91–1.02)	0.98 (0.76–1.25)	
3	0.88 (0.80–0.96)	0.89 (0.74–1.07)	0.90 (0.77–1.06)	0.63 (0.49–0.83)	0.86 (0.81–0.93)	1.07 (0.80–1.43)	
4+	0.67 (0.58–0.76)	0.72 (0.55–0.94)	0.68 (0.54–0.86)	0.71 (0.49–1.03)	0.68 (0.61–0.75)	1.02 (0.69–1.52)	
	$p < 0.001$	$p = 0.01$	$p = 0.007$	$p = 0.006$	$p < 0.0001$	$p = 0.89$	
Risk decrease per birth	10%	9%	9%	16%	10%	−2%	
Age at first birth							
12–19	1.01 (0.93–1.09)	1.01 (0.93–1.09)	1.04 (0.90–1.19)	0.91 (0.71–1.16)	0.99 (0.93–1.06)	0.99 (0.75–1.30)	$p = 0.02$
20–24	1	1	1	1	1	1	
25–29	1.17 (1.09–1.25)	1.17 (1.03–1.34)	1.20 (1.07–1.35)	1.25 (1.03–1.51)	1.18 (1.12–1.24)	1.51 (1.22–1.87)	
30–34	1.27 (1.14–1.42)	1.14 (0.90–1.44)	1.28 (1.05–1.55)	1.31 (0.95–1.79)	1.25 (1.15–1.37)	1.71 (1.21–2.42)	
35+	1.35 (1.10–1.67)	1.11 (0.71–1.74)	1.35 (0.94–1.94)	0.73 (0.34–1.56)	1.27 (1.08–1.49)	2.78 (1.65–4.67)	
	$p < 0.0001$	$p = 0.053$	$p = 0.01$	$p = 0.04$	$p < 0.001$	$p < 0.0001$	
Risk increase per 5 years	9%	9%	9%	11%	9%	30%	

[1]Adjusted for type-specific effects of age, calendar period, parity and age at first birth.–[2]Bilateral cases excluded.–[3]Associations between reproductive history and incidence of the 4 non-central locations were identical (parous status, $p = 0.99$; number of childbirths, $p = 0.47$; age at first birth, $p = 0.87$).–[4]RR for uniparous with a childbirth at age 20–24 vs. nulliparous: non-central, 0.91 (0.84–0.99); central, 0.49 (0.35–0.68).–95% CI, 95% confidence interval.

ductal carcinomas and ER[−] tumours respectively. Tumours located in the central part of the breast, however, were significantly larger at diagnosis than non-central tumours [71% (375/525) vs. 42% (3,822/9,052), $p < 0.001$]. We therefore looked at the association between age at first birth and the incidence of centrally located tumours according to tumour size. In an analysis including only tumours with a size of 2 cm or less, we found the risk of centrally located tumours to increase by 11% per 5-year postponement of the first birth, as compared with 5% in non-central tumours. Including only tumours whose size was more than 2 cm, we found the risk of centrally located tumours to increase by 44%, as compared with 15% per 5 years in non-central tumours. In other words: the increase in risk per 5 years in central compared with non-central tumours is 2.2-fold (=11%/5%) and 2.9-fold (=44%/15%) higher in analyses in which tumour size is taken into account, as compared with the 3.3 (=30%/9%) in the overall analysis, as seen in Table V. Thus, less than ⅓ of the difference in effect of late age at first birth according to location can be explained by differences in tumour size.

DISCUSSION

In this study we looked at the association between reproductive history and the incidence of sub-types of breast cancer according to oestrogen-receptor (ER) status, histology, laterality and location. The study was performed as a prospective analysis on a large population-based cohort, and was based on mandatory reported exposure and outcome information, making information bias on exposure and selection bias on cases unlikely. The estimated effects of reproductive history for each sub-type were adjusted for sub-type-specific age and calendar effects, thus taking into account differential age profiles and secular trends in the diagnosis of the sub-types. The large number of cases, furthermore, allowed us to study the incidence of combinations of sub-types, to evaluate whether differences in the associations between reproductive history and sub-type were independent.

Reproductive history and receptor status

Earlier studies on reproductive risk factors for sub-types of breast cancer, have focused mainly on ER status. Most studies have found nulliparity and late age at first birth to be risk factors for ER[+] tumours only, whereas studies on the effect of additional births have revealed fewer differences (Habel and Stanford, 1993; Stanford et al., 1986; Yoo et al., 1997; Potter et al., 1995). Our finding is in concordance with this, and, in particular, we confirm that late age at first birth affects only the incidence of ER[+] tumours. The pattern was not modified by age, therefore probably not by menopausal status.

There has been discussion as to whether ER status reflects different types of breast cancer or rather different stages in the neoplastic process, with ER[+] tumours gradually becoming ER[−] (Habel and Stanford, 1993). Differences in the association with reproductive history would reflect different risk factors for the various sub-types and, in the latter case, different progression factors between the different stages.

Our analysis cannot differentiate between these 2 interpretations. However, if ER status reflects different types of breast cancer, our finding of a significant association between the incidence of ER[+] tumours and both nulliparity and late age at first birth (i.e., high risk of initiation of a tumour coinciding with nulliparity) would be compatible with the hypothesis that the higher level of oestrogen in nulliparous women can stimulate initiation and promotion of breast tumours.

Reproductive history and histological sub-type

Studies on the association between reproductive history and breast cancer according to histological sub-type have been limited and the results inconsistent (Mausner et al., 1969; Morrison, 1976; LiVolsi et al., 1982; Rosen et al., 1982; Kvåle et al., 1987; Ewertz and Duffy, 1988; Stalsberg et al., 1989; Claus et al., 1993). According to 2 of these studies (LiVolsi et al., 1982; Stalsberg et al., 1989), age at first birth had a stronger effect on (or even restricted to) lobular carcinomas as compared with ductal carcinomas, but this is not supported by 2 other studies (Ewertz and Duffy, 1988; Claus et al., 1993). Our cohort study supported the repeated finding of a significantly stronger effect, but found no evidence of the effect of age at first birth being restricted to lobular carcinomas. Our finding of a stronger association supports the theory according to which additional carcinomas occurring in women with late age at

first birth originate in the lobules rather than ducts by selectively increasing the number of lobular cells at risk (Stalsberg *et al.*, 1989). But higher hormonal sensitivity in the cells from which lobular carcinomas originate may also play a role, since we observed the strong association to be limited to ER^+ lobular carcinomas.

We found the incidence of mucinous carcinomas in parous women to be 36% (24%–53%) of the incidence in nulliparous women. The association is significantly stronger than the association with the incidence of ductal carcinomas, and cannot be explained by differences in ER status or in tumour size. The finding is in line with Stalsberg *et al.* (1989), who observed the incidence of mucinous carcinomas in ever pregnant women to be only 30% of the incidence in never pregnant women ($p < 0.01$). We found a tendency towards a stronger association with age at first birth on the incidence of mucinous carcinomas, which has not been reported.

It should be noted that the present study comprises only patients in the DBCG with available information on reproductive history from the national registries, *i.e.*, women born in 1935 or later, average age at diagnosis being therefore only 44.6 years. This implies that there is a relatively low proportion of lobular and tubular carcinomas, in comparison with other settings, since these tumours are usually diagnosed later in life. For the same reason, the proportion of medullar carcinomas is higher, since these are diagnosed at a relatively early age. However, adjustment for sub-type-specific age effects in all analyses means that there is no bias.

Reproductive history and laterality

It has become a general belief that the incidence of left-sided breast cancer is higher than that of right-sided breast cancers (Weiss *et al.*, 1996): 2 case studies have found a relation between nulliparity and the left-to-right ratio, the study by Ekbom *et al.* (1994) reporting that nulliparous women under 45 years had right dominance, whereas Senie *et al.* (1980) found left dominance in parous women over 40 years. We found no difference in the association with reproductive history and the incidence of left- *vs.* right-sided breast cancer, either overall or in women under or over 45 years of age. Consequently, our study does not support the hypothesis that left-side dominance can be ascribed to reproductive history.

Reproductive history and location

In our study, parous status and age at first birth were much more closely related to the incidence of centrally located tumours than to that of tumours located non-centrally, and the number of additional births was not associated with the incidence of centrally located tumours. These special associations for centrally located tumours were not related to Paget's disease of the nipple or to a particular proportion of lobular or ER^+ tumours in this area of the breast.

We have found that late age at first birth strongly affects, especially, the incidence of late-stage cases, as measured by tumour size (data not shown). Tumours located in the central part of the breast were significantly larger at diagnosis than non-central tumours, probably because they may be more difficult to detect. However, we found that less than $1/3$ of the difference in the association with age at first birth according to location could be explained by difference in tumour size.

Women with a centrally located tumour were on average older than those with a non-central tumour, and the same pattern was found for lobular *vs.* ductal carcinomas and for ER^+ *vs.* ER^- tumours (Table I). For non-central tumours, for lobular carcinomas and for ER^+ tumours, we observed a stronger association with age at first birth (and, in the first 2 types, no effect of additional births). A common explanation for these findings could be an effect modification by age or menopausal status, with a stronger association with age at first birth and no association with number of births in older women, and in younger women a smaller association with age at first birth and a strong association with number of births. However, if anything, the literature points in the opposite direction (Velentgas and Daling, 1994), and in this study we found no effect modification by age, *i.e.*, it was in agreement with the literature.

Conclusion

The known negative effects of nulliparity, low number of additional births and late age at first birth were observed in most sub-types of breast cancer. However, there was no association with number of births and the incidence of lobular carcinomas and centrally located tumours. Furthermore, we found particularly strong associations between late age at first birth and the incidence of lobular and mucinous carcinomas and centrally located tumours. This may indicate different risk-factor profiles for these 3 sub-types, perhaps due to greater sensitivity to hormonal stimuli in cells involved in these types of breast cancer.

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Preterm delivery and risk of breast cancer

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Summary To explore the risk of breast cancer in relation to the length of a pregnancy we tested whether a preterm delivery carries a higher risk of breast cancer than does a full-term delivery. Based on information from the Civil Registration System, and the National Birth Registry in Denmark, we established a population-based cohort of 474 156 women born since April 1935, with vital status and detailed parity information, including the gestational age of liveborn children and stillbirths. Information on spontaneous and induced abortions was obtained from the National Hospital Discharge Registry and the National Registry of Induced Abortions. Incident cases of breast cancer in the cohort ($n = 1363$) were identified through linkage with the Danish Cancer Registry. The period at risk started in 1978 and continued until a breast cancer diagnosis, death, emigration, or 31 December, 1992, whichever occurred first. After adjusting for attained age, parity, age at first birth and calendar period, we observed the following relative risks of breast cancer for different lengths of the pregnancy: < 29 gestational weeks = 2.11 (95% confidence interval 1.00–4.45); 29–31 weeks = 2.08 (1.20–3.60); 32–33 weeks = 1.12 (0.62–2.04); 34–35 weeks = 1.08 (0.71–1.66); 36–37 weeks = 1.04 (0.83–1.32); 38–39 weeks = 1.02 (0.89–1.17); 40 weeks = 1 (reference). Parous women who had a preterm delivery below 32 weeks gestation had a 1.72-fold (1.14–2.59) increased risk of breast cancer compared with other parous women. In conclusion, a preterm delivery of 32+ weeks gestation did not significantly increase a woman's risk of contracting breast cancer. Only for the very small group of women with preterm deliveries of less than 32 weeks gestation did we observe an increased risk.

Keywords: breast cancer; reproductive factors; gestational age; preterm; cohort study; population-based

Major hormones influence the development, proliferation and differentiation of the human breast (Rebar, 1994). Based primarily on animal studies, it has been shown that mammary cells proliferate in the first and second trimester of pregnancy and differentiate in the last trimester (Russo and Russo, 1980). This led Russo and Russo to hypothesize that complete differentiation of the breast cells conveyed by a full-term pregnancy has to be achieved to provide protection against carcinogenic effects. Earlier termination of pregnancy, on the contrary, might increase the risk of breast cancer because proliferation of the breast cells will take place without subsequent differentiation (Russo and Russo, 1980).

Breast cancer risk in women with a history of a short-term pregnancy has primarily been investigated in relation to spontaneous and induced abortions (Kvåle et al, 1987; Adami et al, 1990; Daling et al, 1994; Calle et al, 1995; Michels et al, 1995; Newcomb et al, 1996; Melbye et al, 1997) that occur during the early period of pregnancy. In particular, large prospective studies have not found such women to be at increased risk of breast cancer (Kvåle et al, 1987; Calle et al, 1995; Melbye et al, 1997). In contrast, few studies have addressed the late period of pregnancy and whether a preterm delivery is associated with an increased risk of breast cancer (Choi et al, 1978; Polednak and Janerich, 1983).

In the present study we took advantage of the long tradition for mandatory reporting of pregnancy characteristics and cancer diagnoses in Denmark to address in a prospective study whether

women with preterm delivery are at increased risk of breast cancer compared to other women.

MATERIAL AND METHODS

Registries

We performed a linkage of data from the Danish Civil Registration System (CRS) with the National Birth Registry, the National Hospital Discharge Registry, the National Registry of Induced Abortions and the Danish Cancer Registry. Since April 1968, the CRS has assigned a unique identification number to all residents in Denmark which permits accurate linkage of information from different registries. The CRS also keeps updated information on dates of live births and documents demographic information such as emigration and death.

Since 1973 the National Birth Registry has registered all live-births and stillbirths in Denmark (not including spontaneous and induced abortions). Since 1978, exact (in weeks) gestational age determinations have been included. Gestational age determination is based on information of last menstrual period combined with an early clinical bimanual palpation. In situations of inconsistency between these measures, ultrasound scanning is performed. In the most recent years the use of ultrasound scanning has become widespread and has as such contributed increasingly to the determinations of the gestational age (Sundhedsstyrelsen, 1993). Since 1977, information on spontaneous abortions without specified gestational age has been recorded in the National Hospital Discharge Registry. Information on induced abortions has been recorded in the National Registry of Induced Abortions since reporting became mandatory in 1939. However, information is only available in a computerized format since 1973 (Melbye et al,

1997). The Danish Cancer Registry includes a nearly complete registration of cancer diagnoses on all Danish residents back to 1943 (Storm, 1991).

Subjects

A research database was established from the CRS including all women born in Denmark between 1 April 1935 and 31 March 1978, with information on live-born children. From the National Birth Registry additional information on stillbirths was added as was gestational age-specific information on all births since 1978. Finally, information on spontaneous (since 1977) and induced abortions (since 1973) was added.

Analyses

The possible impact of gestational age at delivery (preterm, or term delivery) on the risk of breast cancer was investigated among parous women in a log-linear Poisson regression model (Breslow and Day, 1987). All women entered the follow-up for breast cancer at the first delivery they had during the period between 1 January 1978 and 31 December 1992, in which gestational age was recorded. Thus, women with pregnancies before 1 January 1978 were included in the study provided they had a delivery during the study period. The period at risk continued until breast cancer diagnosis, death, emigration, disappearance, or 31 December 1992 (at which time the cancer registration was considered complete), whichever occurred first. Person-years at risk were calculated continuously according to the categorical groups of gestational age of the most recent birth in the years 1978–1992, i.e. women with more than one birth between 1978 and 1992 were considered at risk in the period between the first and second birth, according to the gestational age of the first birth; between the second and third birth, according to the gestational age of the second birth; and so on. To evaluate the effect of *ever* having a preterm delivery, an additional analysis was performed where person-years at risk were calculated continuously in categorical groups according to the birth with the lowest gestational age since 1978. Adjustments were made for attained age (1-year intervals), calendar period (5-year intervals), age at first birth (12–19, 20–24, 25–29, 30–34, > 34 years) and parity (1, 2, 3, 4, 5, 6, ≥ 7 births; including stillbirths, preterm and term deliveries). In an additional analysis we adjusted for history of spontaneous and induced abortion and whether the birth was a stillbirth or a multiple birth. Note that information on history of spontaneous and induced abortions, stillbirths and live-births prior to 1 January 1978 was also used in the adjustment. Estimation of breast cancer incidence rate ratios was performed using the SAS procedure PROC GENMOD (SAS Institute, 1996). These rate ratios were used as a measure of the relative risk (RR). Test for trend was performed with gestational age treated as a continuous variable and the median gestational age used as the value for each group. The linear assumption in the trend test was checked by a likelihood ratio test against the model with gestational age as categorical variable. Effect modification was evaluated as a test for interaction between categorical variables.

To assess the possible effect of misclassification due to unregistered gestational age in births prior to 1978 we estimated the percentage of person-years of follow-up and the number of cases in each cell that might be attributed to the 'ever had a delivery with a gestational age less than 32 weeks' category, instead of the

'never' category, and then performed the analysis with the adjusted figures. The percentage of person-years was calculated on the basis of the age-specific cumulative incidence at the baseline of the study, and the number of cases was calculated as the product of the estimated person-years and the rate in the ever category found in the original analysis. The age-specific cumulative incidence of having a delivery with a gestational age less than 32 weeks was calculated using age-specific incidence rates seen in 1983–1992.

RESULTS

Overall, 474 156 parous women were included in the cohort study. In the follow-up a total of 740 794 births were recorded and distributed as follows: 254 458 women (53.7%) had one birth, 178 700 women (37.7%) had two, 35 791 women (7.5%) had three and 5207 women (1.1%) had four or more births. Among these births, 3261 were stillbirths (0.4%) and 37 347 (5.0%) were preterm (< 37 gestational weeks). Preterm births with a gestational age of 32–36 weeks contributed 4.2%, with a gestational age of

Table 1 Distribution of number of breast cancer diagnoses and person-years of follow-up according to age and reproductive history

	preterm delivery				Full-term delivery			
	No. of cases	(%)	Person-years (× 10³)	(%)	No. of cases	(%)	Person years (× 10³)	(%)
Age (years)								
< 35	16	(20)	127	(69)	315	(25)	2507	(70)
35–39	31	(38)	35	(19)	417	(32)	714	(20)
40–44	24	(30)	16	(9)	379	(30)	299	(8)
45–49	8	(10)	5	(3)	147	(11)	72	(2)
50+	2	(2)	1	(0.4)	24	(2)	9	(0.2)
Age at first birth (years)								
< 20	9	(11)	30	(17)	93	(7)	464	(13)
20–24	24	(30)	82	(45)	432	(34)	1728	(48)
25–29	27	(33)	52	(28)	501	(39)	1107	(31)
30–34	18	(22)	15	(8)	191	(25)	254	(7)
35+	3	(4)	4	(2)	65	(5)	48	(1)
Age at latest birth (years)								
< 20	0	(0)	8	(4)	1	(0.1)	105	(3)
20–24	1	(1)	47	(26)	54	(4)	874	(24)
25–29	23	(28)	68	(37)	351	(28)	1449	(40)
30–34	29	(36)	41	(22)	513	(40)	872	(24)
35+	28	(35)	20	(11)	363	(28)	300	(9)
Number of previous births[a]								
0	23	(28)	78	(42)	240	(19)	1281	(36)
1	31	(38)	68	(37)	611	(48)	1609	(45)
2	19	(24)	27	(15)	313	(24)	553	(15)
3+	8	(10)	11	(6)	118	(9)	157	(4)
Previous preterm birth or stillbirth[a]								
Yes	5	(6)	12	(7)	17	(1)	60	(2)
No	76	(94)	171	(93)	1265	(99)	3540	(98)
The delivery was a multiple birth								
Yes	9	(11)	16	(9)	20	(2)	35	(1)
No	72	(89)	167	(91)	1262	(98)	3566	(99)

[a] 'Previous' means prior to the most recent pregnancy.

Table 2 Adjusted[a] relative risk of breast cancer in 474 156 parous women according to gestational age at delivery

Gestational age (weeks)	No. of cases	Person-years (× 10³)	RR (95% CI)
< 29	7	9	2.11 (1.00–4.45)
29–31	13	17	2.08 (1.20–3.60)
32–33	11	26	1.12 (0.62–2.04)
34–35	22	58	1.08 (0.71–1.66)
36–37	82	214	1.04 (0.83–1.32)
38–39	350	949	1.02 (0.89–1.17)
40	552	1526	1
> 40	326	985	1.03 (0.90–1.18)

[a]Adjusted for age, calendar period, parity and age at first birth.

29–31 weeks 0.5%, and with a gestational age of less than 29 weeks 0.3%. The number of women with a preterm delivery was as follows: 32–36 weeks = 29 488 women; 29–31 weeks = 3702 women; < 29 weeks = 2181 women. Parous women represented a total of 3.8 million person-years of follow-up and 1363 of these women developed breast cancer. Table 1 presents a detailed distribution of number of breast cancer diagnoses and person-years of follow-up.

As shown in Table 2, we found a significantly increased relative risk of breast cancer in women with a preterm delivery at < 29 gestational weeks of 2.11 (95% confidence intervals (CI) 1.00–4.45) and at 29–31 gestational weeks of 2.08 (1.20–3.60), which subsequently dropped as follows: 32–33 weeks: RR = 1.12 (0.62–2.04); 34–35 weeks: RR = 1.08 (0.71–1.66); 36–37 weeks: RR = 1.04 (0.83–1.32); 38–39 weeks: RR = 1.02 (0.89–1.17),

40 weeks: 1 (reference). The continued decline in RR observed for preterm deliveries was statistically significant (*P*-trend = 0.04). The trend remained significant after adjustment for history of spontaneous abortion, history of induced abortion, and whether the birth was a stillbirth and/or a multiple birth (*P*-trend = 0.04). A stratified analysis, which was performed to evaluate whether the increased risk of breast cancer was associated both with preterm livebirths and preterm stillbirths, gave the following result with term deliveries as reference: life births with gestational age < 32 weeks: RR = 1.98 (1.24–3.16); stillbirths with gestational age < 32 weeks: RR = 4.62 (0.42–50.9).

The possible effect modification by age of the woman, number of previous births, age at delivery and history of previous preterm births or stillbirths is evaluated in Table 3. None of these characteristics significantly modified the risk association observed with gestational age. However, the number of cases in some of the stratified subgroups became very small. We evaluated whether possible temporal changes in the validity and completeness of the ascertainment of the gestational age had a measurable effect on the results by testing whether there was a significant effect modification by period of delivery. This was not the case (*P* = 0.62).

Comparing parous women *ever* having a delivery of less than 32 gestational weeks with other parous women we found a significantly increased risk of 1.72 (1.14–2.59). When we considered only parous women ever having a delivery less than 32 weeks' gestation, but with the most recent delivery being equal to or longer than 32 weeks' gestation, we found no increased risk when comparing with parous women who had never had a delivery of less than 32 gestational weeks (RR = 0.82; 95% CI: 0.26–2.55). However, this result was based on only three cases of breast cancer in this particular group of women.

Based on the age-specific incidence rates of births with a gestational age less than 32 weeks we estimated that less than 2% will ever experience such a delivery. Taking that into account at the baseline of the analysis the rate ratio between parous women ever

Table 3 Adjusted[a] relative risk of breast cancer in parous women according to gestational age at delivery by age, number of previous births, age at delivery and history of preterm births/stillbirths

	Gestational age					
	≥ 37 weeks		36–32 weeks		< 32 weeks	
	No. of cases	RR (ref.)	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)
Age of woman[b]						
< 40 years	732	1	37	1.21 (0.87–1.69)	10	2.00 (1.07–3.74)
≥ 40 years	550	1	24	0.88 (0.58–1.32)	10	2.11 (1.13–3.95)
Number of previous[c] births[d]						
0	240	1	17	1.14 (0.70–1.87)	6	2.41 (1.07–5.42)
1+	1042	1	44	1.03 (0.76–1.39)	14	1.94 (1.14–3.29)
Age at delivery[e]						
< 30 years	406	1	20	1.20 (0.77–1.89)	4	1.62 (0.60–4.33)
≥ 30 years	876	1	41	1.00 (0.73–1.37)	16	2.22 (1.35–3.64)
Previous[e] preterm birth[f] or stillbirth[g]						
No	1265	1	58	1.06 (0.82–1.38)	18	1.97 (1.24–3.14)
Yes	17	1	3	1.02 (0.30–3.49)	2	3.64 (0.84–15.8)

[a]Adjusted for age of the woman, calendar period, parity and age at first birth. [b]Test for effect modification: *P* = 0.47. A similar lack of effect modification (*P* = 0.73) was found if age of woman was divided by age 50 years. [c]'Previous' means prior to the most recent pregnancy. [d]Test for effect modification: *P* = 0.86. [e]Test for effect modification: *P* = 0.67. [f]Pre-term birth: gestational age < 37 weeks. [g]Test for effect modification: *P* = 0.76.

having a delivery less than 32 gestational weeks and other women increased from 1.72 to 1.73.

DISCUSSION

Based on this large cohort of almost half a million parous women we found reassuring evidence that a preterm delivery of 32+ weeks' gestation does not significantly increase the risk of premenopausal breast cancer. Overall, 84% of all preterm deliveries are of 32+ weeks' gestation. Only for the small group of preterm deliveries of less than 32 weeks' gestation was there a twofold increased risk of breast cancer when comparing with a full-term delivery. This elevated relative risk was obtained in an analysis in which a woman's person-years at risk were calculated continuously according to the gestational age of the most recent birth. In an analysis that compared parous women ever having a delivery of less than 32 gestational weeks with other parous women the risk was 1.7-fold increased. In this last analysis, the preterm birth will not necessarily have been the most recent birth, and we speculate whether the somewhat lower estimate could indicate that a full-term birth following a preterm birth might diminish the effect of a preterm birth on breast cancer risk. We found some support for this assumption in a restricted analysis that estimated the risk in parous women ever having a delivery of less than 32 weeks' gestation but with the most recent delivery being of 32+ gestational weeks. However, this particular analysis has very limited power.

The analysis of parous women *ever* having a delivery with a gestational age less than 32 weeks compared with other women might be subject to some misclassification, since many of the included women may have had preterm births prior to 1978. This misclassification, however, is non-differential, and estimating the effect, we found we could ignore it, as only a very small fraction of women categorized as never having a delivery with a gestational age less than 32 weeks in fact had such a birth prior to 1978.

We used a cohort design for our study based on mandatory reported exposure and outcome information. Nonetheless, some limitations of the study should be acknowledged. Our gestational age-specific RR estimates do not follow a smooth curve, but instead increase rather abruptly below 32 weeks' gestation. This might suggest that the elevated risk of breast cancer among women with a very early preterm delivery was a chance finding. However, another explanation would be that the small number of cases with very early preterm deliveries makes it difficult to assess the true magnitude of the effect. In particular, the estimate obtained among women with a preterm delivery of less than 29 weeks was based on only seven cases of breast cancer and 9000 person-years of follow-up. That said, it is important to note that this estimate did not stand alone but was supported by a similarly increased risk for women with a preterm delivery of 29–31 gestational weeks. We were unable to determine whether the observed risk was due to the preterm delivery per se or the shorter duration of pregnancy. The observation that both women with a preterm stillbirth and women with a preterm livebirth (< 32 weeks) had elevated RR of breast cancer would be in support of the latter but these were very few.

The present study allowed us to consider the influence of potentially confounding factors such as age, age at first birth, parity, multiple births, abortion history and history of stillbirths. However, several factors (smoking history, body mass index, age at menarche and menopause, family history, oral contraceptives,

postmenopausal hormones) that have been suspected as risk factors for breast cancer could not be adjusted for because we lacked the necessary information. The lack of adjustment for such factors would only be important for our results should these factors influence both the occurrence of breast cancer and preterm births. Smoking during pregnancy and high pre-pregnant body weight have been linked to preterm births (Naeye, 1990; Williams et al, 1992). However, there is little evidence for an association between smoking and breast cancer (Palmer and Rosenberg, 1993) and the association between high body mass and premenopausal breast cancer is, if anything, inverse (Hunter and Willett, 1993). Other factors that have been associated with preterm births are low social class and low educational level (Pickering and Deeks, 1991). However, breast cancer risk is associated with high social status and thus we would expect the observed relative risks to be underestimated, rather than the opposite.

We are not aware of any previous cohort study addressing the risk of breast cancer according to week of gestation at delivery. In a case-control study, Choi et al (1978) reported an insignificantly 1.4-fold increased risk of breast cancer in premenopausal women who had a terminated pregnancy of more than 5 gestational months compared to women without such experience. Another case-control study focusing on livebirths, with seven women with a delivery of less than 30 weeks, did not find an increased risk among women with preterm deliveries (Polednak and Janerich, 1983). Stillbirth has not been associated with increased risk of breast cancer, but the available studies have been based on a very limited number of cases and lacked information on gestational length of the pregnancy (Brimton et al, 1983; Rao et al, 1994; Calle et al, 1995).

Studies of spontaneous abortion have generally not revealed significantly positive associations (reviewed in Calle et al, 1995). In a recent study by Newcomb et al (1996), a slightly increased risk of breast cancer was recorded, but the authors cautioned that the finding might be due to recall bias in their case-control design. Most spontaneous abortions take place early in pregnancy and studies have so far lacked detailed information on gestational week at the time of the abortion. Spontaneous abortion may in certain ways be more like a preterm delivery than an induced abortion but they both represent an interruption of pregnancy (Zang, 1996). The results of case-control studies on induced abortion have been inconsistent with risk estimates ranging from moderately elevated to lowered values (Rosenberg et al, 1994). In a large prospective study we found no overall increased risk of breast cancer after an induced abortion, with the exception of the very small group of women with a late second trimester abortion (Melbye et al, 1997).

In conclusion, a preterm delivery did not significantly increase a woman's risk of contracting premenopausal breast cancer, apart from the very small group of women with a preterm delivery of less than 32 weeks' gestation. Despite the large size of this study there were only a few cases of breast cancer in the subgroups representing the very early deliveries and these results should therefore be considered with due caution.

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Food groups, oils and butter, and cancer of the oral cavity and pharynx

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Summary To elucidate the role of dietary habits, a study was carried out in 1992–1997 in the province of Pordenone in Northeastern Italy, and those of Rome and Latina in central Italy. Cases were 512 men and 86 women with cancer of the oral cavity and pharynx (lip, salivary glands and nasopharynx excluded) and controls were 1008 men and 483 women who had been admitted to local hospitals for a broad range of acute non-neoplastic conditions. The validated dietary section of the questionnaire included 78 foods or recipes and ten questions on fat intake patterns. After allowance for education, smoking, alcohol and total energy intake, significant trends of increasing risk with increasing intake emerged for soups, eggs, processed meats, cakes and desserts, and butter. Risk was approximately halved in the highest compared to the lowest intake quintile for coffee and tea, white bread, poultry, fish, raw and cooked vegetables, citrus fruit, and olive oil. The inverse association with oils, especially olive oil, was only slightly attenuated by allowance for vegetable intake. Thus, frequent consumption of vegetables, citrus fruit, fish and vegetable oils were the major features of a low-risk diet for cancer of the oral cavity and pharynx.

Keywords: cancer of the oral cavity; cancer of the pharynx; diet; oil; butter

Cancers of the oral cavity and pharynx are together the fifth most common cancer in the world (Parkin et al, 1993). Although these tumours predominantly affect developing countries, steady increases in mortality have been observed in male cohorts born after 1910–1920, the largest increases having occurred in eastern and southern Europe (La Vecchia et al, 1998). More than 80% of cases of cancer of the oral cavity and pharynx in developed countries should be avoidable by elimination of tobacco smoking and heavy alcohol drinking (Negri et al, 1993). Correlations of these tumours with dietary habits are also, however, among the strongest ones observed for any site of malignancy, although fewer accurate data exist for cancer of the oral cavity and pharynx than, for instance, cancer of the colon-rectum or breast (World Cancer Research Fund, 1997).

Several case-control studies (Marshall et al, 1982; Winn et al, 1984; Notani and Jayant, 1987; McLaughlin et al, 1988; Franco et al, 1989; Rossing et al, 1989; Franceschi et al, 1991; La Vecchia et al, 1991; Oreggia et al, 1991; Gridley et al, 1992; Zheng et al, 1992; 1993; Levi et al, 1998) have consistently found that oral cancer patients have histories of diets low in fruit and vegetables, even after accounting for their high alcohol intake. Protective effects seemed strongest for citrus fruit and vegetables which are likely to be eaten uncooked (McLaughlin et al, 1988).

Increases in risk also seemed to derive from high intakes of foods that represented important sources of calories, such as starchy foods (Franceschi et al, 1991), pulses (Notani and Jayant,

1987), certain meats, especially processed meats, and eggs (Franceschi et al, 1991). These foods can be interpreted as markers of an unbalanced monotonous diet and vary from one place to another. The role of different types of fat on cancer of the oral cavity and pharynx has never been studied in detail (Lipworth et al, 1997), but data on squamous carcinomas of the hypopharynx (Estève et al, 1996) and the oesophagus (Tzonou et al, 1996) raised the possibility that saturated fat may exert an unfavourable influence, while certain vegetable oils be protective.

To further elucidate the role of different foods and dietary fats in cancer of the oral cavity and pharynx, we carried out a case-control study in two Italian areas. The use of a validated food frequency questionnaire allowed us to assess individual fat intake patterns and adjust findings for total energy intake, in addition to a number of non-dietary risk correlates. Furthermore, the large study size made it possible to study dietary correlates separately in four anatomic subsites.

SUBJECTS AND METHODS

A case-control study of cancer of the oral cavity and pharynx was conducted between January 1992 and November 1997 in two Italian areas: the province of Pordenone in Northeastern Italy, and those of Rome and Latina in central Italy.

Cases had histologically confirmed cancer of the oral cavity and pharynx diagnosed no longer than 1 year prior to the interview and with no previous diagnoses of cancer at any site. Overall, 271 subjects with cancer of the oral cavity (219 men and 52 women, median age: 58, range 22–77 years) and 327 with cancer of the pharynx (293 men and 34 women, median age: 58, range 32–76 years) were included (Table 1).

TABLE 2.   Adjusted* Effects of Birth Weight of Latest Offspring on the Maternal Risk of Breast Cancer According to Time Since Latest Birth and Tumor Size at Diagnosis

Birth Weight† of the Latest Offspring	Rate Ratio According to Time Since Latest Birth and Tumor Size at Diagnosis							
	<5 years				≥5 years			
	≤2 cm		>2 cm		≤2 cm		>2 cm	
	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)
≤3 kg‡	51	1	46	1	346	1	240	1
3–3.25 kg	40	1.1 (0.7–1.6)	27	0.8 (0.5–1.3)	261	1.0 (0.8–1.2)	194	1.1 (0.9–1.3)
3.25–3.5 kg	61	1.2 (0.8–1.8)	55	1.2 (0.8–1.8)	328	0.9 (0.8–1.1)	252	·1.0 (0.9–1.2)
3.5–3.75 kg	49	1.1 (0.7–1.6)	52	1.2 (0.8–1.8)	280	0.9 (0.8–1.1)	236	1.1 (0.9–1.3)
>3.75 kg	91	1.2 (0.9–1.7)	93	1.4 (0.9–1.9)	495	1.0 (0.9–1.2)	409	1.2 (1.0–1.4)

* Adjustment was made for attained age, calendar period, age at first birth, number of births, and extremely preterm birth.
† Only singleton births are considered and only mothers with a birth from 1973 and onward are included in these analyses.
‡ Referent category.

Mothers with a multiple birth or a heavy-weighted newborn child are likely to have higher estrogen concentrations (oestradiol, oestriol, and unconjugated oestriol) during pregnancy.[2–4] The increased risk in these mothers during the first 5 years after birth is therefore compatible with the idea that estrogen is involved in the etiology of breast cancer, and the increased incidence of large tumors in mothers with a heavy-weighted newborn child supports the idea that the progression of occult tumors may be affected. We note that the effect on breast cancer risk of a multiple birth is larger compared with a delivery of a relatively heavy child. This finding could be due to a larger difference in hormonal levels in mothers having a multiple vs singleton birth compared with a heavy-weighted vs light-weighted child.

Women with diabetes mellitus may have an increased risk of breast cancer[17,18] and their offspring have a higher average birth weight due to the higher concentrations of different growth factors in these women.[19] Part of the increased risk in mothers with heavy-weighted newborn children could, therefore, also be attributed to a high proportion of diabetics among these mothers.

A few studies of mothers with multiple births have previously reported an increased risk of breast cancer in the early years after a multiple birth.[20–22] These studies, however, have compared the incidence with all other mothers irrespective of the time factor, meaning time since latest birth. Thus these previously published effects cannot be separated from the overall short-term increased risk of breast cancer after a birth as reported by Lambe et al[23] and Albrektsen et al.[24] By analyzing the effect of birth characteristics by time since latest birth, we avoided this problem, and found that indeed there is a higher short-term risk in mothers with a multiple birth or a heavy-weighted newborn child compared with others.

Women with high body mass index (BMI) have an increased risk of breast cancer.[25] Mothers that deliver a heavy-weighted child on average have a higher BMI themselves, which may explain the overall enhanced risk in these mothers. Furthermore, part of the increased incidence of large tumors might be due to difficulties for early detection in these women because of more breast

tissue. However, it cannot explain why the effect is largest in the first 5 years after a birth. Furthermore, most studies indicate that the negative effect of high BMI is restricted to postmenopausal women, whereas in this study most women are in a premenopausal age group in the first 5 years after a birth.

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MULTIVARIATE COMPETING RISKS

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SUMMARY

Competing risks models can be used to compare the effect of risk factors for different causes of death or subtypes of a disease. However, sometimes more than one outcome classification is available and if two such classifications are correlated, one may speculate whether differences in the effect of a risk factor according to one classification simply may be an effect of differences according to the other correlated classification. We introduce in this paper the new concept of *multivariate competing risks* to test formally such a hypothesis. Copyright © 1999 John Wiley & Sons, Ltd.

1. INTRODUCTION

Studies addressing incidence and risk factors for site-specific cancers often operate with only one ultimate cancer diagnosis. However, a more differentiated outcome, that is, specific subtypes of the cancer, may often be of interest. In practice, such differentiated analyses can be performed with follow-up data applying Cox of Poisson regression analyses on each subtype separately. However, in many situations it is desirable to study whether the risk factors have the same effect on the incidence of different subtypes, the purpose being either to study whether the subtypes have the same aetiology or to obtain a better understanding of the causal pathway behind the risk factors. This can be performed as a competing risk analysis testing for identical effects of a risk factor for all or some of the subtypes as discussed for the Cox model by Andersen *et al.*[1] (p. 493ff) and Lunn and McNeil[2] and for Poisson regression by Pierce and Preston.[3]

Sometimes more than one subtype classification is studied. If two such classifications are correlated, one may speculate whether differences in the effect of a risk factor according to one classification simply may be an effect of differences according to the other correlated classification. To evaluate such a hypothesis, we propose the new concept of *multivariate competing risks*.

The plan of the paper is as follows. In Section 2 we introduce an analysis taken from a breast cancer study as a motivating example for the concept of multivariate competing risks which we subsequently describe in Section 3. We illustrate the method on the example in Section 4, and discuss other applications of the method in Section 5.

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2. MOTIVATING EXAMPLE

The concept of multivariate competing risks was developed in the course of analysing a follow-up study of breast cancer. The study was based on information on breast cancer cases from the Danish Breast Cancer Cooperative Group[4] and a population-based cohort of Danish women with information on vital status and reproductive factors.[5,6] In the cohort of 1·5 million women (22·3 million person-years) were identified 10,790 women with breast cancer.

The purpose of the following analysis was to investigate whether a woman's number of (live) births, besides being an important risk factor for breast cancer as such,[7] was also predictive of disease severity at diagnosis, in order to select women for a targeted breast cancer screening. The analysis was performed as a competing risks analysis comparing the effect of number of births on the incidence of breast cancer according to two measures of severity at diagnosis: tumour size ($\leqslant$ 20 mm, 21–50 mm, > 50 mm) and number of positive nodes (no positive nodes, 1–3 positive nodes, and 4 or more positive nodes).

Both tumour size and nodal status reflect different stages rather than different subtypes. A competing risks analysis might therefore not seem to be the obvious approach because a breast cancer with a tumour size larger than 50 mm at diagnosis must have been 10 mm previously, that is, the 'types' do not seem to compete. However, competing risks models are applicable in this setting because the two classifications are measures of severity *at diagnosis* and a case can only have a single level of severity at diagnosis according to a given classification scheme. Nevertheless, such an approach does not allow for differentiation between differences in progression and detection rate, that is, an aetiologically more relevant explanation of why differences may exist. The following analysis is, therefore, primarily an illustration of the use of multivariate competing risks rather than a definitive aetiological analysis of the data at hand.

The competing risks analysis (described in detail in Section 4.1) revealed that number of births had a stronger effect on the incidence of small tumours compared to the effect on the incidence of larger tumours. Similarly, the effect on the incidence of node-negative breast cancers was stronger than the effect on the incidence of node-positive cases. As small tumours tend to be node-negative it is natural to speculate whether the two findings reflect the same phenomenon. An intuitive way to evaluate this hypothesis is to look at the effect of number of births on the incidence of different combinations of tumour size and nodal status, and then see whether the relatively stronger effect on the incidence of small tumours can be found in both node-negative and node-positive cases. The concept of multivariate competing risks analysis formalizes this intuitive idea, and we will now describe the method in detail.

3. MULTIVARIATE COMPETING RISKS MODELS

3.1. Multivariate competing risks models using Cox regression

If the purpose of a study is to evaluate the effect of an exposure on the rates of a specific type of outcome (for example, breast cancer), the rate for individual i is commonly modelled in a log-additive model as $\lambda_i(t) = \lambda_0(t)\exp(\beta x_i)$, with t representing age and x_i being a coded variable representing the exposure for women i. Extension to several exposures and adjustments for cofounders is well known. To ease notation, we drop the index i in the following.

If instead of only one type there are J subtypes of outcome, one can apply a competing risks model, with the cause specific rates modelled as $\lambda_j(t) = \lambda_{0j}(t)\exp(\beta_j x)$, with t being age and $j = 1, \ldots, J$ outcome subtype. In this model the effect of the exposure is different for each subtype

outcome, and the likelihood function factorizes corresponding to j completely separate models. The model with the same effect of the exposure for all outcome subtypes can be stated as $\lambda_j(t) = \lambda_{0j}(t)\exp(\beta x)$. In this model, the likelihood function no longer corresponds to j completely separate models, nevertheless the model can still be analysed using standard Cox regression techniques as described in Andersen et al.[1] (p. 493 ff).

To introduce the multivariate competing risks model, we now describe the situation where two subtype classifications ($j = 1, \ldots, J$ and $k = 1, \ldots, K$) of outcome are being studied simultaneously. As a straightforward extension of the previous model, one can consider the cross-product of the two subtype classifications letting $\lambda_{jk}(t)$ be the rate of having subtypes j and k simultaneously and t representing age. These rates could be modelled as $\lambda_{jk}(t) = \lambda_{0jk}(t)\exp(\beta_{jk} x)$, that is, a model with different baseline hazards and different effects of exposure for all combinations of subtypes. This would be a standard competing risks model. However, a more parsimonious log-additive model would be $\lambda_{jk}(t) = \lambda_{0jk}(t)\exp(\beta^0 x + \beta_j^1 x + \beta_k^2 x)$, where the effect of the exposure is log-additive on both subtype classifications. This model offers a natural means for testing for no differences in effects according to one subtype classification, that is, testing the models $\lambda_{jk}(t) = \lambda_{0jk}(t)\exp(\beta^0 x + \beta_j^1 x)$ or $\lambda_{jk}(t) = \lambda_{0jk}(t)\exp(\beta^0 x + \beta_j^2 x)$. These models for $\lambda_{jk}(t)$ are what we propose to call *multivariate competing risks models* as they can be applied for analysing two or more sets of competing risks, making it possible to test hypotheses about the multivariate effect of risk factors on these sets of competing risks. The models can be analysed using the same techniques as for standard 'univariate' competing risks models, with cause specific rates for every combination of subtypes.

3.2. Multivariate competing risks models using Poisson regression

Under the assumption of piecewise constant baseline rates, the Cox regression model is identical to a Poisson regression model. Poisson regression often provides a more feasible approach in large studies since one may work with abbreviated tables of cases and person-years at risk rather than with the individual data records.[8]

Competing risks analysis using Poisson regression can be performed if an extra dimension in the cross-classification of cases according to the type of disease is created as described for linear models by Pierce and Preston[3] and for log-linear models by Larson.[9] Person-years at risk are independent of this factor. A test for the same effect of a risk factor is then simply a test for no interaction between the risk factor and this new factor.

Multivariate competing risks models can be analysed using Poisson regression following the same arguments and techniques as for 'univariate' competing risks models, that is, by creating an extra dimension according to each of the $J \times K$ combinations of subtypes. However, in order to facilitate the new parsimonious additive models, this extra dimension should be further classified into two new dimensions according to each of two classifications (that is, with J and K levels, respectively). Tests for hypotheses of identical effects of the risk factor according to classification number one can be performed as a test for no interaction between the risk factor and the factor according to classification number one while including an interaction term between classification number two and the risk factor.

4. THE EXAMPLE REVISITED

We now return to the example from Section 2. We first describe the 'univariate' competing risks analyses and thereafter illustrate multivariate competing risks models.

4.1. The 'univariate' competing risks analysis

Owing to the large number of observations, the breast cancer rates were analysed using log-linear Poisson regression models, that is, assuming piecewise constant baseline rates. The effects of number of births according to number of positive nodes were estimated in three independent models of the form:

$$\lambda_j(t) = \lambda_{0j}(t) \exp[\alpha_{\text{period}, j} + \beta_{\text{age 1. birth}, j} + \delta_{\text{no. of births}, j}]$$

with j being the number of positive nodes $(0, 1\text{-}3, 4+)$ and $\alpha_{\text{period}, j}$, $\beta_{\text{age 1. birth}, j}$ and $\delta_{\text{no. of births}, j}$ being the node-specific effects according to levels of calendar period, age at first birth and number of births, respectively. A significant effect of number of births was found for breast cancers with no positive nodes $(p < 0.0001)$ or one, two or three positive nodes $(p = 0.0006)$, whereas there was no effect of number of births on the risk of breast cancer cases with four or more positive nodes $(p = 0.42)$ (Table I). Whether these differences in effect could be due to chance can be answered within the framework of competing risks, that is, by testing whether $\delta_{\text{no. of births}, j} = \delta_{\text{no. of births}}$. Doing so, we found a significant difference between the effects of number of births on the incidence of breast cancer according to the number of positive nodes, that is, a significant interaction between number of births and the dummy variable created according to the number of positive nodes in the breast cancer cases (likelihood ratio test $-2\log Q = 33.07$, d.f. $= 6$, $p < 0.0001$) (Table I).

Similarly, a significant effect of number of births was found for breast cancers with size $\leqslant 20 \, \text{mm}$ $(p < 0.0001)$ or $21\text{-}50 \, \text{mm}$ $(p = 0.012)$, whereas there was no effect of number of births on the incidence of large tumours $(p = 0.98)$ (Table I). As for number of positive nodes, the three effects of number of births were significantly different although the differences were less pronounced (likelihood ratio test $-2\log Q = 13.41$, d.f. $= 6$, $p = 0.04$).

4.2. The multivariate competing risks analysis

Since, the number of positive nodes and tumour size are highly correlated, it is natural to speculate whether the latter finding simply reflects differences according to number of nodes. The effects of number of births for each combination of tumour size and number of positive nodes are presented in Table II. The differences in the effect of number of births according to number of positive nodes that were significant in the 'univariate' competing risks analysis remained significant within constant levels of tumour size ($\leqslant 20 \, \text{mm}$, $p = 0.02$; $21\text{-}50 \, \text{mm}$, $p = 0.02$; $> 50 \, \text{mm}$, $p = 0.38$). However, the data disclosed a tendency to a uniform effect of number of births according to tumour size within a constant level of number of positive nodes (0 nodes, $p = 0.11$; 1-3 nodes, $p = 0.95$; 4 + nodes $p = 0.62$). Application of a multivariate competing risks model makes it possible to make a formal test of whether there is a uniform effect of number of births according to tumour size adjusted for differences according to number of positive nodes.

In this multivariate competing risks model we initially checked whether the differences in effects in Table II could be described as a log-additive effect of differences according to tumour size and differences according to number of nodes, that is, a test of

$$\lambda_{jk}(t) = \lambda_{0jk}(t) \exp[\alpha^1_{\text{period}, j} + \alpha^2_{\text{period}, k} + \beta^1_{\text{age 1. birth}, j} + \beta^2_{\text{age 1. birth}, k} + \delta^1_{\text{no. of births}, j} + \delta^2_{\text{no. of births}, k}]$$

against

$$\lambda_{jk}(t) = \lambda_{0jk}(t) \exp[\alpha^1_{\text{period}, j} + \alpha^2_{\text{period}, k} + \beta^1_{\text{age 1. birth}, j} + \beta^2_{\text{age 1. birth}, k} + \delta_{\text{no. of births}, jk}]$$

Table I. The adjusted effect of number of births on the breast cancer incidence according to number of positive nodes and tumour size

Breast cancer characteristics	Number of births				Likelihood ratio tests for effect (d.f. = 3)	Likelihood ratio tests for interaction (d.f. = 6)
	1 child (ref.)	2 children RR (95%-CI)	3 children RR (95%-CI)	4 or more children RR (95%-CI)		
Positive nodes*						
0 nodes	1	0·97 (0·90–1·05)	0·82 (0·75–0·90)	0·59 (0·51–0·68)	$p < 0.0001$	$p < 0.0001$
1–3 nodes	1	0·98 (0·88–1·09)	0·89 (0·79–1·01)	0·71 (0·59–0·85)	$p = 0.0006$	$-2\log Q = 33.07$
4 + nodes	1	0·98 (0·85–1·13)	1·08 (0·91–1·28)	1·11 (0·89–1·39)	$p = 0.42$	
Tumour size†						
≤ 20 mm	1	0·95 (0·89–1·03)	0·83 (0·76–0·91)	0·61 (0·53–0·70)	$p < 0.0001$	$p = 0.04$
21–50 mm	1	0·98 (0·89–1·08)	0·90 (0·80–1·01)	0·80 (0·68–0·94)	$p = 0.012$	$-2\log Q = 13.41$
> 50 mm	1	1·00 (0·80–1·25)	0·99 (0·76–1·29)	1·06 (0·74–1·52)	$p = 0.98$	

* Adjustment made for node-specific effects of age, calendar period and age at first birth
† Adjustment made for size-specific effects of age, calendar period and age at first birth

Table II. The adjusted* effect of number of births on the breast cancer incidence according to combinations of number of positive nodes and tumour size

Number of nodes	Number of births	Tumour size			Likelihood ratio tests for interaction (d.f. = 6)
		≤ 20 mm RR (95%-CI)	21–50 mm RR (95%-CI)	> 50 mm RR (95%-CI)	
0 positive nodes	1	1	1	1	$p = 0·11$ $-2\log Q = 10·28$
	2	0·95 (0·86–1·04)	1·00 (0·87–1·16)	0·74 (0·43–1·27)	
	3	0·78 (0·69–0·87)	0·86 (0·72–1·02)	1·08 (0·60–1·95)	
	4+	0·56 (0·47–0·67)	0·61 (0·47–0·79)	1·30 (0·62–2·75)	
1–3 positive nodes	1	1	1	1	$p = 0·95$ $-2\log Q = 1·60$
	2	0·97 (0·83–1·12)	0·97 (0·82–1·15)	0·99 (0·65–1·50)	
	3	0·87 (0·73–1·04)	0·87 (0·71–1·06)	0·78 (0·47–1·31)	
	4+	0·65 (0·50–0·85)	0·80 (0·60–1·06)	0·55 (0·24–1·26)	
4 + positive nodes'	1	1	1	1	$p = 0·62$ $-2\log Q = 4·42$
	2	0·99 (0·74–1·33)	0·91 (0·74–1·10)	1·07 (0·79–1·45)	
	3	1·24 (0·90–1·71)	0·98 (0·78–1·23)	1·04 (0·73–1·49)	
	4+	0·98 (0·62–1·55)	1·11 (0·82–1·51)	1·10 (0·68–1·78)	
Likelihood ratio tests for interaction (d.f. = 6)		$p = 0·02$ $-2\log Q = 14·98$	$p = 0·02$ $-2\log Q = 14·95$	$p = 0·38$ $-2\log Q = 6·41$	

* Adjustment made for size and node-specific effects of calendar period and age at first birth and for the effect of age for each of the nine combinations of size and nodal status

with j being number of positive nodes and k the tumour size. This was accepted (likelihood ratio test $-2\log Q = 15\cdot11$, d.f. $= 12$, $p = 0\cdot24$). The underlying assumptions of log-additivity for the effects of calendar period and age at first birth were checked using the same types of test (data not shown).

Finally, we tested whether there were differences in the effect of number of births according to tumour size or number of positive nodes, that is, the hypothesis $\delta^1_{\text{no. of births},j} = \delta^1_{\text{no. of births}}$ and $\delta^2_{\text{no. of births},k} = \delta^2_{\text{no. of births}}$. The estimates based on this multivariate competing risks model clearly demonstrated that the differences according to tumour size can be ascribed to differences according to number of nodes (likelihood ratio test $-2\log Q = 2\cdot29$, d.f. $= 6$, $p = 0\cdot89$). While there were no differences relative to the reference effect for tumour size, there were still noticeable differences for number of positive nodes when adjusting for tumour size (likelihood ratio test $-2\log Q = 22\cdot37$ d.f. $= 6$, $p = 0\cdot001$).

5. DISCUSSION

We have exemplified the concept of multivariate competing risks introduced in Section 3. Using a competing risks model we showed that a woman's number of births is predictive of the severity at diagnosis of breast cancer, measured as tumour size or nodal status. We speculated whether these two findings reflected one phenomenon, and with the use of the multivariate competing risks analysis we were able to confirm formally this hypothesis.

As noted, the example (chosen for illustrative purposes) does not evaluate an aetiological hypothesis as one cannot distinguish between differences in progression and detection rates. An example of a multivariate competing risks analysis of an aetiological hypothesis within breast cancer research would be to compare risk factors for receptor-negative versus receptor-positive breast tumours. Many have found that reproductive risk factors might be stronger for oestrogen receptor-positive than for oestrogen receptor-negative tumours and some have found the same relation using the progesterone receptor status.[10] Progesterone receptor status and oestrogen receptor status are highly correlated. It has, therefore, been speculated whether these two results reflect the same phenomenon.[10] Multivariate competing risks models offer a natural way to test this hypothesis with follow-up data.

Furthermore, it has been suggested that certain combinations of the oestrogen and progesterone receptor status might be more related to reproductive history than others.[11] This can also be studied by a multivariate competing risks model by a goodness-of-fit test for the additive model. However, when we performed these analyses in our data set, the multivariate competing risks analyses turned out to be less useful in this case as we found no strong relation between progesterone receptor status and reproductive history in the 'univariate' competing risks analysis.

In the models described above we have used a multiplicative modelling of competing risks. However, it could be argued that competing risks are intrinsically additive, and that the effects of the two classifications should not be mutually multiplicatively adjusted. An alternative model could, therefore, be to adjust them additively in a more complicated model such as

$$\lambda_{jk}(t) = \lambda_{0jk}(t)\exp[\alpha^1_{\text{period},j} + \alpha^2_{\text{period},k} + \beta^1_{\text{age 1.birth},j} + \beta^2_{\text{age 1.birth},k}](\exp(\delta^1_{\text{no. of births},j})$$

$$+ \exp(\delta^2_{\text{no. of births},k})).$$

This will no longer be a standard log-linear model of the rates but it could be analysed as a Poisson regression model using Epicture.[12]

In conclusion, we have introduced a new type of competing risks models which, we think, may prove relevant in practical situations.

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Reproductive History and Stage of Breast Cancer

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A woman's reproductive history influences her risk of breast cancer. The authors hypothesized that reproductive history also influences stage of disease at the time of diagnosis. The authors analyzed a population-based cohort of 1.5 million Danish women born between 1935 and 1978 for whom individual information on births was available. Between 1978 and 1994, 10,790 incident cases of breast cancer in women under 60 years of age were identified. Nulliparous women compared with parous women and women with a late age at first birth compared with an early age were at significantly increased risk of being diagnosed with a large tumor and with cancer that had spread to regional lymph nodes. However, such an association was not seen for women diagnosed with a small tumor and women with cancer that had not spread to regional lymph nodes. Reproductive history did not appear to influence the time interval from first symptoms to first physician visit ("patient delay") or the time interval from first physician visit to surgery ("doctor delay"). The authors conclude that reproductive history is associated both with incidence of breast cancer and with stage of the disease at diagnosis, indicating possible influences on tumor progression and growth rate. Intensified awareness is warranted to achieve earlier diagnosis among nulliparous women and women with a late age at first childbirth, with the hope of improving their prognosis. *Am J Epidemiol* 1999;150:1325–30.

breast neoplasms; neoplasm staging; reproductive history; risk factors; women

It is well established that a woman's reproductive history influences her risk of breast cancer. In particular, parity and age at first childbirth are considered strongly related to the risk of breast cancer (1). However, studies addressing these issues have almost exclusively dealt with breast cancer as a single entity. Thus, little is known about the possible effect of these reproductive factors on tumor biology (tumor progression, metastatic potential, etc.) as reflected in stage of the disease at diagnosis.

We hypothesized that parity and age at first childbirth not only are related to the risk of developing breast cancer but also are associated with the stage of breast cancer at diagnosis. We used a large population-based cohort with detailed information on reproductive history and tumor characteristics to evaluate whether parity and age at first birth are related to tumor size or axillary nodal spread at diagnosis.

MATERIALS AND METHODS

Population registries

Since April 1, 1968, the Civil Registration System in Denmark has assigned an individually unique national registration number to all citizens. This number permits accurate linkage of information obtained from different registries. The Civil Registration System also keeps updated information on vital status, emigration, and dates of live births.

The Danish Breast Cancer Cooperative Group started a series of national prospective studies in 1978 to systematically evaluate breast cancer treatment programs. A detailed description of the group's breast cancer registry has been given elsewhere (2, 3). The Cooperative Group collects detailed information on breast cancer cases at diagnosis, including the size of the tumor and the number of positive nodes. During a limited time period (1977–1981), the Cooperative Group collected additional information such as whether the tumor had been discovered by the woman herself, the date on which the woman experienced the first symptom(s) of her disease, and the date of the woman's first consultation with a medical doctor (4).

Through a linkage between the Danish Breast Cancer Cooperative Group and the Danish Cancer Registry, the Cooperative Group was found to have information on 94 percent of all breast cancer cases reported to the Danish Cancer Registry. The Danish Cancer Registry is considered close to complete regarding incident cases of malignant neoplasms diagnosed in Denmark since 1943 (5); world-standardized breast cancer rates in Denmark during the periods 1978–1982, 1983–1987, and 1988 onward were 64.8, 69.5, and 74.6 per 100,000 women, respectively (6).

Study cohort

A research parity database was established from the Civil Registration System that included all women born between April 1, 1935, and March 31, 1978, as described previously (7, 8). Based on each person's identifiable number from the Civil Registration System, a linkage was performed with the Danish Breast Cancer Cooperative Group data to obtain information on invasive primary breast cancers registered during the period January 1, 1978–September 30, 1994.

Statistical analyses

The possible impact of reproductive history on the incidence of breast cancer of a specific size or a particular nodal status was investigated in a follow-up study in which data were analyzed by log-linear Poisson regression (9). Each stage-specific subtype of breast cancer was analyzed separately. All women entered follow-up for each of the stage-specific breast cancer diagnoses on January 1, 1978, or on their 12-year birthday, whichever came last. The at-risk period continued until first diagnosis of breast cancer (at whatever stage), death, emigration, or September 30, 1994, whichever occurred first. Pregnancies occurring after a diagnosis of breast cancer were not included in the study. Incidence rate ratios are referred to here as relative risks. Adjustment was made for attained age (12–24, 25–29, 30–34 , ..., 50–54, and >54 years), calendar period (1978–1982, 1983–1987, 1988–1992, and 1993–1994), parity (0, 1, 2, 3, and ≥4 live births), and age at first live birth (nulliparous and 12–19, 20–24, 25–29, 30–34, and >34 years). All variables were treated as time-dependent variables. The effects of the confounders were allowed to differ according to stage, making it possible to take into account the fact that temporal trends and other effects could differ by size and nodal status. Testing for effect modification by attained age was performed with age categorized as <45 years versus ≥45 years. Analyses were performed using the SAS procedure PROC GENMOD (10).

The relations between reproductive history and factors associated with tumor detection, such as whether the woman had discovered the tumor herself (yes/no), the time interval from first symptom to first physician visit in days (patient delay), and the time interval from first physician visit to surgery in days (doctor delay), were evaluated by means of the Mann-Whitney and χ^2 tests.

RESULTS

Incidence

In total, 1,529,512 women were included in the cohort. Of these, 1,000,276 women (65.4 percent) had a total of 2,071,415 births before the end of follow-up: 254,694 women (25.5 percent) had one birth, 494,697 (49.5 percent) had two, 193,250 (19.3 percent) had three, and 57,635 (5.7 percent) had four or more. A total of 10,790 primary invasive breast cancers diagnosed before 60 years of age were detected in this cohort during 22.3 million person-years of follow-up. Table 1 gives the distribution of cases and person-years by age, calendar period, parity, and age at first birth.

TABLE 1. Distribution of cases of breast cancer and person-years of follow-up by age, calendar period, and reproductive history, Denmark, 1978–1994

	Cases		Person-years of follow-up
	No.	%	
Age (years)			
12–29	158	1.5	10,399,000
30–39	2,054	19.0	5,973,000
40–49	6,072	56.3	4,665,000
≥50	2,506	23.2	1,234,000
Calendar period			
1978–1982	1,390	12.9	5,850,000
1983–1987	2,734	25.3	6,657,000
1988–1992	4,656	43.2	7,245,000
1993–1994	2,010	18.6	2,519,000
Parous status			
Nulliparous	1,295	12.0	9,501,000
Parous	9,495	88.0	12,770,000
Age (years) at first birth			
12–19	1,472	15.5	2,362,000
20–24	4,437	46.7	6,480,000
25–29	2,693	28.4	3,164,000
30–34	710	7.5	648,000
≥35	183	1.9	116,000
No. of births			
1	1,910	20.1	3,469,000
2	4,892	51.5	6,188,000
3	2,112	22.2	2,390,000
≥4	581	6.1	723,000

Overall, we documented a significantly lower incidence of breast cancer among ever parous women compared with never parous women (relative risk = 0.87; 95 percent confidence interval: 0.82, 0.92). Among parous women, we found a significantly increasing incidence of breast cancer with increasing age at first birth ($p < 0.0001$) and decreasing parity ($p < 0.0001$) (table 2).

Table 2 shows the associations between these reproductive factors and breast cancer risk according to tumor size. Ever parous women had a significantly lower incidence of larger tumors than nulliparous women; for tumors less than or equal to 20 mm in diameter, we found no such association. In other terms, nulliparous women had a significantly increased risk of being diagnosed with a large tumor compared with parous women (relative risk = 1.69; 95 percent confidence interval: 1.37, 2.04). Among ever parous women, age at first birth was largely unrelated to the incidence of breast tumors less than or equal to 20 mm in size. In contrast, increasing age at first birth was positively associated with risk for larger tumors. The protective effect of multiparity was significantly stronger for small tumors (≤20 mm) than for larger tumors. Indeed, we found no association between number of births and risk of breast tumors above 50

mm in diameter (table 2). Similar associations with reproductive history were found when breast cancer cases were classified by nodal status instead of by tumor size (no positive nodes, 1–3 positive nodes, and ≥4 positive nodes; data not shown). To evaluate whether our results were modified by age, particularly by menopausal status, we performed a test for interaction with age categorized as <45 years versus ≥45 years. Our analysis did not show any effect modification by attained age.

The associations shown in table 2 are further illustrated in figures 1 and 2. Here the predicted breast cancer *rates* (based on the model from table 2) are calculated by tumor size at diagnosis for women aged 50–54 years in 1993–1994, according to their reproductive history. In figure 1, tumor size-specific rates of breast cancer in nulliparous women are compared with rates in uniparous women according to their age at the birth. Having one's first child at a young age slightly increases a woman's risk of being diagnosed with a small tumor, whereas the risks of medium and large tumors are reduced after the first birth. The reduction in medium and large tumors becomes smaller the older the woman is at the time of childbirth. For women aged ≥35 years at their first birth, there is even a small increase in risk. The incidence of tumors less than 21 mm in diameter at

TABLE 2. Effects of parous status, age at first birth, and number of births on overall risk of breast cancer and on risk according to tumor size, Denmark, 1978–1994

| | Overall relative risk | | Relative risk according to tumor size* | | | | | | | | |
| | | | <21 mm | | | 21–50 mm | | | >50 mm | | |
	RR†	(95% CI†)	No.	RR	(95% CI)	No.	RR	(95% CI)	No.	RR	(95% CI)
All women											
Parous status‡											
Nulliparous	1		576	1		491	1		122	1	
Parous	0.87	(0.82, 0.92)	5,019	1.03	(0.94, 1.12)	3,187	0.76	(0.69, 0.84)	585	0.59	(0.49, 0.73)
p for difference§	<0.0001			0.56			<0.001			<0.0001	
Parous women only											
Age (years) at first birth¶											
12–19	0.99	(0.93, 1.05)	808	1.02	(0.94, 1.11)	458	0.91	(0.82, 1.01)	87	0.94	(0.74, 1.21)
20–24	1		2,378	1		1,484	1		262	1	
25–29	1.19	(1.13, 1.24)	1,385	1.12	(1.05, 1.20)	944	1.26	(1.16, 1.36)	160	1.23	(1.01, 1.51)
30–34	1.27	(1.17, 1.37)	364	1.18	(1.05, 1.32)	233	1.27	(1.10, 1.46)	58	1.86	(1.38, 2.51)
≥35	1.33	(1.14, 1.55)	84	1.10	(0.88, 1.37)	68	1.50	(1.17, 1.93)	18	2.42	(1.47, 3.98)
p for difference§	<0.0001			0.0032			<0.0001			<0.0001	
No. of births#											
1	1		1,009	1		637	1		122	1	
2	0.97	(0.92, 1.02)	2,626	0.95	(0.89, 1.03)	1,633	0.98	(0.89, 1.08)	287	1.00	(0.80, 1.25)
3	0.88	(0.82, 0.94)	1,103	0.83	(0.76, 0.91)	704	0.90	(0.81, 1.01)	129	0.99	(0.76, 1.29)
≥4	0.70	(0.63, 0.77)	281	0.61	(0.53, 0.70)	213	0.80	(0.68, 0.94)	47	1.06	(0.74, 1.52)
p for difference§	<0.0001			<0.0001			0.012			0.98	

* Numbers of cases might not sum to the total number because of missing information on tumor size for some cases.
† RR, relative risk; CI, confidence interval.
‡ Adjusted for age and calendar period.
§ Likelihood ratio test for differences in the relative risks.
¶ Adjusted for age, calendar period, and number of births.
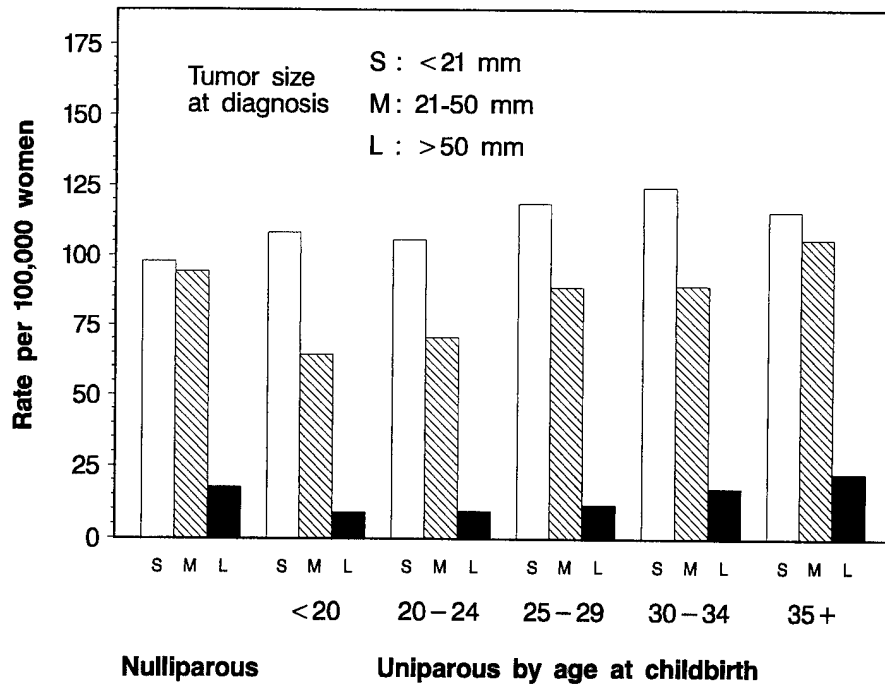# Adjusted for age, calendar period, and age at first birth.

FIGURE 1. Predicted breast cancer rates by tumor size at diagnosis in nulliparous and uniparous Danish women aged 50–54 years in 1993–1994, according to age at first childbirth. Prediction is based on the model from table 2 (Denmark, 1978–1994); cases with missing information on tumor size are not included.
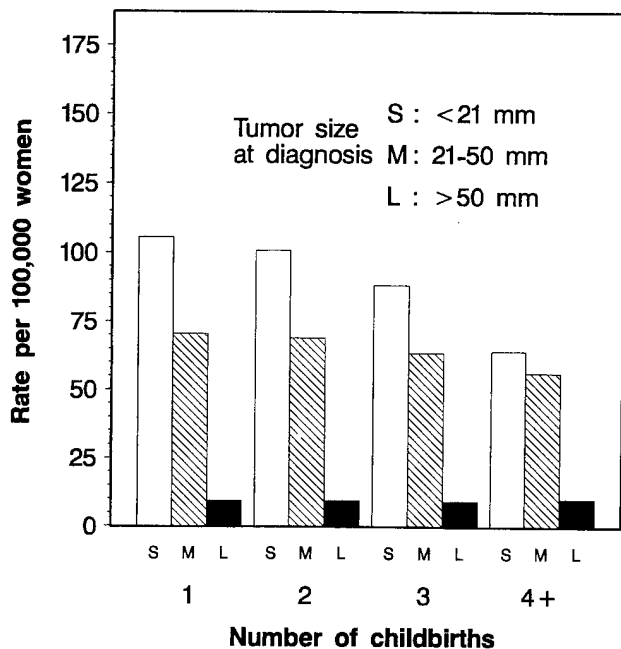
diagnosis is only slightly increased the older the woman is at her first birth. Therefore, much of the overall increase in risk with increasing age at first birth can be attributed to the fact that the reduction in the incidence of tumors with a diameter of 20–50 mm after a birth is smaller the older the woman is at her first birth. Although the *relative* increase in risk with increasing age at first birth is highest in the women with tumors larger than 50 mm (as table 2 shows), the *absolute* contribution to the overall risk is small.

Figure 2 illustrates the effect of having additional births beyond the first. The rates are calculated for women who were 20–24 years of age at their first birth. This restriction affects only the level of the rates, not the shape of the figure. Figure 2 shows that additional births beyond the first generally do not affect the incidence of large tumors and only slightly reduce the incidence of medium-sized tumors. The overall reduction in breast cancer risk with additional births is therefore attributable to a reduction in the incidence of small tumors.

FIGURE 2. Predicted breast cancer rates by tumor size at diagnosis in parous Danish women aged 50–54 years in 1993–1994 and aged 20–24 years at their first childbirth, according to number of births. Prediction is based on the model from table 2 (Denmark, 1978–1994); cases with missing information on tumor size are not included.

Diagnostic delay

For women whose cancer was diagnosed during the period 1978–1982, additional information had been obtained about whether the woman discovered the

tumor herself, about the time interval between the first symptoms' being observed by the woman and her first visit to a physician (patient delay), and about the time interval between the first physician visit and the time of definitive surgery or biopsy (doctor delay) (4). Overall, 93.3 percent of the women had discovered the tumor themselves, and among these women the median patient delay was 9 days. The median doctor delay was 29 days.

A more detailed presentation of the data is given in table 3. We evaluated the associations between the three tumor detection-related variables and the reproductive variables presented in table 2. There was no significant association in any of the nine tests (table 3).

DISCUSSION

This study showed that parity and age at first birth are associated not only with the incidence rate of breast cancer but also with the stage of the disease at diagnosis. Whereas nulliparous women versus parous

TABLE 3. Percentage of self-discovered tumors and median patient delay and doctor delay among Danish women diagnosed with breast cancer between 1978 and 1982, according to reproductive history

	Self-discovered tumor* (%)	Median patient delay† (days)‡	Median doctor delay§ (days)¶
Parous status			
Nulliparous	94	15	34
Parous	93	9	28
p for difference	0.78	0.09	0.14
Age (years) at first birth			
12–19	93	10	28
20–24	93	8	29
25–29	93	9	27
30–34	93	8	27
≥35	92	10	25
p for difference	0.99	0.98	0.88
No. of births			
1	92	7	28
2	93	7	28
3	93	13	29
≥4	97	13	31
p for difference	0.62	0.27	0.75

* Information was available for 87% (1,215/1,390) of the cases.
† Time interval from the first symptom to the first physician visit.
‡ Information was available for 76% (1,055/1,390) of the cases.
§ Time interval from the first physician visit to breast cancer surgery.
¶ Information was available for 87% (1,203/1,390) of the cases.

women and women with a late age at first childbirth compared with an early age were at similar risks of having breast cancer diagnosed at an early stage (small tumor, no metastatic spread), nulliparous women and women with a late first birth were at significantly increased risk of being diagnosed with advanced breast cancer (large tumors, extensive metastatic spread to regional lymph nodes). In contrast, multiparity was protective against being diagnosed with a small tumor but not against being diagnosed with a large tumor. These results can be ascribed to differences in tumor progression rates and/or differences in detection rates. Obviously, a large tumor must at some point have been small. Under the assumption that certain tumors grow more rapidly than others, some tumors will stay in the category of small tumors for a shorter time before they move on to become medium-sized and eventually large tumors. Thus, according to one interpretation, nulliparous women and women with a late age at first birth who are at particularly high risk of being diagnosed with large tumors may have tumors with rapid growth potential.

A rival explanation would be that associations exist between reproductive factors and the probability of early tumor detection. For example, differences in detection rates might arise if breast self-examination is more difficult for nulliparous women compared with parous women or more difficult for women with a late age at first birth versus an early age at first birth. The breast tissue of a nulliparous woman is firmer and more homogenous than the breast tissue of a parous woman, which might make detection of a tumor more difficult. However, it is equally conceivable that the nodularity present in a parous woman's breast would make it difficult to distinguish glandular tissue from tumor tissue. Thus, the extent of which and direction in which reproductive factors may influence detection of tumors is difficult to predict. Differential use of mammography according to reproductive history could also cause differences in time of detection. However, the vast majority of women in our study were below age 50. In Denmark, mammography is offered only to women aged 50 years or older and only in a few parts of the country.

Finally, behavioral differences according to reproductive history could cause differences in time of detection. For example, parous women and those considering pregnancy may be more frequently in contact with the medical care system, leading to shorter delays in detection in comparison with nulliparous or older women. However, the differences in the effects of reproductive history were the same regardless of age. Furthermore, based on detailed referral information on a subset of the women included in this study, we found

no evidence of an association between delay in referral or delay in diagnosis and the reproductive factors in question. Therefore, the most likely explanation for our findings is that a woman's reproductive status influences both her risk for tumor development and the biologic features of the tumor, notably its growth potential.

Our prospective analysis was performed in a large population-based cohort, which made selection and information bias very unlikely. A potential limitation of our study was the lack of data on other reproductive factors, such as age at menarche and age at menopause. However, the confounding introduced by lack of adjustment for these variables should have been limited (11). Temporal trends in breast cancer incidence might differ according to tumor characteristics. We took this into account by allowing for different effects of calendar period in the different stage-specific analyses. The cohort included only women who were under age 60 years at the end of follow-up. Therefore, our results were obtained primarily among premenopausal women. However, the effects of reproductive history were the same regardless of age, indicating that the effects may be applicable to both pre- and postmenopausal women.

It is well established that having advanced breast cancer at the time of diagnosis (large tumor, lymphatic spread) is associated with a particularly poor prognosis. Thus, the association with more advanced disease observed for nulliparous women and women with a late age at first birth also gives them a higher risk of lethal disease. In a large cohort of women who had undergone breast cancer treatment, we previously investigated whether the prognostic effect of parity and age at first birth also had an independent effect on these women's survival (12). We found in that study an independent negative prognostic effect of a late age at first birth but no prognostic effect of number of births. To evaluate the independent effect on the *prognosis* of breast cancer in that study, we adjusted for differences in tumor size and nodal status at the time of diagnosis (in addition to age, histologic grading, treatment regimen, and other factors) (12). Taken together, the two studies illustrate how reproductive risk factors have a further negative effect on the progression rate besides the effects seen as differences in tumor size and nodal status at diagnosis.

In conclusion, these data provide novel evidence that a woman's reproductive status may also influence her stage of breast cancer at diagnosis and thereby her long term disease-specific survival. In particular, nulliparous women and women who give birth to their first child at a late age are at increased risk of being diagnosed with large tumors with extensive metastatic growth and a poor prognosis. Regardless of the underlying biologic mechanism, these results support the development of initiatives to achieve earlier detection of breast cancer, perhaps through a combination of increased awareness and more frequent mammography, in this subset of women who tend to develop more lethal breast cancer.

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Factors influencing the effect of age on prognosis in breast cancer: population based study

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Abstract

Objective To investigate whether young age at diagnosis is a negative prognostic factor in primary breast cancer and how stage of disease at diagnosis and treatment influences such an association.
Design Retrospective cohort study based on a population based database of patients with breast cancer containing detailed information on tumour characteristics, treatment regimens, and survival.
Setting Denmark.
Subjects 10 356 women with primary breast cancer who were less than 50 years old at diagnosis.
Main outcome measures Relative risk of dying within the first 10 years after diagnosis according to age at diagnosis after adjustment for known prognostic factors and expected mortality.
Results Overall, young women with low risk disease who did not receive adjuvant treatment had a significantly increased risk of dying; risk increased with decreasing age at diagnosis (adjusted relative risk: 45-49 years (reference): 1; 40-44 years: 1.12 (95% confidence interval 0.89 to 1.40); 35-39 years: 1.40 (1.10 to 1.78); <35 years: 2.18 (1.64 to 2.89). However, no similar trend was seen in patients who received adjuvant cytotoxic treatment. The increased risk in younger women who did not receive adjuvant treatment compared with those who did remained when women were grouped according to presence of node negative disease and by tumour size.
Conclusion The negative prognostic effect of young age is almost exclusively seen in women diagnosed with low risk disease who did not receive adjuvant cytotoxic treatment. These results suggest that young women with breast cancer, on the basis of age alone, should be regarded as high risk patients and be given adjuvant cytotoxic treatment.

Introduction

Women diagnosed with breast cancer in their 20s and 30s seem to have a poorer prognosis than women diagnosed in middle age.[1-7] The reason for this unusual pattern is unclear. Young women with breast cancer are more likely to have affected lymph nodes, be negative for oestrogen receptors, and have tumours that are large with a high grade of anaplasia [1-3] Thus, the poorer outcome could at least partly be due to differences in these important prognostic factors, although many, though not all, studies retain a negative effect after adjustment for such confounding factors.[1 8-19] It is unknown to what extent adjuvant cytotoxic treatment might influence this association.

We examined the effect of age on breast cancer survival adjusted for expected mortality using Denmark's large and very complete population based breast cancer registries. These include detailed information on clinical presentation, postoperative treatment, and follow up status for women with breast cancer. Our main objectives were to determine whether the poor prognosis reported among young women was independent of common prognostic factors and to what extent this pattern might be affected by treatment.

Subjects and methods

Population database

In 1977, the Danish Breast Cancer Cooperative Group (DBCG) started nationwide prospective studies on treatment of breast cancer.[20] Three programmes have so far been launched: DBCG 77 (patient accrual from 1977-82), DBCG 82 (patient accrual from 1982-9), and DBCG 89 (patient accrual since 1989). Primary clinical and histopathological data and data on postoperative treatment and status at follow up visits have all been registered by the Danish Breast Cancer Cooperative Group based on specific forms submitted by departments of surgery, pathology, and oncology in Denmark. Linkage between the Danish Breast Cancer Cooperative Group register and the Danish cancer registry, which is considered almost complete regarding reporting of breast cancer diagnoses among residents in Denmark,[21] showed a 94% concordance (unpublished result).

Patient records in the Danish Breast Cancer Cooperative Group registry were linked with the Danish civil registration system registry to obtain complete information on deaths. Since 1968, the civil registration system registry has assigned a unique identification number to all residents in Denmark. Individual information is kept under this personal identification number in all national registries, permitting accurate linkage of information in different registries. The civil registration system registry keeps updated files on dates of childbirth and death. A detailed description of the information included in this registry is given elsewhere.[22]

Recent studies have shown that age at first birth and short interval between last birth and diagnosis of breast cancer may affect the prognosis of breast cancer.[23 24] Information on childbirth history was available for women born since 1 April 1935.

Treatments

Patients were classified as either low or high risk according to histopathological criteria. Detailed information on allocation of risk groups is given elsewhere.[23] For all three programmes, the primary surgical treatment of patients was total mastectomy plus axillary dissection (90% of the population) or lumpectomy with axillary dissection. Standard adjuvant cytotoxic chemotherapy was used in all three pro-

Table 1 Postoperative adjuvant treatment given during 1977-96 to Danish premenopausal women with high risk breast cancer

Treatment protocol	Treatment randomisation
DBCG 77	Radiotherapy or
	Radiotherapy plus levamisol or
	Radiotherapy plus cyclophosphamide or
	Radiotherapy plus CMF
DBCG 82	CMF or
	CMF plus radiotherapy or
	CMF plus tamoxifen
DBCG 89:	
Oestrogen receptor positive	CMF or
	Castration
Oestrogen receptor negative	CMF or
	CEF or
	CMF plus pamidronate or
	CEF plus pamidronate

CMF=cyclophosphamide plus methotrexate plus fluorouracil.
CEF=cyclophosphamide plus epirubicin plus fluorouracil.

grammes.[20 25] Table 1 gives a summary of the adjuvant treatment.

Patients with bilateral breast cancer or inflammatory cancer, distant metastases, contraindications to the planned postoperative treatment, or who were not treated according to the surgical guidelines were not allocated to any of the protocols.

Statistical analysis

Women who had breast cancer diagnosed between January 1978 and 1 July 1996 were included and followed up for 10 years after diagnosis or until 1 July 1996, whichever came first, with respect to survival. The study was restricted to premenopausal women aged younger than 50 at the time of diagnosis.

The overall death rate was modelled by a sum of two terms. The first term was the age and calendar specific expected mortality as a known time dependent offset. Expected mortality was obtained from life tables for the total female population in Denmark in five year age groups and five year calendar periods.[26] The second term in the overall model was the exponential function of a linear expression including the categorical variables age at diagnosis (five year groups), tumour size (≤ 2 cm, >2-5 cm, >5 cm), number of positive nodes (0, 1-3, 4-9, ≥ 10), histological grading (I, II and III, non-ductal carcinomas), protocol allocation (allocated, not treated according to surgical guidelines, not allocated for other reasons), and year of diagnosis (1977-81, 1982-88, 1989-96). This model can be viewed as a log-linear model of the observed death rate minus the expected death rate—that is, a log-linear model of the excess death rate. The expected number of deaths due to breast cancer amounts to only a small proportion of all expected deaths.[26] Therefore, the adjusted relative risks were interpreted as relative risks of death due to breast cancer. Poisson regression was chosen instead of Cox regression to facilitate additive adjustment for expected mortality.

We also did multivariate analyses without adjusting for expected mortality, which allowed us to use both Poisson and Cox regression. The two approaches gave identical estimates of the relative risk. All tests in the Poisson regression analyses were performed as likelihood ratio tests with Epicure.[27] Tests for difference in the age effect in low risk patients compared with

high risk patients receiving cytotoxic treatment were performed by including an interaction term between age and risk group. Association between age at diagnosis and tumour characteristics was analysed by χ^2 tests.

Results

By 1 July 1996, 10 356 premenopausal women aged younger than 50 with primary breast cancer were registered with the Danish Breast Cancer Cooperative Group. Our cohort represented a total of 52 432 person-years of follow up. Table 2 shows the distribution of patients according to tumour characteristics, protocol allocation, and age at diagnosis. Compared with older patients, patients aged younger than 35 at diagnosis were at higher risk of being node positive (51% (404/795) v 46% (4061/8854); P = 0.02). The proportion of patients with histological grading I was significantly lower in patients aged younger than 35 compared with older patients (18% (122/668) v 32% (2321/7303); P < 0.001).

To evaluate the independent effect of age at diagnosis on survival from breast cancer, we performed a multivariate analysis that included age at diagnosis, tumour size, axillary nodal status, histological grading, year of treatment, protocol allocation, and expected mortality (table 3). Women aged 45-49 years were chosen as the reference category because they constituted the largest group around the time of menopause. Compared with this group, women in the two age groups less than 40 years at diagnosis were at significantly increased risk of dying (table 3). Women younger than 35 had the worst prognosis, with a

Table 2 Distribution of 10 356 premenopausal women with primary breast cancer operated on in Denmark during 1977-96 according to tumour characteristics, risk group allocation, and age at diagnosis. Values are numbers (percentages)

	Age at diagnosis (years)			
	<35 (n=867)	35-39 (n=1733)	40-44 (n=3354)	45-49 (n=4402)
Tumour size (cm):				
≤ 2	431 (49.7)	948 (54.7)	1769 (52.7)	2322 (52.8)
>2-5	330 (38.1)	595 (34.3)	1169 (34.9)	1652 (37.5)
>5	69 (8.0)	133 (7.7)	278 (8.3)	291 (6.6)
No information	37 (4.3)	57 (3.3)	138 (4.1)	137 (3.1)
No of positive nodes:				
0	391 (45.1)	886 (51.1)	1691 (50.4)	2216 (50.3)
1-3	259 (29.9)	478 (27.6)	910 (27.1)	1258 (28.6)
4-9	114 (13.1)	174 (10.0)	397 (11.8)	497 (11.3)
≥ 10	31 (3.6)	76 (4.4)	127 (3.8)	144 (3.3)
No information	72 (8.3)	119 (6.9)	229 (6.8)	287 (6.5)
Histological grading:				
I	122 (14.1)	351 (20.3)	812 (24.2)	1158 (26.3)
II and III	546 (63.0)	1017 (58.7)	1785 (53.2)	2180 (49.5)
Non-ductal carcinoma*	199 (23.0)	365 (21.1)	757 (22.6)	1064 (24.2)
Oestrogen receptor status†:				
Positive	198 (51.2)	469 (57.8)	1086 (65.9)	1634 (71.0)
Negative	189 (48.8)	342 (42.2)	561 (34.1)	667 (29.0)
Risk group:				
Low	315 (36.3)	733 (42.3)	1423 (42.4)	1920 (43.6)
High	349 (40.3)	677 (39.1)	1319 (39.3)	1715 (39.0)
Not treated according to guidelines‡	143 (16.5)	231 (13.3)	443 (13.2)	496 (11.3)
Not allocated for other reasons§	60 (6.9)	92 (5.3)	169 (5.0)	271 (6.2)

*Includes women with no information available on histological grading.
†Information available for 5146 (49.7%) women.
‡Patients not allocated because surgical treatment did not follow guidelines.
§Patients not allocated because of medical contraindications, bilateral or inflammatory breast cancer, or distant metastases.

Table 3 Adjusted relative risk of dying after diagnosis of primary breast cancer according to age at diagnosis, tumour characteristics, and protocol allocation in 9541 breast cancer patients* diagnosed during 1978-96

Variables	Adjusted relative risk (95% CI)†
Age at diagnosis (years):	
<35	1.46 (1.27 to 1.70)
35-39	1.26 (1.12 to 1.42)
40-44	1.07 (0.97 to 1.19)
45-49	1 (reference)
Tumour size (cm):	
≤2	1 (reference)
>2-5	1.78 (1.61 to 1.97)
>5	2.31 (2.00 to 2.67)
No of positive nodes:	
0	1 (reference)
1-3	1.80 (1.62 to 2.01)
4-9	3.44 (3.05 to 3.89)
≥10	4.71 (3.96 to 5.59)
Histological grading:	
I	1 (reference)
II and III	2.44 (2.12 to 2.81)
Non-ductal carcinoma‡	1.12 (1.00 to 1.43)
Protocol allocation:	
Allocated	1 (reference)
Not treated according to surgical guidelines	1.11 (0.95 to 1.28)
Not allocated for other reasons§	2.61 (2.26 to 3.01)

*815 patients (7.9%) excluded because of missing information on tumour size or nodal status.
†Adjusted for age at diagnosis, tumour characteristics, protocol allocation, year of diagnosis, and expected mortality.
‡Includes patients with no information on histological grading.
§Medical contraindications, bilateral or inflammatory breast cancer, or distant metastases.

1.46-fold increased risk of dying. The results were not changed by adjustment for oestrogen receptor status in the subgroup of patients for whom this information was available (data not shown).

To evaluate the effect of adjuvant cytotoxic therapy in relation to age at diagnosis, we allowed for an interaction between age at diagnosis and low risk patients (none of whom received adjuvant treatment, n = 4329), versus high risk patients (all of whom received adjuvant cytotoxic treatment, n = 2824; figure). Among patients who did not receive adjuvant cytotoxic treatment, there was a highly significant increased risk of dying with decreasing age (adjusted relative risk: 45-49 years: 1 (reference); 40-44 years: 1.12 (95% confidence interval 0.89 to 1.40); 35-39 years: 1.40 (1.10 to 1.78); <35 years: 2.18 (1.64 to 2.89). A similar trend was not observed in young patients receiving adjuvant cytotoxic therapy (high risk disease) (see figure). The negative effect of young age among women without adjuvant cytotoxic treatment was significantly more pronounced than that observed in the group of treated patients (test for effect modification: P = 0.02).

In further analyses we looked at the effect of treatment among node negative women (table 4). In line with the findings above, only young women in the group that received no treatment were at increased risk; no increased risk was observed among women who received adjuvant cytotoxic treatment. A similar pattern was observed when the analysis was restricted to women with small tumours at diagnosis (≤2 cm) or women with large tumours (>2 cm).

We have previously shown that age at first childbirth and time since last birth are independent prognostic factors for death from breast cancer.[23][24] Complete information on reproductive history was available for 3373 low risk patients (77.9%). The estimated prognostic effect of age at diagnosis was not significantly altered by adjusting for age at first childbirth or time since last birth (data not shown).

Discussion

In agreement with previous studies, we found that breast cancer in young women has a particularly poor prognosis.[1][4-19] Younger women are at high risk of having axillary lymph node disease and tumours with high histopathological grading and of being oestrogen receptor negative.[1-3]

Part of the explanation for young women having more advanced and aggressive disease at diagnosis has been suggested to be the increased potential for a delayed diagnosis.[17][28] Detecting tumours in the breasts of young women is difficult because of the density of the mammary glands, and this problem is particularly pronounced among pregnant and lactating women.[29] Our detailed information on tumour characteristics at diagnosis enabled us to adjust for the effect of factors such as tumour size, nodal status, and histological grading and therefore judge more clearly the independent effect of age. Furthermore, we had complete reproductive history for a subset of the women and could therefore include the previously reported negative prognostic effect of a recent childbirth in our multivariate analyses. However, none
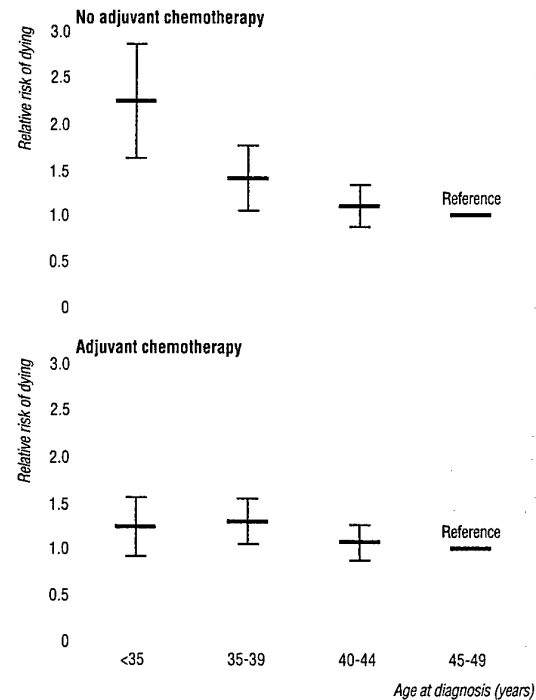
Adjusted relative risk of dying after diagnosis of primary breast cancer according to age at diagnosis among 4329 low risk patients who received no adjuvant treatment (top) and 2824 high risk patients who received adjuvant cytotoxic treatment (bottom). Women aged 45-49 at diagnosis were used as reference. Bars indicate 95% confidence intervals. Relative risk was adjusted for tumour size, nodal status, histological grading, year of diagnosis, and expected mortality

Table 4 Adjusted relative risk (95% confidence interval) of dying according to age at diagnosis and treatment in node negative women and women with tumour size ≤2 cm and >2 cm

Age at diagnosis (years)	Node negative*		Tumour size ≤2 cm		Tumour size >2 cm	
	No adjuvant treatment	Adjuvant cytotoxic treatment	No adjuvant treatment	Adjuvant cytotoxic treatment	No adjuvant treatment	Adjuvant cytotoxic treatment
<35	2.1 (1.6 to 2.8)	0.6 (0.1 to 5.5)	2.8 (1.9 to 4.0)	1.3 (0.9 to 2.1)	1.5 (1.0 to 2.4)	1.2 (0.9 to 1.6)
35-39	1.4 (1.1 to 1.7)	0.9 (0.3 to 3.5)	1.4 (1.0 to 1.9)	1.3 (0.9 to 1.9)	1.4 (1.0 to 2.0)	1.3 (1.0 to 1.6)
40-44	1.1 (0.9 to 1.4)	0.7 (0.2 to 2.3)	1.1 (0.8 to 1.5)	1.1 (0.8 to 1.5)	1.1 (0.8 to 1.5)	1.1 (0.9 to 1.3)
45-49†	1	1	1	1	1	1

*Only node negative women were considered in the analysis as all node positive women received adjuvant cytotoxic treatment.
†Reference group.

of these adjustments changed the overall result that young age at time of diagnosis is associated with a particularly poor prognosis. This argues in favour of breast cancers among young women tending to be biologically more aggressive than those diagnosed in older women but does not indicate how these cancers respond to adjuvant cytotoxic chemotherapy. However, other results suggest that tumours in young women respond adequately to chemotherapy. A meta-analysis of 133 randomised trials including 75 000 women with high risk breast cancer found the relative benefit of adjuvant cytotoxic chemotherapy to be larger in patients younger than 50 years compared with patients older than 50.[30]

Treatment of younger women

Henderson and Patek have argued against accepting young age alone as a criterion for adjuvant treatment.[31] The international consensus panel on the treatment of primary breast cancer came to a similar conclusion in 1995,[32] but has recently changed its recommendation to include women younger than 35, although no scientific evidence to back this decision was presented.[33] To evaluate the role of postoperative adjuvant cytotoxic treatment in relation to age at diagnosis we allowed for an interaction between age at diagnosis and low risk patients who received no adjuvant treatment versus high risk patients who received adjuvant cytotoxic treatment. We found that the negative effect of young age was almost exclusively seen in women classified as

What is already known on this subject

Most previous studies indicate that young age at diagnosis of breast cancer is an independent negative prognostic factor

No study has evaluated whether the negative effect of young age is influenced by adjuvant cytotoxic treatment

What this paper adds

This large population based study shows that the negative effect of young age occurs almost exclusively among those not receiving adjuvant treatment

Age did not have a significant effect among women who received adjuvant cytotoxic treatment

Young age should be considered as a sole criterion for allocating breast cancer patients to adjuvant cytotoxic treatment

having low risk disease, being non-significant in high risk patients who received cytotoxic adjuvant treatment. This finding remained when the comparison of women who did and did not receive adjuvant cytotoxic treatment was restricted to node negative patients and patients with the same tumour size. This raises the question of whether the negative effect of young age seen in low risk patients is due to lack of adjuvant cytotoxic treatment. Our results cannot be taken as direct evidence that young patients classified as having low risk disease will benefit from adjuvant cytotoxic treatment. However, Fisher et al recently showed that women with low risk disease do benefit from adjuvant cytotoxic treatment and that the greatest benefit is seen in premenopausal women.[34] Therefore, we feel confident that the low risk tumours associated with a poor prognosis in young women will respond to adjuvant cytotoxic treatment leading to a better prognosis for this group of women.

The relative risk of dying was adjusted for expected mortality, which includes death from breast cancer. In some age categories, particularly among young women, this leads to an underestimation of the disease-specific risk because death from breast cancer accounts for up to 15% of the total mortality in young women.[26] Thus, the prognosis for young compared with middle aged women is probably worse than we estimated. However, this approach did not introduce an age differential bias when comparing the age specific effects in women receiving no treatment with those receiving adjuvant treatment.

In conclusion, we found that diagnosis of breast cancer at a young age was associated with an increased risk of death, with women younger than 35 at diagnosis having the worst prognosis of all age groups. The age effect was not significant among women who received adjuvant cytotoxic treatment, but was highly significant among low risk women who received no adjuvant treatment. These results suggest that all young women with breast cancer should be regarded as high risk patients and be offered adjuvant cytotoxic treatment.

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Commentary: much still to learn about relations between tumour biology, prognosis, and treatment outcome in early breast cancer

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What is it about breast cancer in women under 40 that is independently associated with worse prognosis? And what biological factors could explain both the poor prognosis and the disproportionately improved outcome seen after adjuvant chemotherapy? Do these tumours have special characteristics that can account for both these observations?

Possible mechanisms

Two biological processes could be implicated. The first involves changes in the ability of tumour cells to maintain the correct DNA sequence and to survive DNA damage caused by chemotherapy and radiotherapy. The second involves underlying molecular changes that promote rapid tumour proliferation.

The p53 protein acts to safeguard the integrity of the genetic code. If DNA is damaged and a cell proliferates without repair, mutations are passed on to daughter cells. Rapid acquisition of multiple mutations can lead to early onset aggressive cancers. Under normal circumstances the p53 protein prevents this by arresting the cell cycle to allow repair of damaged DNA or by promoting cellular suicide (apoptosis). A mutation in the p53 gene disrupts this normal DNA housekeeping, and cells can continue to proliferate unabated despite the presence of damaged DNA. Similarly, if the p53 protein is not functional the ability of cells to recognise and respond to damage induced by chemotherapy or radiotherapy may be reduced, potentially allowing tumour cells to survive cancer treatment.

The cell membrane receptor p185 is also involved in the control of cellular proliferation. It is encoded for by the gene c-erbB-2. When this receptor is activated, cell proliferation is stimulated. In many breast cancers c-erbB-2 is overexpressed, leading to increased cellular proliferation.

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Adjustment for Age at First Birth in Etiologic Studies of Breast Cancer Involving Exposures That May Affect Age at First Birth

To the Editor:

In a recent letter, Sharpe[1] argues that in our study of the association between induced abortion and breast cancer risk,[2] we should not have adjusted for age at first birth in certain situations. Here we show that without adjustment for age at first birth, we would get a confounded result.

Sharpe suggests that women with a history of induced abortion on average might have a first birth at a later age. Therefore, he claims, age at first birth is an intermediate variable in the analysis of the association between induced abortion and breast cancer risk in women with an induced abortion before the first birth. Nevertheless, the scientific question in our analysis was whether there is a direct *biological* relation between having an induced abortion and development of breast cancer. For age at first birth to be an intermediate variable in such an analysis, it should be related to a step in the potential causal biological pathway between induced abortion and breast cancer. That women with a history of induced abortion tend to have a first birth at a later age can hardly constitute such a biological effect. Instead it reflects different social behavior among these women. Similarly, an association between, for example, higher education and breast cancer risk would reflect a behavioral association between education and having a late age at first birth and not a direct biological causal

pathway from education to breast cancer. In other words, lack of adjustment for age at first birth would introduce a spurious effect that would reflect the well-known biological effect of late age at first birth rather than a potential biological effect of induced abortion.

Nevertheless, we can assure Sharpe that adjustment for age at first birth was of no importance for the conclusion in our analysis. Overall we find no effect of induced abortion 1.00 (0.94–1.06) when adjusting for age, calendar period, parity, and age at first birth.[2] Dropping adjustment for age at first birth we get 1.00 (0.94–1.06), and adjusting for age only we get 0.99 (0.93–1.04).[3] Therefore, even when we do not adjust for the behavioral effect through late age at first birth, there is no effect of induced abortion on the risk of breast cancer.

A related misunderstanding is to argue that induced abortion enhances the risk of breast cancer because primigravida women having an induced abortion by definition postpone the first birth that they would otherwise have had. Nevertheless, this effect is again a biological effect of the first birth and not of the induced abortion. What we show in our study is that after an induced abortion the women will have the same risk of breast cancer as before the pregnancy, that is, there is no biological effect of induced abortion.

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Testosterone Level as a Potential Selection Bias for Semen Donors in Assessing Population Fertility

To the Editor:

Studies assessing time trends in male fertility have relied on the results of analyses of donor-collected semen samples as an outcome measure.[1,2] The characteristics of men who become semen donors are not well understood, however. A number of studies have addressed psychosocial characteristics and motivation in semen donors, highlighting the roles of altruism and financial incentives.[3,4] But semen quality and sperm production are regulated by a variety of hormones, and the possible physiological and hormonal differences that may exist between men in the general population and men who donate semen have not been explored. Testosterone, for example, in addition to being essential for normal sperm production, is also an important determinant of be-

Short Communication

Gender of offspring and long-term maternal breast cancer risk

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Summary Gender of offspring is influenced by maternal hormonal level during pregnancy, which is blieved to influence the subsequent maternal breast cancer risk. However, analysing national birth and cancer registrations in a cohort of 998 499 women, we found no association between gender of offspring and subsequent breast cancer risk. © 2000 Cancer Research Campaign

Keywords: gender; pregnancy; breast cancer incidence; population-based cohort study

Childbirth induces a short-term increase and a long-term decrease in a mother's breast cancer risk (Lambe et al, 1994; Albrektsen et al, 1995*a*). Hormonal levels during pregnancy may influence both effects. This has been investigated by looking at the maternal breast cancer risk following a pregnancy with characteristics associated with elevated hormonal levels (Enger et al, 1997; Troisi et al, 1998; Wohlfahrt and Melbye, 1999). The maternal breast cancer risk according to gender distribution of offspring has for the same reason attracted interest since gender differences in the maternal level of serum alfa-fetoprotein, human chorionic gonadotrophin (hCG) and sex hormone-binding globulin have been reported that might also be related to maternal breast cancer risk (reviewed in Hsieh et al, 1999). A large Norwegian cohort study with 3937 cases found no association between breast cancer risk and gender distribution of offspring (Albrektsen et al, 1995*b*), but recently a Swedish case–control study including 2328 cases found that deliveries of male offspring had a protective effect (Hsieh et al, 1999). The mechanisms behind the hormonal influence on the short-term increase and long-term decrease in breast cancer risk following childbirth are believed to be different (Adami et al, 1998), and it is therefore important to investigate the effects separately. In a recent, large cohort study including 9495 cases we have found no modification by gender of offspring of the short-term effect (Wohlfahrt and Melbye, 1999). In the present study we investigated whether this is also true for the long-term effect of childbirth.

MATERIALS AND METHODS

Since 1 April 1968, the Civil Registration System (CRS) in Denmark has assigned an individually unique national registration number to all citizens. This number permits accurate linkage of information from different registries. The Civil Registration System also keeps updated information on gender and dates of live

births, emigration and vital status. Information on still-births was available from the National Birth Register. To identify multiple deliveries we looked for children born to the same mother within 2 days. A research parity database was established from the CRS including all women born between 1 April 1935 and 31 March 1978, as earlier described (Melbye et al. 1997; Westergaard et al, 1997). Based on the person-identifiable CRS number, a linkage was performed with the Danish Breast Cancer Group's registry (DBCG) (Andersen and Mouridsen, 1988; Kroman et al, 1997) giving information on registered invasive breast cancers in the period from 1 January 1978 to 30 September 1994. The DBCG's registry was found to contain information on 94% of all breast cancer patients reported to the Danish Cancer Registry, which has nearly complete registration of all incident cases of malignant neoplasms diagnosed in Denmark since 1943 (Storm, 1991). The impact of gender on the incidence of breast cancer was investigated in a follow-up study analysed by log-linear Poisson regression models (Breslow and Day, 1987). All women entered the follow-up for breast cancer diagnoses on 1 January 1978, or on the date of their first childbirth, whichever came last. The period at risk continued until breast cancer, death, emigration or 30 September 1994, whichever occurred first. Incidence rate ratios are referred to as relative risks. In all analyses adjustment was made for age (< 30, 31, 32, ... 57, 58), calendar period (1978–82, 1983–87, 1988–92, 1993–94) and age at first birth (< 20, 20–24, 25–29, 30–34, 35+). The gender of n birth was categorized as ($n - 1$ parous or less, boy, girl). The first parameter in the gender variables were redundant when the gender variables for all births were included and these parameters were therefore set to zero.

RESULTS

In all we observed 9495 cases of breast cancer during 12.8 million years of follow-up. All the following analyses are restricted to 5 or more years after latest birth in mothers with a history of only single births in order to focus on long-term effects of single births. With this restriction we observed 8607 cases during 8.7 million years of follow-up. Using 10 years instead of 5 years gave similar results. The mother's risk of breast cancer decreased significantly with number of births: 1 birth: 1 (reference); 2 births: 1.0 (0.9–1.0);

Table 1 Relative maternal risk[a] of breast cancer 5 or more years after latest birth according to gender distribution of offspring

Number of girls	Number of boys					
	0	1	2	3	4+	All
0	–	1.0 (0.9–1.1)	1.0 (0.9–1.1)	0.9 (0.8–1.1)	0.6 (0.4–0.8)	1.2 (1.1–1.2)
1	1 (ref.)	0.9 (0.9–1.0)	0.9 (0.8–1.0)	0.7 (0.6–0.9)	0.5 (0.3–0.9)	1 (ref.)
2	1.0 (0.9–1.1)	0.9 (0.8–0.9)	0.8 (0.6–0.9)	0.5 (0.4–0.8)	0.2 (0.1–0.7)	0.9 (0.8–1.0)
3	0.8 (0.6–0.9)	0.7 (0.6–0.9)	0.7 (0.4–1.0)	0.2 (0.1–0.9)	0.9 (0.3–2.8)	0.7 (0.7–0.8)
4+	0.7 (0.4–0.1)	0.4 (0.2–0.9)	1.3 (0.7–2.4)	0.4 (0.1–2.7)	– (no cases)	0.7 (0.5–0.9)
All	1.1 (1.1–1.2)	1 (ref.)	1.0 (0.9–1.0)	0.9 (0.8–1.0)	0.6 (0.4–0.7)	

[a]All relative risks are adjusted for attained age, calendar period and age at first birth and with 95% confidence interval. The effects of number of boys and number of girls are furthermore mutually adjusted.

Table 2 Relative maternal risk[a] of breast cancer 5 or more years after latest birth according to gender of offspring in 1st to 6th birth

Offspring birth order	Gender of offspring	
	Boy	Girl
1	1 (ref.)	1.0 (1.0–1.0)
2	1 (ref.)	1.0 (0.9–1.0)
3	1 (ref.)	0.9 (0.9–1.0)
4	1 (ref.)	1.0 (0.9–1.2)
5	1 (ref.)	1.0 (0.7–1.6)
6	1 (ref.)	1.0 (0.4–2.7)

[a]All relative risks are adjusted for attained age, calendar period, age at first birth and gender of 1st to 6th birth and with 95% confidence interval.

3 births: 0.9 (0.8–0.9); 4 births: 0.7 (0.6–0.8); 5+ births: 0.5 (0.4–0.7). Table 1 shows the risk of breast cancer according to the gender distribution of the mother's offspring. We observed that women with many compared to few boys, and women with many compared to few girls, had a lower breast cancer risk. However, the effects are similar and can be described more simply by the total number of births. This can be seen by the very similar estimates within the diagonals from left-bottom to right-top, i.e. within strata of similar parity (Table 1, not including the All category). Within the parity-specific strata the distribution of boys and girls does not modify the risk. The pattern was the same in women younger than 45 years of age and in women aged 45 years or older. In an alternative approach we estimated the gender difference in the long-term effects of the 1st to 6th birth (Table 2). The effect of 1st to 6th birth was not modified by the gender of the offspring.

DISCUSSION

Our study shows that gender of offspring does not modify the effect of a childbirth on the breast cancer risk. This is true for both the short-term increase and the long-term decrease of breast cancer risk after a childbirth. The gender modification of the long-term effect was investigated by studying breast cancer risk 5 or more years after the latest birth according to the gender distribution of offspring as well as the effects of each birth. The long-term decrease in risk following a childbirth is believed to originate from permanent changes in the susceptibility of the stem cells, changes that perhaps partly are determined by the hormonal level during pregnancy (Adami et al, 1998). We therefore used these approaches as it is most plausible that a potential gender-induced modification of the long-term effect would be an effect of the gender of all previous births some years after the latest birth, i.e. after the most marked effects of these transient negative effects of the births.

The short-term effect of a childbirth is, on the other hand, believed to be due to hormonally induced growth of premalignant and malignant tumours. A study of the gender modification of the short-term effect should therefore either focus on the latest birth in a short time-interval after the latest birth or the short-term effects of each of the births separately. Studying the gender modification of the short-term effect according to gender distribution of all births disregarding the order of appearance and only restricting to young women, as in the study by Hsieh et al of the gender effect in 'childbearing ages' (Hsieh et al, 1999), is therefore most likely going to obscure the true short-term effect. As argued above, such an approach is more appropriate in the study of long-term effects. We have recently looked at the effect of gender of the most recent birth within the first 5 years following birth (Wohlfahrt and Melbye, 1999) and observed no modifying effect of gender of offspring. Based on these findings in a large population-based cohort study we conclude that gender of offspring modifies neither the short- nor the long-term effect of breast risk following childbirth. Our findings do not necessarily imply that the hormones related to gender are of no importance in the aetiology of breast cancer, but probably illustrate that the gender differences in hormonal levels during pregnancy are small compared with the hormonal changes induced by a pregnancy irrespective of gender.

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Age at Any Birth Is Associated with Breast Cancer Risk

Jan Wohlfahrt and Mads Melbye

The period before the first birth is traditionally viewed as particularly critical for a woman's breast cancer risk. Nonetheless, the importance of early timing of a woman's first compared with subsequent births is not well understood. In the present study we examine this question using a population-based cohort of 1.5 million Danish women born between 1935 and 1978. Between 1968 and 1994, 13,049 incident cases of breast cancer were identified in the Danish Cancer Registry. According to our results, a woman's breast cancer risk is related to her age at any of her births. The risk increase per 5 year's increase in maternal age at first, second, third, and fourth birth was 9%, 7%, 5%, and 14%, respectively. For fifth and sixth births it was 5%. We observed a risk reduction after any birth occurring before 30 years of age (in uniparous women before 25 years of age). These effects were strongest more than 10 years after birth. Thus, our study shows that early timing of any additional birth induces an additional long-term reduction in maternal risk of breast cancer; that is, early reproductive years, rather than just the nulliparous years, constitute the critical period. (Epidemiology 2001;12:●●●-●●●)

Keywords: breast cancer risk, reproductive history, cohort study, population-based.

It is well established that childbirth affects a woman's breast cancer risk. Traditionally, the timing of the first birth has been considered to be of particular importance, that is, earlier age at first birth reduces the risk of breast cancer. This thinking derives from the assumption that breast cells are particularly prone to carcinogenic stimuli before a first pregnancy and that maturation and protection of breast cells takes place at the time of a first pregnancy.[1] High parity among parous women, however, may further reduce the risk of breast cancer, an effect that is attributed to a maturation of breast cells not affected by the first birth. This reduction in risk after subsequent births can be substantial,[2,3] and it is therefore intriguing that the timing of the first birth should be considered more critical than the timing of subsequent births. Presently, little is known on this subject because few datasets are large enough to estimate the effects of age at each birth simultaneously. In the present study, we take advantage of the large cohort of women in population-based national registries in Denmark to in-vestigate the influence of the age at first relative to subsequent births on the development of breast cancer.

Subjects and Methods

POPULATION REGISTRIES

Since April 1, 1968, the Civil Registration System in Denmark has assigned an individually unique national registration number to all citizens. This number permits accurate linkage of information from different registries. The Civil Registration System also keeps updated information on dates of livebirths, emigration, and vital status. Additional information on stillbirths was available from the National Birth Registry. The Danish Cancer Registry is considered close to complete regarding incident cases of malignant neoplasms diagnosed in Denmark since 1943.[4]

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STUDY COHORT

A research database on parity was established from the Civil Registration System. It includes all women born between April 1, 1935, and March 31, 1978, as earlier described.[5,6] On the basis of personal identification numbers from the Civil Registration System, we linked data with the Danish Cancer Registry, which had information on invasive primary breast cancers in the period from April 1, 1968, through September 30, 1994.

STATISTICAL ANALYSES

We investigated the impact of age at birth on the incidence of breast cancer in a follow-up study the results of which were analyzed using log-linear Poisson regression models.[7] All women entered the follow-up for

breast cancer diagnoses on April 1, 1968, or on their 12th birthday, whichever came later. The period at risk continued until breast cancer, death, emigration, a seventh birth, or September 30, 1994 (end of follow-up), whichever occurred first. The effect of age at birth was analyzed using two different approaches. In one approach we performed a parity-stratified analysis including age at the most recent and previous births (categorized as <25, 25–29, 30–34, ≥35), age (quadratic splines with knots: 30, 35, 40, 45, 50, 55),[8] and calendar period. The parsimonious modeling of age in the stratified analyses is due to the lower number of cases compared with the overall analyses. In a second approach, we used models that included information from all parity strata. In these analyses, we adjusted for single-year categories of age and 5-year categories of calendar period (1968–1972, 1973–1977, 1978–1982, 1983–1987, 1988–1992, and 1993–1994). In the following models these categorical variables and an intercept are represented by the term c_1 (age, period). All variables were treated as time-dependent variables. To estimate the change in risk after first to fourth birth, we used the following model for the logarithm of the incidence rate (λ):

$$\log(\lambda) = c_1(age, period) + \sum_{k=1}^{4} \alpha_k x_k \quad (1)$$

where $x_k = 1$ if parity ≥ k and $x_k = 0$ otherwise. α_k represents the change in risk (on the log scale) after kth birth. In other words, the logarithm of the effect of reproductive history in, for example, biparous women is in this model represented by $\alpha_1 + \alpha_2$, where α_1 represents the change in risk after the first birth and α_2 the change in risk after the second birth. To see how the risk changes after the first to fourth birth varied with age at the first to fourth birth, we used the following model:

$$\log(\lambda) = c_1(age, period) + \sum_{k=1}^{4} \sum_{l=1}^{4} \beta_{kl} y_{kl} \\ + c_2(5th \ and \ 6th \ births) \quad (2)$$

l = 1–4 represents the categories (<25, 25–29, 30–34, and ≥35, respectively), and $y_{kl} = 1$ if parity is k or larger and age at kth birth is in the lth age-at-birth category; y_{kl} = 0 otherwise. The term c_2 (fifth and sixth births) represents the effects of fifth and sixth births and is explained below. β_{kl} represents the change in risk after a kth birth occurring in the lth age-at-birth category. The effect of reproductive history in, for example, biparous women with a first birth before 25 years of age and a second after 35 years age is in this model represented by $\beta_{11} + \beta_{24}$, where β_{11} represents the change in risk after a relatively early first birth and β_{24} the change in risk after a relatively late second birth. As a parsimonious alternative, we estimated the change in risk per increase in age at birth by:

$$\log(\lambda) = c_1(age, period) + \sum_{k=1}^{4} \sum_{l=1}^{4} (\varepsilon_k x_k + \chi_k z_{kl}) \\ + c_2(5th \ and \ 6th \ births) \quad (3)$$

where z_{kl} = the lth age level if parity is k or larger and age at kth birth is in lth age-at-birth category; otherwise, z_{kl} = 0. The categories <25, 25–29, 30–34, and ≥35 were assigned the levels 22.5, 27.5, 32.5, and 37.5, respectively. $5\chi_k$ represents the increase in risk per 5-year increase in age at kth birth. Owing to small numbers, we used this expression for fifth and sixth births in all analyses (except model 1); that is, the effects of age at fifth and sixth births were represented by trends using

$$c_2(5th \ and \ 6th \ birth) = \sum_{k=5}^{6} \sum_{l=1}^{4} (\varepsilon_k x_k + \chi_k z_{kl}).$$

To estimate the general increase per 5 years in age at first to fourth birth, we substituted χ_k with δ:

$$\log(\lambda) = c_1(age, period) + \sum_{k=1}^{4} \sum_{l=1}^{4} (\varepsilon_k x_k + \delta z_{kl}) \\ + c_2(5th \ and \ 6th \ births) \quad (4)$$

with δ representing the general effect for first to fourth birth. In a more elaborate model, we investigated how the changes after the first to fourth birth are modified by time since the first to fourth birth using the following modification of model 2:

$$\log(\lambda) = c_1(age, period) \\ + \sum_{k=1}^{4} \sum_{l=1}^{4} \sum_{m=1}^{2} \beta_{klm} u_{klm} \\ + \sum_{m=1}^{2} c_{2m}(5th \ and \ 6th \ births) \quad (5)$$

with m = 1 and 2 representing the categories <10 years and ≥10 years, respectively, and $u_{klm} = 1$ if parity is k or larger, age at kth birth is in lth age-at-birth category, and time since kth birth is in the mth time-since-birth category; u_{klm} = 0 otherwise. β_{klm} represents the change in risk after a kth birth occurring in lth age-at-birth category in the mth time-since-birth category after kth birth. c_{2m} represents trend estimates for fifth and sixth birth for each time-since-birth category. Equivalent extensions of models 3 and 4 were used to estimate trends when stratifying by time since birth. The same models were used with m representing attained age or attained parity. Estimation of the effect of time since latest birth with adjustment for age and age at first birth when including uniparous women has been discussed by Heuch et al.[9] Finally, we estimated the effect of age at latest birth extending model 2 to:

$$\log(\lambda) = c_1(age, period) + \sum_{k=1}^{4} \sum_{l=1}^{4} (\beta_{kl} y_{kl} \\ + \beta_l^{latest} y_{kl}^{latest}) + c_2(5th \ and \ 6th \ births) \quad (6)$$

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TABLE 1.   Relative Risk* (RR) of Breast Cancer According to Age (Years) at First to Fourth Birth Stratified by Parity†

	Uniparous Women			Biparous Women			Triparous Women			4,5,6 Parous Women		
	No.	RR	95% CI	No.	RR	95% CI	No.	RR	95% CI	No.	RR‡	95% CI
Age at first birth												
<25§	944	1		3,605	1		1,978	1		631	1	
25–29	808	1.17	1.06–1.28	1,846	1.10	1.03–1.18	486	1.08	0.96–1.23	76	1.11	0.83–1.48
30–34	432	1.24	1.11–1.39	377	1.14	1.00–1.30	49	1.15	0.82–1.60	8	2.00	0.79–5.06
≥35	166	1.29	1.10–1.53	41	0.96	0.68–1.33	3	1.57	0.46–5.31	0		
Age at second birth												
<25§				1,304	1		1,144	1		464	1	
25–29				2,685	1.04	0.97–1.11	1,080	1.06	0.96–1.17	230	1.24	1.00–1.52
30–34				1,493	1.14	1.04–1.25	267	1.25	1.04–1.50	19	0.63	0.34–1.19
≥35				387	1.29	1.12–1.49	25	1.28	0.80–2.04	2	0.78	0.17–3.59
Age at third birth												
<25§							218	1		214	1	
25–29							929	1.09	0.94–1.27	345	0.98	0.80–1.22
30–34							1,016	1.18	1.00–1.39	124	0.88	0.64–1.20
≥35							353	1.14	0.93–1.39	32	1.63	0.99–2.66
Age at fourth birth												
<25§										46	1	
25–29										208	1.05	0.74–1.48
30–34										285	1.27	0.87–1.86
≥35										176	1.45	0.96–2.19

* Estimated within strata of parity with adjustment for age, calendar period and other effects presented in the column.
† Estimating in model 5 with adjustment for parity and m equal to attained parity gave results very similar to those presented in Table 1. The deviance from this model is 6711.5. Comparing with deviance from model 2 (6732.9) substantiates the conclusion that the effect of age at nth birth does not depend on the attained parity.
‡ Further adjusted for the effects of age at fifth and sixth birth as in model 2.
§ Reference category.

with $y_{kl}^{latest} = 1$ if parity is precisely k and age at kth birth is in lth age-at-birth category; $y_{kl}^{latest} = 0$ otherwise. y_{kl} and y_{kl}^{latest} are identical for k-parous women. As the variables differ in other strata, however, it is possible to perform meaningful estimations of β_{kl} and β_l^{latest} simultaneously, assuming that they represent two distinct etiologic effects.

Results

Overall, 1,529,414 women were observed during 31.2 million person-years of follow-up (mean follow-up 20.4 years; range, <1–26.5 years). Of these, 13,049 women were diagnosed with breast cancer before the end of the study with the following parity status: 1,599 were nulliparous, 2,350 were uniparous, 5,869 were biparous, 2,516 were triparous, 583 had four children, and 132 had five or six children. At the seventh birth women were excluded from follow-up. Age during follow-up ranged from 12 to 59 years. Among cases, 10,281 women were younger than 50 years at diagnosis and 2,768 were 50 years or older.

Table 1 shows the estimated effect of age at first birth in uniparous women; the estimated effect of age at first and second births in biparous women; the estimated effect of age at first, second, and third births in triparous women; and the estimated effect of age at first, second, third, and fourth births in women with four children. These stratified analyses show that the woman's age at first to fourth birth is associated with breast cancer risk and that the associations are also observed after subsequent births.

The overall relative risks after the first, second, third, and fourth births are 0.98 [95% confidence interval (95% CI) = 0.92–1.05], 0.90 (95% CI = 0.86–0.95),

0.86 (95% CI = 0.83–0.91), and 0.81 (95% CI = 0.75–0.88), adjusted for age and calendar period (model 1). Table 2 illustrates how these effects are affected by age at birth; that is, it shows the effect of the first to fourth birth on the maternal risk of breast cancer according to age at birth. Compared with nulliparous women, a first birth induced a decreased risk of breast cancer if the woman was less than 25 years of age at the time of giving birth. A second, third, and fourth birth induced a reduced breast cancer risk among women less than 30 years of age at the time of giving birth compared with uniparous, biparous, and triparous women, respectively (model 2).

To evaluate whether age at any birth was equally important for breast cancer risk, we furthermore compared the increase in risk according to increase in maternal age at first to fourth birth. The risk of breast cancer increased by 9% per 5-year increase in age at first birth and 7%, 5%, and 14% per 5-year increase in age at second, third, and fourth births, respectively (model 3). The general increase per 5 years in age at first, second, third, and fourth births was 8% (model 4). The general increase per 5 years in age at fifth and sixth births was 5% (12%–26%). Including only age at first birth and adjusting for number of births, the trend for age at first birth was 13% per 5 years. The associations with age at second, third, and fourth births were not due to residual confounding introduced by the categorization of age at first birth in four groups. Adjusting for age at first birth in 1-year categories, the increases per 5 years in age at second, third, and fourth births were 7%, 4%, and 14%, respectively (Table 2, modified model 3). We found no interaction between the effects of age at different births. Among women with a first birth before 25 years of age,

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TABLE 2. Relative Risk* (RR) of Breast Cancer after First to Fourth Birth According to Age (Years) at Birth

	No. of Cases	Overall* RR‡	Overall* 95% CI	<10 Years RR‡	<10 Years 95% CI	≥10 Years RR‡	≥10 Years 95% CI	<50 Years RR‡	<50 Years 95% CI	≥50 Years RR‡	≥50 Years 95% CI
				According to Time since Birth†				According to Attained Age†			
Age at first birth											
<25	7,158	0.91	0.85–0.98	0.96	0.82–1.12	0.91	0.84–0.97	0.93	0.86–1.00	0.87	0.74–1.00
25–29	3,216	1.01	0.94–1.08	1.10	0.97–1.23	0.98	0.91–1.06	1.02	0.94–1.11	0.95	0.81–1.11
30–34	866	1.08	0.99–1.18	1.11	0.97–1.26	1.05	0.95–1.17	1.12	1.02–1.24	0.93	0.77–1.14
≥35	210	1.10	0.95–1.27	1.00	0.82–1.21	1.19	0.96–1.46	1.05	0.88–1.25	1.23	0.93–1.62
Nulliparous§‖	1,599	1		1		1		1		1	
Increase per 5 years		9%	5–12%	2%	−5–10%	9%	5–13%	9%	5–12%	9%	2–16%
Age at second birth											
<25	2,912	0.88	0.82–0.94	1.09	0.90–1.31	0.86	0.80–0.93	0.88	0.82–0.95	0.87	0.75–1.02
25–29	3,995	0.92	0.87–0.97	1.05	0.95–1.17	0.89	0.84–0.95	0.93	0.87–0.99	0.88	0.77–1.00
30–34	1,779	1.00	0.94–1.07	1.06	0.96–1.16	0.97	0.90–1.04	1.01	0.94–1.09	0.96	0.83–1.10
≥35	414	1.09	0.97–1.21	1.05	0.92–1.21	1.10	0.94–1.29	1.15	1.02–1.30	0.88	0.69–1.11
Uniparous§‖	2,350	1		1		1		1		1	
Increase per 5 years		7%	4–11%	0%	−6–7%	6%	2–10%	9%	5–13%	2%	−4–10%
Age at third birth											
<25	432	0.84	0.74–0.94	0.99	0.63–1.56	0.83	0.73–0.94	0.86	0.75–0.98	0.76	0.58–0.99
25–29	1,274	0.89	0.83–0.95	0.96	0.81–1.13	0.88	0.81–0.95	0.89	0.82–0.97	0.86	0.74–1.00
30–34	1,140	0.94	0.88–1.00	1.01	0.91–1.13	0.92	0.85–0.99	0.97	0.90–1.05	0.85	0.74–0.98
≥35	385	0.97	0.87–1.07	0.95	0.83–1.09	1.00	0.86–1.17	0.94	0.84–1.07	1.04	0.85–1.27
Biparous§‖	5,869	1		1		1		1		1	
Increase per 5 years		5%	0–10%	−1%	−10–9%	5%	−1–11%	4%	−1–10%	8%	−1–19%
Age at fourth birth											
<25	46	0.76	0.54–1.07	0.37	0.05–2.73	0.78	0.55–1.11	0.72	0.49–1.07	0.89	0.45–1.75
25–29	208	0.77	0.65–0.91	0.77	0.50–1.19	0.78	0.65–0.92	0.76	0.63–0.92	0.79	0.58–1.09
30–34	285	0.92	0.81–1.05	0.84	0.66–1.07	0.96	0.83–1.12	0.93	0.80–1.09	0.90	0.70–1.15
≥35	176	1.03	0.89–1.21	1.01	0.82–1.24	1.09	0.87–1.37	1.03	0.86–1.23	1.06	0.80–1.42
Triparous§‖	2,516	1		1		1		1		1	
Increase per 5 years		14%	4–26%	18%	−4–46%	15%	3–30%	15%	3–29%	11%	−7–34%
General increase per 5 years per birth¶		8%	6–9%	2%	−2–5%	7%	6–9%	8%	6–10%	6%	3–9%

* The age at birth-specific estimates are estimated using model 2 (deviance = 6732.9), parity-specific estimates of the increase in risk with increasing age at birth using model 3 (deviance = 6735.9), and general increase in risk with increasing age at birth using model 4 (deviance = 6738.4). Including only age at first birth (in four categories) and adjusting for number of births, the deviance was 6777.0.

† For age at first birth is time since birth the time since first birth, for age at second birth the time since second birth, and so on. The general increase in risk with increasing age at birth was estimated using model 5 with a general trend as in model 4 [deviance = 6715.0 (time since model) and 6725.4 (attained age model)].

‡ Adjusted for age, calendar period and age at first to sixth birth.

§ For simplicity references are labeled as nulli-, uni-, bi- and triparous. Only a subgroup is the actual reference group. For instance, the reference group for "age at second birth" = 25–29 is only uniparous women with an "age at first birth" in the categories <25 or 25–29.

‖ Reference category.

¶ Estimating the general effect for first to sixth birth (instead of first to fourth birth) gave the same result.

the increases in risk after second, third, and fourth births were 6%, 4%, and 14%, respectively.

The effects of age at birth differed according to time since birth (Table 2), that is, the effect of age at *n*th birth differed according to time since *n*th birth. The first 10 years after birth there was only a minor effect of age at birth (2% risk increase per 5 years), whereas 10 or more years after birth the effect of birth was modified by the age at birth (7% risk increase per 5 years) (model 5). The ratio between the trends was 1.07/1.02 = 1.05 (95% CI = 1.01.–1.10). Comparing the overall trends according to attained age, we found only a minor difference: for <50 years, 8% risk increase per 5 years, and for ≥50 years, 6% risk increase per 5 years (Table 2, model 5). The ratio between the trends was 1.08/1.06 = 1.02 (95% CI = 0.99–1.05).

In additional analyses we examined whether effects of each childbirth could explain a possible effect of age at latest birth (model 6), that is, whether there was an additional effect of age at latest birth besides the effects of each birth. Adjusting only for age at first birth, we found a strong effect of age at latest birth [<25 years, 1

(reference category); 25–29 years, 1.02 (95% CI = 0.96–1.07); 30–34 years, 1.12 (95% CI = 1.05–1.21); ≥35 years, 1.25 (95% CI = 1.13–1.38); increase per 5 years, 8% (95% CI = 4–11%)]. However, after additional adjustment for the effect of age at subsequent births, we found no effect of age at latest birth [<25 years, 1 (reference category); 25–29 years, 0.98 (95% CI = 0.91–1.06); 30–34 years, 1.02 (95% CI = 0.92–1.14); ≥35 years, 0.99 (95% CI = 0.79–1.24); increase per 5 years, 0% (95% CI = −5%–4%)].

We only had complete information on livebirths, but information on stillbirths occurring from 1973 to 1993 was available. Including these births in the analyses gave similar results [for example, general increase per 5 years: 8% (95% CI = 6%–9%)].

Discussion

Women with low age at first birth are at reduced risk of breast cancer; that is, women with a short nulliparous period (defined as the period between menarche and first birth) have a low risk of breast cancer later in life.[1]

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This observation has led to the hypothesis that the nulliparous period represents a critical time window in a woman's life during which breast cancer may be initiated.[10] Although subsequent births are known to reduce breast cancer risk, it is not well understood whether the timing of these childbirths during reproductive life is of importance. Our data show that the timing of all childbirths, and not only the first, affects a woman's breast cancer risk. We found that all childbirths result in a long-term reduction in maternal breast cancer risk if the woman delivers at an early age, whereas childbirths occurring later in a woman's life evidently induce no reduction in risk. This effect was observed irrespective of parity and attained age. These findings suggest that the early reproductive years, rather than just the initial nulliparous period, are the critical time window and that the risk of negative long-term effects initiated during this time window is reduced by any early childbirth, with age at first birth not being more important than age at subsequent births.

The lower risk in women with a young age at birth was not due to their relatively long time since birth. Thus, after stratification for time since birth, we still found an effect of age at birth. We did find, however, that the effect of age at birth was modified by time since birth. There was essentially no effect of age at birth the first 10 years after birth, perhaps owing to a time delay in the effect of the birth. Thus, our data suggest that the effect of the age at birth primarily is a long-term effect.

Few previous studies have focused on the independent effect of age at births subsequent to the first. The results have been conflicting; Some have found effects of age at second birth,[11-14] whereas others observed no effect of subsequent births.[15,16] These studies, however, have included fewer observations, and analyzing the independent effect of each birth requires the inclusion of many cases in the study to achieve sufficient precision. Lack of precision might explain why some of these smaller studies reported an effect of age at first birth and age at second birth but not for other births. Recently a large study found an effect of any birth, although with a stronger effect of age at first birth compared with subsequent births.[17] This study and most previous studies, however, have examined differences in risk increase per increase in maternal age, that is, trend differences. In contrast, our study included more than 13,000 cases, which allowed us to study not only the trend differences but also to use a statistical approach that could identify the age-at-birth categories associated with a reduced risk, taking into account latency period and short-term effects after a birth. This latter approach was essential to identify a potential critical period in a woman's life.

An alternative interpretation of our data is that early age at first birth is important combined with shortness of interbirth intervals; that is, that early timing of subsequent births is important because of short interbirth time intervals. Nonetheless, the fact that we do not find any interaction between the effects of age at different births does not support this interpretation. Regardless of interpretation, however, it seems evident that the timing of any birth is important.

Although our study emphasizes the effects of childbirths subsequent to the first, our observations give no support to the idea that age at latest birth has any special importance. Studies in Norway and Brazil have previously found an effect of age at latest birth.[3,18,19] None of these studies, however, took into account the ages at intermediate births in their analyses, as pointed out by Hsieh et al.[20] We initially observed a strong effect of age at latest birth when only adjusting for age at first birth, but after adjustment for the effects age at first and subsequent births, there was no independent effect of age at latest birth. Therefore, in addition to the lack of a biological rationale for an effect of age at latest birth, we think that these findings are artifacts representing the effects of age at first and subsequent births observed in the present study.

Our findings are not likely to be due to information or selection bias, as the study was performed as a prospective analysis on a large population-based cohort and was based on mandatorily reported information on reproductive history and breast cancer. A limitation of the study, however, was the lack of possible confounder information such as oral contraceptive use and infertility (and the associated treatment). We had no information on menopausal status, which might modify the long-term effect of age at birth. Nonetheless, we observed almost the same pattern in women less than and more than 50 years of age, which suggests that the hormonal changes during menopause do not affect the long-term risk reduction conferred by early reproduction.

In conclusion, our study shows that we should modify the traditional view that age at first birth and number of births are the main reproductive long-term risk factors for breast cancer. Our data suggest that the fundamental factor behind the association between a woman's reproductive history and breast cancer risk is simpler; that is, that early timing of any additional birth induces an additional long-term reduction of maternal risk of breast cancer. Thus, the effect of an early first birth is no stronger than that of subsequent births.

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α-Fetoprotein Levels in Maternal Serum During Pregnancy and Maternal Breast Cancer Incidence

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Background: A full-term pregnancy is associated with a reduced risk of breast cancer, but the underlying biologic mechanism has not been elucidated. During pregnancy, maternal serum levels of α-fetoprotein, an estradiol-binding protein, rise sharply. In culture, α-fetoprotein inhibits the growth of estrogen-sensitive cells, including estrogen-sensitive breast cancer cells. Thus, we investigated whether a high level of α-fetoprotein in maternal serum during pregnancy is associated with a reduced risk of breast cancer. *Methods:* From a population-based cohort of 42 057 pregnant women in Denmark, enrolled in an α-fetoprotein-screening program from 1978 through 1996, we obtained a complete reproductive history, vital status, and a possible diagnosis of breast cancer (in 117 women) to the end of follow-up on September 1, 1998. *Results:* During pregnancy, women with an α-fetoprotein level greater than or equal to the median value had a 41% lower risk of breast cancer than women with an α-fetoprotein level below the median value (relative risk [RR] = 0.59; 95% confidence interval [CI] = 0.41–0.85). RRs for breast cancer by mother's age at childbirth were as follows: 29 years or younger, RR = 0.21 (95% CI = 0.08–0.56); 30–34 years, RR = 0.61 (95% CI = 0.32–1.14); 35–37 years, RR = 0.96 (95% CI = 0.49–1.89); and 38 years or older, RR = 0.71 (95% CI = 0.29–1.75) (*P* for trend = .02). Further analyses suggested that high levels of α-fetoprotein were associated with a reduced incidence of aggressive disease. The most striking finding was that women with high levels of serum α-fetoprotein, compared with women with low levels of serum α-fetoprotein, showed a particularly reduced incidence of large tumors (>2 cm; RR = 0.24 [95% CI = 0.11–0.50]). *Conclusion:* A high level of α-fetoprotein in maternal serum during any pregnancy is associated with a low overall incidence of breast cancer and, in particular, with a low incidence of advanced breast cancer at diagnosis. This association appears particularly strong for a pregnancy occurring at a young age. [J Natl Cancer Inst 2000;92:1001–5]

Reproductive factors—in particular, the number and timing of births—are well-established risk factors for breast cancer (*1–3*). The biologic mechanism of how a full-term pregnancy affects maternal breast cancer risk is not fully understood. Although the most popular hypothesis is that the differentiation of mammary gland cells induced in the late stages of a pregnancy reduces a woman's risk of breast cancer (*4*), alternative explanations must be considered. A pregnancy is accompanied by a steep rise in the levels of estrogens (*5*) and α-fetoprotein (*6*) in maternal serum. α-Fetoprotein is a glycoprotein that is produced by the fetal liver and yolk sack (*7*) and that is transmitted into the maternal circulation via the placenta (*8*) as well as the amniotic fluid and amniotic membranes (*9*). Thus, the increased production of fetal α-fetoprotein is followed by a rise in the level of α-fetoprotein in maternal serum. In animal models, both naturally occurring and recombinant human α-fetoproteins bind estradiol (*10–13*) and suppress the estrogen-dependent growth of breast cancer cells (*14,15*). Thus, α-fetoprotein appears to possess biologically important anticarcinogenic properties (*16,17*).

In this study, we have examined the relationship between α-fetoprotein levels in maternal serum during pregnancy and the subsequent maternal risk of breast cancer in a cohort of 42 057 Danish women who gave birth during the period from 1978 through 1996.

MATERIALS AND METHODS

For this investigation, we linked data from the National Civil Registration System (CRS), the National Birth Registry, and the Danish Breast Cancer Cooperative Group (DBCG) with data from a population-based screening of α-fetoprotein levels in maternal serum. This study was approved by the Scientific Ethics Committee and by the National Data Protection Board of Denmark.

Population Registries

Since April 1, 1968, the CRS has assigned a unique identification number to all residents of Denmark. This number permits information from differ-

ent registries to be linked. The CRS contains dates of any live births (which allows reconstruction of reproductive history for each woman) and dates of emigration and deaths (*18*). Information on stillbirths was available from the National Birth Registry.

α-Fetoprotein Assessment

Measuring the level of α-fetoprotein in maternal serum has been offered to all women receiving antenatal care in three Danish counties since 1978, and a screening program was introduced in 1980. Serum samples used for screening α-fetoprotein in maternal serum were taken during the second trimester of each pregnancy, before any amniocentesis was performed. Gestational age was recorded in completed weeks of pregnancy, estimated from ultrasound examination or from the date of the last menstrual period. For each pregnancy from an individual woman, the α-fetoprotein level in maternal serum was standardized by dividing the level by the median level in all measurements from singleton births taken at the same gestational age in the same calendar year (which gives multiples of the median [MoM]). For women who gave birth to more than one child during this period, several measurements of α-fetoprotein levels were available. Prepregnancy weight was available for only a subset of these women.

The median value of α-fetoprotein in maternal serum for all pregnancies was 40 000 IU/L of serum. The median value for the first pregnancy was also 40 000 IU/L of serum, and it was 37 000 IU/L of serum for two or more pregnancies. The median was 40 000 IU/L of serum for first pregnancies occurring at age 24 years or younger to age 34 years; it decreased to 35 000 IU/L of serum for first pregnancies at age 35 years or greater. Median levels of α-fetoprotein according to the age of the mother during a pregnancy in which α-fetoprotein was measured were as follows: 29 years or younger, 40 000 IU/L; 30–34 years, 39 300 IU/L; 35–37 years, 35 000 IU/L; and 38 years or older, 36 000 IU/L.

Patients With Breast Cancer

Women who developed breast cancer were identified by linkage with the DBCG because this reg-

ister was complete until September 1, 1998. The DBCG collects detailed information on the breast cancer diagnosis, including tumor size, lymph node status, and receptor status *(19,20)*. In a linkage between the DBCG Registry and the Danish Cancer Registry, the DBCG Registry was found to contain information on 94% of all patients with breast cancer reported to the Danish Cancer Registry *(3)*. Reporting of cancer to the Danish Cancer Registry has been mandatory in Denmark since 1943 *(21)*.

Design and Statistical Analysis

A cohort of 42 057 Danish women was retrospectively established to include all women born in Denmark during the period from April 1, 1935, through March 31, 1978 (since information on reproductive history could be obtained for these women by linkage with the CRS), who gave birth to their first child during the period from 1978 through 1996 and for whom an α-fetoprotein measurement was available for at least the first birth.

In this cohort, 117 women developed breast cancer by the end of follow-up on September 1, 1998. Women contributed person-time (years under observation) from the date of their first screening for α-fetoprotein to a diagnosis of invasive breast cancer, death, emigration, or the end of follow-up on September 1, 1998 (at which date the DBCG was considered complete), whichever occurred first. Relative risk (RR) estimates were obtained by modeling incidence rates of breast cancer with the use of a log-linear Poisson regression model.

Because the relationship between α-fetoprotein levels in maternal serum and the incidence of breast cancer appeared to be nonlinear, α-fetoprotein levels were categorized into four categories by quartiles of the MoM, using cut points based on the distribution of person-years. If a woman had more than one pregnancy during the study period and if α-fetoprotein was measured for more than one pregnancy, her α-fetoprotein information was updated when the new value was available. The study variable representing a woman's α-fetoprotein level is the value from her latest pregnancy, and the variable, therefore, is time dependent and can change after each pregnancy. Complete α-fetoprotein data for all pregnancies were available for 50% of the cohort. If α-fetoprotein levels were missing for a pregnancy after the first, the latest value was carried forward until the next pregnancy with an available value. Mothers of twins (or other multiple births) have at least twice the level of serum α-fetoprotein during pregnancy, compared with a mother carrying only one child, and probably also a different risk of breast cancer. In our analysis, this potential confounding effect of multiple births was avoided by excluding data from multiple-child pregnancies but including data from their other single-child pregnancies for these women.

Analyses were adjusted for attained age in 5-year intervals (≤34 years, 35–39 years, 40–44 years, and ≥45 years), calendar period (before 1993 versus 1993 and after), age at birth of first child (≤24 years, 25–29 years, 30–34 years, and ≥35 years), and parity (one child versus two children or more). The attained age is the age at any time during follow-up; for patients with breast cancer, this is their age at diagnosis. All variables were treated as time-dependent variables in the analyses. To evaluate the potential effect modification of the association of

α-fetoprotein with breast cancer, we also conducted analyses stratified by attained age, maternal age at first child's birth, age at latest pregnancy with an α-fetoprotein measurement, time since latest pregnancy with an α-fetoprotein measurement, and number of pregnancies. For the stratified analyses, α-fetoprotein levels in maternal serum that were the median value or higher were compared with levels that were less than the median.

Tests for the trend of the RR of breast cancer in women with α-fetoprotein levels of one MoM or more compared with those of less than one MoM across the levels of the stratification variables were performed by including an interaction term between α-fetoprotein and the stratification variable. The various categories of the stratification variables in these trend analyses were represented by the median value of the categories.

The association between the level of α-fetoprotein in maternal serum from the latest pregnancy and survival after a diagnosis of breast cancer was analyzed by Cox's proportional hazards method, with adjustment for tumor size, number of positive lymph nodes, age at diagnosis, and protocol allocation. The vital status of study participants was followed from the date of breast cancer diagnosis to October 1, 1998.

RESULTS

The 42 057 women who fulfilled the inclusion criteria for this study contributed 379 287 person-years of follow-up and 79 531 pregnancies. Values of α-fe-

toprotein in maternal serum were available for 70% of these pregnancies. In this population, the median age of the women at their first child's birth was 26 years (which is representative of the Danish female population for the period studied). Of these women, 117 developed invasive breast cancer during follow-up. About 96% of patients were 50 years of age or younger when their cancer was diagnosed.

Table 1 shows the distribution of factors characterizing the population at risk including the number of cases of breast cancer and person-years of follow-up. Table 2 shows the association between α-fetoprotein levels in maternal serum and the risk of breast cancer. Women in the highest two quartiles (quartiles 3 and 4) of serum α-fetoprotein levels had a statistically significantly lower incidence of breast cancer than women in the lowest two quartiles (quartiles 1 and 2). Women in the two highest quartiles of serum α-fetoprotein levels had about half the risk of breast cancer as women with serum α-fetoprotein levels that were just below the median, after adjustment for age, calendar period, parity, and maternal age at first child's birth. Adjusting only for age and

Table 1. Distribution of factors characterizing the population at risk, including number of patients with breast cancer and person-years of follow-up

	No. of patients	Follow-up, person-years* (thousands)
Attained age,† y		
≤34	23	263.7
35–39	45	73.8
40–44	17	30.2
≥45	32	11.6
Age at first child's birth, y		
≤24	14	139.5
25–29	34	153.7
30–34	34	59.2
≥35	35	26.7
Age at child's birth with α-fetoprotein measurement,‡ y		
≤29	24	236.4
30–34	40	98.9
35–37	34	28.2
≥38	19	15.7
No. of childbirths		
1	63	209.0
≥2	54	170.2
Time since latest childbirth, y		
<2	18	135.4
2–4	39	135.8
5–9	40	87.8
≥10	20	20.3

*Person-year = years of observation.

†Age at any time during follow-up; for patients with breast cancer, attained age is age at the time of diagnosis of breast cancer.

‡Time-dependent variable.

Table 2. Relative risk (RR) of breast cancer according to serum levels of α-fetoprotein (AFP) during the second trimester of pregnancy

AFP*	No. of patients	RR (95% CI)†
<0.8 MoM (quartile 1)	32	0.74 (0.46–1.20)
0.8–0.99 MoM (quartile 2)	34	1 (referent)
1–1.29 MoM (quartile 3)	28	0.51 (0.31–0.85)
≥1.3 MoM (quartile 4)	23	0.49 (0.29–0.83)

*AFP levels in maternal serum were standardized to gestational age by dividing the absolute value by the median value across all singleton live births, for each gestational week and for each calendar year to give multiples of the median (MoM).

†RR and 95% confidence interval (CI) were adjusted for age, calendar period, parity, and age at first birth.

calendar period produced virtually identical results. Adjusting for prepregnancy weight did not alter the results; therefore, the results are presented unadjusted for this variable. When we restricted the cohort to women for whom we had complete information on serum α-fetoprotein levels for each birth during follow-up, the RR estimates relative to quartile 2 (referent quartile) were 0.91 (95% CI = 0.51–1.64) for quartile 1, 0.56 (95% CI = 0.29–1.08) for quartile 3, and 0.46 (95% CI = 0.25–0.86) for quartile 4.

In Table 3, we present the association between α-fetoprotein levels and the RR of breast cancer, stratified by maternal

age, maternal age at first child's birth, maternal age at child's birth with measurement of α-fetoprotein in corresponding pregnancy, number of children born, and time since latest child's birth. An α-fetoprotein level equal to the median value or greater was associated with a 41% decreased risk of breast cancer compared with a level less than the median. The association between α-fetoprotein levels in maternal serum and the incidence of breast cancer was even stronger among younger women and among women with a pregnancy before age 30 years, whereas it was the same for the first and subsequent pregnancies.

Table 4 presents the association between α-fetoprotein levels in maternal serum and the risk of specific tumor characteristics. The difference with respect to estrogen receptor status was rather modest. However, the reduction in the incidence of breast cancer in women with high serum α-fetoprotein levels was more strongly associated with tumors with a positive lymph node status (RR = 0.48; 95% CI = 0.30–0.79) than with tumors with a negative lymph node status (RR = 0.70; 95% CI = 0.39–1.25). Similarly, the reduction was also more strongly associated with tumors larger than 2 cm (RR = 0.24; 95% CI = 0.11–0.50) than with smaller tumors (RR = 0.83; 95% CI = 0.52–1.33). In other words, the most striking finding was that women with high serum α-fetoprotein levels, compared with women with low levels of serum α-fetoprotein, showed a particularly reduced incidence of large tumors, i.e., those greater than 2 cm.

Of the 117 women who developed breast cancer, 22 died before October 1, 1998. The RR of dying was 0.70 (95% CI = 0.22–2.24) for patients who had an α-fetoprotein level that was greater than

Table 3. Serum levels of α-fetoprotein (AFP) and relative risk (RR) of breast cancer stratified by age and reproductive variables

Stratification variable	No. of patients		RR for AFP ≥1 MoM versus AFP <1 MoM (95% CI)†	P for trend
	AFP <1 MoM*	AFP ≥1 MoM		
Overall	66	51	0.59 (0.41–0.85)	
Attained age,‡ y				
≤34	19	4	0.17 (0.06–0.51)	
35–39	25	20	0.65 (0.36–1.16)	
40–44	8	9	0.85 (0.33–2.19)	
≥45	14	18	0.86 (0.43–1.73)	.01
Age at first child's birth, y				
≤24	11	3	0.22 (0.06–0.80)	
25–29	21	13	0.51 (0.25–1.01)	
30–34	17	17	0.80 (0.41–1.57)	
≥35	17	18	0.70 (0.36–1.35)	.11
Age at child's birth with AFP measurement,§ y				
≤29	19	5	0.21 (0.08–0.56)	
30–34	23	17	0.61 (0.32–1.14)	
35–37	15	19	0.96 (0.49–1.89)	
≥38	9	10	0.71 (0.29–1.75)	.02
No. of childbirths				
1	36	27	0.53 (0.32–0.88)	
≥2	30	24	0.66 (0.39–1.13)	.57
Time since latest childbirth, y				
<2	10	8	0.65 (0.26–1.65)	
2–4	25	14	0.45 (0.23–0.87)	
5–9	23	17	0.54 (0.29–1.01)	
≥10	8	12	1.06 (0.43–2.58)	.39

*AFP levels in maternal serum were standardized to gestational age by dividing the absolute value by the median value across all singleton live births, for each gestational week and for each calendar year as multiples of the median (MoM).

†RR and 95% confidence interval (CI) were adjusted for age, calendar period, parity, and age at first birth (unless the variable was stratified).

‡Age at any time during follow-up; for patients with breast cancer, this is age at the time of diagnosis of breast cancer.

§Time-dependent variable.

Table 4. Serum levels of α-fetoprotein (AFP) and relative risk (RR) of breast cancer stratified by estrogen receptor status, lymph node status, and tumor size

| Stratification variable | No. of patients* | | RR for AFP ≥1 MoM versus AFP <1 MoM (95% CI)‡ |
	AFP <1 MoM†	AFP ≥1 MoM	
Estrogen receptor status			
Negative	21	19	0.69 (0.37–1.28)
Positive	32	24	0.57 (0.34–0.97)
Lymph node status			
Negative	24	22	0.70 (0.39–1.25)
Positive	41	26	0.48 (0.30–0.79)
Tumor size, cm			
≤2	33	36	0.83 (0.52–1.33)
>2	29	9	0.24 (0.11–0.50)

*Number of patients do not add to 117 because of missing information on tumor characteristics for some patients.

†AFP levels in maternal serum were standardized to gestational age by dividing the absolute value by the median value across all singleton live births, for each gestational week and for each calendar year as multiples of the median (MoM).

‡RR and 95% confidence interval (CI) were adjusted for age, calendar period, parity, and age at first child's birth.

or equal to the median value compared with patients who had a level less than the median.

DISCUSSION

In a large population of more than 42 000 Danish women, we found that a high level of α-fetoprotein in maternal serum during the second trimester of pregnancy was associated with a statistically significantly reduced incidence of breast cancer. This finding confirmed our expectation, which was mainly based on the antiestrogenic properties of α-fetoprotein. The association between high α-fetoprotein levels in maternal serum and a reduced incidence of breast cancer was particularly strong among women with a pregnancy at a young age.

The current study is, to our knowledge, the only one in which repeated measures of α-fetoprotein in maternal serum are available for consecutive pregnancies. To our knowledge, the only previous epidemiologic study in which the association between α-fetoprotein levels in maternal serum and breast cancer has been considered was a case–control study nested in the Californian Child Health and Development Studies (CHDS) *(22)*. In this study of 573 women, third-trimester blood samples were taken during the period from 1959 through 1966 and subsequently frozen; later, levels of α-fetoprotein were determined in these samples. These blood samples were taken during only one pregnancy; thus, α-fetoprotein levels in maternal serum were available for only that pregnancy (at arbitrary birth order). No overall association was found

in that study *(22)* between α-fetoprotein levels in maternal serum for that one pregnancy and the risk of breast cancer. The authors did, however, report a reduced risk of breast cancer for women with high α-fetoprotein levels in maternal serum during the one index pregnancy, provided the women's first pregnancy occurred when they were young. We also found that the lowest RR of breast cancer was among women whose first pregnancy occurred when they were younger than 24 years old. In contrast to the findings in the previous study, however, we did not find an increased risk of breast cancer associated with high α-fetoprotein levels in maternal serum among women who were older than 26 years during their first pregnancy.

We (Wohlfahrt J: unpublished data) and others *(23)* have previously shown that the effect of pregnancy on the risk of breast cancer approximates a short-term increase in risk followed by longer term protection. In the present study, the association between α-fetoprotein levels in maternal serum and a reduced incidence of breast cancer was particularly strong among women with a pregnancy at a young age. In these women, a recent pregnancy is associated with an increased risk of breast cancer that is greater than the risk for nulliparous women. Consequently, high levels of α-fetoprotein might be associated not with a reduction in the risk of breast cancer but, rather, with a smaller increase in the risk of breast cancer. For obvious reasons, our study did not include nulliparous women. However, we found no difference in the

association between breast cancer risk and high levels of α-fetoprotein according to years since the last childbirth *(see* Table 3). The short-term effect is, therefore, probably not related to the α-fetoprotein level in maternal serum during pregnancy.

There are a number of possible explanations for the difference in the overall association between breast cancer risk and α-fetoprotein levels reported by the CHDS group *(22)* and by us. First, in the CHDS cohort, the α-fetoprotein level was available from only one pregnancy and, thus, that index pregnancy could represent any pregnancy. In contrast, we had several measurements of α-fetoprotein levels in maternal serum; we measured α-fetoprotein levels in maternal serum for all first pregnancies and for the majority of the other pregnancies. If there were important differences among α-fetoprotein levels in maternal serum from different pregnancies, we would capture their influence more effectively with our updated analyses. Furthermore, α-fetoprotein in maternal serum was measured during the third trimester in the CHDS cohort and during the second trimester in our study. As is acknowledged in the study by the CHDS group *(22)* as a potential limitation of their cross-sectional approach, the consistency of third-trimester α-fetoprotein levels in maternal serum between subsequent pregnancies has not been studied *(24)*. In addition, the majority of patients with breast cancer in the CHDS were postmenopausal at diagnosis, whereas most of our patients with breast cancer were diagnosed premenopausally. Our conclusions of a positive association between a high α-fetoprotein level in maternal serum during pregnancy and a lower incidence of breast cancer cannot be extrapolated to postmenopausal women on the basis of our data. Finally, although both studies had a large number of patients with breast cancer overall, the number of patients in the stratified analyses was relatively small, leaving room for some variability between results.

Our cancer registry included detailed information on characteristics of the breast cancer at the time of diagnosis, which allowed a more detailed analysis of the association of risk of breast cancer with α-fetoprotein level in maternal serum. There was a clear tendency among women with high levels of serum α-fetoprotein to have a particularly low incidence of breast tumors that had ag-

gressive characteristics at the time of diagnosis, such as large tumor size and positive lymph node status. The most striking finding was that women with high levels of α-fetoprotein, compared with women with low levels of serum α-fetoprotein, showed a particularly reduced incidence of large tumors, i.e., tumors larger than 2 cm (RR = 0.24; 95% CI = 0.11–0.50). In line with these results, women with high levels of serum α-fetoprotein also appeared to have a better overall survival than women with low levels, even after adjustment for important characteristics influencing survival. This finding, however, was based on a limited number of deaths and should be viewed with due caution.

The prospective nature of our cohort design limited the potential for biases related to differential misclassification and selection. Thus, all covariate information was obtained independently of the exposure and was not dependent on recall. In our analysis, we were not able to adjust for a number of known risk factors for breast cancer, such as family history of breast cancer, height, body mass index, age at menarche, and age at menopause. Because the majority of our patients with breast cancer were diagnosed before age 50 years, confounding by menopausal status is unlikely. As reported by the CHDS group, we found no confounding effect of prepregnancy weight. Thus, it is unlikely that adjustment for height or body mass index would alter the results. The Danish population is very homogeneous, and the vast majority are Caucasian. The present investigation only included women born in Denmark; therefore, the study population almost exclusively represents Caucasians. Thus, confounding by race is not an issue.

In conclusion, we found that a high α-fetoprotein level in maternal serum during the second trimester of pregnancy was associated with a subsequent reduction in the overall incidence of breast cancer and, in particular, with a low incidence of advanced breast cancer among primarily premenopausal women. This association appeared strongest if the woman's pregnancy occurred at a young age. The present findings are potentially important. First, they may offer a complementary explanation as to why the risk of breast cancer is lower in parous women than in nulliparous women. Moreover, our results indicate that even time-limited exposure to high levels of α-fetoprotein can lower the risk of breast cancer overall and of advanced breast cancer in particular. If confirmed in future studies, and given the availability of recombinant α-fetoprotein, these findings may open up new venues for the prevention of breast cancer. However, any practical implications await a better understanding of whether the observed association of a reduced risk of breast cancer with high levels of α-fetoprotein reflects a direct effect of α-fetoprotein on tumor carcinogenesis or the effect of another substance closely interacting with α-fetoprotein.

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NOTES

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The Authors Reply:

In 1997 we published a paper showing no association between induced abortion and subsequent breast cancer risk in a population-based cohort study based on national registries including 1.5 million women with 10,246 cases of breast cancer.[1] Recently we indicated the importance of adjusting for age at first birth in this study[2] as a response to a letter by Sharpe.[3] We argued that otherwise the biological effect of age at first birth would wrongly be attributed to induced abortion.[2] Brind *et al* state in the first paragraph of their letter that they concur with this conclusion, but they use the rest of the letter to repeat their critique of our abortion study,[4] a critique that we previously have commented on point by point.[5]

They argue that we should have used the same approach as in our recent publication concerning pre-term birth and breast cancer risk.[6] They thereby disregard, however, the fact that in the pre-term study the focus is on the importance of gestational age at delivery among parous women only. The same approach is not applicable in the abortion study because it would imply studying the effect of induced abortion in a cohort including only women who have had an induced abortion. Therefore, to call the methodology used in the pre-term study "corrected" compared with the abortion study methodology is simply incorrect.

Their insistence on not adjusting for birth-cohort is just as wrong as insisting on not adjusting for age at first birth. Without adjustment for birth-cohort effects, differences in risk behavior according to birth-cohort might be ascribed incorrectly as an effect of induced abortion. If this is the type of nonspecific effects they would like to attribute to induced abortion, as mentioned in their first paragraph, we can only disagree. In other words, adjusting for birth-cohort does not underestimate the biological effect of induced abortion on breast cancer risk, but rather excludes the possibility of over-estimation due to differences in risk factors other than induced abortion between birth-cohorts.

For these reasons we stand firm by our results showing no association between induced abortion and breast cancer risk.

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Risk of late stage breast cancer

following a childbirth

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Running head: Risk of late stage breast cancer following a childbirth

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ABSTRACT

A pregnancy may lead to hormone-induced growth of breast tumors. We investigated whether women in the first years following childbirth had a higher incidence of breast cancer and in particular a higher incidence of late stage tumors (i.e., large tumor, node positive or histologic grading II+III). The study was based on a population-based cohort of 1.5 million Danish women born between 1935-1978 with individual information on births. Between 1978 and 1994, 10,790 incident cases of breast cancer were identified in a nationwide cancer registry. Overall, uniparous and biparous mothers experienced a transient increased risk that did not appear to be attributable to delayed cancer diagnosis. The risk of being diagnosed with a tumor with a diameter larger than 5 cm was on average 53% higher the first 10 years after birth compared to later. The risk of tumors less than 2 cm was not significantly associated with time since latest birth. In conclusion, after a childbirth mothers experience a transient increased risk of breast cancer and in particular a relatively high risk of late stage disease. This finding suggests that pregnancy related factors transiently induce a high growth rate in cells that are already malignant and induce new tumor growth.

Keywords: breast cancer, reproductive history, cohort study, tumor size

It is well established that the birth of a child decreases a mother's long-term risk of breast cancer (1). However, several studies have found that her risk of breast cancer may be elevated in the immediate years following childbirth (2,3,4,5). This latter observation has been thought explained by a growth enhancing effect of the hormonal changes occurring during pregnancy on malignant or premalignant cells (4). If correct, the pregnancy promoted tumors should have particularly rapid growth and therefore be likely on average to be diagnosed at a later stage, i.e. the transient increase should be especially pronounced for the rate of late stage breast cancer. To evaluate this hypothesis of pregnancy-induced rapid growth of occult tumors we studied the overall rate of breast cancer in the years following a birth and in particluar, the rate of late stage tumors taking advantage of the detailed registration of breast cancer characteristics in Denmark.

SUBJECTS AND METHODS

Study cohort

Since April 1, 1968, the Civil Registration System (CRS) in Denmark has assigned an individually unique national registration number to all citizens. Based on this number the CRS keeps updated information on dates of live births, emigration and vital status. The CRS-number also permits accurate individual-based linkage of information from other registries. We used the CRS-registry to establish a national parity database including all women born between April 1, 1935, and March 31, 1978 as earlier described (6,7). To be able to study breast cancer rates during pregnancy, we added information on induced and spontaneous abortions and gestational age of births from the

National Registry of Induced Abortions, the National Discharge Registry and the Danish National Birth Registry.

Detailed information on registered invasive primary breast cancers in the period January 1, 1978 to September 30, 1994 including the size of the tumor, number of positive nodes and histological grading was obtained from the Danish Breast Cancer Cooperative Group (DBCG) registry. DBCG initiated a series of national prospective studies in 1977 to systematically evaluate breast cancer treatment programs. A detailed description of this registry has been given elsewhere (8,9). During a limited time period (1977-81), the DBCG collected additional information such as the date at which the woman experienced the first symptom(s) of her disease, and the date of her first consultation with a medical doctor (10). Through a linkage between the DBCG registry and the Danish Cancer Registry, the DBCG registry was found to contain information on 94 percent of all breast cancer patients reported to the Danish Cancer Registry. The Danish Cancer Registry is considered close to complete regarding incident cases of malignant neoplasms diagnosed in Denmark since 1943 (11).

Statistical analyses

The impact of time since birth on the incidence of breast cancer with a specific tumor characteristic was investigated in a follow-up study analyzed using log-linear Poisson regression models (12). Each stage specific diagnosis of breast cancer was analyzed separately. Both tumor size, nodal involvement and histologic grading are used as indicator of stage. All women entered follow-up for each of the stage specific breast cancer diagnoses on January 1, 1978 or on their 12-year birthday whichever came last. The period at risk continued until breast cancer (whatever stage), death, emigration, or Sep-

tember 30, 1994 whichever occurred first. Incidence rate ratios are referred to as relative risks. All variables were treated as time-dependent variables. Calculations were performed using the SAS procedure PROC GENMOD (13). Adjustment was made for age (quadratic splines with knots: 30,35,40,45,50,55) (14) and calendar period (1978-82,1983-87,1988-92,1993-94). Using age in 1-year categories in the overall analysis had no impact on the conclusions. In the analysis of time since latest birth we furthermore adjusted for age at first birth (nulliparous, 12-19, 20-24, 25-29, 30-34, >34) and parity (nulliparous,1,2,3,4+). Estimation of the effect of time since latest birth with adjustment for age and age at first birth when including uniparous women has been discussed by Heuch et al (15). Test for effect modification by parity (1,2,3+) was performed as a test for interaction between categorical variables.

In an alternative approach we compared the mother's risk with what would have been her risk had she not delivered a child. This was done according to time since each delivery categorized as: <2 years, 2-3 years, 4-5 years, 6-7 years, 8-9 years, 10+ years, i.e. four time-dependent variables representing time since 1st ,2nd, 3rd and 4th birth were included in the model. In these analyses women were followed until a possible fifth birth. In addition to age and calendar period we also adjusted for age at 1st, 2nd, 3rd and 4th birth. As we only found minor insignificant effects of age at 1st to 4th birth the first 10 years after birth, we only added the effects of age at 1st to 4th birth in the model 10 years after birth. This was done by further categorizing the category "10+ years" in each of the four "time since birth"-variables according to age at birth (12-24 years, 25-29 years, 30-34 years, 35+ years). In other words, the "short-term" effect of a birth (<10 years) was categorized according to time since birth, and the "long-term" effect (=10 years) according to age at birth.

In an additional analysis we estimated the rate of breast cancer during pregnancy using a similar approach including additional information on interrupted pregnancies and gestational age at delivery. A woman was considered pregnant from the time of conception (estimated by gestational age) until birth or time of interruption of the pregnancy. Her parous status during pregnancy was the number of births prior to the pregnancy.

To evaluate whether an increased risk after childbirth could be ascribed to delayed diagnosis we estimated the cumulative difference between the observed number of incident breast cancer cases in newly pregnant nulliparous and uniparous women in the cohort according to time since latest birth and the predicted number of cases had they not had the latest birth. The prediction was based on a model including age, calendar period, parity and age at first birth. The deficit of cases in these women during pregnancy was estimated using the distribution of person-years in uni- and biparous women with less than one year since latest birth (multiplied by 9/12) assuming that they were one year younger and have had one childbirth less.

RESULTS

Overall, 1,529,512 women were included in the cohort. A total of 10,790 primary invasive breast cancers were observed during 22.3 million person-years of follow-up. Of these 1,295 women were nulliparous at time of diagnosis.

The association between time since latest birth and the incidence of breast cancer is shown in Table 1. Overall, there was a small but significant association between time since latest birth and the breast cancer rate (p=0.0002). After adjustment for the differences in age and other confounders the risk was highest (1.16-fold) 2-3 years

after delivery compared to 10-14 years after. Table 1 furthermore shows the association between the time interval since latest birth and the risk of breast cancer by tumor size at diagnosis. The rate of large tumors was significantly associated with the time interval since latest birth (*p*=0.002), for instance, women with 2-3 years since latest birth had a 2.27-fold (95 percent confidence interval 1.49-3.44) higher risk of breast cancer compared to women with 10 to 14 years since latest birth. Overall the risk of being diagnosed with a tumor with a diameter larger than 5 cm was 53 percent higher the first 10 years after birth compared to later. There was no association between time since latest birth and the rate of small tumors (<21mm) (*p*=0.17). The rate of medium sized tumors (21-50 mm) was only slightly associated with the time since latest birth (*p*=0.06). The association between time since latest birth and large breast cancer was not modified by parity (*p*=0.56). We found similar patterns of an increased risk of tumors with adverse features when the cases were divided according to nodal status or histological grading (Table 2).

In an alternative approach we compared a mother's risk with what would have been her risk had she not delivered a child. In the first 10 years after the first and second birth the breast cancer risk was increased by ratios of 1.07 (0.97-1.19) and 1.07 (0.98-1.15) compared to nulliparous and uniparous women, respectively. Overall, the increase the first 10 years after first and second birth was 1.07 (1.01-1.13) and according to time since birth: <2 years: 0.97 (0.82-1.15), 2-3 years: 1.13 (0.99-1.29), 4-5 years: 1.08 (0.96-1.21), 6-7 years: 1.11 (1.00-1.22), 8-9 years: 1.06 (0.97-1.15). In the first 10 years after the third and fourth birth there was no increased risk (RR=0.99 (0.90-1.08) and 0.89 (0.74-1.07), respectively). As illustrated in Figure 1A and 1B we performed the same analyses according to tumor size at diagnosis. During the first 10 years after the second and third birth, mothers had up to a 2-fold higher risk of being diagnosed with a tumor larger than 50 mm. The relative risk the first 10 years after fourth birth compared

with triparous women was 1.34 (0.74-2.43) for being diagnosed with tumors larger than 5 cm and 0.88 (0.72-1.08) for being diagnosed with tumors 5 cm or smaller. The same type of analysis was not informative for uniparous women because there was an overall higher rate of late stage tumors in nulliparous women as mentioned in the discussion.

Based on 20 cases of breast cancer detected in *pregnant* women during 706,234 years of follow-up, we estimated that the rate of breast cancer in pregnant women was 72 percent (95 percent confidence interval from 51 percent to 84 percent) lower than expected. To evaluate whether this lower rate of breast cancer during pregnancy could explain the increased risk of breast cancer in the first years after first and second birth we estimated the cumulative difference between the observed number of incident breast cancer cases in the newly pregnant nulliparous and uniparous women in the cohort and the expected number of cases had they not had the latest birth. During pregnancy, we estimated that there was a total deficit of around 31 breast cancer cases compared with non-pregnant women. However, in the first 10 years after birth the women who had been pregnant experienced an excess of 88 cases. Assuming that the deficit of cases during pregnancy was exclusively due to postponed diagnosis it could only account for the excess during 4 to 5 years after birth.

For a subgroup of women diagnosed in the period from 1978 to 1982 we had information about the time interval between the first symptoms observed by the woman and her first visit to her doctor (patient's delay), and between the first visit to her doctor and the time of definitive surgery or biopsy (doctor's delay) (*10*). There was no significant relationship between the two measures of diagnostic delay and the time since latest birth when compared by a Mann-Whitney test. Within five years after a birth the median patient's and doctor's delay was 12 and 29 days, respectively. During

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5 to 9 years after a childbirth the similar figures were 11 days and 30 days, respectively, and in the subsequent years the figures were 7 days and 28 days, respectively.

DISCUSSION

The present study documented that a mother's age-adjusted risk of breast cancer is highest the first 10 years following the latest birth and in particular that their risk of late stage tumors is significantly elevated. That the overall breast cancer risk is increased after a childbirth has been observed before (2,3,4,5), but that in particular the risk of late stage breast cancer is elevated is a novel observation that may give further insight to the mechanisms behind the increased risk.

The high rate of late stage breast cancer in the first years following a birth could be due to delayed diagnosis/surgery of breast cancer during pregnancy. Either because of difficulties in detecting the tumor during pregnancy or because breast surgery was postponed to after the delivery. A delayed diagnosis/surgery due to pregnancy would result in larger tumors after the delivery, but the breast cancer rate during pregnancy should also be correspondingly low. In concordance with three previous studies (4,16,17) we observed a 72 percent lower risk of breast cancer during pregnancy. Some of this lower rate might very well be explained by a "healthy women" effect, but, even if we assumed that the lower rate during pregnancy should exclusively be explained by delayed diagnosis, we found that such a diagnostic delay only could account for an excess of cases equivalent to e.g. the observed increased breast cancer rate in the first four or five years after first and second delivery. Thus, delayed diagnosis/surgery due to pregnancy did not appear to explain the entire excess of cases in the years following pregnancy.

We furthermore investigated whether the higher rate of late stage breast cancers after the first years could be due to delayed detection because of woman's primary attention being devoted to childcare during the first years after delivery. However, based on detailed referral information on a subset of the women in this study we found no elevated diagnostic delay in women diagnosed in the these years after a childbirth compared to later years. Altogether, delayed diagnosis during pregnancy and delayed detection in the first years following birth appeared unable to explain the significantly elevated age-adjusted risk of late stage breast cancer in the first 10 years after a birth.

Part of the higher rate of late stage breast cancer in the first 10 years after a birth is probably explained by cases diagnosed in the first 10 years being initiated before the birth, whereas the malignant process in cases diagnosed after the first 10 years more likely are initiated after the birth where the rate is reduced by the protective effect of an additional birth. However, if this was the only explanation for the higher rate of breast cancer after a delivery we would anticipate that uniparous mothers in the first years after childbirth had the same *overall* risk as nulliparous women (and likewise when comparing biparous with uniparous women) or maybe even a lower risk in the first years due to a "healthy women" effect. Nevertheless, in additional analyses we observed that uniparous and biparous women had a slightly increased overall breast cancer risk in the first 10 years after the latest birth when compared with women with one birth less. Such analyses suggest that a mother transiently has a higher risk compared to what would have been her risk had she not delivered a child and therefore directly support the idea that pregnancy related factors, e.g. the elevated hormonal level during pregnancy, transiently increase a mother's overall risk of breast cancer by stimulating high growth rate in already malignant cells and/or inducing a new tumor growth.

An enhanced tumor growth following a birth might just mean that relatively indolent tumors are accelerated and therefore discovered sooner, but at the same stage as without a growth rate change. However, performing the same kind of analysis on the rate of late stage tumors we observed an even more dramatic transient increase of the rate of late stage breast cancer after a second and third delivery. During the first years following the second or third birth we observed a more than 2-fold higher risk of late stage cancer when comparing with women everything else equal but the latest birth. This novel observation should not alarm the average pregnant women as the rate of late stage breast cancer is very small. In other words, the absolute effect is small and therefore has no direct implications for primary prevention, but the finding is of etiologic interest because it supports the hypothesis that the transient increased risk of breast cancer after birth is due to an increased growth rate in malignant and premalignant cells that to some extent leads to discovery at a later stage compared to what would have been the case had the women not delivered a child.

The stage-specific analysis should be considered with due caution. We have previously shown that the rate of late stage tumors in general is much higher in nulliparous compared with parous women, which can either be because a woman's reproductive history influences the time of detection or it affects the progression rate of the tumor (18). The lack of a transient increased risk of late stage breast cancer after the first birth is most likely due to this generally lower risk of late stage breast cancer in uniparous compared to nulliparous women. Because of this phenomenon we have in this paper also focused on comparisons between mothers with the same number of births, thereby excluding differences in the rate of late stage breast cancer attributable to parity per se. Using this approach we observed the same association between the time since latest birth and the rate of late stage breast cancer irrespective of parity, which suggests

that regardless of number of prior births, a recent pregnancy transiently increases the number of late stage cases of breast cancer.

We have used two different analytic approaches with different features. In one approach we compare the risk in mothers at a given time-interval after the birth compared to what would have been her risk had she not delivered a child. With such an approach one is able to estimate the transient increase, which is of etiological interest. However, as one estimates the combined effect of the birth and the time interval one cannot determine whether differences in the effects according to stage is related to the birth per se, the time interval or both. In the other approach we avoid this problem by comparing the risk according to time since latest birth between women with the same number of births. This approach can, however, not be used to estimate the transient increase (19) and it has furthermore been argued that one cannot estimate the effect of time since latest birth in uniparous women while adjusting for age at first birth and age (5). However, estimation in uniparous is possible when including nulliparous and assuming a common age effect for all women (15). A recent paper reveals that this approach is reasonable (20). Nevertheless we have in Table 1 provided results where uniparous are excluded.

The study was performed as a prospective analysis on a large population-based cohort and was based on mandatory reported exposure and outcome information making information bias on exposure and selection bias on cases unlikely. As noted by Hsieh et al (3) the cohort follow-up design is more powerful than a case-control design when studying a time-dependent variable as time since latest birth, since all births are included in the study and not just the last birth. A limitation of the study was the lack of data on other reproductive breast cancer risk factors such as age at menarche, age at menopause, family history and use of exogenous hormones.

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In conclusion, a recent childbirth results in a transiently increased risk of breast cancer in the mother and in particular a relatively high risk of late stage disease. These findings suggest that pregnancy related factors transiently stimulate high growth rate in already malignant cells and induce new tumor growth.

ACKNOWLEDGMENTS

LEGEND TO FIGURE:

Figure 1. Effect of second birth on the risk of being diagnosed with a breast tumor 50 mm (triangles) and >50 mm (dots) by time since second birth. The exact estimates for =50 mm are: <2 yr: 0.94 (0.73-1.21), 2-3 yr: 1.11 (0.90-1.35), 4-5 yr: 1.02 (0.86-1.22), 6-7: 1.14 (0.98-1.31), 8-9: 1.05 (0.92-1.19) and for >50 mm: <2 yr: 1.29 (0.59-2.83), 2-3 yr: 1.88 (1.05-3.38), 4-5 yr: 1.38 (0.80-2.38), 6-7: 1.26 (0.78-2.03), 8-9: 1.02 (0.65-1.61). B) Effect of third birth on the risk of being diagnosed with a breast tumor =50 mm (triangles) and >50 mm (dots) by time since third birth. The exact estimates for =50 mm are: <2 yr: 0.79 (0.57-1.09), 2-3 yr: 0.76 (0.57-1.01), 4-5 yr: 0.92 (0.74-1.15), 6-7: 1.00 (0.83-1.21), 8-9: 1.01 (0.85-1.19) and for >50 mm: <2 yr: 0.70 (0.22-2.20), 2-3 yr: 2.39 (1.31-4.36), 4-5 yr: 1.79 (0.96-3.33), 6-7: 0.73 (0.32-1.67), 8-9: 1.01 (0.54-1.88).

TABLE 1. Adjusted* relative risk of breast cancer overall and according to time since latest birth by tumor size at diagnosis‡, Denmark, 1978-1994.

Years since latest birth	Overall†			Tumor size at diagnosis‡					
				<21mm		21-50 mm		>50 mm	
	Person-years (10³)	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)
<2	1,794	211	1.02 (0.87-1.19)§	97	0.91 (0.73-1.14)	77	1.16 (0.89-1.50)	14	1.14 (0.63-2.07)
2-3	1,479	303	1.16 (1.02-1.32)	139	1.04 (0.86-1.26)	102	1.21 (0.97-1.52)	35	2.27 (1.49-3.44)
4-5	1,191	369	1.13 (1.00-1.27)	170	1.03 (0.86-1.22)	122	1.16 (0.94-1.42)	35	1.80 (1.21-2.69)
6-7	1,080	486	1.14 (1.03-1.27)	243	1.13 (0.98-1.31)	149	1.09 (0.90-1.30)	39	1.53 (1.05-3.23)
8-9	1,022	591	1.08 (0.99-1.19)	290	1.04 (0.91-1.19)	202	1.14 (0.97-1.34)	40	1.22 (0.85-1.76)
10-14	2,414	1993	1 (reference)	1038	1 (reference)	659	1 (reference)	118	1 (reference)
15+	3,791	5542	0.92 (0.86-0.98)	3042	0.93 (0.85-1.01)	1876	0.90 (0.81-1.00)	304	0.95 (0.74-1.21)
Homogenity test			$p=0.0002$		$p=0.17$		$p=0.06$		$p=0.002$

* Adjusted for overall and tumor size specific age, calendar period, parity and age at first birth.

†When excluding uniparous women from the analysis we found a similar association: <2: 1.05 (0.88-1.26), 2-3: 1.15 (0.99-1.33), 4-5: 1.16 (1.03-1.32), 6-7: 1.13 (1.01-1.27), 8-9: 1.07 (0.97-1.19), 10-14: 1 (reference), 15+: 0.91 (0.85-0.97).

‡ Number of cases by tumor size do not add up to number of cases overall due to missing information on tumor size for some cases (7%).

§0 yr: 0.92 (0.74-1.15), 1 yr: 1.10 (0.90-1.33).

TABLE 2. Adjusted* relative risk of breast cancer according to time since latest birth by nodal status and histological grading at diagnosis, Denmark, 1978-1994.

Years since latest birth	Nodal status at diagnosis†				Histological grading at diagnosis‡			
	Node negative		Node positive		I		II+III	
	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)
<2	88	0.85 (0.67-1.08)	109	1.01 (0.75-1.36)	30	0.77 (0.52-1.14)	137	1.13 (0.93-1.38)
2-3	140	1.06 (0.88-1.29)	139	1.28 (1.00-1.63)	55	1.07 (0.79-1.44)	178	1.19 (1.00-1.41)
4-5	163	0.99 (0.83-1.17)	184	1.34 (1.08-1.65)	74	1.09 (0.84-1.41)	213	1.17 (1.00-1.36)
6-7	260	1.21 (1.05-1.39)	202	1.11 (0.91-1.36)	95	1.02 (0.82-1.28)	287	1.24 (1.08-1.42)
8-9	281	1.01 (0.88-1.16)	266	1.27 (1.08-1.51)	134	1.07 (0.88-1.30)	321	1.11 (0.98-1.26)
10-14	1020	1 (reference)	862	1 (reference)	490	1 (reference)	1014	1 (reference)
15+	2972	0.96 (0.89-1.05)	2364	0.89 (0.79-1.00)	1500	0.91 (0.80-1.02)	2770	0.98 (0.90-1.07)
Homogenity test		p=0.06		p=0.0002		p=0.33		p=0.03

* Adjusted for nodal and grading specific effects of age, calendar period, parity and age at first birth.
† Number of cases according to nodal status do not add up to number of cases overall (Table 1) due to missing information on nodal status (4%).
‡ Histological grading is only registered for ductal carcinomas. Number of ductal cases with missing information on histological grading were 4%.

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INFLUENCE OF TUMOR LOCATION ON AXILLARY NODAL STATUS AND BREAST CANCER PROGNOSIS

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ABSTRACT

Background: Axillary lymph node dissection is the most important staging procedure forming the basis for choice of treatment for breast cancer patients. However, lymph drainage from the breast is not restricted to the axillary lymph nodes and the risk exists that lymphatic spread is neglected when only the axillary lymph nodes are examined. We investigated the influence of tumor location on axillary nodal status and prognosis.

Methods: We used a population-based registry which since 1977 has collected detailed information regarding clinical and histopathological presentation, postoperative therapy and follow-up status on Danish women with breast cancer.

Results: Overall, 35,319 patients with primary breast cancer were included in the study. Irrespective of tumor size, women with tumors close to the axilla (lateral tumors) were significantly more often classified as node positive (p<0.001). Compared to women with a tumor in the upper lateral quadrant, women with other tumor locations had between 15% and 21% increased risk of dying of their disease. Among women with no apparent metastatic spread to axillary lymph nodes, survival was 30% worse for women with a tumor in the upper medial quadrant compared to the upper lateral quadrant.

Conclusions: Survival is significantly better for women with a tumor in the upper lateral quadrant than tumors located elsewhere in the breast. Better staging of the tumor and extensive surgery to dissect lymph nodes for staging purposes out into the axilla are likely explanations for the superior survival of women with such tumor location. This suggests that a more aggressive treatment of tumors in other locations might increase these women's chance of survival.

Key words: Breast cancer, prognosis, staging procedures, lymph nodes, tumor location,

population-based.

INTRODUCTION

Axillary lymph node status is the single most important prognostic factor in primary breast cancer and the significance of a proper axillary dissection both with regard to staging and local tumor control is well established (1). Recent efforts to optimize the existing staging system with the sentinel node lymphadenectomy have put renewed focus on the prognostic importance of nodal status in breast cancer (2-5).

From anatomical studies it is known that lymphatic drainage from the breast goes not only to the axillary lymph nodes, but also to the internal mammary, the supraclavicular nodes, and to lymph nodes outside these locations (6;7). Today's emphasis on axillary nodal status raises an important clinical question as to whether some women with breast cancer are misclassified as low-risk patients because axillary dissection does not reveal spread of the disease to the lymphatic system. Recently, Zucali et al. (8) reported that women with medially located tumors were less likely to be classified as having node positive disease compared with other women with breast cancer. In spite of this, these women had a reduced chance of survival compared with women with lateral tumors.

In the present study we extended this line of investigation on the prognostic effect of tumor location based on a large and very detailed population-based registration of breast cancer patients in Denmark.

MATERIAL AND METHODS

Registries

In 1977, the Danish Breast Cancer Cooperative Group (DBCG) started nationwide prospective studies on treatment of breast cancer (9). The primary surgical treatment of

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patients allocated in treatment protocols included total mastectomy plus axillary clearance (90% of the population), or lumpectomy with axillary dissection. Patients were classified as having either low-risk disease or high-risk disease according to histopathological criteria. Low-risk patients were observed without further adjuvant treatment apart from radiotherapy to the residual breast of women who had breast conserving surgery. High-risk patients were allocated to adjuvant chemotherapy and/or radiotherapy. Guidelines for risk group allocation and treatment have been described in detail elsewhere (9-12).

Primary clinical and histopathological data and data concerning postoperative therapy and status at follow-up visits are all registered by the DBCG based on specific forms submitted by the participating departments of surgery, pathology and oncology. Location of the tumor was determined based on an indication made by the surgeon on a figure (Figure 1). When a tumor was located in the borderline between two areas, it was assigned to one of the two areas by randomization according to date of birth.

The Danish Civil Registration System (CRS) was established in 1968 and since then a unique identification number has been assigned to all residents in Denmark. Individual information is kept under the personal identification number in all national registers permitting accurate linkage of information between different registries. The CRS registry keeps updated files on vital status including dates of death and emigration. A detailed description of the information included in this registry is given elsewhere (13).

Subjects

Permission to perform the study was obtained in advance from the National Scientific Ethics

Committee and the Data Protection Board. Information on patients in the DBCG-registry was linked with the CRS-registry to obtain information on vital status. The study was restricted to women less than 70 years at diagnosis, because the DBCG in the DBCG 82 program restricted the data collection to this group of women. Women included in the DBCG-program since 1977 and diagnosed with breast cancer before September 1, 1998, were followed from time of diagnosis until date of death, emigration, or October 1, 1998, whichever occurred first.

Statistical analysis

Associations between tumor characteristics and location were evaluated by chi-square statistics. The association between location and survival was investigated using Cox proportional hazard regression with adjustment for axillary nodal status (0, 1-3, 4-9, ≥10 positive nodes), tumor size (≤2cm, >2 cm and up to 5 cm, >5 cm) histologic grading (I, II-III, non-ductal carcinomas, and patients without information on histologic grading), year of diagnosis (1977-1981, 1982-1988, 1989-1998) and protocol allocation (allocated, not treated according to surgical guidelines, not allocated for other reasons). Test for effect modification was performed as test for interaction between categorized variables. All analyses were performed with the use of SAS (14).

RESULTS

By September 1, 1998, 35,319 women with primary breast cancer less than 70 years of age were registered in the DBCG. The cohort represented a total of 237,364 person-years of follow-up. Distribution of patients according to tumor characteristics and tumor site is given in Table 1. Compared with laterally located tumors, tumors located medially tended to be smaller (p<0.001) and the chance of nodal involvement was significantly reduced (p<0.001). Tumors with central location were found to be larger (p<0.001), associated with higher risk of

nodal involvement (p<0.001), and with lower chance of having histologic grading I (p<0.001) compared to laterally located tumors.

In order to further analyze tumor characteristics according to the tumor location in the four quadrants, women with central tumors and women without information on tumor location or nodal status were excluded, leaving 27,234 women for further analysis. Nodal status according to tumor site is given in Figure 2, and further details on tumor site, tumor size and nodal status is given in Table 2. The chance of being axillary node negative was significantly greater for women with medial tumors compared with lateral tumors in the subgroup with tumors ≤ 2 cm (p<0.001) and women with tumors being > 2 cm and ≤ 5 cm (p<0.001). The same trend was seen for the group of women with large tumors (> 5 cm), but the differences did not reach significance (p=0.38).

The independent prognostic effect of tumor location was analyzed by performing a multivariate analysis including tumor size, nodal status, histologic grading, age at diagnosis, protocol allocation, year of treatment, and tumor site. Compared to women presenting with a tumor in the upper lateral quadrant, women with other tumor locations had significantly impaired prognosis (Table 3). Axillary nodal status did not modify the negative prognostic effect among women with lower lateral and lower medial tumors. However, the negative effect of tumor location in the upper medial quadrant was almost exclusively restricted to women classified as axillary nodal negative (upper medial node negative RR=1.30, 95 percent confidence interval, 1.20 to 1.40; upper medial node positive RR=1.08, 95 percent confidence interval, 0.996 to 1.16). The differences in prognosis according to tumor location were not modified by tumor size (p=0.77, data not shown).

DISCUSSION

The present study shows that the prognosis in breast cancer patients differs significantly according to tumor location. Compared to women with tumors in the other three quadrants, women with tumors located in the upper lateral quadrant clearly had the best survival. They were, however, also the group of women most likely to be diagnosed with metastatic spread to the axillary lymph nodes. In contrast, women with tumors in the upper medial quadrant had the worst prognosis but were the least likely to be diagnosed with axillary node positive tumors. An explanation for these seemingly contradictory associations is that treatment allocation according to axillary lymph node spread is insufficient. Thus, a proportion of women with tumors in the upper medial quadrant and with no spread to axillary nodes most likely had lymphatic dissemination of their disease to lymph nodes outside the axilla, and thus should have been allocated to a more aggressive treatment program than the one given to them. Support for this view is given by our finding that women with upper medial and lateral tumor locations had similar survival when restricting the analysis to those with positive axillary nodes whereas survival was 30 percent worse among women with upper medial compared to upper lateral tumors among those classified as axillary node negative. The internal mammary lymphnodes have been found the most important destination of lymph drainage outside the axilla (15). It seems likely that more accurate diagnosis and surgical treatment of the internal mammary nodes could lead to improved prognosis for patients with tumors located in the upper medial quadrant of the breast. The impact on survival after treatment of the internal mammary nodes in women with medially located tumors is the subject of an ongoing EORCT trial (16).

Compared to tumors in the upper lateral quadrant we also found an impaired survival for women with lower medial and lower lateral tumors. However, for these tumor locations

survival was independent of axillary nodal status. This observation indicates that other factors than nodal misclassification and consequently wrong allocation to existing treatment protocols should be considered.

It is documented that a proper axillary dissection is important not only regarding staging of the disease but also with respect to the local tumor control (1;17). Hence, women with tumors in the upper lateral quadrant are likely to have the most complete surgical management of the tumor burden when mastectomy/lumpectomy and axillary dissection is the standard treatment. Compared with these patients, women with other tumor locations must be expected to have a higher risk of having residual tumor tissue after surgical treatment. Thus, incomplete removal of tumor tissue among women with tumors located away from the axilla may explain why survival disadvantage is observed also among certain groups of axillary node positive patients who receive adjuvant treatment.

Some centers have evaluated whether more extended operations including internal mammary chain dissection can improve survival of the patients (18-22). Based on these studies between 6% and 9% (some old studies even up to 19%) of the patients have been found to have metastases in the internal mammary chain without axillary nodal involvement. Although some authors found a beneficial effect of the extended operation for women with medial tumors, the overall conclusion was that due to increased morbidity of the intensive procedure, it was not found recommendable.

Recent studies on sentinel node procedures have revealed that about three percent of breast cancer patients without positive axillary lymph nodes have metastatic nodes outside the axilla (3;15) . The present study underlines that axillary nodal staging is insufficient in a proportion

of women with breast cancer. The sentinel node technique may offer an attractive opportunity to identify women with primary lymph drainage to lymph nodes outside the axilla and thus lead to changed treatment procedures for some women. However, based on the present results such altered procedures may primarily be beneficial to women with upper medially located tumors. Unfortunately, a better classification of nodal status does not appear to remove the differential survival for all tumors in the breast. The differences in survival according to tumor location are substantial and suggest that other factors of prognostic importance need be considered. It is unlikely that the biology of the tumors should differ based on tumor location in the breast. Rather, factors such as differences in the surgical efficacy of removing metastatic tissue might show important for the differential survival according to tumor location observed in the present study.

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Table 1. Distribution according to tumor characteristics and location of 35,319 breast cancer patients operated in Denmark 1977-1998.

| | Tumor location n (%) | | | | | |
| | Lateral | | Medial | | Central | Not indicated |
	Upper	Lower	Upper	Lower		
Total No	17,659	4,559	5,987	2,212	2,584	2,318
Positive nodes						
0	8,960 (50.7)	2,301 (50.5)	3,704 (61.9)	1,233 (55.7)	868 (33.6)	844 (36.4)
1-3	4,964 (28.1)	1,302 (28.6)	1,351 (22.6)	567 (25.6)	683 (26.4)	507 (21.9)
4-9	2,214 (12.5)	562 (12.3)	488 (8.2)	206 (9.3)	527 (20.4)	240 (10.4)
≥ 10	785 (4.5)	161 (3.5)	142 (2.4)	67 (3.0)	263 (10.2)	90 (3.9)
No information	736 (4.2)	233 (5.1)	302 (5.0)	139 (6.3)	243 (9.4)	637 (27.5)
Tumor size						
≤ 2 cm	8,717 (49.4)	2,476 (54.3)	3,289 (54.9)	1,256 (56.8)	741 (28.7)	830 (35.8)
> 2 cm, ≤ 5 cm	6,560 (37.2)	1,525 (33.5)	2,028 (33.9)	714 (32.3)	1,009 (39.1)	643 (27.7)
> 5 cm	1,074 (6.1)	189 (4.2)	209 (3.5)	70 (3.2)	515 (19.9)	139 (6.0)
No information	1,308 (7.4)	369 (8.1)	461 (7.7)	172 (7.8)	319 (12.4)	706 (30.5)
Histologic Grading						
I	4,521 (25.6)	1,220 (26.8)	1,679 (28.0)	580 (26.2)	517 (20.0)	457 (19.7)
II + III	9,275 (52.5)	2,342 (51.4)	3,118 (52.1)	1,148 (51.9)	1,349 (52.2)	858 (37.0)
ND *	3,863 (21.9)	997 (21.9)	1,190 (19.9)	484 (21.9)	718 (27.8)	1,003 (43.3)

* Patients with non ductal carcinomas and patients without available histologic grading

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Table 2. Distribution of nodal status according to tumor size and quadrant location of tumor. Presentation of 27,234* breast cancer patients with laterally or medially located tumors operated in Denmark 1977-1998.

	Tumor quadrant location n (%)			
	Lateral		Medial	
	Upper	Lower	Upper	Lower
Tumor ≤ 2 cm				
Positive nodes				
0	5,519 (64.9)	1,483 (61.8)	2,360 (74.2)	821 (68.2)
1-3	2,236 (26.3)	673 (28.1)	631 (19.8)	285 (23.7)
4-9	595 (7.0)	192 (8.0)	156 (4.9)	73 (6.1)
>10	152 (1.8)	51 (2.1)	32 (1.0)	25 (2.1)
Tumor > 2 cm, ≤ 5 cm				
Positive nodes				
0	2,651 (41.6)	642 (43.7)	1,047 (53.6)	320 (46.8)
1-3	2,126 (33.4)	478 (32.5)	586 (30.0)	230 (33.6)
4-9	1,198 (18.8)	270 (18.4)	244 (12.5)	105 (15.4)
>10	398 (6.2)	79 (5.4)	75 (3.8)	29 (4.2)
Tumor > 5 cm				
Positive nodes				
0	234 (22.7)	31 (17.3)	51 (25.5)	18 (28.1)
1-3	294 (28.6)	60 (33.5)	57 (28.5)	18 (28.1)
4-9	297 (28.9)	69 (38.5)	62 (31.0)	18 (28.1)
>10	204 (19.8)	19 (10.6)	30 (15.0)	10 (15.6)

* Patients with central tumors or missing information on tumor location, tumor size, or nodal status were excluded.

Table 3. Adjusted relative risk of dying according to tumor quadrant location and axillary nodal status among Danish women with primary breast cancer operated 1977-1998

		Adjusted relative risk of dying (95% CI)*		
		All† (n=27,234)	Node + (n=12,057)	Node – (n=15,177)
Lateral	Upper	1 ref.	1 ref.	1 ref.
	Lower	1.15 (1.09-1.22)	1.15 (1.07-1.24)	1.16 (1.05-1.27)
Medial	Upper	1.17 (1.11-1.24)	1.08 (0.996-1.16)	1.30 (1.20-1.40)
	Lower	1.21 (1.11-1.31)	1.21 (1.09-1.35)	1.21 (1.07-1.37)

*Relative risk of dying (95% confidence intervals) adjusted for number of positive nodes, tumor size, histologic grading, age at diagnosis, year of treatment, and protocol allocation.
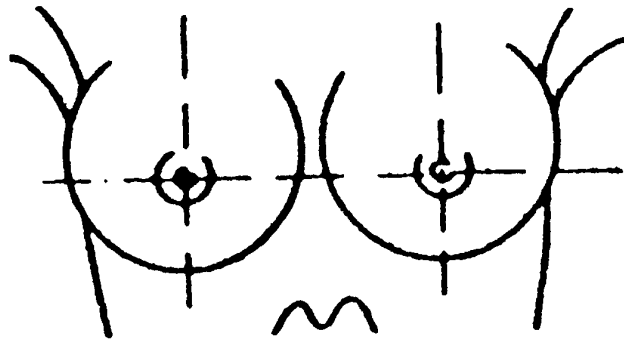†Patients with central tumors or missing information on tumor size or nodal status are excluded.

Figure 1. Surgeon's figure for location of the tumor.

Figure 2. Percentage of tumors with negative axillary nodal status according to quadrant location of tumor among 27,234 women with non central tumor localization aged less than 70 years operated in Denmark 1977-1997.

Breast cancer risk after a childbirth

in young women with family history

Jan Wohlfahrt, Jørgen H Olsen, Mads Melbye

Abstract

The increased risk of breast cancer in women with family history of breast cancer (FHBC) might be reduced by early childbirths. However, a childbirth induces a transient increase in risk in the first 5 to 10 years, which collide with family cases that tend to be diagnosed at a relatively young age. To investigate this short-term change in risk we used a population-based cohort of 1.5 million Danish women. Between 1968 and 1990, 2,770 incident cases of breast cancer below 40 years of age were identified in the Danish Cancer Registry whereof 276 (10%) had a FHBC. The first five years after a birth the short-term increase in risk was 30% (3%-64%) larger in women with FHBC than without FHBC. After the first 5 years we observed no difference in the effect of a birth between women with and without FHBC. In conclusion, the adverse short-term effect of a childbirth is stronger in women with FHBC, which corresponds with the hypothesis that a childbirth induces growth potential in occult tumors.

Word count: abstract 170, text: 2110

Abbreviation: FHBC: family history of breast cancer.

Introduction

Women with a family history of breast cancer (FHBC) have an increased risk of breast cancer (Pharoah *et al*, 1997). A potential way to modify this risk could be by early childbirths. To investigate this possibility previous studies have focused on the protective effect of many childbirths and young age at first birth (see discussion for references). However, in the last decade there has been a growing acknowledgment of the increased risk of breast cancer in the first 5-10 years after a birth (Lambe *et al*, 1994; Albrektsen *et al*, 1995). This effect collide with the period where women with FHBC have a relative higher breast cancer risk, i.e. before the age of 40 years (Pharoah *et al*, 1997), and the adverse effect might therefore be stronger in women with FHBC. To investigate the short-term effect of a childbirth in women with FHBC we used population-based register data from the Danish population with information on family history. By using register based information on family history we avoided differential recall in cases that could otherwise cause bias.

Material and Methods

Study population and ascertainment of cases

A research parity database was established from the Civil Registration System (CRS). It includes all women born in Denmark between April 1, 1935, and March 31, 1978, as earlier described (Melbye 1997, Westergaard 1997). Based on the personal identification number from the Civil Registration System, we linked data with the Danish Cancer Registry, which

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has information on invasive primary breast cancers since 1943. As earlier described were 2860 women born 1935 or later diagnosed with breast cancer in the period 1943 to 1990 before the age of 40 years (Olsen et al, 1999). The research parity database includes 2770 of these cases (i.e. excluding cases diagnosed Jan 1, 1935 to Marts 31, 1935 and cases born outside Denmark).

Identification of mothers

The method for identification of mothers of the women in the cohort differed according to mother's birth cohort. For women with a mother born in April 1, 1935 or later the mother could be identified in the CRS. For women with a mother born before April 1, 1935 the identity of the mother was not necessarily available from the CRS. For cases among these women the mother was identified from parish registries as described in Olsen et al (Olsen et al, 1999). The identity of the mothers was found in 94% of the cases (Olsen et al, 1999). Breast cancer cases among mothers were identified in the Cancer Registry with follow-up to end of 1993.

Determination of person-years of follow-up

For women with a mother born in April 1, 1935 or later the mothers identity was known for all women, and it was therefore possible to directly calculate person-years of follow-up in strata according to both FHBC and other factors. For women with a mother born before April 1, 1935 the identity of the mother was only known for cases. The distribution of person-years of follow-up in these women was therefore estimated on the basis of the distribution among women with a mother born April 1, 1935 or

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later. This was done by estimating, for each strata according to other factors, the proportion of persons-years of follow-up contributed by women with FHBC using logistic regression (with adjustment for age, parity and age at first birth) based on the person-years distribution in women with a mother born April 1, 1935 or later. To test the robustness of these imputations of person-years of follow-up we alternatively scaled the estimated proportions by a factor 5 and as a second alternative used the average proportion regardless of age and reproductive history. Using these two alternative approaches did not change the conclusion, e.g. the general relative increase in risk the first 5 years after a birth in women with compared to without FHBC was found to be 1.30 using the logistic regression approach, and 1.30 and 1.27 in the two alternatives. The main effect of FHBC might be modified by mothers birth cohort (due to differences in mothers mean age). Therefore, as we used a birth-cohort dependent imputation procedure, we did not estimate the main effect of FHBC on a woman's breast cancer risk. However, it is much less likely that this should affect the estimation of the interaction between FHBC and reproductive history which is the focus of this paper.

Statistical methods

We investigated the impact of 1st, 2nd and 3rd birth on the incidence of breast cancer according to FHBC in a follow-up study using log-linear Poisson regression models (Breslow and Day, 1987). The impact of 1st (2nd and 3rd) birth was modeled as a comparison between uniparous and nulliparous (biparous versus uniparous, triparous versus biparous) according

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to time since and age at 1st (2nd and 3rd) birth. A more formal statistical description of the model is given elsewhere (Wohlfahrt and Melbye, 2001). As described in that paper the effect of age at birth only affect the breast cancer risk more than 10 years after birth, the risk in the first 10 years after birth is therefore not stratified according to age at birth (Wohlfahrt and Melbye, 2001). FHBC, i.e. family history of breast cancer, was a constant variable defined as having a mother with breast cancer diagnosed before the end of 1993. All women entered the follow-up for breast cancer diagnoses on April 1, 1968, or on their 12th birthday, whichever came latest. The period at risk continued until breast cancer, 40th birthday, death, emigration, or December 31, 1990 (end of follow-up), whichever occurred first. Adjustment was made for age (one year categories), calendar period (5 years categories), an interaction between having a fourth birth (yes/no) and FHBC, and an interaction between mother's birth cohort (<1935, 1935) and FHBC. When adjusting for the interaction between FHBC and age, the age factor was modeled by quadratic splines with knots (age=30,35) (Greenland 1995). Common effects for 1st, 2nd and 3rd, for example the effect in the first 5 years after birth, were estimated by substituting the three related indicator variables (0/1) in the model by their sum.

Results

In all 2,770 cases of breast cancer were observed during 22.7 mill person-years of follow-up. Among the cases 276 (10%) had a mother with breast cancer. Table 1 shows the distribution of number of cases and distribution

of person-years of follow-up in women with and without a family history of breast cancer (FHBC) according to age and number of births.

In table 2 is shown the effect of 1st, 2nd and 3rd birth on breast cancer risk according to family history of breast cancer. The relative risk of breast cancer in the first 5 years after the first birth compared to nulliparous is 1.5 in women with FHBC and 1.1 in women without FHBC, i.e. the relative risk is 1.4-fold higher in women with FHBC. The same figure for the 2nd and 3rd birth is 1.2 and 1.2, and the general estimate for 1st, 2nd and 3rd birth is 1.30 (95-confidence interval: 1.03-1.64). In other words the increased risk the first 5 years after birth is 30% higher in women with compared to without FHBC. Performing the same analysis of the relative risk of breast cancer 5 to 9 years after birth, compared with women with a birth less, the general effect is 1.02 (0.84-1.23). Including an interaction between age and FHBC the two estimates were 1.30 and 1.01.

More than 10 years after birth the relative risk is categorized according to age at birth. The relative risk of breast cancer more than 10 years after birth in women that were 25 to 29 years at first birth compared to nulliparous was 0.7 in women with FHBC and 1.0 in women without FHBC, i.e. the relative risk in woman with FHBC was 0.7-fold that of women without FHBC. The same figure for 2nd and 3rd birth was 0.9 and 1.4. The general estimate for 1st, 2nd and 3rd birth was 0.89 (0.67-1.19). When performing the same analysis for women younger than 25 at

childbirth the general estimate obtained was 0.93 (0.78-1.12). Including an interaction between age and FHBC the two estimates were 0.87 and 0.93.

Discussion

In a comprehensive review of 74 studies from the period from 1935 to 1995 on the association between family history of breast cancer (FHBC) and breast cancer the authors found based on meta-analysis that the strongest association with family history was among young women (Pharoah *et al*, 1997). Factors interacting with FHBC in the older ages might therefore be different from the factors acting in younger women and studies in post-menopausal can not necessarily be used to predict the effect modifications in pre-menopausal women. The situation is further complicated by the fact that the effect of reproductive history might be modified by age (Andrieu *et al*, 2000). Our study concentrates on pre-menopausal women and is by far the largest study among women 40 years or younger.

The focus in previous studies on the interaction between FHBC and reproductive history has been on number of births and age at first birth. Some studies have found an interaction with age at first birth (Dupont and Page, 1987; Negri et al, 1988; Byrne *et al*, 1991; Sellers *et al*, 1992; Colditz *et al*, 1993; 1996) or parity (Negri *et al*, 1988; Parazzini et al, 1992; Colditz *et al*, 1996), some found no interaction with age at first birth (Brinton *et al*, 1982; Parazzini *et al*, 1992; McCredie *et al*, 1997; Magnusson *et al*, 1998; Andriu et *al*, 1998) or parity (Bain et al,1980; Colditz et al,1993; Sellers *et al*, 1992;1993; McCredie *et al*, 1997; Andrieu et al 1998). However, a

dominant reproductive risk factor in the young years is the short-term increase in risk following a childbirth, and we have therefore in our study focused on the negative short-term effect of a childbirth.

We found that the increase in risk the first 5 years after a childbirth was stronger in young women with compared to without FHBC. More than five years after a childbirth the protective effect was equal to the effect seen in other young women, or if anything even larger. Our finding is in correspondence with the 50% larger transient increased effect seen in women with FHBC in the Nurses' Health Study (Colditz et al, 1996) . Furthermore, a small Swedish study found an increased risk of pregnancy related breast cancer among carriers of *BRAC1* and *BRAC2* compared with a references population (Johansson et al, 1998). In other words, although FHBC can reflect genetic factors, shared environmental factors or both, and although only a small fraction of women with FHBC are *BRAC1* or *BRAC2* carriers, our results might be applicable to this particular group.

One interpretation is related to the hypothesis that the short-term increase after childbirth is due to a childbirth induced increase in growth potential in occult tumors. Young women with family history have a higher risk of having occult breast tumors during the reproductive years (due to their higher risk in the young years) and the impact of the growth inducing effect might therefore be stronger in women with FHBC. However, although the relative risk the first 5 years after birth is high, the absolute rate in the young years is relative low and the actual excess number of

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cases the first five years is therefore small. The importance of our study is therefore primarily related to the etiologic interpretation of breast cancer in relation to the mechanism of the short-term effect of a childbirth.

The study of the effect of FHBC is related to several technical issues. Firstly, family history of breast cancer when assessed by questionnaire can be subject to recall bias (Floderus and Mack, 1990). This type of information bias is avoided in our study by using register data. Secondly, there might be some non-differential misclassification due to the fact that a mother can develop breast cancer after follow-up. However, it is unlikely that this should affect the interaction with reproductive history. A limitation of the study was the lack of detailed confounder information in the registries. One potential confounder could be the lower average age at diagnose in women with FHBC, that would give them higher likelihood of being diagnosed few years after a birth. However, we found the same result when allowing for different age effects according to FHBC. Our findings are not likely to be due to selection bias as the study was performed as a prospective analysis on a large population-based cohort and was based on mandatorily reported information on reproductive history and breast cancer.

In conclusion, women with family history had a stronger adverse short-term effect of a childbirth compared to others, which corresponds with the hypothesis that a childbirth induce growth potential in occult tumors.

Acknowledgements

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Table 1. Number of cases and distribution of person-years of follow-up in women with and without family history of breast cancer (FHBC) according to attained age and parity.

	with FHBC			without FHBC		
	no.	%cases	%pyrs	no.	%cases	%pyrs
Attained age						
12-20	1	0.4%	25.9 %	5	0.2%	30.1 %
20-24	2	0.7%	17.8%	23	0.9%	19.3 %
25-29	27	9.8%	20.3%	211	8.5%	18.8 %
30-34	81	29.4%	20.1%	706	28.3%	17.6%
35-39	165	59.8%	16.0%	1,549	62.1%	14.1 %
Parity						
0	42	15.2%	50.2%	353	14.3%	54.0 %
1	61	22.1%	17.3%	492	19.7%	16.1 %
2	124	44.9%	23.1%	1165	46.6%	21.3%
3	44	15.9%	7.5%	397	15.9%	6.8%
4+	5	1.8%	1.9%	87	3.5%	1.7%

Table 2. Relative risk* (RR) of breast cancer after 1st, 2nd and 3rd birth compared to women with one birth less according to time since and age at birth by family history of breast cancer (FHBC).**

	with FHBC		without FHBC	
	no.	RR (95%-CI)	no.	RR (95%-CI)
Time since and age at 1st birth				
Nulliparous	42	1 (ref.)	353	1 (ref.)
<5 yr after 1st birth	38	1.5 (0.9-2.4)	218	1.1 (0.9-1.3)
5-9 yr after 1st birth	62	1.0 (0.6-1.6)	529	1.1 (0.9-1.3)
10 yr after 1st birth, 12-24 yr at 1st birth	114	0.9 (0.6-1.5)	1,138	1.0 (0.8-1.1)
10 yr after 1st birth, 25-29 yr at 1st birth	20	0.7 (0.4-1.3)	256	1.0 (0.9-1.3)
Time since and age at 2nd birth				
Uniparous	103	1 (ref.)	845	1 (ref.)
<5 yr after 2nd birth	49	1.3 (0.9-2.0)	342	1.1 (0.9-1.2)
5-9 yr after 2nd birth	60	1.1 (0.7-1.6)	610	1.1 (0.9-1.2)
10 yr after 2nd birth,12-24 yr at 2nd birth	35	0.7 (0.4-1.3)	410	0.9 (0.7-1.0)
10 yr after 2nd birth,25-29 yr at 2nd birth	29	0.9 (0.6-1.6)	287	1.0 (0.8-1.2)
Time since and age at 3rd birth				
Biparous	227	1 (ref.)	2,010	1 (ref.)
<5 yr after 3rd birth	19	1.1 (0.7-1.8)	153	0.9 (0.7-1.0)
5-9 yr after 3rd birth	21	1.2 (0.7-1.9)	206	1.0 (0.8-1.2)
10 yr after 3rd birth,12-24 yr at 3rd birth	2	0.4 (0.1-1.8)	63	0.9 (0.7-1.3)
10 yr after 3rd birth,25-29 yr at 3rd birth	7	1.1 (0.5-2.4)	62	0.8 (0.6-1.0)

* Adjusted for age, calendar period and subsequent births.
** -2logL in the model is 4352.4. In the model with common effect for 1st, 2nd and 3rd birth -2logL is 4367.3 (with 12 parameters less).

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