Is the biology of breast cancer changing? An exploration of breast cancer incidence and molecular epidemiology in Scottish women

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Increasing incidence of breast cancer: distinguishing between the effects of birth cohort and a national breast screening programme. **Brown SB,** Morrison DS, Cooke TG. Breast Cancer Res Treat 2009 Aug. vol . 116, pp. 603-7 (Epub 2008 Oct 15)

Is the biology of breast cancer changing? A study of hormone receptor status 1984-1986 and 1996-1997. **Brown SBF,** Mallon EA, Edwards J, Campbell FM, McGlynn LM, Elsberger B, Cooke TG. Br J Cancer 2009 Mar. vol. 100, pp. 807-10 (Epub 2009 Feb 17)

Breast cancer incidence trends in deprived and affluent Scottish women. **Brown SBF,** Hole DJ, Cooke TG. Breast Cancer Res Treat. 2007 Jun. vol.103, pp. 233-8 (Epub 2006 Oct 11)

Published abstracts:

Is the biology of breast cancer changing? A study of hormone receptor status 1984-1986 and 1996-1997. **Brown SB**, Mallon EA, Edwards J, Campbell FM, McGlynn LM, Elsberger B, Cooke TG. European Journal of Surgical Oncology (EJSO) 2008 vol. 34 pp. 1172 (P25)

Increasing incidence of breast cancer: distinguishing between the effects of birth cohort and a national breast screening programme. **Brown SB**, Morrison DS, Cooke TG. Journal of Pathology 2007 vol. 213 Supplement 1 (P23)

Trends in breast cancer incidence in Scotland: influence of screening and deprivation. **Brown S**, Hole DJ, Cooke TG European Journal of Surgical Oncology 2007 vol. 31 pp.1084 (P33) and Breast Cancer Research and Treatment 2007 vol. 94 Supplement pp.147 (P3068)

List of Abbreviations

ANOVA = Analysis of Variance statistic

AP1 = Activated protein 1 transcription factor

ASCO = American Society of Clinical Oncology

ATAC= Arimidex, Tamoxifen, Alone or in Combination

B = B coefficient (slope coefficent) of regression equation

BMI = Body mass index

CDC = Centers for Disease Control, United States

CI = confidence interval

D = optical density of radiographic film (equal to $\log_{10}$ of the ratio of incident to transmitted photons, with 'useful' range being 0.5-2.5)

DAB = 3,3-diaminobenzidine

Depcat = deprivation category

DNA = deoxyribonucleic acid

DPX = dibutylphthalate and xylene

DPX = dibutylphthalate and xylene

EDTA = ethylenediaminetetraacetic acid

e = mathematical constant e

ER = Oestrogen receptor

GP = General Practitioner

H&E = haematoxylin and eosin

HER-1= The Human Epidermal Growth Factor 1 gene

HER-2 = The Human Epidermal Growth Factor 2/neu gene (also known as Erb-B2)

HRP = horseradish peroxidase

HRT = Hormone replacement therapy

ICD = International Classification of Disease

IHC = Immunohistochemistry

ISD (Scotland): Information and Statistics Division of the Scottish Executive

l = litre

LBA = Ligand Binding Assay

Abbreviations Continued

m = milli

n = sample size/populatiom

NHS = National Health Service

p = either proportion or 'p-value' depending on context

PR = Progesterone receptor

QUASAR = Quick And Simple And Reliable trial of chemotherapy in colorectal cancer

RR = Relative risk

SDR = Standardised Detection Ratio

SEER = Surveillance, Epidemiolgy and End results programme encompassing data from 9 states in the United States of america

SERM = Selective Oestrogen Receptors Modulators

SPSS = Statistical Package for the Social Sciences, SPSS Incoporated

TMA = Tissue Microarray

Tris = tris(hydroxymethyl)aminomethane

UK = United Kingdom

US(A) = United States of America

v = version

WHO = World Health Organisation

μ = micro

-ve = negative

+ve = positive

Summary

Breast cancer is the most common female cancer in Scotland, in common with many Western countries. This thesis aimed to analyse changes in the incidence and molecular epidemiology of breast cancer in Scotland. Part 1 concentrated on epidemiological research, with data derived from various agencies, and Part 2 on a laboratory project aimed at looking at changes in the molecular profile of breast cancers in two cohorts of patients in Glasgow.

The period between 1987 and 1994 in which coverage of the country by the breast screening programme gradually increased was expected to raise incidence rates as seen in studies in Scandinavia and elsewhere; after 1994, incidence rates should have returned to normal in women aged 55-64, with incidence in women aged 50-54 remaining slightly above pre-existing rates. An observed/expected analysis of breast cancer incidence rates after 1994 was performed; this showed a 58% increase in rates in women aged 50-54 above that which would have been expected had the trends continued as expected in the absence of screening. In 55-59 year olds and 60-64 year olds there were 42% and 40% increases, respectively, above expected rates.

Reproductive risk factors such as low parity and late age at first pregnancy are important risk factors in breast cancer. Reproductive risk factors are likely to affect the 'birth-cohort' incidence of breast cancer but the temporal effects of breast screening make this difficult to interpret. Breast cancer incidence in Scotland by year of birth was examined using a Lexis diagram. In women aged 50-54, 55-59 and 60-64, breast cancer incidence rates increased by birth cohort during the presence of the prevalent round of screening, a finding which is likely to have been due to detection of large numbers of asymptomatic tumours. However, in women who were offered screening after the prevalent round, incidence continued to rise with successive birth year, suggesting a contribution from risk factors. This is the first study of birth cohort incidence of breast cancer and its relation to screening (published in Breast Cancer Research and Treatment).

The contribution of screening and risk factors to breast cancer incidence in Scotland was also assessed. A small rise in screening uptake between 1990 and 2001

and an increase in standardised detection ratio may indicate that screening improvements could be contributing to increasing incidence. The number of first pregnancies to women in Scotland aged 35-59 has risen from several hundred in 1976 to 2000 in 2001. A plot of completed family size in Scotland against maternal birth year shows that a steadily declining trend has been developing since the 1935 birth cohort. Based on data from the Scottish Health Surveys, the percentage of women with a BMI of over 25 has increased from 47.2% to 57.3% between 1995 and 2003. Mean BMI in women has increased from 25.7 to 26.9 over the same period. It is likely that the observed changes have contributed to changes in breast cancer incidence in Scotland. Using prescription and population data, the prevalence of HRT use in women aged 40-64 in Scotland was estimated; this estimated prevalence has increased from 13.8% in 1993 to 17% in 2001. It is difficult to know if this small increase in prevalence of HRT could have influenced breast cancer epidemiology.

A study of breast cancer incidence by deprivation quintile showed that breast cancer incidence between 1991 and 2000 rose in all quintiles. Interaction analysis suggested that breast cancer incidence is rising to the same extent in deprived and affluent women. The risk factor analyses above were also applied to women of different socioeconomic standing (the results were published in Breast Cancer Research and Treatment).

A laboratory project was carried out to assess whether increasing survival from breast cancer could be a result of changing molecular epidemiology. This project was an comparison of the prevalence of breast cancers which were ER, PR and Her2 positive and of different grades in two cohorts of Glasgow patients, from 1984-86 and 1996-1997. The application of current molecular techniques to stored tissue aimed to improve the quality of data compared to previous studies based on clinical databases using heterogeneous techniques. There were significant differences in grade distribution of tumours in the two cohorts ($p=0.009$) with fewer grade 1 and more grade 3 tumours in the second cohort. Further study showed the grade difference to be exerted by the tumours in screened women in the second cohort with there being no difference in grade between symptomatic patients in the two groups. 64.2% of the tumours in cohort 1 and 71.5% of the tumours in cohort 2 were ER

positive (p=0.042); this is also likely to be a clinically significant difference. The difference between the cohorts appeared to be exerted by high percentage of screen-detected tumours in cohort 2 being ER positive; however this finding still supports a theory of changing biology. 44.9% of the tumours in cohort 1 and 49.9% of tumours in cohort 2 were PR positive (p=0.181). 21.5% of tumours in cohort 1 and 20.6% of tumours in cohort 2 were Her-2 positive; this was not a significant difference. An increase in ER positivity was seen in all age groups in the study, though multivariate analysis did suggest a contribution from a higher number of women over 60 in the more recent cohort. Kaplan-Meier analysis showed survival to be higher in the second cohort than the first. There was a significant difference in survival between ER positive and negative patients. Cox's regression was performed; as expected this showed a multifactorial contribution to increases in survival in these cohorts rather than it being entirely due to changes in ER status. However the changes in ER status shown in a population of Glasgow patients over time may mean that the results of clinical trials carried out in many years ago need to be interpreted with caution when applying them to the women of today. The results of this project were published in the British Journal of Cancer.

Overall, the epidemiological studies within this thesis shed an important new light on the factors contributing to breast cancer incidence in Scotland, with a major finding being a significant association between birth cohort and breast cancer incidence suggesting a significant impact being made by reproductive risk factors. This hypothesis is supported by the analysis of risk factor trends in Scotland undertaken within the thesis. The laboratory study has shown a significant lowering in grade and increase in ER positive status of tumours in a cohort of Glasgow women over time; while the changes are statistically explainable by known effects of a breast screening programme on tumour detection they could still represent a true change in biology. The results of all the studies contained in the thesis could have significant implications for future health service planning.

Chapter 1: Introduction

1.1. Breast cancer incidence trends

Changes in breast cancer incidence in the UK and relation to breast screening

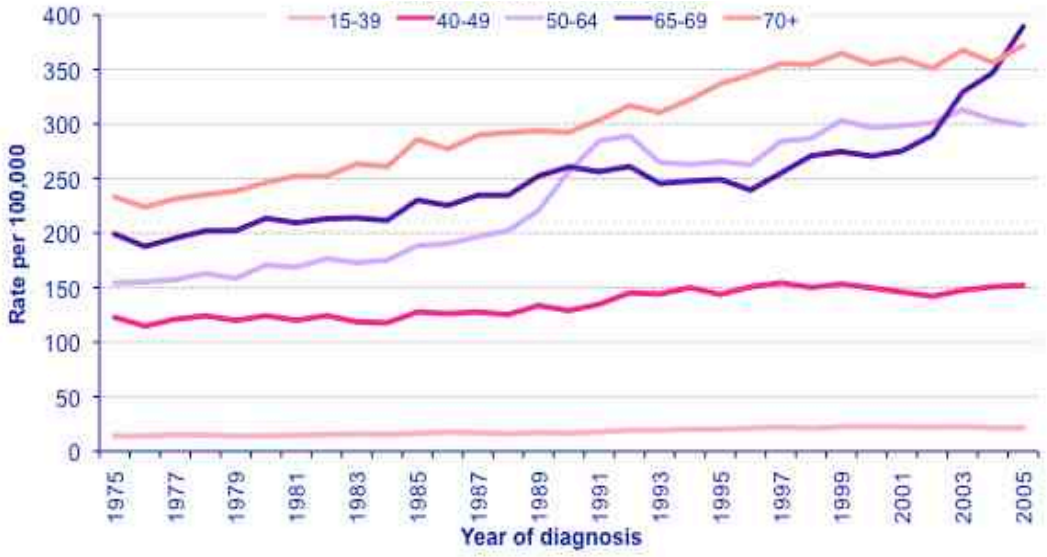
Breast cancer is the most commonly diagnosed cancer in women in the UK, with over 40,000 cases diagnosed annually (Cancer Research UK 2007). The epidemiology of breast cancer has been dynamic over the last 30 years, undergoing increases in incidence and reductions in mortality.

In 1995 Quinn and Allen described the changes in incidence of breast cancer in England and Wales up to 1992. From 1979 to 1987 the overall incidence had increased by about 2% every year, from 74 to 86 per 100,000 per year. A sharp increase, however, was seen from 1988 to 1991, with the annual rate of increase more than doubling, to 4.5% per year. In 1988, the NHS national mammographic screening programme began, and the impact of a screening programme on disease incidence makes interpretation of trends from 1988 onwards more complex. The NHS breast screening programme invites women over the age of 50 for screening mammograms 3-yearly, stopping at age 64 (although certain areas have also piloted screening for women aged 64-70 and 40-49). The first ever 'round' of screening - the time taken for all eligible women in the country to be invited for screening – would have contributed to an markedly increased incidence of breast cancer by picking up pre-existing asymptomatic tumours, a well recognised phenomenon in breast cancer epidemiology (Møller et al. 2005; Olsen et al. 2003; Schouten et al. 2002; Zahl, Strand, & Maehlen 2004). In England the prevalent round of screening was completed by 1995 (Blanks et al. 2000) and in Scotland by 1994 (Scottish Health Statistics 2000). In 1992 the prevalent round had not yet been completed, but incidence rates in women aged 50-64 were still 25% higher than in 1987 (Quinn & Allen 1995). Incidence rates had risen slightly from 1987 in 65-69 year olds, but in women older and younger than screening age there had been only slight fluctuations

in incidence over this period. Published data for Scotland up to 1992 demonstrate that trends were similar to those in England and Wales (Brewster et al. 1996).

Breast cancer incidence data for the UK for 1975-2005 (Cancer Research UK 2009) showed a large rise in incidence in the screened age group following the start of the breast screening programme, and then a slight fall towards the end of the prevalent round, as predicted above (see figure 1.1 below). Incidence rates remained stable thereafter until 1997, at which point they began to rise again. There has been a levelling off of incidence rates in women of screening age since 2003. In women younger than screening age, rates have remained relatively stable over this whole period. A rise can be seen in incidence in women aged 65-69 that has developed steadily since 1979 and especially since 2001 which cannot be explained by the screening programme. Incidence in women aged 70 and over increased up to 1997 at which point it levelled off.

Figure 1.1: age-specific female breast cancer incidence rates, UK, 1975-2005 (Cancer Research UK 2009)



After the completion of the prevalent round of a screening programme, it has been postulated that the overall incidence rate of breast cancer should fall to a point higher than before screening was introduced but not markedly higher (Schouten et al. 2002). Women aged 50-53 will always be undergoing 'prevalence' screening – although screening begins at age 50, women in different primary care catchment areas are invited sequentially which takes 3 years, so that some women are 53 by the time they are invited. Screens in these women will raise the incidence rate but other women, having had a negative screen, will only develop tumours at a rate that reflects what the incidence rate would have been in the absence of screening, even if they are detected at an early stage on a subsequent screen rather than symptomatically, and should not contribute to incidence rate rises. If the background incidence rate is stable then the overall incidence rate should theoretically remain stable. In fact, Feuer and Wun in their model of breast cancer incidence in the screening era (Feuer & Wun 1992) suggest that once screening has reached steady state, the overall breast cancer incidence rate will fall back to exactly the rate before screening began because the tumours that are screen-detected each year will effectively be removed from the potential 'pool' of tumours that would have been detected the following year and this balances out any extra tumours detected by screening.

As can be seen in the data above, the incidence rate of breast cancer in women aged 50-64 is continuing to rise rather than level off as expected. In this thesis I will demonstrate that a similar pattern is developing in Scotland. In establishing reasons for this increased incidence rate, consideration must be made as to the possible contribution of changes in screening and the possible contribution of a continued rise in background incidence rate; incidence rates in England & Wales were rising 2% per year before screening began (Quinn & Allen 1995).

Impact of screening on incidence figures: worldwide experience.

In 2007 Michael Waller and others (2007) studied the effect of screening on breast cancer incidence in England and Wales between 1971 and 2000. They constructed a Poisson age-period-cohort model, based on breast cancer incidence and population data and the proportion of women attending each screening opportunity, with the

model then generating incidence rate ratios corrected for age, period and cohort. The authors found that the incidence of breast cancer was higher in women attending screening for the first time, as expected (the rate ratio was 1.73). Incidence was also 18-35% higher in screening attenders at subsequent screening rounds. However, the authors felt that continued rises in breast cancer incidence during the periods of 'subsequent' screening were evidence that the background incidence of breast cancer would have been increasing in the absence of the screening programme.

In Limburg in the Netherlands, the expected changes in incidence with the prevalent round were seen; however, a second peak of incidence was seen after this, with rates up to 45% higher than pre-screening levels. The authors postulated that a combination of improvements in screening and increasing background incidence explained these findings (Schouten et al. 2002). In Norway and Sweden, the introduction of screening raised incidence rates by over 50% (Zahl, Strand, & Maehlen 2004); a slightly increasing background incidence may have contributed but the authors concluded that the majority of the increase is due to screening and represents overdiagnosis of cancers which would have otherwise not presented in the patient's lifetime.

Studies of breast cancer incidence in the Nordic countries (Sweden, Denmark, Finland, Iceland, Sweden, Norway) confirmed the initial increase in rates following the initial round of screening, slightly higher incidence rates while women remain in the screening programme, and a decrease in rates as women leave the programme (Moller et al. 2005). The authors performed an age-period-cohort analysis which suggested that the increase in risk for women born in 1920 to 1940 was largely explainable by screening. Jonsson et al (2005) studied breast cancer rates in Sweden following the introduction of screening. They adjusted rates in each age group for the effect of advanced time of diagnosis and found that this accounted for all the excess incidence since the screening programme began. Another study of rates in Denmark (Olsen et al. 2003) confirmed the expected prevalence peak and found that incidence rates after the prevalence round were only very slightly higher than before screening had been introduced.

There have been several studies of incidence and screening in the United States. Studies of incidence patterns within the nine SEER (Surveillance, Epidemiology and

End Results) programme registry states (Feuer & Wun 1992; White, Lee et al 1990; Wun, Feuer, & Miller 1995) suggest that the use of screening mammography rather than risk factor changes are largely responsible for increases in breast cancer incidence in middle-aged women, although the relative contributions varied depending on the age range of the women. Feuer & Wun (1992) assessed how much of the breast cancer incidence rises in the United States up to that point could be explained by increases in the uptake of screening mammography. The authors felt that that the studies that had gone before failed to take into account the lead time (preclinical screen-detectable time) of breast cancer and how this varies between age groups, and also failed to extrapolate pre-existing secular incidence trends. The authors started by studying breast cancer incidence rates in different age ranges in women in the nine SEER registry states between 1940 and 1987. They found that there had been a rise in breast cancer incidence rates from 1982 onwards which was greater than the extrapolated pre-existing secular trend. Screening had been available from 1970 onwards, but there was evidence to suggest that uptake before 1982 had been minimal and that the effects of screening were likely to be seen from 1982 onwards. They went on to study incidence between 1981 and 1987 more closely. For each different age range they calculated the percentage of tumour diagnoses which were attributable to symptom-detected diagnoses, screen-detected tumours in women having a first screen after 1982 and screen-detected tumours in women having had a subsequent screen after 1982, based on data available on uptake of screening, screening detection ratios and time for breast cancers. Their calculations revealed that in all age groups those tumours that were screen-detected after 1982 accounted for all of the incidence of breast cancer above the extrapolated pre-existing secular trends.

‘Overdiagnosis’ in breast screening

One other prediction is that once a screening programme is developed there will be a decrease in incidence rates in the age range above the screening age – i.e., women who have had a last negative screen and stop being screened. If they develop cancers these will take time to develop into symptomatic tumours (the ‘lead time’)

and thus women aged just above the screening age group should present in smaller numbers than before.

Zahl et al. (2004) found that introduction of screening to Norway increased incidence rates in the 50-69 age group by 50%. They showed that there was no corresponding decline in incidence rates in women 70-74, concluding that one third of invasive cancers in the 50-69 age group would not have been detected in the patient's lifetime. They suggest that 'overdiagnosis' i.e. diagnosis of slow-growing cancers that would not become evident in the patient's lifetime, is a bigger factor in breast cancer epidemiology that first thought. Overdiagnosis has long been recognised as a factor in the epidemiology of prostate cancer, but its contribution in breast cancer remains controversial. Duffy et al. suggest that overdiagnosis is likely to be as little as 5% (Duffy et al. 2005); in a recent study comparing cumulative lifetime rates of breast cancer in screened women and controls Zackrisson et al. (2006) argue that overdiagnosis is at least 10% , with several correspondents arguing it may be even higher, up to 25% (Gotzsche 2006; Welch, Schwartz, & Woloshin 2006; Zahl & Maehlen 2006).

It could be argued that if overdiagnosis was a major problem, screening programmes would not have had the effects on reducing mortality described in the Swedish and UK trials (Rayter & Kutt 2004). One alternative explanation for the failure of incidence rates to 'fall away' in the first group after screening ended is that these women may have elected to continue being screened; the NHS breast screening programme allows women to self-refer for mammograms over screening age. However it is doubtful whether rates of self-referral would be high enough to explain the lack of drop-off in incidence rates.

Changes in screening in the UK

Since 1988 there have been major changes in the screening programme that have considerably advanced its performance. One mark of the performance of a screening programme is the standardised detection ratio: the ratio of incidence of screen detected tumours to background incidence rates – the standardised detection ratio or SDR. The European Community guidelines on mammography state that this should be over 3 for a first screen and 1.5 for a subsequent screen although the NHS breast

screening programme targets are 1.1 for both. The SDR of the breast screening programme overall has only been above 1 since 1996/97, and increased 36% from 0.83 to 1.13 between 1994 and 1999, suggesting major improvements (Blanks, Moss, & Patnick 2000). Important steps have been a change from one to two view mammography for prevalent screens, film density has been standardised at 1.4-1.8D, implementation of quality assurance procedures and the establishment of a skill base of radiographers and radiologists (Blanks, Moss, & Patnick 2000). Improvements in screening since the prevalent round of screening was completed may be contributing to a continued rise in incidence rates, particularly in those women undergoing a first or 'prevalent' mammogram, as more small asymptomatic tumours will be revealed. For women undergoing subsequent screens, improvements in detection rate will result in fewer interval cancers but may not have such a great effect on incidence rates.

Rates of uptake of screening invitations will also contribute to the effects of a screening programme on incidence. Percentage of uptake of invitations in the NHS breast screening programme in the UK has remained more or less constant at 75% over time. (Blanks, Moss, & Patnick 2000)

1.2. Potential reasons for an increasing 'background' incidence of breast cancer

It is important to exclude an artefactual increase in incidence rates caused by changes in data registration. Peto (Peto et al. 2000) has suggested that current recorded incidence rates are dominated by large artefactual increases but Coleman (Coleman 2000) argues that cancer registration practices (as opposed to death certification practices) have remained more or less unchanged and that recorded incidence rates should be accurate. Figures here have been age standardised, usually to the European population, thus compensating for any change in the age structure of the population.

Reproductive and hormonal factors are particularly important in the development of breast cancer. There have been major changes in fertility patterns in

Western societies and it is possible that such changes could be contributing to rising breast cancer rates.

Reproductive, hormonal and other risk factors

Reproductive risk factors for breast cancer

Established risk factors for breast cancer include early menarche and late menopause, late age at first pregnancy, the oral contraceptive pill and hormone replacement therapy and postmenopausal obesity. In 1980 Korenman published his 'oestrogen window' hypothesis (Korenman 1980). He hypothesised an endocrine contribution to breast cancer based on previous studies showing the protective effect of early age at first pregnancy and negative effect of early menarche, late menopause and nulliparity. He postulated that breast cancer was induced by carcinogens in a susceptible breast and that duration of oestrogen exposure determined risk.

Indeed, it has been shown that oestrogen exposure stimulates breast tissue epithelial proliferation and hence DNA synthesis, increasing the chance of a potentially cancer-causing mutation being amplified. That initial mutation may itself be caused by certain carcinogenic metabolites of oestrogen (Mitrunen & Hirvonen 2003; Russo & Russo 1998). Numerous retrospective and prospective studies of serum oestradiol level and breast cancer risk have shown higher rates of postmenopausal breast cancer in women with the highest serum oestradiol levels, with relative risks in the prospective trials up to double in the highest quintile of oestradiol levels compared to the lowest (Dorgan et al. 1997; Key et al. 1996; Key et al. 2003; Thomas et al. 1997; Thomas, Reeves, & Key 1997; Toniolo et al. 1995). For women of the same age and with the same childbearing pattern, relative risk of breast cancer is higher in those who are postmenopausal than those who are premenopausal (RR 0.81 for menopause at age 50-54) or perimenopausal (RR 0.77) (Collaborative Group on Hormonal Factors in Breast Cancer 1997). Risk of breast cancer increases with increasing age at menopause – relative risk increases overall by about 2.8% for each year older at menopause (Collaborative Group on Hormonal Factors in Breast Cancer 1997). However, Korenman's theory of prolonged oestrogen exposure does not explain certain features such as the strong protective

effect of early age at first pregnancy, and it is clear that there are more complex mechanisms involved.

Pike et al. (1983) proposed that breast tissue 'ages' at different rates - i.e. exhibits different proliferation rates - in different stages of life. He proposed that the rate of breast tissue ageing decreases after first pregnancy; so women who have first pregnancy at a late age have had a prolonged period with a high breast tissue proliferation rate and hence greater risk of developing breast cancer in the future. Late age at first pregnancy has been shown to confer higher lifetime risk of breast cancer than nulliparity (Colditz 2005; Kampert, Whittemore, & Paffenbarger, Jr. 1988; Pathak, Osuch, & He 2000; Pathak & Whittemore 1992; Rosner, Colditz, & Willett 1994). Pike (1983) explained this by a brief large rise in breast tissue ageing rate around the time of first pregnancy; with early age at first pregnancy this is offset by the longer period of reduced proliferation rate but with late age at first pregnancy no such offset occurs, causing risk to be higher than in nulliparous women. His model appeared to fit the epidemiological data.

Many studies have also confirmed an increased relative lifetime risk of breast cancer with decreasing total parity and nulliparity (Colditz 2005; Kampert, Whittemore, & Paffenbarger, Jr. 1988; Pathak, Osuch, & He 2000; Pathak & Whittemore 1992; Rosner, Colditz, & Willett 1994). Several authors have modified the Pike model to produce a model that explains various epidemiological phenomena including the effects of total parity on breast cancer risk. (Colditz 2005; Kampert, Whittemore, & Paffenbarger, Jr. 1988; Pathak & Whittemore 1992) Within these models, risks in older and younger women can be shown to be very different; there is a demonstrable 'crossover age' before which the effects of nulliparity, parity and late age at first pregnancy on breast cancer risk are in fact opposite. The crossover age is influenced by age at first pregnancy and parity and can be shown to be anywhere between 40 and 65 years of age. Cumulative (lifetime) incidence is affected by the same factors as incidence after the crossover time, with data from the large Nurses Health Study (Rosner, Colditz, & Willett 1994) which involved 1,212,855 person-years of follow-up showing cumulative incidence of breast cancer at age 70 20% lower, 10% lower and 5% higher for parous versus nulliparous women for first birth at age 20, 25 and 35 respectively. A woman with multiple births at young age could

have up to a 50% lifetime relative risk reduction compared to a woman with late age at first pregnancy.

Henderson et al hypothesised that an increased number of regular ovulatory cycles increased the risk of developing breast cancer, and that women with early menarche developed ovulatory cycles much sooner after menarche (Rosner, Colditz, & Willett 1994). Both theories are supported by the work of others (Apter & Vihko 1983; Butler et al. 2000; MacMahon et al. 1982; Olsson, Landin-Olsson, & Gullberg 1983; Wu et al. 1996). One possible explanation for the detrimental effect of regular cycles is that most of the mitotic activity in the breast occurs in the luteal phase (Ferguson & Anderson 1981; Nazario et al. 1995), and that as the duration of luteum is constant in most women from month to month, a woman with regular short cycles will spend more time in luteum (i.e. with 'susceptible' breast tissue) than women with irregular longer cycles.

Oral contraceptives

The Collaborative Group on Hormonal Factors in Breast Cancer analysed data from epidemiological studies in 53 297 women with breast cancer and 100 239 women without breast cancer to assess the effects of combined oral contraceptives on breast cancer risk. Women currently using combined oral contraceptives or who had stopped using them less than 10 years before, had an increased risk of breast cancer (The Collaborative Group on Hormonal Factors in Breast Cancer 1996). Women who had stopped using them 10 or more years previously had no significantly increased risk of breast cancer. Increasing duration of use and age at first use had little effect on relative risks, although first use under 20 did increase the relative risk of breast cancer being diagnosed at a young age.

Hormone replacement therapy

The effects of hormone replacement therapy on breast cancer risk are likely to be related to their effect of artificially delaying menopause and contributing to cumulative oestrogen exposure. The Collaborative Group on Hormonal Factors analysed 54 epidemiological studies involving 52,705 women with breast cancer and 108,411 women without breast cancer to assess the effects of hormone replacement therapy use on breast cancer risk (The Collaborative Group on Hormonal Factors in Breast Cancer 1997). They estimated that in North America and Europe, the

cumulative excess number of breast cancers diagnosed per 1000 women for women starting HRT at 50 and using for 5,10,15 years is 2 (C.I. 1-3) , 6 (3-9) and 12 (5-20) respectively. In current-users of HRT or women who had recently stopped using HRT, who had used it for less than 5 years, the relative risk of breast cancer increased by a factor of 1.023 with each year of use; relative risk of breast cancer was 1.35 for HRT use for duration of use of 5 years or longer. Women who had stopped using HRT more than 5 years previously did not have a significantly increased risk of breast cancer. Cancers diagnosed in users of HRT were less advanced clinically than those in non-users. There appeared to be little difference between the different types of hormonal therapy in their effects on breast cancer risk. However the results of the Million Women Study (Beral 2003) suggested that risk of breast cancer was higher for combined HRT than oestrogen-only HRT.

The Million Women Study was a non-randomised cohort study, following one million women aged 50-64 and relating the use of HRT in the 828923 postmenopausal women in the study to outcomes including breast cancer incidence and death. Current users of HRT had greater risk of breast cancer than never-users; risk for current users was 1.21 for those who had used HRT for under 5 years and 1.34 for those who had used HRT for over 5 years. In contrast to the findings of the Collaborative Group (The Collaborative Group on Hormonal Factors 1997), women who had stopped using HRT as recently as under a year previously were not shown to be at increased risk of breast cancer.

Obesity

Extensive study has been made of obesity and its importance in breast cancer. Conventionally, a body mass index of 18.5-24.9 is classed as 'normal', 25-29.9 as 'overweight' and a BMI of 30 or over as 'obese'. Pooled analysis of seven major prospective cohort studies in 2000 (van den Brandt et al. 2000) concluded that high BMI had a significant positive association with postmenopausal breast cancer risk – with the opposite being true for premenopausal breast cancer risk. To quantify this further, for each 4 kg/m^2 increase above 'low' BMI in postmenopausal women the relative risk of breast cancer increased by a factor of 1.02. The RR for a postmenopausal woman with a BMI of 33 kg/m^2 or more compared with women with a BMI of less than 21 kg/m^2 was 1.27 (95 percent CI: 1.03-1.55). In

postmenopausal women, circulating oestrogens are mainly produced from adipose tissue via the enzyme aromatase. Excess adipose tissue in postmenopausal women increases serum oestradiol levels (Kaye et al. 1991), and this is one mechanism by which obesity may lead to breast cancer. Indeed in a meta-analysis of seven prospective studies of BMI and breast cancer risk, adjusting for serum oestradiol markedly reduced the effect of rise in BMI on relative risk of breast cancer (from 1.19 to 1.02 for a 5 kg/m2 increase in BMI) (Key et al. 2003). The breast adipose tissue is especially rich in aromatase, and therefore local oestrogen production by the breast may be carcinogenic (Miller 1991). There has been recent interest into other mechanisms by which obesity may promote breast cancer; this includes interest in the adipocytokines (cytokines produced by adipose tissue) such as leptin. Leptin levels are increased in obesity; leptin has been shown to induce aromatase activity in breast cell lines and to stimulate growth of ER positive breast cancer cell lines (Rose, Gilhooly, & Nixon 2002 ; Rose, Komninou, & Stephenson 2004).

Alcohol

Large cohort studies have shed light on the influence of alcohol consumption on risk of breast cancer. Data from the Million Women cohort study in the UK suggests that for every additional alcoholic drink regularly consumed per day, the increase in breast cancer incidence up to age 75 is 11 cases per 1000 women (Allen et al. 2009). In the Women's Health study, the relative risk of breast cancer with alcohol consumption of 30g or over of alcohol consumed per day was 1.43 in comparison to no alcohol at all (Zhang et al. 2007). Various animal studies have shown that alcohol can induce breast tumours in mice; postulated mechanisms include effects on oestrogen metabolism, effects on folate metabolism, direct mutagenesis and oxidative stress (Dumitrescu & Shields 2005). Alcohol consumption is associated with more frequent, shorter menstrual cycles and increased serum oestrogen metabolites (Cooper et al. 1996). In addition alcohol can contribute to obesity, which as discussed above is an independent risk factor for breast cancer.

Dietary Factors

In 2007 the World Cancer Research Fund assessed the available evidence on dietary factors in the aetiology of breast cancer (World Cancer Research Fund 2007). They found that meta-analysis of case-control data suggested a significantly increased risk

of postmenopausal breast cancer with high dietary fat intake, probably as a result of its contribution to increased aromatase activity (see the section on obesity above). Analysis of the data on all other aspects of dietary intake showed that the studies were of insufficient rigour to allow a comparison to be made.

Smoking

The association of smoking with risk of other cancers has long been documented but many years of study of breast cancer and smoking have resulted in studies with conflicting outcomes and methodological flaws , as reviewed by Terry et al. in 2002. More recent studies have attempted to try to clarify the association. A large cohort study into smoking and breast cancer risk was carried out in California between 1995 and 2000 (Reynolds et al. 2005) While an isolated study, it was hailed as being a thorough, detailed prospective cohort study with large numbers. The hazard ratio of breast cancer in those women who smoked compared to never-smokers was 1.32.

Exercise

There is increasing evidence that increasing physical activity in the postmenopausal period could substantially reduce the risk of breast cancer (Monninkhof et al. 2007; Neilson et al. 2009). It is difficult to elucidate, however, whether this is simply as a result of the parallel decrease in BMI that results from exercise, because all of the proposed mediators (e.g oestradiol activity, insulin resistance) of physical activity on breast cancer risk mediate obesity and breast cancer risk in the same way (Neilson et al. 2009). Several ongoing randomized controlled trials are attempting to determine whether physical activity is an independent risk factor.

Trends in reproductive / hormonal factors

As in many Western societies, fertility patterns in the UK are changing. This is probably a result of social changes, such as couples actively delaying starting a family until later, changing attitudes towards women and work, the availability of the oral contraceptive pill and possibly the rise in the number of stepfamilies. Mean age at first pregnancy is increasing in the UK; there was a 110% increase in rates of pregnancies in women aged 35-39 between 1978-80 and 1997/98 (Population Trends 2000). Parity (as measured by completed family size) has been

decreasing with increasing birth cohort from the 1930s onwards (Office of National Statistics 2004); parity had been rising before this, reflecting the 1960s 'baby boom'. Prescriptions of the oral contraceptive pill in the UK rose immediately from the introduction of the pill in 1961 (Population Trends 2000). The numbers of prescriptions reached a peak in the late 1970s to early 80s, and then fell slightly, levels of use thereafter remaining fairly stable, with distribution of age of use remaining fairly consistent.

Hormone replacement therapy has been used for relief of menopausal symptoms since the 1930s. Numbers of prescriptions more than doubled between 1973 and 1976 but fell again, until the late 1980s when they began to rise again (Townsend 1998). Numbers of prescriptions remained fairly stable between 1998 and 2001 (Townsend & Nanchahal 2005) but there is evidence that usage has fallen since 2002, probably due women's increased awareness of its risks (Parkin 2009). Estimates of population prevalence calculated from prescription data suggested that prevalence in England in 45-64 year-olds rose from 2.2% in 1987 to 21.7% in 1994 (Townsend 1998). The same study estimated that prevalence in Scotland in the same age group increased from 1% in 1987 to 20.4% in 1994. Another study of HRT use among a database of several million patients in England showed that the prevalence of current HRT use in women aged 45-64 rose from 18.6% to 27.7% between 1992 and 1998 (Bromley, de Vries, & Farmer 2004). In all studies, use of HRT was maximal among 50-54 year olds. Most women take HRT for at least 6 months and many much longer. In one study 77% of women who started HRT in 1995 took it for a year, 52% of women took it for at least 3 years, and 46% for at least 4 years (Bromley, de Vries, & Farmer 2004). The same study suggested a trend towards increasing duration of use; 89% of women starting HRT in 1998 took it for at least a year, compared to 77% of those starting in 1995.

In this thesis I will investigate the magnitude of such changes in Scotland and how they may have affected breast cancer risk.

Birth Cohort Incidence of Breast Cancer and Risk Factor Trends

It is interesting to look at birth cohort incidence and how it relates to trends in reproductive factors. Birth cohort incidence is the incidence of breast cancer over

time in women born in a particular 5-year range. Birth cohort incidence ratios are useful as women of each birth cohort will overall have had a similar risk experience to the rest of their cohort; the individual influences on each cohort as they age theoretically gives the women within it a unique risk factor profile. Risk factor influences on each cohort include exposure to the oral contraceptive, population fertility patterns and exposure to hormone replacement therapy.

Swerdlow and Dos Santos Silva (dos Santos Silva & Swerdlow 1995) looked at the relationship between incidence and mortality in the UK of breast cancer and population reproductive factors, up until 1985. Birth cohort incidence trends become more difficult to analyse after this point, as screening will raise incidence rates in several birth cohorts once the women reach screening age, making the contribution of risk factors difficult to assess. Trends in average completed family size did appear to reflect the cohort risk of breast cancer. Average completed family size fell from 3.3 per woman born in 1875 to 2.0 in women born in the early 1930s, coinciding with a marked increase in breast cancer relative risk for successive birth cohorts over this time. Family size then rose with successive birth cohorts to 2.4 for 1935 birth cohorts, coinciding with a reduction in risk. However between the 1935 and 1950 cohorts completed family size fell with successive cohorts but cohort incidence also fell.

Elsewhere, Swerdlow showed that birth cohort incidence in Scotland gradually increased until the birth date in the late 1930s, and risk decreased with more recent year of birth up to 1987 (Swerdlow et al. 1998). Robertson and Boyle also found a reduction in incidence of breast cancer in more recent birth cohorts in their 1997 study, and they speculated that these women could have been healthier than cohorts that had gone before as a result of dietary changes.

Studies carried out elsewhere, such of that by Chia et al in Singapore and Sweden (Chia et al. 2005), showed that birth cohort incidence continued to rise after the 1935 birth cohort, and it is possible that the relatively young age of the later birth cohorts in these earlier studies confounded the incidence figures, despite the age-standardisation used in the study. An age-period-cohort analysis carried out using breast cancer incidence data for Hong Kong revealed that the increase in incidence was principally a cohort effect, with relative risk increasing in a linear fashion with

successive birth cohorts; there was an up to 3-fold increase in risk in women born in the 1960s compared to 1900 (Leung et al. 2002).

A relationship between age at first pregnancy and incidence rates was not evident in Swerdlow and dos Santos Silva's study (dos Santos Silva & Swerdlow 1995). The percentage of women having a first child at ages under 25 increased from 40% of women born in 1920 to 60% of women born in 1945 but cohort cancer incidence increased rather than decreased. 45% of those born in 1955 had a first child at ages under 25; however breast cancer cohort incidence declined rather than increased as would be expected with this trend towards later age of first pregnancy. Trends in nulliparity bore little relation to trends in incidence. The study by Chia et al. (2005) demonstrated a relationship between cohort incidence of breast cancer and temporal trends in age at first pregnancy in Singapore but not in Sweden.

Temporal Incidence of Breast Cancer and Risk Factor Trends

A study by Coombs et al. calculated the potential contribution of HRT use to breast cancer incidence in Australia (Coombs et al. 2005).They based the study on the epidemiological theory that a small individual risk (such as that provided by HRT use in terms of breast cancer risk) can become magnified into a significant population risk if the prevalence of the risk factor is high enough. They calculated the proportion of breast cancers in Australia every year that were attributable to HRT use; this proportion was estimated to be 9%. Their analyses revealed that reduction in HRT prescribing in terms of restricting use to women under 65 and limiting length of prescription could reduce breast cancer incidence by up to 5.7%. They concluded that when HRT prevalence is high the effect of the increased breast cancer risk is relatively high.

1.3. Breast cancer mortality and survival

In the Eurocare study of cancer registries published in 1995 (Quinn & Allen 1995), England and Scotland were ranked 8th and 10th respectively out of 12 countries in Europe in terms of survival from breast cancer. However, survival now appears to be increasing and mortality to be decreasing in both countries, despite increasing incidence. Coleman (2000) showed 5 year survival in England and Wales to have

steadily improved across all age groups since 1970. The greatest increase was in women aged 50-69 at diagnosis, in whom 5-year survival increased from by 7% from 1989 to 1993. Figures from the Office of National Statistics (Quinn & Allen 1995) showed overall 5 year survival to have improved by 6% between 1986-90 and 1991-93. Survival in women aged 50-69 increased by 7-10%, compared to survival increases of 1-4% in younger and older women.

Peto (2000) had reported the fall in mortality from breast cancer in UK women between 1987 and 1997 aged 20-49, 50-69 and 70-79 to be 22%, 22% and 12% respectively. Coleman (2000) showed standardised mortality figures adjusted for changes in coding, and showed a better estimate to be a drop of 21%, 21% and 7% respectively. Quinn (Quinn & Allen 1995) showed that in women aged 55-69 mortality changed little throughout the 1980s but fell sharply after 1990; in 1994 the rate for these women was 12% lower than in 1987. The standardised mortality rate for all ages from breast cancer in Scotland decreased by 23% between 1975 and 2000 (figures from Scottish Executive Information & Statistics Division).

For mortality statistics, changes in death registration practice and revisions of ICD coding could potentially interfere with trends in mortality. For example, between 1984 and 1993 the Office of Population Censuses and Surveys in England and Wales used a different interpretation of the WHO rules on determining underlying cause of death, reverting to the old method in 1993. It is important to determine whether mortality rates are calculated from data on all-cause mortality or mortality due to breast cancer.

Explanations for increases in survival and mortality

Postulated reasons for decreasing mortality include screening, better treatment, shift to lower stage and grade tumours at time of diagnosis, and changes in receptor status of tumours. Several studies in the UK have attempted to assess the relative contribution of factors to the decreasing mortality rates and increasing survival rates; all acknowledge the complex interaction between different factors.

Since the advent of both screening and increased ‘breast awareness’ in women, lead-time bias (longer time between diagnosis and death because of early detection but no true increase in survival) and length bias (slower growing tumours

preferentially detected by screening) have become confounding factors when considering increasing survival figures for breast cancer. Improved survival over time may not reflect improved cancer treatment, instead simply representing the lead-time bias of tumours detected early. Mortality rates, however, should definitely decrease with improvements in cancer treatment and detection (Welch, Schwartz, & Woloshin 2000).

Breast cancer is a progressive disease; the aim of service mammography screening is to detect breast cancers at an early stage, when there is less chance that micrometastases have occurred and there is more chance of definitive treatment effecting a cure. Several reviews of randomised controlled trials have suggested screening mammography could reduce breast cancer mortality by about 20% for screened women over a period of 10 years (Hackshaw 2003; Nystrom et al. 2002) Duffy et al. (2002) suggested the reduction could be 40%. 16 year mortality figures from the UK Trial of Early Detection of Breast Cancer revealed a 27% lower mortality from breast cancer in screened women (1999). One Cochrane Review of studies, however, suggests that the available evidence does not support a breast-cancer specific mortality reduction (Olsen & Gotzsche 2001) as a result of inconsistencies in aspects such as recording of cause of death., although the authors of the studies reviewed have disputed these findings. Nevertheless, as the prevalent round of screening was not complete until 1995, many of the deaths in the 1990s will have been in women already diagnosed with breast cancer before attending screening, and so the impact of screening on mortality statistics so far is likely to be much less than 20%. As time goes on however, fewer patients will have been diagnosed before the screening era and the effect on mortality may become clearer.

The changing management of breast cancer has undoubtedly contributed to improvements in survival, particularly in terms of pharmacological management. The use of adjuvant therapies such as hormone therapy in the form of tamoxifen, which markedly reduced recurrence rates in women with ER positive tumours (EBCTCG 2005) has become widespread in its availability and clinical application over the past two decades, having been introduced in the early 1980s. Similarly the availability and application of various forms of chemotherapy has increased markedly over this time; with chemotherapy clearly improving survival when used in

appropriate clinical situations, especially younger women and women with ER negative disease this will have undoubtedly have contributed to improvements in survival and mortality. In recent years the use of aromatase inhibitors in specific clinical instances has increased, although there will have been little contribution to population survival figures over the time periods in question here.

Another important factor has been the reorganisation of breast cancer services, with women being managed by multidisciplinary teams of surgeons and oncologists, surgery being carried out by surgeons specialising in breast cancer surgery, and the availability of consensus guidelines. There is evidence that these trends have positively influenced survival; Gillis and Hole showed a 17% reduction in risk of death for patients treated by specialist breast surgeons as opposed to non-specialists (Gillis & Hole 1996).

Attempts have been made to quantify the contributions made of each of these factors to increasing survival. Blanks et al (Blanks et al. 2000) designed a model based on expected mortality rates without screening, to determine the contribution of screening to falling mortality. They estimated 30% of the reduction in mortality in 1998 was due to screening, with 70% due to other factors. Thomson et al. (2004) assessed reasons for an 11% increase in 8-year survival in Scotland between 1987 and 1993. They suggested that 47% of the improvement was directly explicable by screening, with a further 33% due to clinicopathologic factors (change in stage, grade, ER status). Treatment factors did not appear to contribute to survival in their model. Bradburn et al. (1998) showed improved survival for postmenopausal women. Their analysis suggested that treatment factors explained much of the improvement; changes in prognostic factors did not contribute. Stockton et al. (1997) found an odds ratio of 1.5 of being diagnosed stage I/II in 1986-89 compared with 1982-85. They estimated 44% and 60% of the improvement in survival to be due to stage shift in patients under 50 and 50-64 respectively. They felt this stage shift was unlikely to be due to screening, as screening was still in its infancy in 1989, and more likely to be due to breast awareness. Elkin et al. (2005), in a study of changes in tumour size distribution, estimated that within-stage size migration explained 61% of the improvement in survival of the patients in the study. Pisani & Forman (2004) found that almost all the improvement in survival from their patients from 1982-90

to 1991-99 was accounted for by adjusting for stage. They did note a small improvement in stage specific survival for all stages except IV.

1.4 Is the biology of breast cancer changing?

The most striking feature of the epidemiology of breast cancer in the UK described above is the improvement in mortality and survival despite increasing incidence, especially in middle-aged women. Screening has undoubtedly contributed to incidence changes, with its impact so far on survival and mortality less clear; improvements in treatment may have contributed to reduced mortality even in the face of the increased incidence. There is also growing evidence that the excess of incidence of breast cancer noted in recent years may be countered by an increasing incidence of tumours which are less 'aggressive' and have intrinsically better prognosis. This would help to explain reductions in mortality and improvements in survival.

Detecting a general shift in tumour biology of breast cancers could have profound implications for the treatment of breast cancer, as treatment decisions are often made on the basis of longstanding clinical trial evidence. If we know that tumour characteristics have changed since the publication of these trials, it may be that we must interpret results of previous trials with caution before applying them to our patients.

Evidence for changing biology

Changes in receptor status

Studies in Marin County in San Francisco, an area with a homogenous population of a relatively high socio-economic status, suggest that incidence rate increases in breast cancer in women seen there appears to consist of an increasing rate of development of oestrogen receptor positive tumours, with the rate of receptor-negative tumours staying fairly constant (Benz, Clarke, & Moore 2003)

Few studies of changing oestrogen receptor status have been carried out in the UK. Bradburn and others 1998) looked retrospectively at ER and PR status of tumours in

Guy's Hospital Breast Unit. They showed an increase in percentage of ER positive tumours from 46% to 66% between 1975-79 and 1985-89, and an increase in percentage of PR positive tumours from 24% to 48% between 1975-79 and 1985-89. In this study, the number of tumours who did not undergo testing for hormone receptors decreased markedly between the two time periods; therefore it is possible that increased receptor positivity simply represented increased detection. Henley et al (2005) detailed a comparison of selected patients treated in Glasgow from 1980-88 and from 1996-2001; 49% of patients in 1980-88 and 78% of patients in 1996-2001 were ER positive.

A recent major study by Glass (2007) calculated incidence rates of breast cancer within women in a health-plan and plotted the incidence of ER positive and ER negative disease; they found dramatic differences in incidence rate trends by ER status. Rates of ER positive disease followed the overall incidence pattern for breast cancer, with a 5% annual increase from 1980 to 1983, an 18.9% annual increase from 1983 to 1986 and a 2.1 % increase from 1986 to 2001; ER negative disease showed a different pattern, with an annual decrease of 2.1% between 1980 and 1995, 3.7% form 1995 to 1999 and 9.8% from 1999 to 2006.

Li et al (2003) studied receptor status of tumours in the SEER registry (USA) between 1992 and 1998. While they too noted a decrease in the number of tumours of unknown status, they then looked specifically at those tumours of known status. The percentage of those with known status that were ER+ increased from 75.4 to 77, and PR+ from 65 to 67.7. Their data also suggested that the increase in ER positive tumours was limited to early stage disease. They felt that a possible explanation was that ER status may be affected by use of HRT and that using HRT may result in increased contact with health services and thus early detection of disease.

In a study of ER status in 11195 tumours in the US between 1973 and 1992, the percentage of ER + tumours increased significantly across the period (Pujol et al. 1994). They did find ER positive status was commoner in older women, in smaller tumours and was unrelated to nodal status but the rise persisted after adjusting for age, tumour size and type of assay, showing that there was a true rise in ER positive tumours. A study by Celentano et al. (1998) of trends in ER status by

birth cohort found a decreasing odds ratio of ER positivity with successive birth cohorts although there appeared a small increase and decrease in odds ratio before and after 1941-45 birth cohort.

The main confounding factor in many of these studies is that most have combined data from ligand binding assays and immunohistochemistry, with the more recent patients in their studies having had oestrogen receptor status determined by IHC (although Glass (2007) noted that there had been no changes in laboratory procedures in their study period). Detection of oestrogen receptors was formerly carried out using ligand binding assays such as the dextran-charcoal method which measured absolute levels of oestrogen receptor in the 'cytosol'- the supernatant following ultracentrifugation of tissue - but now immunohistochemistry has been almost universally adopted. Immunohistochemistry has been accepted as the more sensitive and specific method of analysis (Allred et al. 1990), as there is no interference with receptors by endogenous or exogenous circulating oestrogen. In addition IHC is easier to perform, less expensive, safer, applicable to a wide variety of samples , and microscopy of the specimen allows identification of ER positive cells in a low cellularity specimen and identification of ER positive benign epithelium (Allred et al. 1990; Allred et al. 1998; Harvey et al. 1999; Pertschuk & Axiotis 1999). But most importantly, given the use of oestrogen receptor in clinical breast cancer management, oestrogen receptor status established by immunhistochemistry has also been shown to predict response to endocrine therapy better than ligand binding assays (Allred et al. 1990; Allred et al. 1998; Harvey et al. 1999). Several studies found no straightforward relationship between tumour ER content by LBA and the ratio of ER positive to ER negative cells at IHC, and it is likely to be this ratio which is most important for predicting endocrine responsiveness and survival. The direct concordance between the two methods in various trials was reviewed by Allred (Allred et al. 1990); in permanent sections the concordance of the methods ranged between 82% and 96%, with the discordance most likely due to ligand binding being 'false positive'.

Another potential source of artefact (Pujol et al. 1994) in studies could be an improvement in transit time and transit methods to laboratories from theatre (for example specimens being placed directly in formalin), with faster transit times

reducing protein breakdown and resulting in better detection of hormone receptors. It should be noted that there still remains a great deal of heterogeneity within different laboratories as to the antibodies used for ER determination, scoring system and cutoffs used to determine positivity and type of sample (frozen or paraffin) (Layfield et al. 2003; Pertschuk & Axiotis 1999)

Changes in grade

No studies have been made of population changes in tumour grade. However several studies have suggested a trend toward lower grade in tumours detected at screening (Anderson et al. 2004; Crisp et al. 1993; Duffy et al. 1991; Tabar et al. 1999)

Changes in size

One recent large study within the SEER registry showed that within each stage category, the proportion of small tumours had increased significantly over time (Elkin et al. 2005).

Screening and changing biology

The epidemiology of breast cancers could be different in the screening era, with a higher percentage of slow growing, better prognosis, cancers in women of screening age, thus affecting survival and mortality figures. However, as noted above, the effects of screening on survival are potentially subject to lead-time bias. Length bias - detection by prevalence screening of slower growing tumours which spend a longer time in the preclinical 'sojourn period'- is another potential confounding factor when assessing the biology of breast cancers in the screening era. Any studies of the effects of screening on the biology of breast cancer have to take account of potential bias.

As noted in the section 'Explanation for improvements in mortality & survival', phenotypic drift has been postulated to occur (Tabar et al 1999), with differentiation being lost as tumours progress. Due to the intrinsic intratumour heterogeneity of breast cancers, areas of the tumour that are higher grade may grow more rapidly than the rest of the tumour and so a tumour that starts out as mostly low grade with a tiny area of high grade tumour may eventually become a tumour that is mostly high

grade. Screening may therefore interrupt this process and detect tumours when they are of low grade. The results of several studies (Anderson et al. 2004; Crisp et al. 1993; Duffy et al. 1991; Nordèn et al. 1997) showing lower grade in screening detected cancers, even when women undergoing prevalence screening are excluded due to the high potential for length bias (ie preferential detection of slow-growing tumours) in this group, have been used to support the theory of phenotypic drift (Tabar et al. 1999).

The large study by Elkin et al (Elkin et al. 2005) of changes in tumour size over time did not collect data on the screening status of the tumours in the study. However it is very likely that the advent of screening resulted in the changes demonstrated. Studies of the characteristics of tumours detected by screening have shown them to be of smaller size than non-screen-detected tumours (Cortesi et al. 2006; Duffy et al. 1991; Ernst et al. 2002; Klemi et al. 1992) the resulting increase in survival being the basis for the introduction of mammographic screening programmes.

Several comparative studies have shown that screen-detected tumours are more likely to be oestrogen receptor positive than negative. A comparative cohort study by Klemi et al. (Klemi et al. 1992) showed a relative risk of ER negativity of 0.29 after controlling for tumour size. Another, population registry-based, study showed screen-detected tumours were significantly more likely to be ER positive. (Ernst et al. 2002) However other studies have shown no difference in hormone receptor status between screen-detected and symptomatic tumours (Nordèn et al. 1997).

A comprehensive review of the literature on stability of ER expression throughout progression of breast cancer concluded that ER remained a stable phenotype (Robertson 1996); therefore there is no evidence to suggest phenotypic drift of oestrogen receptor status. It appears, then, that a trend towards increasing incidence of tumours with intrinsically better prognosis may be contributed to by screening in the case of grade and size but not receptor status.

1.5. How do biological differences in breast cancer influence prognosis?

Oestrogen receptor (ER)

ER is a nuclear transcription factor which regulates the expression of several genes involved in control apoptosis and cell proliferation, both in normal breast epithelium and in breast cancers. It is activated by the binding of oestrogen to the hormone binding domain. After this binding the receptor dimerises to another ER receptor, and this dimmer in turn binds to oestrogen responsive elements in the promoter regions of the target genes. These include ps2, progesterone receptor (as discussed below) and the bcl2 apoptosis inhibitor. There also appears to be some interaction with genes that do not contain oestrogen responsive elements such as the AP1 transcription factor family (Osborne 1998).

Detection of the presence of oestrogen receptor was formerly carried out by ligand binding assays, in which case cytosolic oestrogen was measured in fmol per mg of protein. Cut-offs for positivity were generally based on the limits of detection of the various assays, ranging from 3 fmol/mg (Harvey et al. 1990) to 5 fmol/mg (Allred et al. 1990) to 10 fmol/mg (Aamdal et al. 1984). In several of the original studies into the endocrine responsiveness and outcome of tumours whose ER status was determined by IHC (Allred et al. 1990; Allred et al. 1998; Pertschuk et al. 1990) 10% of cells staining positive was used as a cut-off for ER positivity. This cut-off was shown to give adequate separation between positive and negative groups in terms of prognosis (Ogawa et al. 2004) and has been adopted by many laboratories worldwide (Allred et al. 1998; Layfield et al. 2003). However, in a study of 1900 patients where ER positivity was taken as 1% or more positively staining cells, Allred (Allred et al. 1998) found that this still correlated with endocrine responsiveness and hence improved disease-free survival; nevertheless few clinicians would use 1% as a cut-off for positivity.

Oestrogen receptor positive tumours are generally of lower grade than receptor negative tumours (Fisher et al. 1981; Maynard et al. 1978; Millis 1980; Stierer et al. 1993) and have been shown to have a lower proliferation rate than receptor negative

tumours, as shown in a study of S-phase fractions in 127,000 tumours (Wenger et al. 1993). While ER positive tumours can be shown to have less 'aggressive' histopathological features, and the presence of the ER allows treatment with endocrine therapy, the issue of whether ER positive tumours have an intrinsically better prognosis is less clear. The most useful studies in this regard are studies with long term follow up and survival analysis in women who received surgical treatment with no adjuvant therapy, and there are few such studies.

Gelbfish et al. (1988) studied 204 patients with primary operable breast cancer whose receptor status was determined by dextran-charcoal and who received no adjuvant therapy. There was found to be no significant difference in disease-free interval between ER positive and negative patients. Butler et al (1985) followed 556 node-negative patients who received only surgery. They found no significant difference in both overall and disease-free survival between the ER negative and ER positive patients. Coradini et al. (2000) analysed 1793 patients who had node negative tumours and no systemic therapy, and who had at least 10 years of follow-up. They concluded that ER positivity was not correlated with survival in the first few years of follow-up but thereafter gave a higher risk of relapse. Similarly, multivariate analysis by Hilsenbeck (1998) showed a pattern of initial lack of prognostic ability changing later on to poorer prognosis with ER positivity; while their patients were a heterogeneous group who had different adjuvant treatments, they stated that the same results were seen in their analysis of a subgroup of untreated patients

Far more than its intrinsic effects on prognosis, the importance of oestrogen receptor lies in its ability to predict response to adjuvant endocrine therapy in the form of tamoxifen or aromatase inhibitors. Tamoxifen is an antioestrogen that has oestrogenic properties. It inhibits expression of oestrogen-regulated genes. It is mainly cytostatic, slowing cell proliferation, although it has also been shown to be pro-apoptotic (Osborne 1998). A recent meta-analysis of all tamoxifen trials (EBCTCG 2005) confirms shows that women with ER positive tumours derive significant benefit from 5 years of tamoxifen as regards recurrence reduction and longterm survival whereas ER negative women do not. In the group of 30,000 women who were ER positive or ER unknown, 5 years of tamoxifen treatment

provided a recurrence reduction of 47%, and a mortality reduction of 26% at 10 years of follow-up. In a further 8000 women who were ER negative, tamoxifen had no significant effects on mortality and recurrence. Therefore, ER positive cancers are of 'better prognosis' than ER negative cancers due to the effects of hormonal therapy.

This does not mean that all oestrogen receptor positive patients will respond to endocrine therapies; between 30 and 40% of ER positive tumours are estimated to be refractory to tamoxifen from the start. Indeed, most ER positive tumours do become tamoxifen resistant over time. There has been much recent interest in translational research into the roles of various signal transduction pathways in de novo and acquired tamoxifen resistance. The development of aromatase inhibitors has therefore given new hope in the endocrine treatment of oestrogen receptor positive cancers. Aromatase inhibitors reduce circulating oestrogen levels and their use as adjuvant therapy (in postmenopausal women only) reduces recurrence rates (Winer et al. 2002). The results of the ATAC trial suggested improved disease free survival with anastrozole over tamoxifen, although it remains unclear whether postmenopausal women should receive aromatase inhibitors as first line therapy (Howell et al. 2005; Winer et al. 2002). There are indications that aromatase inhibitors should be used as first line therapy when certain characteristics of the tumour which suggest intrinsic tamoxifen resistance, such as HER-2 positivity and PR negativity, are present (Winer et al. 2002).

Progesterone receptor (PR)

The progesterone receptor gene is under the control of oestrogen receptor. It was initially hypothesised by Horwitz and McGuire in 1975 that its presence suggested that its oestrogen receptor was functional, and that the tumour was likely to respond to endocrine therapies. It has indeed been shown that adjuvant tamoxifen is less effective in ER +ve , PR –ve tumours than in ER +ve PR+ve tumours, with up to double the recurrence rate in the PR-ve tumours (Bardou et al. 2003; Howell et al. 2005; Winer et al. 2002). However in the ATAC trial the patients with ER+ve, PR –ve tumours responded almost as well to anastrozole as ER+ve PR+ve tumours

(Howell et al. 2005), suggesting that many PR negative tumours may in fact still have a functioning oestrogen receptor. Similarly ER has been shown to be transcriptionally active in some ER+ve PR-ve tumours (Cui et al. 2005).

Various mechanisms for loss of PR have been studied such as hypermethylation of the PR promoter or genetic loss at the PR gene (Cui et al. 2005). However, these mechanisms do not explain the aforementioned resistance to selective oestrogen receptor modulators (SERMs) such as tamoxifen. A recent study of over 40,000 breast cancers (Arpino et al. 2005) showed that ER +ve PR -ve patients express higher levels of HER-1 and HER-2 than ER +ve PR+ve patients and display more aggressive characteristics. The authors hypothesise that reduced PR expression may be a marker of aberrant growth signalling, and it is this altered signalling that suppresses PR and also causes tamoxifen resistance to develop. In a later paper the hypothesis is detailed that hyperactive growth factor signalling not only represses PR expression directly by inhibiting PR promoter activity, but promotes tamoxifen resistance by causing ligand-independent activation of ER-responsive genes or causing a shift to membrane initiated signalling which is associated with increased tamoxifen agonist activity (Cui et al. 2005). Other postulated mechanisms of tamoxifen resistance in ER+ve PR-ve tumours include altered ER coregulator levels, or the ER may be unable to bind DNA (Cui et al. 2005)

The findings of reduced efficacy of SERMs in women with the ER+ve PR-ve phenotype have important implications for endocrine adjuvant therapies. Some members of the 2005 American Society of Clinical Oncology Technology Assessment panel (Winer et al. 2002) felt that such patients should be given an aromatase inhibitor as first-line adjuvant therapy instead of tamoxifen whilst others disagreed. For premenopausal women, options may include degradation of ER by oestrogen receptor degraders such as fulvestrant. Preclinical trials have suggested that combined use of a SERM and a growth factor inhibitor may in the future be the treatment of choice for ER+ve PR-ve tumours (Winer et al. 2002).

As to whether progesterone positivity has an effect on prognosis independently of the use of endocrine therapies, the analysis of Coradini et al. (2000) of outcome patients receiving no adjuvant therapy revealed that high progesterone receptor levels correlated with reduced risk of relapse which persisted over time.

HER-2

The presence of HER-2 , while not a hormone receptor, has prognostic significance. The HER-2/neu gene, also known as the Erb-B2 gene, allows the expression of the HER-2/neu protein, which is a transmembrane tyrosine kinase growth factor receptor. It is closely related to three other growth factor receptors, HER-1,-3 and -4. Studies have shown that the amplification of the HER-2 gene and overexpression of the HER-2 protein correlates with poor outcome in terms of overall and disease-free survival, with HER-2 positive breast cancer being universally recognised as an aggressive form of the disease (Chang et al. 1999; Gusterson et al. 1992; Rilke et al. 1991; Ross & Fletcher 1999; Slamon et al. 1987; Witton et al. 2003). The differences between HER-2 +ve and negative patients appear to be most marked in node positive patients (Rilke et al. 1991), and survival differences appear to level out after 4-5 years (Rilke et al. 1991). HER-2 overexpression is associated with high grade, lymphocytic infiltration, high mitotic index and p53 mutation (Menard et al. 2001; Menard et al. 2003).

HER-2 positive tumours are more likely to be ER negative than ER positive(Konecny et al. 2003). However, in those tumours that are ER positive, overexpression of HER-2 has been linked with de novo tamoxifen resistance (Carlomagno et al. 1996; De Laurentiis et al. 2005; Dowsett et al. 2001; Elledge et al. 1998; Horiguchi et al. 2005; Menard et al. 2001; Menard et al. 2003; Tovey et al. 2005; Winer et al. 2002; Wright et al. 1992). Overexpression of growth factors like HER-2 can directly modulate ER activity independently of oestrogen or cause a switch towards membrane-based signalling which allows tamoxifen to act as an agonist and inhibits its apoptotic ability (Arpino et al. 2005; Cui et al. 2005). Some have therefore suggested that ER positive, HER-2 positive patients should be considered for aromatase inhibitor therapy as a first line; the ASCO 2005 guidance (Winer et al. 2002) was that there was inadequate clinical data to support this. The advent of a monoclonal antibody to HER-2 in the form of trastuzumab (Herceptin) has allowed some modification of this poor outcome, with a disease free survival improvement of up to 50% at 3 years when used adjuvantly (Piccart-Gebhart et al. 2005; Romond et al. 2005). HER-2 protein is detected by immunohistochemistry and conventionally scored as 0, 1+, 2+ or 3+, with 2+ being indeterminate and requiring

determination as positive or negative with fluorescent in-situ hybridization which tests for actual gene amplification (Bartlett, Mallon, & Cooke 2003; Bartlett et al. 2001; Dowsett et al. 2003; Garcia-Caballero et al. 2006)

Grade

The relationship between the morphology and degree of differentiation of breast cancers and the outcome of the patients was first noted by Greenhough in 1925 (Going et al. 2001). In the 1950s Bloom and Richardson developed a grading system assessing presence of tubule formation, nuclear pleomorphism and numbers of mitoses, classified into grades 1-3, and found that grade correlated with prognosis. The Nottingham modification of the Scarff-Bloom-Richardson grading, which retains the three main histological features described by Bloom, was introduced in 1987 and is widely used in the UK; however, a variety of different grading systems are used worldwide. The use of grade as an independent prognostic factor has remained controversial despite the fact that many studies have confirmed the correlation of grade and prognosis.

When constructing their (now widely used) Nottingham Prognostic Index, The Nottingham Breast Group performed multivariate analysis to establish those factors that were independently related to prognosis, and found grade to be an independent significant factor (Galea et al. 1992; Haybittle et al. 1982). In a 1997 review of the literature, Roberti et al. (1997) found that low grade predicted either higher recurrence-free or overall survival in almost all the studies. Included in this was a study of 22,616 cases of breast cancer (Henson et al. 1991) which showed 93% 5-year survival in the grade 1 group and 65% 5-year survival in the grade 3 group. Another study of 1537 node-positive women in an adjuvant therapy trial demonstrated that grade retained its significance in a multivariate analysis (Davis et al. 1986). Reasons for the reluctance outside the UK to accept grade as a prognostic factor may include the lack of standardisation and reproducibility and concern that, despite the results of multivariate analyses, it may not be a truly independent risk factor and thus not give any additional prognostic information over tumour size or stage (Burke & Henson 1997; Going et al. 2001).

1.6 Why might breast cancer biology be changing?

It is possible that a change toward hormone receptor positive cancers reflects increasing exposure of women to certain factors which not only increase the risk of breast cancer but preferentially increase the risk of ER positive or negative cancers. There is no evidence thus far that this is due to changes in hormonal exposures. An exhaustive systematic review by Althuis et al. (2004) evaluated the results of 40 cohort and case-control studies of risk factors for hormone receptor positive and negative breast cancer. For late age at first pregnancy, the strongest associations were with increased risk of ER positive and ER+/PR+ cancers. For total parity, the reduction in breast cancer risk with increasing parity was most closely linked with ER positivity; there was no link with joint receptor positivity and only one study showing a reduced risk of PR positive disease. Older age at menarche was not linked with ER or PR status in those studies that assessed receptors separately, although studies of joint positivity suggested a link with reduced risk of ER+PR+ disease.

A consistent association of postmenopausal obesity and ER+PR+ status was demonstrated. Among the large prospective cohort studies, the Iowa Women's Health Study (Potter et al. 1995) showed an association with ER+, PR+ and ER+PR+ disease and the Nurses' Health Study (Colditz et al. 2004) showed that only PR positivity was independently correlated with raised BMI. There was weak evidence that ever-use of combined oral contraceptives was associated with increased risk of ER negative phenotype; the inclusion of older women in many studies may have affected the validity of this result. Most of the studies did not find any evidence of increased breast cancer risk with use of hormone replacement therapy; the Nurses' Health Study (Potter et al. 1995), however, did show an increased risk of ER positive tumours in past users of HRT. Breast-feeding, smoking, alcohol, family history and premenopausal obesity were not associated with receptor status differences.

As noted earlier, changes in all these factors are occurring; not only will this increase risk of breast cancer but it could potentially selectively increase the risk of ER positive and potentially PR positive disease.

1.7 Deprivation and its relation to incidence, mortality, screening trends

It has long been recognised that breast cancer rates are higher in areas of higher socio-economic status (Faggiano et al. 1997; Ketcham & Sindelar 1975; van Loon et al. 1995). The reasons behind this have not been conclusively proven, but differences in risk factors such as reproductive factors and body mass index have been suggested. There have been suggestions that the incidence gap between affluent and deprived in terms of breast cancer incidence is in fact narrowing, with more and more deprived women developing breast cancer (Population Trends 1997; Dano et al. 2003) although these observations were made in cohort study data rather than population data. There are also inequalities in survival, with women of lower socio-economic status having significantly lower survival rates (Thomson et al. 2001). Torgerson et al (1994) postulated that socio-economic differences in body mass index could explain survival differences.

The survival differences do not appear to be due to deprived women presenting with tumours of higher stage or grade. For example Carnon et al. (1994) showed that 32% of women in the most affluent group presented with tumours under 2cm compared with 31% of the least affluent group and Brewster et al. (2001) examined cancer registry data and found no difference in tumour stage at presentation.

The only prognostic factor that possibly differs between affluent and deprived women is a tendency towards more oestrogen receptor negative tumours. Thomson et al. (2001) showed that in deprived women under 65 there were significantly higher rates of ER negative tumours and lower rates of ER positive tumours than in affluent women. However, they did note that although the distribution of unknown status tumours was no different between the groups, one third of the women in their study had a tumour status that was unknown. Furthermore, they did not feel that ER status difference explained survival differences; they estimated that only 4% of the 22% difference in survival between groups was explicable by ER status.

There has been much recent interest in the epidemiology of Marin County in the Bay area of San Francisco. This county has significantly higher breast cancer rates per 100,000 than the rest of San Francisco and in many parts of the United States (Benz, Clarke, & Moore 2003; Hwang et al. 2005; Prehn et al. 2002; Prehn & West 1998; Robbins, Brescianini, & Kelsey 1997; Wrensch et al. 2003). Its population is demographically homogeneous and it has a high proportion of women of high socio-economic status. In fact it has been shown that rates in Marin County are similar to rates in other areas of California with a similarly high socio-economic status (Prehn & West 1998).

The affluence of the women in Marin County may be a marker for exposure to certain risk factors over time which promote the development of breast cancer. Several studies have attempted to elucidate such risk factors. Robbins et al (1997) analysed incidence rates in the San Francisco Bay Area and elsewhere in the SEER registry, correcting them for the presence of identifiable risk factors, such as age at first pregnancy, age at menarche, alcohol use, parity and breastfeeding duration. They concluded that the high incidence in the San Francisco Bay area could be completely explained by regional risk factor differences. Wrensch et al. performed a case-control study of women in Marin County and found the only difference in risk factors between cases and controls to be that of a difference in alcohol consumption (Wrensch et al. 2003).

Deprived women in the UK (Gatrell et al. 1998) and the US (CDC 2005) take up invitations for screening in lower numbers than affluent women; reasons may include differences in awareness and attitudes to health, availability of services and access to public transport. There is also some suggestion from cross-sectional studies that women of lower socioeconomic status are less likely to use hormone replacement therapy (Lawlor, Smith, & Ebrahim 2004).

The excess breast cancer incidence in the population of Marin County includes a higher than expected number of ER + PR+ cases (Benz, Clarke, & Moore 2003). As discussed above, particular risk factor profiles may contribute to development of oestrogen receptor positive breast cancers, and it is notable that these risk factors (low parity, late age at first pregnancy) are typical of the women in Marin County. Therefore the theory of a difference between the receptor status of tumours in

women of different socio-economic categories is plausible and may be explained by risk factor profiles.

1.8 Aims of This Thesis

This thesis presents research carried out on two separate but closely related projects; an epidemiological study of influences on breast cancer incidence in Scotland, and a laboratory project studying the grade and receptor status of breast cancers in Scotland over time. The aim of the thesis as a whole was an exploration of influences on breast cancer incidence and molecular epidemiology in Scotland. Each of the chapters 2 (Materials and Methods) and 3 (Results) is divided into Part 1 (Epidemiology project) and Part 2 (Laboratory project).

Aims: Part 1 - Breast Cancer Incidence in Scotland and Its Influences

On the background of the changing breast cancer incidence patterns in the UK, the following research aimed to establish:

- trends in breast cancer incidence in Scotland, by age group and birth cohort up to 2003, particularly concentrating on women aged 50-64
- the potential influence of screening on incidence trends aged 50-64 in Scottish women over this period: both temporally and by birth cohort
- incidence rates within women of different socioeconomic standing over this period
- the potential contribution of breast cancer risk factors to these incidence trends

Aims: Part 2 - Is The Biology Of Breast Cancer Changing? A Study Of Hormone Receptor Status And Grade 1984-86 And 1996-97

The project was designed in order to identify any changes that have occurred in the biology of breast cancers over time.

The primary aim of the study was:

To compare the percentage of breast cancer patients from two Glasgow hospitals in two separate time periods (1984-1986 and 1996-1997) who had tumours which were

1) oestrogen receptor positive
2) of high and low pathological grade
3) progesterone receptor positive
4) HER-2 positive

Previous studies of changes in receptor status over time have retrospectively studied database records of receptor positivity, with the inherent problem of heterogeneity of receptor testing over time; this is the first such study to apply immunohistochemistry to use archival tissue to establish the prevalence receptor positivity. Studies have show stability of antigenicity over time in archived tissue blocks (Camp et al. 2000, Shibata et al. 1988).

Secondary aims included:

- To study the distribution of hormone receptor positivity and pathological grade between different socio-economic groups and how this may have changed over time
- To study and compare the distribution of hormone receptor positivity and pathological grade in screen-detected and symptomatic tumours.
- To assess the contribution of these changes to the survival of the patients

Chapter 2: Materials and Methods

Materials and Methods Part 1: Breast Cancer Incidence in Scotland and Its Influences

This section explores trends in breast cancer incidence in Scotland, by age group and birth cohort up to 2003, particularly concentrating on women aged 50-64, i.e. those women routinely invited for screening by the NHS Breast Screening Programme in Scotland during this period. A specific study of breast cancer incidence by year of birth in these women was performed (section 2.2); as discussed above, birth cohort cancer incidence rates can provide information on the likely contribution of risk factors to incidence, and this particular analysis was the first such analysis to try to incorporate the effects of a screening programme into the calculations. Further exploration of changes in the screening programme was also performed (section 2.3, section 2.6). The difference in incidence trends in different socioeconomic categories was performed (section 2.5), and the potential contribution of known breast cancer risk factors (hormone replacement therapy, BMI, alcohol, fertility patterns) to these incidence trends was also undertaken (section 2.4, section 2.7).

General Notes on the Epidemiological Data and Statistical Methods Used

Data on breast cancer incidence in Scottish women were obtained from the Scottish Executive's Information and Statistics Division (ISD), as discussed in more detail in the sections below. The ISD manages the Scottish Cancer Registry, which is responsible for collecting information on all new cases of malignant tumours in Scotland. Since 1997 a computer system with linkage to hospital discharge codings and pathology records has been used to obtain data on cancer cases along with the relevant prognostic criteria and demographic data of the patients, and frequent validation checks are carried out to ensure data completeness. The Cancer Registry was independent between 1958 and 1997, and during this time a system of compulsory notification by clinicians of cancer cases was in place while the lack of

computer linkage is likely to have reduced the completeness of the data, regular validation audits were carried out and the Registry has long had a reputation for the accuracy of its data. Other data obtained from ISD Scotland as described below includes data on prescriptions and breast screening. The Scottish Breast Screening Programme has always rigorously maintained and analysed data about the activity of its service, including screening invitations, numbers of mammograms performed and details of findings on these, and this data is all passed to ISD.

Data on births in Scotland is kept by the General Register Office for Scotland; with the compulsory registration of a birth at a local register office birth, the parents' occupations and ages are recorded and all data passed directly to the General Register Office where it is immediately checked and now digitised ensuring data completeness. The Register Office staff add further information to those data, for example comparing with previous certificates to determine whether a birth is the mother's first or not.

Some data on lifestyle-based risk factors is taken from the Scottish Health Surveys of 1995, 1998 and 2003 (Bromley et al. 2005; Dong & Erens 1997; Shaw et al. 2008). These ground-breaking cross-sectional surveys studied around 6000 male and female subjects in Scotland, recording detailed information on lifestyle factors based on anthropometric recordings and self-reporting; extensive statistical analyses were performed and the authors of each study compared their results to the studies before to give an estimate of trends. In 1995 the subjects were aged 16-64, 16-74 in 1998 and 16 and over in 2003. These data have been extrapolated extensively by the Scottish Executive and other researchers to represent the health and lifestyle of Scotland as a whole. The benefit of such projects is to gain information that would be impossible to collect from the population of Scotland as a whole; while greater computer recording by GP surgeries is now providing more data about the health and lifestyle of patients, a large proportion of the population will rarely attend a GP. Using the data requires extrapolating to represent the Scottish population, and this assumption may be invalid if there has been a selection bias in the subjects assessed by the Survey. In order to minimise the risk of this selection bias, extensive statistical advice was used to ensure representativeness, with stratified cluster sampling being carried out. Additionally, while the data has been divided into age

categories there is no data on the menopausal status of the women in the study, information which may have been of relevance for this thesis.

A note on the regression analyses used in this thesis: as incidence rates are a form of count data, they should follow a Poisson distribution. The Poisson distribution assumes that the events are integers that are not less than 0, they occur singly and independently of time since the last event, and the probability of an event occurring in a given time interval is constant over time. Poisson regression is the corresponding model used to describe the association between explanatory variables and count or rate data. A limitation of Poisson regression, however, is that regression on rate data requires the absolute population denominators and these may not be readily available from routine data that are expressed as rates per 100 000 population. A useful technique is the Normal approximation to the Poisson for large values (> 30) of the Poisson parameter, which allows one to use the log-transformed rates in a linear regression and does not require absolute denominator data - that is, routinely available rates per n population can be used. The Normal approximation produces very similar regression coefficients to Poisson regression particularly where counts are large, as is the case for nationally published breast cancer incidence rates. Therefore, linear regression on log-transformed rates was used to produce all regression analyses of incidence data in this thesis. Simple regression analyses were used when appropriate, e.g. percentage uptake of screening invitations.

2.1. Temporal Breast Cancer Incidence Trends in Scotland

Incidence rates of breast cancer in women younger than and older than screening age in the UK have shown little variation over time and it is the age groups offered screening who have seen the biggest changes in incidence. Therefore, a detailed study of breast cancer incidence trends in Scotland in the age ranges 50-54, 55-59 and 60-64, that is, women who are offered screening mammography, and women of 65-69, that is, women just above screening age, was felt to be of most value in elucidating influences on incidence.

The breast cancer incidence rates per 100,000 women for each year from 1975 to 2003, divided into 5 year age ranges, were obtained from the website of the

Scottish Executive's Information and Statistics Division. To assess the magnitude of the changes in incidence in women aged 50 to 69 in this study, a plot was constructed and then an 'observed versus expected' analysis (as described by Smith et al. 2008) was performed to estimate what breast cancer trends in Scotland were likely to have been in the absence of the screening programme and compare them to current trends.

"**Observed versus Expected" Analysis**

Observed trends:

The mean incidence rate for 1999-2003 was calculated, to represent the 'observed' incidence rate in 2001, with a time range being more reliable than a single year

Expected number of cases:

The annual percentage increase in cases per 100,000 per year between 1975 and 1987 (when the screening programme began) for each of the four age groups (50-54, 55-59, 60-64 and 65-69) was calculated by performing linear regression analysis of log-transformed incidence rates using SPSS statistics package version 14.0; annual percentage increase was determined by 100 x ($e^{B} - 1$) where B is the B coefficient of the regression equation. The expected number of cases for 2001 – the midpoint of the 1999-2003 range - was then determined by sequentially increasing the 1987 rate by the annual percentage increase determined above for each of the intervening 14 years.

The percentage increase in incidence rate was calculated by dividing expected (E) by observed (O), minus 1 and multiplied by 100%.

2.2. Birth Cohort Incidence of Breast Cancer in Scotland and the Influence of the Screening Programme

Age and year specific breast cancer incidence rates per 100,000 were supplied for women aged 45 to 69 years from 1977 to 2003 inclusive by the Information and Statistics Division. The data were placed into a Lexis diagram (appendix 1), a format which allows easy identification of the birth year represented by each age within

each calendar year. The mean incidence rate for women aged 50 to 64 was calculated for each birth year cohort from 1920 to 1949. Mean incidence rates were then calculated for each birth year for the age ranges 45-49, 50-54, 55-59, 60-64 and 65-69. The time points at which the women in each cohort and age range became eligible for screening in the prevalent round (1987-1994) and the time points between which ALL women in the cohort and age range would have been eligible for screening in the prevalent round were identified. For example, women born in 1933 were aged 50-54 between 1983 and 1987. Some of the 50-54 year old age group who were born in 1933 were thus eligible for screening when the round began in 1987, albeit only those aged 54. In the 1934 birth cohort, only 53- and 54-year olds were eligible for screening. The 1937 birth cohort was thus the first in which all 50-54 year olds were eligible for screening in the prevalent round. Similarly, as women in the oldest ages of any age-group reach the end of the prevalent round in 1994 they will then go on to be screened during the established ("incident") round of screening. Thus the 1940 cohort was the last in which all 50-54 year olds would have been part of the prevalent screening round.

Breast cancer incidence rates per 100,000 were plotted against birth year. Mean incidence rates were then plotted against birth year separately for each age range, with the region of the graph representing all women in the cohort being offered screening being highlighted. 95% confidence intervals (CI) for the differences in breast cancer incidence rates were calculated using a method based on that described by Altman and others for differences in proportions for paired samples (Altman et al. 2000):

p = incidence rate / 100,000 (1 = rate at end of period, 2 = rate at start)
n= population (100,000)
1.96 = constant used based on 95% confidence interval, as listed in Altman

Difference in rates =

$$p1 - p2 \pm 1.96 \times \sqrt{\frac{p1(1-p1)}{n}} + \sqrt{\frac{p2(1-p2)}{n}} \times 100{,}000$$

The differences in rates (with confidence intervals) was calculated for each age range for:

1) the rate in the cohort representing the start of the study period to the rate in the cohort where women began to be offered screening

2) the rate in the cohort where women began to be offered screening as part of the prevalent round and the cohort where women began to be offered screening as part of the 'incident round' of screening (this therefore includes cohorts where some or all women would have been offered screening in the prevalent round)

3) the rate in the cohort where women began to be offered screening as part of the 'incident round' to the cohort representing the end of the study period.

A Lexis diagram (Appendix 1) was used to identify the points at which women were entering or leaving screening periods.

2.3 Breast Screening Data

The Scottish Breast Screening Programme was introduced in 1987. All eligible women had been invited for a first screen – that is, the 'prevalent round' had been completed, by 1994. In 2003/04 a phased extension of age range or routine invitation to 50-70 years began. Figures studied here, however, are from before this age extension began.

As detailed above, improvements in the breast screening programme may artificially raise incidence rates by improving the ability of the screening programme to detect cancers, and increasing uptake rates may also increase incidence rates as more women have a 'prevalent' screen. Self-referrals and GP referrals for screening are not covered by figures on uptake rates, and changes in these figures may similarly affect incidence rates. In particular, if numbers of older women self-referring for screening increase, this could have an effect on incidence rates in older women. Information on the history of the screening programme in Scotland was

obtained from ISD Scotland's website. Information on percentage uptake of screening invitations, self-referrals for screening and standardised detection ratio over time was obtained by contacting the Scottish Executive's Information and Statistics Division (ISD). The author went on to further analyse these trends using log-linear regression on SPSS v14.

2.4. Risk factor trends

Information of fertility trends was obtained from the General Register Office for Scotland and ISD Scotland. This comprised completed family size data (as a marker for parity) and data on births at different maternal ages, both temporal trends and trends by maternal age. Further analysis by constructing graphs and performing linear regression (SPSS v14.0) was carried out by the author.

Specific attention was paid to trends in numbers of women having their first birth at ages 35-39. Having a first birth over the age of 35 increases breast cancer risk; the data were available divided into 4-year age groups and as it was felt that grouping the data from different age groups together was not epidemiologically sound, the 35-39 age group was analysed. The author also calculated first births to women aged 35-39 as a percentage of all first births.

Data on trends in body mass index and prevalence of obesity over time were obtained using the published Scottish Health Surveys of 1995, 1998 and 2003 (Bromley et al. 2005; Dong & Erens 1997; Shaw et al. 2008). Without having the full dataset it was not possible to further statistically analyse BMI trends over time and so here a simple reporting of changes in mean BMI was made by the author. In 1995 the subjects were aged 16-64, 16-74 in 1998 and 16 and over in 2003, so a comparison was made between women who were aged 16-64 only.

Data on trends in oral contraceptive use were not felt to be relevant in this analysis; as risk is thought to disappear 10 years after last use, and these women are unlikely to have used oral contraceptives less than 10 years previously. The only data available from ISD Scotland on hormone replacement in Scotland was total numbers of prescriptions from 1993 to 2003. Therefore estimation of prevalence was

made by the author in a similar fashion to the calculations of Townsend when estimating prevalence in England and Wales (Townsend 1998). Trends in alcohol use in Scottish women over time had been assessed by the authors of the Scottish Health Surveys (Bromley et al 2005; Dong & Erens 1997; Shaw et al 2008) and these trends are detailed here.

2.5 Incidence trends in different socioeconomic categories

Statistics of breast cancer incidence by socioeconomic status have been collected by the Information and Statistics Division. Each patient is assigned a deprivation quintile. Quintiles of deprivation were calculated by ISD Scotland using the Carstairs and Morris index. This index is calculated on the basis of the results of the most recent census (in the case of these data, the 1991 census) and is based on four main indicators: lack of car ownership, Registrar General Social Class, overcrowded households and male unemployment. Index scores are then divided into quintiles in a weighted manner so that 20% of the population fall into each quintile. 'Deprivation categories' were the original categories proposed by Carstairs and Morris and were used until the recent adoption of the quintile method. Categories 1 to 7 were based on index scores and were designed to retain the discriminatory value of each category rather than divide the population into equal groups. Since 2001, deprivation quintiles have been calculated on the basis of the more complex Scottish Index of Multiple Deprivation. This is based on 37 separate indicators within 7 headings (current income, employment, health, education, geographic access, crime and housing) for each postcode, which are used in a weighted manner to give a 'score' to an area.

In order to analyse breast cancer incidence trends in different socio-economic groups it is important to calculate trends on the basis of similarly categorised data and so rates from 1991 to 2000, which were categorised into deprivation quintiles on the basis of Carstairs and Morris index, were used. At any rate, such data are more 'stable' when analysed several years down the line.

Breast cancer incidence rates from 1991 to 2000 for the five quintiles of deprivation were obtained from ISD. Unfortunately age-specific data are not kept by ISD. Linear regression of log-transformed rates was performed on the rates of breast cancer in each deprivation quintile; p-values for each regression equation were assessed to look for the presence of a significant trend. Then in order to see whether the slopes differed significantly from one another, analysis of interaction was performed using the univariate ANOVA in SPSS v14.

2.6 Breast screening and socioeconomic status

Data were obtained from the Scottish Breast Screening Programme via ISD Scotland on percentage uptake of screening invitations by deprivation category (1-7), divided into one- year periods from 1990-1991 to 2001-2002. A graphical representation of trends was made, which suggested that uptake was increasing over time in all deprivation categories. It was important to assess not only whether percentage uptake was increasing in each category, but also whether it was increasing to the same degree in each category. Therefore linear regression (using SPSS v14) was performed of the rates over time for each deprivation category and an interaction term (using univariate ANOVA) was added into the analysis. The p value for the interaction term gives an understanding of whether the rates are increasing to the same extent in all deprivation categories.

2.7. Risk factor trends and socioeconomic status

The General Register Office for Scotland supplied data on numbers of first births at various maternal ages by deprivation category (1-7) from 1975 to 2003. In view of the particular influence on incidence of first pregnancy over the age of 35, particular attention was paid to the maternal age category 35-39. Data on completed family size and nulliparity by deprivation category were not calculated by the General Register Office for Scotland or ISD Scotland.

A graphical representation carried out by the author of numbers of births to women of maternal age 35-39 divided into deprivation category suggested that births

at late maternal age in all deprivation categories was increasing in a linear fashion, but with a widening gap as a result of many more affluent women than deprived women having first birth over 35 in recent years. In order to confirm this, linear regression of log-transformed numbers of births was performed by the author using SPSS v14, with univariate ANOVA to test for interaction. The p-value for the interaction term was assessed to identify whether rates of late first birth were increasing to the same degree in all categories.

Data on BMI and socio-economic status was obtained from the Scottish Health Surveys of 1995, 1998 and 2003. In Health Surveys 1995 and 1998, Registrar General Social Class was used to classify socio-economic status but in 2003 the Scottish Index of Multiple Deprivation was used. The association of BMI and obesity prevalence and social class in each study year was analysed by the author (Sylvia Brown) using Spearman's correlation with SPSS v 14.0. Trends in oral contraceptive and hormonal therapy use by deprivation category were not available from any agency.

Methods Part 2: Laboratory Project - Is the biology of breast cancer changing? A study of hormone receptor status and grade 1984-86 and 1996-97

2.8 Study Design Summary

The project was designed as a laboratory based study comparing archival tumour samples from every female patient who had surgical treatment for breast cancer in one of the two study periods. The study periods 1984-86 and 1996-1997 were chosen, and patients from Glasgow Royal Infirmary and Glasgow Western Infirmary included in the study, having been identified from the Scottish Cancer Registry. A database was constructed and archival tissue from each patient obtained. New sections were taken and prepared by the author for grade analysis (which was performed by Dr. Elizabeth Mallon at Glasgow Western Infirmary) and samples were taken to put into tissue microarrays (TMAs). Sections were taken from the TMAs and immunohistochemistry performed for oestrogen receptor, HER-2 and progesterone receptor. Positivity for each of these receptors was assessed by visual assessment using the weighted histoscore method. Statistical analysis was then carried out to compare pathological grade and positivity between the two time cohorts. Analysis of the influence of socioeconomic status and screening status on pathological and molecular tumour characteristics was also undertaken.

2.9 Patient Selection

The study period was chosen as Scottish Cancer Registry data existed for these women, the period was before screening had been introduced, and a suitable length of time was present between this and the later study period. By using patients from Glasgow Royal Infirmary whose catchment area mostly consists of patients of deprived and intermediate socioeconomic status, and the Western Infirmary which includes intermediate and affluent patients, a wide range of socioeconomic

categories would be present in the study, allowing analysis of association of receptor status, pathology and socioeconomic status to be undertaken. In 1984 tamoxifen was offered to ER positive women as adjuvant therapy in Glasgow hospitals.

A sample size calculation was undertaken to ensure that the study was sufficiently powered to detect a difference in receptor positivity. A sample size adequate to detect a 10% difference in oestrogen receptor positivity was felt to be the most important criterion. Sample size was calculated using the following formula (Whitley & Ball, 2002):

$$n = \frac{2 \times [p1 \times (1-p1)] + [p2 \times (1-p2)]}{(p1-p2)^2} \times Cp,power \text{ (a constant based on power and p)}$$

where p1 is old cohort predicted percentage and p2 new cohort. If the older cohort oestrogen receptor positivity level is predicted to be 60% (from pilot studies at Glasgow Royal Infirmary) and the newer cohort to be 70%, a total of 712 patients are required to show a 10% difference in receptor status with a power of 80% and level of significance of 0.05. As the cohorts were expected to be of unequal size (cohort 2: cohort 1 ratio expected to be 1.5:1) as a result of the increase in incidence of breast cancer, an adjustment had to be made to estimate the sample number in each group. Using a formula detailed by Whitley and Ball (2002), the required sample size in cohort 1 was estimated to be 296 and the sample size in cohort 2, 446.

2.10 Ethical Approval and Consent

Ethical approval was obtained from the Glasgow East Local Research Ethics Committee 1 on December 17th 2004. Women from 1998 onwards at Glasgow Royal and Western Infirmaries signed a consent form before surgery which specifically stated consent for use of tumour samples in research. Before this, consent for tissue use in research was not specifically requested. Ethical approval for this study was granted without the need to retrospectively acquire consent from the patients or their next of kin, in the light of data from the QUASAR colorectal radiotherapy study suggesting that most women would not object to use of tumour samples for research,

and in view of the difficult and potentially traumatising nature of a request to the next of kin for use of their relative's tumours sample.

2.11 Construction of Database

The Scottish Cancer Registry had collected data from every patient with breast cancer in the period 1984-1986 who had been seen at Glasgow Royal and Glasgow Western Infirmary. Those women who had a clinical diagnosis only or core biopsy diagnosis only were excluded from the analysis (this was evident from the Cancer Registry data and confirmed by a search of stored pathology records). An additional 41 patients had Registry data suggesting that they had undergone breast cancer surgery but search of stored pathology records revealed either only core biopsy pathology or no pathology record at all. These patients may have had an operation at a different hospital or the pathology record may have been lost, and therefore these patients could not be included in the study. This gave a database of 423 patients who were operated on for breast cancer between 1984-1986 in Glasgow's Royal and Western Infirmaries.

At this time in Glasgow pathologists often did not report the pathological size of the tumour. In addition, the practice of axillary clearance as the only surgical technique used in the axilla meant that more women were denied axillary surgery on the grounds of age or fitness than would be refused today. This meant that many of these patients do not have full pathological data to allow us to retrospectively assess their prognosis. 120 of these patients had incomplete pathological data, with tumour size and/or nodal status data missing. These patients were nevertheless included in the study, as their exclusion would preclude the direct comparison of two full cohorts of patients.

Patients from 1996 and 1997 were obtained from the Glasgow Breast Cancer Audit, maintained by Greater Glasgow Health Board. 653 patients were suitable for inclusion in our study database. Only 12 of these had incomplete pathology data.
This gave a total of 1076 patients. A computer database was formed in SPSS version 14. Each patient was assigned a database number to allow anonymisation, and data for each patient included: age, hospital, deprivation category (based on Carstairs index data calculated from the 1981 or 1991 census), tumour size, nodal status,

method of tumour detection (screen-detected or symptomatic), cause of death if appropriate, survival status at time of last follow-up, survival time from diagnosis and pathology number. Pathology numbers for 1996-7 patients were included in the Glasgow Audit database; pathology numbers for 1984-6 patients were obtained by searching paper pathology records. Names and dates of birth were not included. The database was securely stored on a password protected server.

2.12 Tumour Retrieval

Once the pathology number was obtained for each patient, a paraffin-embedded tumour block was sourced from storage. For the 1984-1986 cohort of patients, the block which appeared most likely to contain tumour was chosen. For the 1996-1997 cohort, blocks containing tumour were already identified by an orange block casing. For some patients, either no block at all could be retrieved or no tumour-containing block could be found.

Of 1076 patients 123 tumour blocks could not be found.

1984-1986: 100 blocks were missing (24% of 423): 323 in study 'cohort 1'

1996-1997: 76 blocks were missing (11% of 653): 577 in study 'cohort 2'

2.13 Mercuric chloride: Testing possible interference with IHC

After retrieving specimens from 1984-1986 it became evident that specimens from this time period at the Western Infirmary had been fixed with mercuric chloride which was previously used to enhance H&E staining. As there was little literature on the possible effects of mercuric chloride, immunohistochemistry for oestrogen receptor was performed on whole sections from ten tumours and compared with negative and positive controls. Receptor immunohistochemistry did not appear to be affected by the presence of mercuric chloride in the specimens.

2.14 Haematoxylin and eosin staining

A 4μm-thick section was taken from each block (sectioning carried out by Miss Fiona Campbell of the Endocrine Group at Glasgow Royal Infirmary). Haematoxylin and eosin (H&E) staining was carried out to allow microscopy to confirm the presence of tumour, allow grading of the tumour and allow marking of tumour areas for tissue microarray construction.

H&E protocol

Materials:

Deparaffinisation and rehydration:

- Xylene
- 99% industrial grade methylated spirits
- 90% industrial grade methylated spirits
- 70% industrial grade methylated spirits

Staining:

- Mayer's Haematoxylin
- Scott's Tap Water substitute (3.5 g sodium bicarbonate, 20g magnesium sulphate, 1 litre distilled water)
- Eosin (1 spoonful calcium chloride dihydrate added at the start of each protocol)
- Calcium chloride dihydrate

Dehydration:

- 70% industrial grade methylated spirits
- 90% industrial grade methylated spirits
- 99% industrial grade methylated spirits
- Xylene

Mounting:

- DPX (dibutylphthalate and xylene)
- 22mm x 40 mm glass coverslips

Protocol:

Tissue sections were dewaxed in xylene by dipping 3 times for 2 minutes each time, and then rehydrated by immersing in graded alcohol (two separate periods of 2 minutes in 99% methylated spirits and 2 minutes in each of 90% and 70% methylated spirits).

Tissue sections then underwent Haematoxylin and Eosin staining. Initial staining was performed by placing slide trays in Mayer's haematoxylin for 3 minutes, then running water for 30 seconds and then Scott's tap water substitute until the slides were stained dark blue. After a further running water step, the slide trays were placed in Eosin for 10 minutes and then rinsed. Dehydration was then performed by placing tissue sections in graded methylated spirits, initially 30 seconds in 70% methylated spirits, then 1 minute in 90% and finally 2 separate periods of 1 minute in 99% methylated spirits. Finally, 3 separate 2 minute periods of immersion in xylene were carried out.

Mounting:

Coverslips were then mounted onto each slide using a drop of DPX fixative. Slides were left to dry overnight.

2.15 Grading

H&E slides of whole sections from the tumours were sent to Dr. Elizabeth Mallon, consultant histopathologist at the Western Infirmary in Glasgow, to establish pathological grade. This established that the same pathologist for all the tumours to allow valid comparison of all the tumours had calculated in the same way and the grade. The Nottingham grading system (Elston-Ellis modification of the Scarff-Bloom-Richardson system) was used to grade the tumours; this modification has increased reproducibility of grading (Dalton et al. 2000; Elston & Ellis 1991; Going et al. 2001). This grading system classifies tumours as Grade 3 (high grade), Grade 2 (intermediate grade) and Grade 1 (low grade) based on a cumulative score of tubule formation, mitotic count and nuclear pleomorphism as below:

Tubule formation:

Majority of tumour (>75%)	1
Moderate degree (10-75%)	2
Little or none (<10%)	3

Mitotic count:

0-9 mitoses/ 10 high power fields	1
10-19 mitoses/ 10 hpf	2
≥ 20 mitoses/ 10 hpf	3

Nuclear Pleomorphism:

Small regular uniform cells	1
Moderate nuclear size and variation	2
Marked nuclear variation	3

Combined histologic grade

Total score 3-5	Grade 1 (low)
Total score 6-7	Grade 2 (intermediate)
Total score 8-9	Grade 3 (high)

An initial check was made on microscopy to confirm the presence of tumour, as certain of the older blocks were not automatically identified containing tumour. For those that did not contain tumour, further blocks were obtained and the sectioning, H&E and microscopy process was repeated. The presence of lymphovascular invasion and the type of cancer (lobular or ductal) was also noted. 862 tumours were graded in this way. The study population undergoing immunohistochemistry was larger than this due to further tumour blocks having been retrieved after the grading part of the project had been completed.

2.16 Immunohistochemistry

Construction of Tissue Microarrays

Tissue microarrays are a convenient and widely used technique, allowing immunohistochemistry to be performed on samples from multiple patients simultaneously. At least 20 studies have shown that the sample of tumour taken for use in a tissue microarray (generally a cylindrical core 0.6mm in diameter) is representative of the sample as a whole (Simon, Mirlacher, & Sauter 2003). Camp et al. validated the use of tissue microarrays in breast cancer (Camp et al. 2000). They noted that expression of oestrogen receptor, progesterone receptor and HER-2 is heterogeneous from place to place in breast cancers. They still found, however, that using only two needle cores from a tissue block accurately represented antigen expression on a whole tissue section with 95% accuracy. Cores were taken by the author from areas confirmed as invasive carcinoma by a pathologist. Zhang et al (2003) suggested that even a single core could be sufficiently representative of the tumour as a whole. Additionally, tissue microarray technology means that all the specimens in the microarray are processed at one time under identical laboratory conditions, and also results in much less damage to original archival samples, allowing them to be used many more times than would previously have been the case.

An initial decision was made to take three cores from each tumour sample, as described by Camp et al. (Camp, Charette & Rimm 2000). A pathologist (Dr. Elizabeth Mallon, consultant histopathologist at the Western Infirmary in Glasgow) marked 2mm areas of invasive tumour on whole sections to ensure the cores were taken from the correct areas. Two separate areas of invasive tumour were marked in each whole section. Two 0.6mm cores were taken from one area and one from the other area giving a total of three cores. Cores were inserted into paraffin blocks; each of the three cores from the same tumour was inserted into a separate block resulting in the tissue microarrays being reproduced in triplicate. Cores were inserted into each block in an asymmetric pattern, which facilitated the correct orientation of sections at the time of microscopy. A grid format was used to record the study

number of each sample inserted into the TMA. 18 tissue microarrays in total were constructed.
Despite careful sampling in triplicate a small number of tumour blocks were not represented by a tumour-containing core (as this was discovered on microscopy of the TMAs at the end of immunohistochemistry, the tumour could not be re-sampled).

Sections 4μm in thickness were cut from each TMA to be used in the study.

Standardisation

In order to eliminate the effect of alteration in laboratory conditions, sections from every TMA underwent each individual immunohistochemistry protocol at the same time. Fresh sections were taken from each TMA on the day of the study in order to avoid the risk of loss of antigenicity (Fergenbaum, 2004).

Oestrogen Receptor Immunohistochemistry

Materials
Slide rehydration and dehydration:
Xylene
99% industrial grade methylated spirits
90% industrial grade methylated spirits
70% industrial grade methylated spirits
Antigen retrieval:
Optimisation steps:
Tris-EDTA solution ph8.0 (Sigma EDTA 0.37g, Sigma Tris Base 0.55g , 1 litre distilled water)

Tris-EDTA solution pH 9.0 (Sigma EDTA 0.37g, Sigma Tris Base 1.21g, 1 litre distilled water

Citrate buffer pH 6.0 (3.84g anhydrous citric acid in 1.8 litres distilled water)

Final protocol: Tris-EDTA solution ph8.0

Endogenous peroxidase blocking agent:

Hydrogen Peroxide 3% (40mls 30% hydrogen peroxide in 400mls distilled water)

Primary antibody:

Novocastra Oestrogen Receptor Clone 6F11 (mouse anti-human)

Final protocol dilution 1:50

Dako antibody diluent

Secondary antibody:

Dako Envision Chem-Mate

(Dextran –labelled Goat anti-mouse/rabbit antibody with horseradish peroxidase)

Visualisation reagent:

Dako DAB (3,3-diaminobenzidine)

Dako DAB substrate buffer (Tris-Hcl)

Wash buffer:

Optimisation steps: Tris-buffered saline

Counterstain:

Mayer's haematoxylin

Distilled water

Mounting:

DPX (dibutylphthalate and xylene)

22mm x 40 mm glass coverslips

Antigen retrieval steps and antibody optimisation steps were performed using TMAs containing ER positive tumour samples; the final protocol was carried out using the study TMA sections.

Slide preparation:

Tissue sections were dewaxed in xylene by dipping slide trays 3 times for 2 minutes each time, and then rehydrated by immersing in graded alcohol (two separate periods of 2 minutes in 99% methylated spirits and 2 minutes in each of 90% and 70% methylated spirits).

ER Antigen retrieval optimisation

A test battery was performed in order to find the antigen retrieval process and antibody concentration which gave optimum tissue staining: that is, strong staining with minimal non-specific 'background' staining. No difference in staining was seen between those samples that had been fixed in formaldehyde and those that had been fixed in mercuric chloride throughout the optimisation steps.

Optimisation of antigen retrieval was performed using each of Tris-EDTA pH 8.0, Tris-EDTA pH 9.0 and Citrate buffer (pH 6.0) as buffers in combination with each of the following heat induction methods: water bath at 100 degrees centigrade, microwave pressure cooker for 1 minute, microwave pressure cooker for 2.5 minutes and the microwave pressure cooker for 5 minutes.

In the pressure cooker steps, the solution was heated in a microwave until boiling and placed in a pressure cooker along with the prepared slides. The pressure cooker was placed in the microwave until pressure was reached and then heated at pressure for the desired length of time. The pressure cooker was removed from the microwave, the lid was removed and the slides allowed to cool down in the antigen retrieval solution for 20 minutes.

In the water bath steps, the slides and retrieval solution were placed in a Coplin jar in water bath at to 100 degrees centigrade and heated for 20 minutes; the jar was removed from the water bath and left to cool for 20 minutes.

The optimum staining was found to be with EDTA pH 8.0 using the microwave pressure cooker technique for 5 minutes.

ER Immunohistochemistry Optimisation

Immunohistochemistry optimisation steps were then carried out using different concentrations of primary antibody. While oestrogen receptor staining is a well-established technique, optimisation before the protocol is always preferable due to inter-laboratory variation in conditions. After antigen retrieval using EDTA pH 8.0 with the microwave pressure cooker technique for 5 minutes as above, tissue sections were placed in TBS and stirred continuously for 5 minutes. The blocking of any endogenous peroxidase in the tissue was ensured by placing the sections in 400mls hydrogen peroxide 3% for 10 minutes. The slides were placed in TBS, which was stirred continuously for 5 minutes. Primary antibody (clone 6F11) was diluted in antibody diluent to strengths of 1 in 50, 1 in 100, 1 in 200 and 1 in 400. For each dilution, 200 µl of solution was placed onto an ER positive slide (a water-impermeable barrier (Dako pen) having been placed around the tissue). 200µl of antibody diluent was then added to a breast tumour section to act as a negative control. The slides were then left for 1 hour at room temperature.

Slides were placed in TBS, which was stirred continuously for 5 minutes, and were then placed in fresh TBS for a further 5 minutes. 200µl of HRP-labelled anti-mouse secondary antibody were placed on each of the optimisation slides and left for 30 minutes at room temperature. Slides were placed in TBS which was stirred for 5 minutes, and were then placed in fresh TBS for a further 5 minutes. For visualisation, DAB and substrate buffer were mixed in a ratio of 20µl DAB to every 1ml of substrate buffer. 100µl of the resulting chromogen solution was pipetted onto each slide and left for 6 minutes before washing the slides in distilled water. Slides were placed in haematoxylin as a counterstain for 30 seconds before washing in distilled water. Dehydration was then performed by placing tissue sections in graded methylated spirits, initially 30 seconds in 70% methylated spirits, then 1 minute in 90% and finally 2 separate periods of 1 minute in 99% methylated spirits. Finally 3 separate 2-minute periods of immersion in xylene were carried out. Coverslips were placed on the slides using DPX.

The optimum primary antibody concentration was found to be 1:50.

Final Oestrogen Receptor Protocol

After antigen retrieval using EDTA pH 8.0 with the microwave pressure cooker technique for 5 minutes as above, tissue sections were placed in TBS and stirred continuously for 5 minutes. The blocking of any endogenous peroxidase in the tissue was ensured by placing the sections in 400mls hydrogen peroxide 3% for 10 minutes. The slides were placed in TBS, which was stirred continuously for 5 minutes

200 µl of 1:50 primary antibody solution were pipetted onto each of the study TMA slides and one positive control (an ER-positive full tumour section). 200 µl of antibody diluent alone were pipetted onto a breast tumour section to act as a negative control. The slides were then refrigerated overnight to allow maximum antibody binding (as recommended by colleagues at another laboratory who used this technique with clone 6F11).

Slides were placed in TBS, which was stirred continuously for 5 minutes, and were then placed in fresh TBS for a further 5 minutes.

200µl of HRP-labelled anti-mouse secondary antibody were placed on each of the study TMA slides and control slides and they were left for 30 minutes at room temperature.

Slides were then placed in TBS, which was stirred for 5 minutes, and were then placed in fresh TBS for a further 5 minutes. For visualisation, DAB and substrate buffer were mixed in a ratio of 20µl DAB to every 1ml of substrate buffer. 100µl of the resulting chromogen solution was pipetted onto each slide and left for 6 minutes before washing the slides in distilled water. Slides were placed in haematoxylin as a counterstain for 30 seconds before washing in distilled water. Dehydration was then performed by placing tissue sections in graded methylated spirits, initially 30 seconds in 70% methylated spirits, then 1 minute in 90% and finally 2 separate periods of 1 minute in 99% methylated spirits. Finally 3 separate 2 minute periods of immersion in xylene were carried out. Coverslips were then placed on the slides using DPX.

Progesterone Receptor Immunohistochemistry

Materials:

Slide rehydration and dehydration:

Xylene

99% industrial grade methylated spirits

90% industrial grade methylated spirits

70% industrial grade methylated spirits

Antigen retrieval:

Optimisation:

Tris-EDTA solution ph8.0 (Sigma EDTA 0.37g, Sigma Tris Base 0.55g , 1 litre distilled water)

Tris-EDTA solution pH 9.0 (Sigma EDTA 0.37g, Sigma Tris Base 1.21g, 1 litre distilled water

Citrate buffer pH 6.0 (3.84g anhydrous citric acid in 1.8 litres distilled water)

Final protocol:

Citrate buffer pH 6.0

Endogenous peroxidase blocking agent:

Hydrogen Peroxide 3% (40mls 30% hydrogen peroxide in 400mls distilled water)

Primary antibody :

Dako Progesterone Receptor Clone 636 (mouse anti-human)

Final protocol dilution: 1:50

Dako antibody diluent

Secondary antibody:

Dako Envision Chem-Mate

(Dextran –labelled Goat anti-mouse/rabbit antibody with horseradish peroxidase)

Visualisation reagent:

Dako DAB (3,3-diaminobenzidine)

Dako DAB substrate buffer (Tris-Hcl)

Wash buffer:

Optimisation steps: Tris-buffered saline

Final protocol: Tween – tris buffered saline for Autostainer

Counterstain:

Mayer's haematoxylin

Distilled water

Mounting

DPX (dibutylphthalate and xylene)

22mm x 40 mm glass coverslips

A test battery was performed in order to find the antigen retrieval process and antibody concentration which gave optimum tissue staining. Optimisation was performed on stock tissue microarrays containing strongly progesterone receptor–positive cores with the immunohistochemistry protocol performed manually. No difference in staining was seen between those that had been fixed in mercuric chloride throughout the optimisation steps.

In the final protocol the immunohistochemistry was performed using the study tissue microarrays and the Dako AutoStainer.

Slide preparation:

Tissue sections were dewaxed in xylene by dipping slide trays 3 times for 2 minutes each time, and then rehydrated by immersing in graded alcohol (two separate periods of 2 minutes in 99% methylated spirits and 2 minutes in each of 90% and 70% methylated spirits).

Progesterone Antigen Retrieval Optimisation

Optimisation of antigen retrieval was performed using each of Tris-EDTA pH 8.0, Tris-EDTA pH 9.0 and Citrate buffer pH 6.0 as buffers in combination with each of the following heat induction methods: water bath at 100 degrees centigrade, microwave pressure cooker for 1 minute, microwave pressure cooker for 2.5 minutes and the microwave pressure cooker for 5 minutes.

In the pressure cooker steps, the solution was heated in a microwave until boiling and placed in a pressure cooker along with the prepared slides. The pressure cooker was placed in the microwave until pressure was reached and then heated at pressure for the desired length of time. The pressure cooker was removed from the microwave, the lid was removed and the slides allowed to cool down in the antigen retrieval solution for 20 minutes.
In the water bath steps, the slides and retrieval solution were placed in a Coplin jar in water bath at to 100 degrees centigrade and heated for 20 minutes; the jar was removed from the water bath and left to cool for 20 minutes.

Optimal antigen retrieval was found to be citrate pH 6.0 heated for 5 minutes with the microwave- pressure cooker technique.

Progesterone Immunohistochemistry Optimisation

While progesterone receptor immunohistochemistry is a well-validated technique, antibody optimisation was required due to the potential for variation in laboratory conditions. After antigen retrieval using citrate pH 6.0 with the microwave pressure-cooker technique for 5 minutes as above, tissue sections were placed in TBS and stirred continuously for 5 minutes. The blocking of any endogenous peroxidase in the tissue was ensured by placing the sections in 400mls hydrogen peroxide 3% for 10 minutes. Primary antibody (PR clone 636) was diluted in antibody diluent to strengths of 1 in 50, 1 in 100, 1 in 200 and 1 in 400. For each dilution, 200 µl of solution was placed onto an ER positive slide (a water-impermeable barrier (Dako pen) having been placed around the tissue). 200µl of antibody diluent was then added to a breast tumour section to act as a negative control slide. The slides were then left for 1 hour at room temperature. Slides were placed in TBS, which was stirred continuously for 5 minutes, and were then placed in fresh TBS for a further 5 minutes. 200µl of HRP-labelled anti-mouse secondary antibody were placed on each of the optimisation slides and left for 30 minutes at room temperature. Slides were placed in TBS which was stirred for 5 minutes, and were then placed in fresh TBS for a further 5 minutes. For visualisation, DAB and substrate buffer were mixed in a

ratio of 20µl DAB to every 1ml of substrate buffer. 100µl of the resulting chromogen solution was pipetted onto each slide and left for 6 minutes before washing the slides in distilled water. Slides were placed in haematoxylin as a counterstain for 30 seconds before washing in distilled water. Dehydration was then performed by placing tissue sections in graded methylated spirits, initially 30 seconds in 70% methylated spirits, then 1 minute in 90% and finally 2 separate periods of 1 minute in 99% methylated spirits. Finally 3 separate 2 minute periods of immersion in xylene were carried out. Coverslips were placed on the slides using DPX.

The optimal staining was found to be a 1:50 dilution.

Final Progesterone Receptor Immunohistochemistry Protocol

After antigen retrieval using citrate pH 6.0 with the microwave pressure-cooker technique for 5 minutes as above, tissue sections were placed in TBS and stirred continuously for 5 minutes.

The Dako AutoStainer was then used to perform the steps of immunohistochemistry from peroxidase blocking onwards. In addition to the study TMA sections, a section was added to act as a negative control slide and a PR positive tumour section was used as a positive control. On each slide a waterproof barrier was drawn around the tissue.

Set-up:

The autostainer was set up to ensure the primary antibody step lasted for 1 hour and secondary antibody step 30 minutes. Reagents were added to the wells of the autostainer in appropriate amounts for each slide: hydrogen peroxide 3% (200µl per slide) to block endogenous peroxidase, primary antibody (PR clone 636) at 1:50 dilution (200µl per slide), secondary antibody (HRP-labelled anti-mouse, 200µl per slide) and chromogen solution for visualisation (20µl DAB per 1ml substrate buffer, 100µL per slide). Antibody diluent (200µl) was added to the negative control slide instead of primary antibody. Tween-tris-buffered saline was used as wash buffer.

Once the immunohistochemistry had been carried out, slides were placed in haematoxylin for 30 seconds as a counterstain before washing in distilled water.

Dehydration was then performed by placing tissue sections in graded methylated spirits, initially 30 seconds in 70% methylated spirits, then 1 minute in 90% and finally 2 separate periods of 1 minute in 99% methylated spirits. Finally 3 separate 2 minute periods of immersion in xylene were carried out. Coverslips were then placed on the slides using DPX.

HER-2 Immunohistochemistry

The protocol used for HER-2 immunohistochemistry was the well-validated Dako Herceptest.

Materials:

Slide rehydration and dehydration:
Xylene
99% industrial grade methylated spirits
90% industrial grade methylated spirits
70% industrial grade methylated spirits

Antigen retrieval:
Citrate buffer pH 6.0 (3.84g anhydrous citric acid in 1.8 litres distilled water)
Endogenous peroxidase blocking agent:
Hydrogen Peroxide 3%
Primary antibody :
Dako Anti-HER-2 antibody (rabbit anti-human)
Dako antibody diluent for negative control
Secondary antibody:
Envision Chem-Mate
(Dextran –labelled Goat anti-rabbit/mouse antibody with horseradish peroxidase)

Visualisation reagent:

Dako DAB (3,3-diaminobenzidine)

Dako DAB substrate buffer (Tris-Hcl)

Wash buffer:

Optimisation steps: Tris-buffered saline

Final protocol: Tween – tris buffered saline for Autostainer

Counterstain:

Mayer's haematoxylin

Distilled water

Mounting:

DPX (dibutylphthalate and xylene)

22mm x 40 mm glass coverslips

Slide preparation:

Tissue sections were dewaxed in xylene by dipping slide trays 3 times for 2 minutes each time, and then rehydrated by immersing in graded alcohol (two separate periods of 2 minutes in 99% methylated spirits and 2 minutes in each of 90% and 70% methylated spirits).

HER-2 Antigen Retrieval

Slides were heated in a glass jar in citrate pH 6.0 in a water bath at 100 degrees centigrade for 20 minutes; they were then removed and left to cool in the citrate for 20 minutes.

HER-2 Immunohistochemistry

After antigen retrieval using citrate pH 6.0 with the water bath technique as above, tissue sections were placed in TBS and stirred continuously for 5 minutes. The Dako Autostainer was used to perform the steps of immunohistochemistry from peroxidase blocking onwards. In addition to the study TMA sections a breast tumour section

was added to act as a negative control and a HER-2 positive control slide (supplied by Dako) was used. On each slide a waterproof barrier was drawn around the tissue.
Set-up:
The autostainer was set up to ensure the primary antibody step lasted for 1 hour and secondary antibody step 30 minutes. Reagents were added to the wells of the autostainer in appropriate amounts for each slide: Hydrogen peroxide 3% to block endogenous peroxidase (200µl per slide), primary antibody (Dako Anti-HER-2), 200µl per slide, secondary antibody (HRP-labelled anti-mouse, 200µl per slide), chromogen solution for visualisation (20µl DAB per 1ml substrate buffer, 100µL per slide). Antibody diluent (200µl) was added to the negative control slide instead of primary antibody. Tween-tris-buffered saline was used as wash buffer. Slides were placed in haematoxylin for 30 seconds as a counterstain before washing in distilled water.

Dehydration was then performed by placing tissue sections in graded methylated spirits, initially 30 seconds in 70% methylated spirits, then 1 minute in 90% and finally 2 separate periods of 1 minute in 99% methylated spirits. Finally 3 separate 2 minute periods of immersion in xylene were carried out. Coverslips were then placed on the slides using DPX.

2.17 Scoring

ER/PR scoring
Each sample was studied under a microscope at 40x power. After confirming the presence of tumour a weighted histoscore was calculated to assess the level of nuclear staining. The weighted histoscore is a semiquantative method of assessing strength of staining, calculating a score based on percentage staining of cells and a graded intensity scale (0=no staining , 1= weak staining, 2 =moderate to strong staining, 3= very strong staining). It was originally developed in the late 1980s (Katz et al. 1990) and its validity in assessing receptor status and prognosis

confirmed by its use in various other studies, particularly in those carried out at Glasgow Royal Infirmary (Cannings et al. 2007; Fraser et al. 2003; Kirkegaard et al. 2005; Kirkegaard et al. 2007; Tovey et al. 2004; Witton et al. 2003).

Histoscore was calculated as follows:

Histoscore = ((% cells staining at 0) x 0)+ ((% cells staining at 1) x 1) + ((%cells staining at 2) x 2) + ((% cells staining at 3) x 3)

Histoscores were averaged for the three cores. In the case of ER staining, 140 of the tumours had only two scoreable cores as there was little tissue left in one of the tissue microarrays by the time of the final study. 'Negative' staining was qualified as histoscore below 10. Interobserver variability was assessed by 10% of samples being independently scored by a second experienced scorer (this task was performed variously by Drs. Joanne Edwards, Beatrix Elsberger and Liane McGlynn of Glasgow Royal Infirmary). Discordance (conventionally accepted as a difference between histoscores of > 50) was resolved by observers simultaneously re-scoring the disputed samples.

The intraclass coefficient for interobserver variability was calculated using SPSS version 14.

HER-2 scoring:

A weighted histoscore was calculated for HER-2 staining. The intensity of membrane staining was graded as 0=no membrane staining, 1=incomplete membrane staining, 2=weak or moderate complete staining, 3=strong complete staining. Histoscore was calculated as follows: ((% cells staining at 0) x 0)+ ((% cells staining at 1) x 1) + ((%cells staining at 2) x 2) + ((% cells staining at 3) x 3). HER-2 positivity was taken as a score of 90 or over (Witton et al. 2003). Interobserver variability was assessed by 10% of samples being independently by a second experienced scorer (Kirkegaard et al. 2006). Discordance (conventionally accepted as a difference between histoscores of > 50) was resolved by observers simultaneously re-scoring disputed samples.

2.18 Statistical Analysis

Comparison of the demographics of the two groups was carried out using chi-squared analysis performed on SPSS v. 14 (Fisher's exact test was used in one case where numbers were small). The grade of each case was inserted into the previously constructed SPSS v.14 database (containing all the other prognostic and demographic data). For each case and for each stain, a mean histoscore for each tumour was calculated from the three scores. This score was then inserted into the SPSS database. Statistical analysis was then performed using this software.

Comparison of the molecular profiles in the groups was carried out using chi-squared analysis for hormone receptor or grade status, t-test to compare mean receptor levels and Mann-Whitney test to compare median receptor levels. A multivariate analysis was performed using binary logistic regression to assess whether age, screening and deprivation affected the percentage of ER positive tumours in the groups. Initial tests suggested there was not a direct linear relationship between ER and age, but ER positivity rates rose from age 60 onwards and so 'age over or under 60' as a categorical variable was included in the regression.

2.19 Survival Analysis

A comparison of survival between the older and newer cohorts was performed to establish if our cohorts reflected the changing population-based survival from breast cancer seen in Scotland (as in the introduction to this thesis). Data supplied by the Scottish Cancer Registry and the Glasgow Breast Cancer Audit included status at last review (Breast Cancer Audit patients) or status at 31st December 2003 (Cancer Registry patients), survival time in days from diagnosis to last follow-up or death if applicable, and cause of death if applicable. Data on recurrences was unfortunately not available. This allowed survival analysis to be performed using the Kaplan-Meier method using SPSS v.14. Survival analysis was performed for all patients in the original database regardless of whether or not they were entered into a TMA; for the ER status comparisons data on those patients who had been entered into the

TMA and had ER status determined were analysed. A 5-year survival analysis was thought to be the most appropriate way to compare the two groups and so those patients whose survival was longer than 5 years (1825 days) were considered as being alive at the 1825-day stage. The log-rank test was used to assess the significance of the differences in survival.

Survival comparisons performed were:

- Overall survival in cohort 1 vs cohort 2
- Disease-specific survival in cohort 1 vs cohort 2
- Disease-specific survival in ER positive vs ER negative patients in the whole study
- Disease-specific survival in ER positive vs ER negative patients in cohort 2
- Overall survival in cohort 1 vs cohort 2, symptomatic patients only
- Disease-specific survival in cohort 1 vs cohort 2, symptomatic patients only
- Disease-specific survival in screen-detected vs symptomatic patients in whole study
- Disease-specific survival in lymph node positive vs negative patients overall and in each cohort

Cox's proportional hazard regression was performed in a stepwise fashion. Cohort, ER status, HER-2 status, grade, nodal status, tumour size, age, screen-detected status, and deprivation category were inserted into the model. The final model excluded those variables in which a difference of in survival could be explained by one or more other variables, and generated hazard ratios for death from breast cancer for each variable after adjusting for the others.

Kaplan-Meier survival curves estimate the probability of survival beyond a certain time. The y-axis shows the proportion of the total group remaining with the x-axis showing time in days, and each downward step in the curve represents an event, i.e. a death. However, each event does not cause a downward step of equal magnitude, because the curves take into account censoring which in effect represents 'loss to follow-up'. With each censoring, i.e. loss of a patient to follow-up, a death represents a greater proportion of the total group and hence the downward step gets

larger. Kaplan-Meier curves assume that censored events are not related to cancer prognosis and that the probability of survival is the same early and late in a study. The log-rank test is a statistical test used to test the hypothesis that there is no difference in survival between the groups, and shares the same assumptions as the Kaplan-Meier curve. Cox's proportional hazard regression calculates the effect of changes in predictor variables on survival curves and hence whether a variable is a significant independent predictor of survival or not. The main assumption of this form of regression is that curves based on two predictor variables will each have a hazard function (i.e risk of death) that is relative and proportional to the other curve throughout. This is usually the case in terms of variables influencing cancer survival.

Chapter 3: Results and Analysis

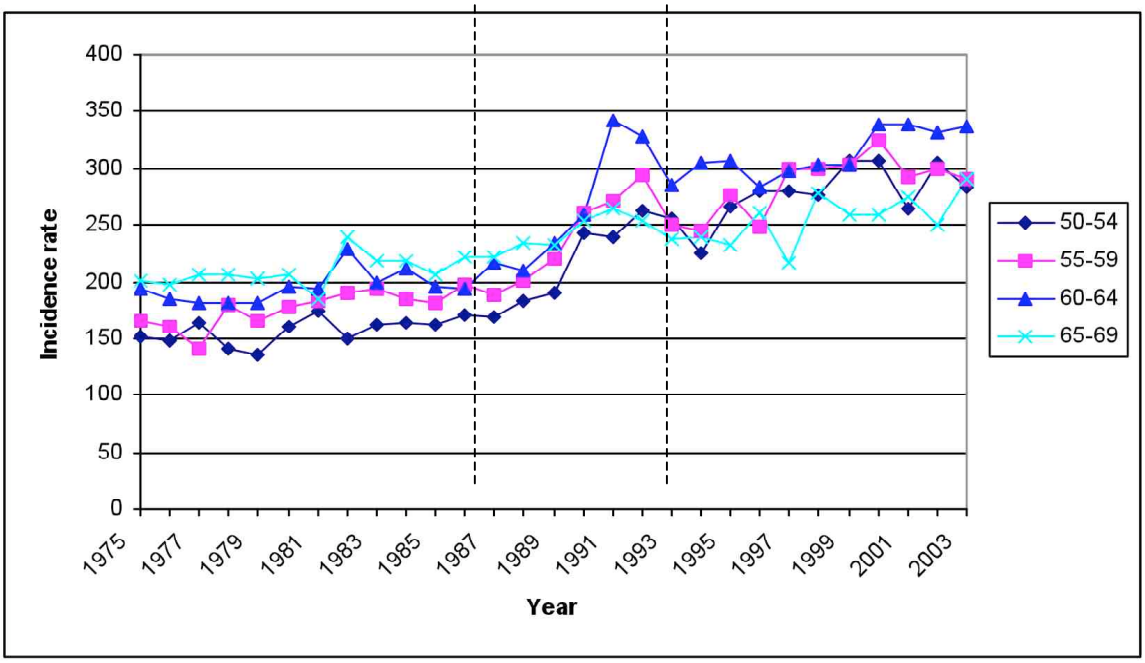
Results Part 1: Epidemiology Project

3.1 Breast cancer incidence rates in Scotland

In 1975, 2213 cases of breast cancer in females were registered in all age groups in Scotland. In 2003, 3823 cases of female breast cancer were registered. The incidence rate of breast cancer in Scotland in 1975 adjusted to European standard population was 79.6 cases per 100,000 women and in 2001 it was 111.2 cases per 100,000 women.

In women under the age of 49 the incidence of breast cancer remained fairly constant over the time period of interest, and as expected rates were higher with successive age groups. In women aged 70-74, 75-79 and 80-84 incidence rates were higher with successive age group and had been rising since 1975, with a downturn in incidence since around 1998. Incidence rates on women of 85 and over were the highest of all; in this group the incidence of breast cancer rose between 1982 and 1985 before staying fairly constant with some year on year variation, with another rise incidence between 1991 and 1996 and a decrease in incidence after this. Incidence rates in women aged 50-64 showed the greatest changes of all, with rates rising to the extent that they were higher than in women in the 70-74 age group. A thorough analysis of incidence trends in the age groups affected by screening, along with the age group just above this, is shown below in figure 3.1.

Figure 3.1: Breast cancer incidence rates in Scottish women in age groups 50-54, 55-59, 60-64 and 65-69, between 1975 and 2003 (per 100,000 women) Dotted lines mark 1987 and 1994 – the years when screening began in Scotland and when the prevalent round was completed.



In women of screening age and just above, incidence rates appeared to have been rising even before the introduction of screening; this is confirmed by linear regression below. From 1987 there was a sharp rise in incidence rates in women of screening age, as would be expected as a screening programme undergoes its 'prevalent round'. Women aged 65-69 have no such rise. The incidence rates reach a peak and begin to fall just before the end of the prevalent round; again this peak and fall replicates the expected pattern for a screening programme (Schouten, de Rijke, Huveneers, & Verbeek 2002). However, the prediction for after the completion of the prevalent round is that rates should remain just higher than and parallel to the underlying background incidence rate. However we see that rates in women of screening age are much higher and are continuing to increase at a steeper rate than would have been expected if trends up to 1987 had continued. A thorough analysis

of rates in the age groups affected by screening is undertaken below and in the discussion.

Observed vs Expected Analysis

Table 3.1: Comparison of observed mean rate in 1999-2003 and 2001 predicted rate

Age Group	1975 actual incidence rate per 100,000 per year	1999-2003 mean rate (O)	2001 predicted rate (E)	% difference
50-54	151.1	293.0	185.7	+58
55-59	165.8	302.1	212.5	+42
60-64	193.4	329.6	234.7	+40
65-69	200.8	266.9	238.6	+18

3.2 Breast screening data

Screening uptake

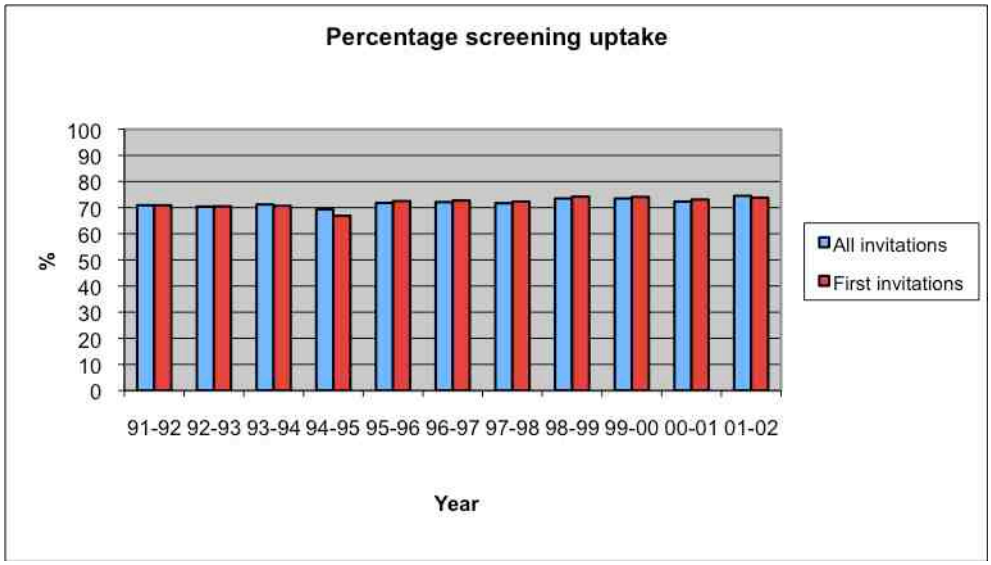


Figure 3.2: Percentage uptake of screening invitations for all invitations and first invitations by financial year

There was a small but significant trend towards increasing breast screening uptake between 1991-92 and 2001-02, from 70.9 to 74.5% of invited women (p=0.02). In women being invited for a first screen the uptake increased from 70.9 to 73.8% (p=0.017).

Self-referrals

As a percentage of all screening appointments, the level of self-referrals gradually increased throughout the prevalent round as the screening programme became more established, rising from 2.5% in 1991-2 to 3.9% in 1994-5. However in the intervening years, percentage self-referrals have continued to increase gradually to the extent that 10.5% of all screening appointments in 2001-2 were self-referrals. Unfortunately it is not known what percentage of these self or GP referrals are from women older than screening age, as other common reasons for self-referral for screening include GP concern, patient concern and family history.

Trends in standardised detection ratio

The standardised detection ratio of the screening programme in Scotland (rate of invasive cancers detected divided by number expected for the background incidence rate) for first screens has been above the target SDR of 1.0 since 1993/4-1995/6. The

SDR has increased from 1.1 to 1.5 since the prevalence 'round' of screening was completed, again suggesting that continued improvements in the screening service in Scotland could be continuing to improve incidence rates. For second ('incident') screens the SDR has been above the target of 1.0 since 1992/3-1994/5. The SDR has increased, but by less than for first screens, to 1.2 since the prevalent completed. Improvements in 'incidence' screening should reduce the number of cancers developed between screening periods (interval cancers), on the basis that some interval cancers are true interval cancers but some may have been missed on screening mammograms.

3.3 Birth cohort analysis

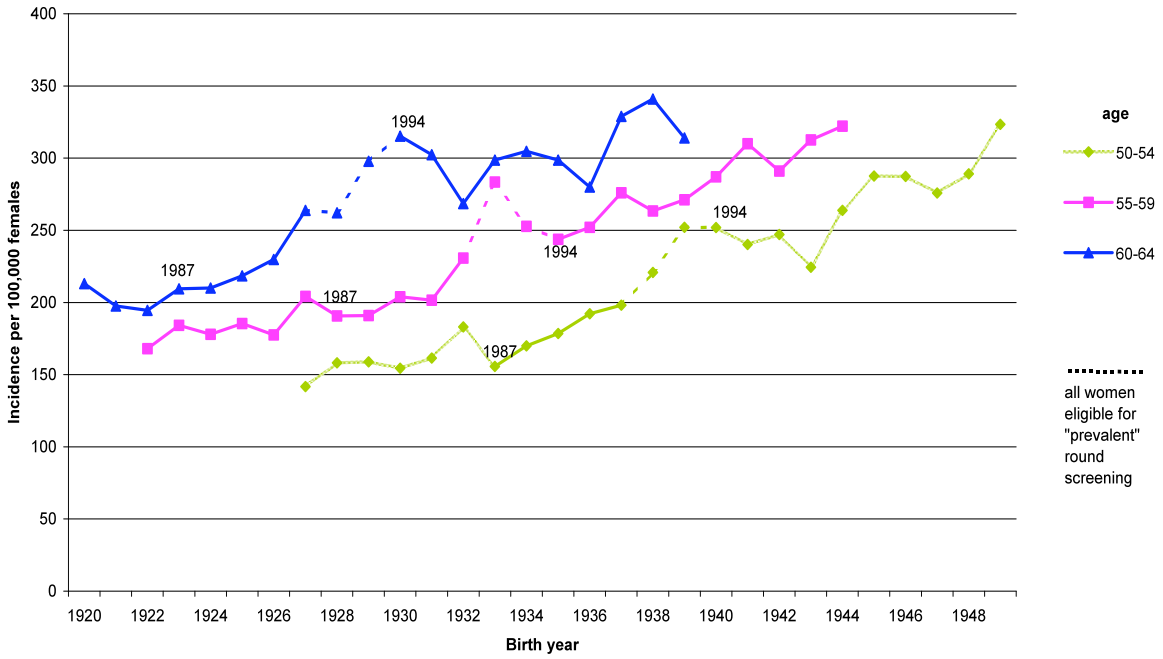
Table 3.2 demonstrates breast cancer incidence rates in women aged 50-64 by birth year cohort, with incidence rates grouped by 5-year age group, as derived from the Lexis diagram described in the materials and methods section. Dark shaded areas represent cohorts where some or all women would have been offered screening as part of the prevalent round; lighter shading either indicates where some or all women were either offered no screening at all (earlier cohorts) or were offered screening as part of the incident round of screening (later cohorts). Cohorts where all women would have been offered screening have a bold border.

Table 3.2 Breast cancer incidence rates in women aged 50-64 by birth year cohort, with incidence rates grouped by 5-year age group, as derived from the Lexis diagram

Birth year	Age range				
	45-49	50-54	55-59	60-64	65-69
1920				213.13	210.48
1921				197.58	226.58
1922			167.98	194.59	267.49
1923			184.15	209.53	254.43
1924			178.05	210.02	250.88
1925			185.43	218.53	258.03
1926			177.55	229.85	253.80
1927		141.73	204.20	263.77	240.47
1928		158.23	190.60	262.17	252.55
1929		158.83	191.00	297.88	259.49
1930		154.42	203.95	315.22	240.25
1931		161.55	201.65	302.44	233.88
1932	159.91	183.02	230.79	268.58	263.74
1933	166.42	155.71	283.40	298.69	246.12
1934	166.00	169.87	252.74	304.76	243.40
1935	161.84	178.55	243.82	298.65	
1936	167.04	192.25	252.00	279.23	
1937	158.99	198.13	275.97	328.91	
1938	161.80	220.87	263.42	341.00	
1939	167.53	252.07	271.17	314.65	
1940	162.76	251.90	287.00		
1941	169.60	240.15	310.02		
1942	160.58	247.03	291.04		
1943	179.82	224.38	312.66		
1944	169.71	263.82	322.20		
1945	177.90	287.54			
1946	184.82	287.26			
1947	181.54	275.86			
1948	187.06	289.18			
1949	175.32	323.46			

The data are plotted in figure 3.3 below; the dotted lines represented the central portion of the dark shaded boxes in table 1 where all women in the cohorts should have been offered screening.

Figure 3.3: Breast cancer incidence in women aged 50-64 years in 1977-2003 by year of birth



The incidence of breast cancer was higher with increasing age group between 50 and 64 years. In 50-54 year olds and 55-59 year olds there were small increases in incidence with birth cohort year before the screening programme began. In all age groups there were significant rises in incidence throughout the duration of the prevalent round. The fall in incidence that was expected after the majority of Scottish women had been screened at least once was not observed. Large rises in breast cancer incidence with increasing birth cohort continued after the screening

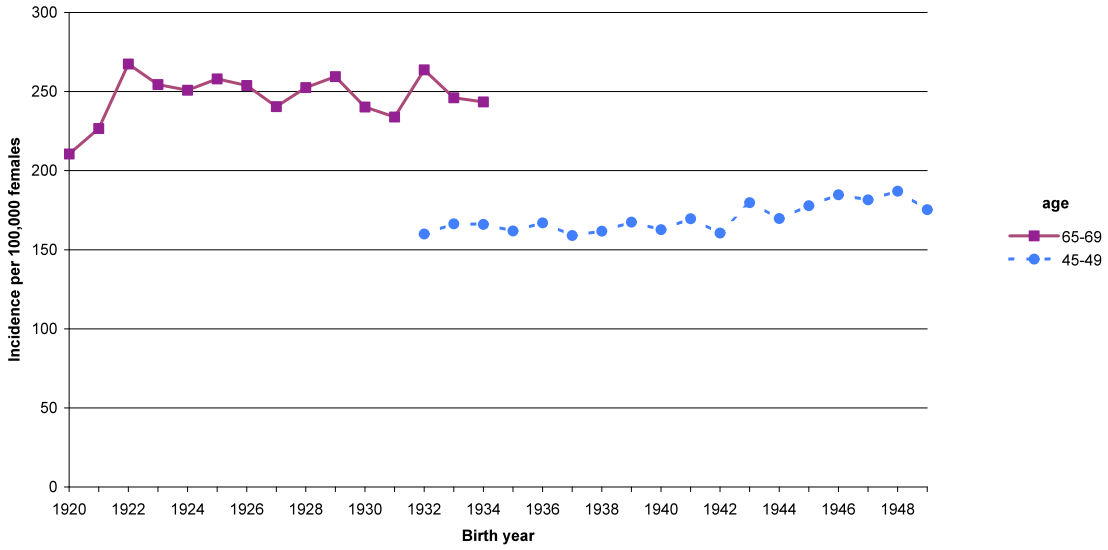
programme had been fully established (although as described below, the confidence intervals were wide). Rising incidence with increasing birth cohort occurred in parallel across all three age categories.

In 50-54 year olds, cohort incidence changed from 141.7 to 183.0 per 10^5 in the years before 1987 (a rise of 41.3 per 10^5 , 95%CI 6.1 to 76.5) ; from 155.7 to 263.8 per 10^5 (a rise of 108.1 per 10^5, 95%CI 68 to 148.2) in cohorts where some or all women were eligible for screening in the prevalent round; and from 287.5 to 323.5 per 10^5 (a rise of 35.9 per 10^5, 95%CI -12.45 to 82.9) in cohorts where women were offered screening in the established screening programme. In 55-59 year olds, cohort incidence changed from 168.0 to 204.2 per 10^5 in the years before screening (a rise of 36.2 per 10^5, 95%CI -1.5 to 73.9) ; from 190.6 to 271.17 per 10^5 (a rise of 80.5 per 10^5, 95%CI 38.5 to 122.7) in cohorts where some or all women were eligible for the prevalent round; and from 287 to 322.2 per 10^5 (a rise of 35.2 per 10^5, 95%CI -13.1 to 83.5) in cohorts who were entering screening during the 'incident round'. In 60-64 year olds, cohort incidence changed from 213.1 to 194.6 per 10^5 in the years before screening (a decrease of 18.5 per 10^5, 95%CI –17.4 to 54.4); from 209.53 to 304.8 per 10^5 (a rise of 95.2, 95%CI 50.8 to 146) in cohorts where some or all women were eligible for screening in the prevalent round; and from 298.7 to 314.7 per 10^5 in the cohorts beginning screening after the prevalent round was complete (an increase of 16 per 10^5, 95%CI –32.4 to 64.4).

Figure 3.4, below, shows breast cancer incidence in the same birth cohorts presented in Figure 2.4**,** but for women in the 5-year age groups below and above the screening ages. There was no appreciable change in incidence in 65-69 year olds from the 1922 to the 1948 birth cohort. Among 45-49 year olds, there was little change in incidence until 1942 and a small but non-significant rise thereafter from 160.6 to 175.3 (a rise of 14.7 cases per 10^5 female population, 95% CI -21.1 to 50.5) in the 1948 cohort.

3.4. Risk factor trends

Figure 3.4: Breast cancer incidences in women aged 45-49 and 65-69 years in 1977-2003, by year of birth and age



Late age at first pregnancy

Figure 3.5 below demonstrates the changing pattern of age at first pregnancy within Scotland over the past 25 years. From just under 12,000 first pregnancies to women 20-24 in 1976, the number has fallen gradually to under 6000 in 2001; however the numbers of pregnancies to women aged 30-34 have tripled, and women aged 35-59 has risen from 431 in 1976 to almost 2000 in 2001.

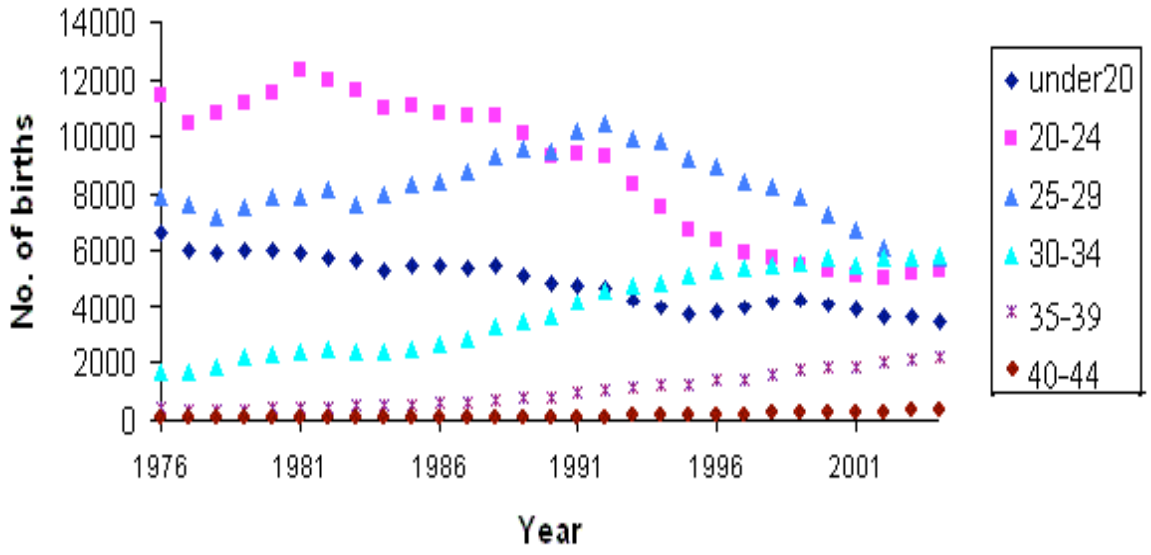


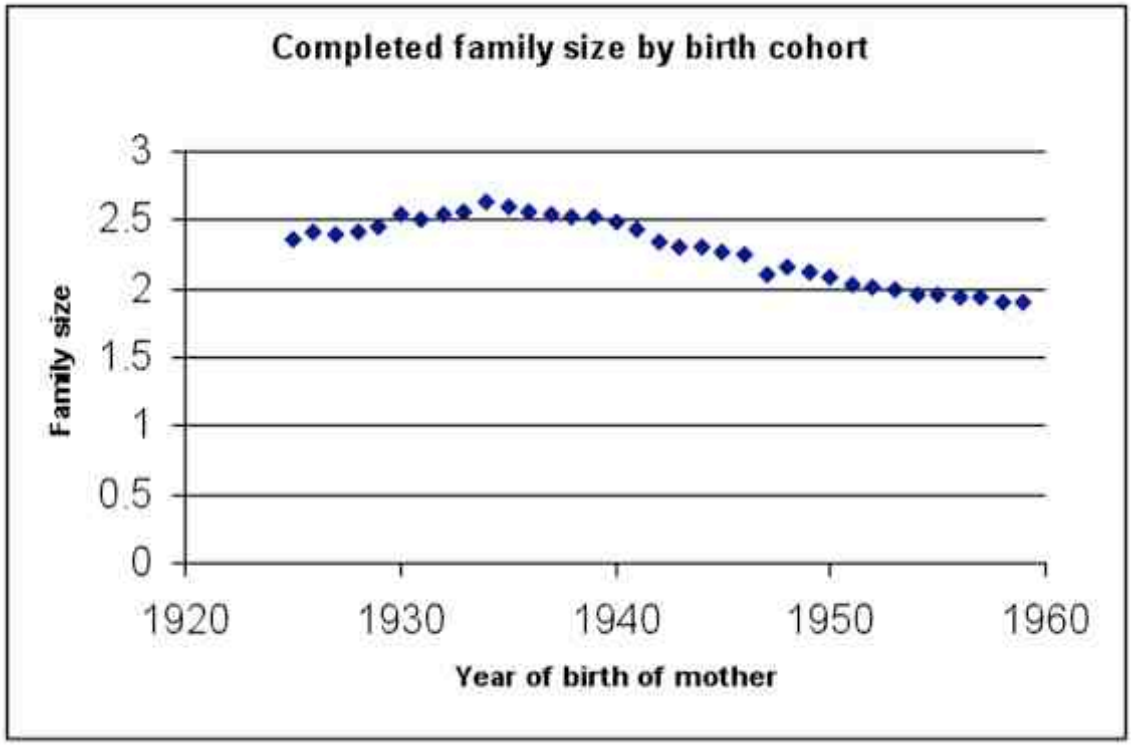
Figure 3.5: Numbers of first births at different maternal ages in Scotland over time

Figure 3.6, below, demonstrates how this compares overall to the numbers of first births in Scotland over time; in 1976 1.4% of all first births were in women aged 35-39 but by 2001 this had increased to 9%, with the rates increasing slowly up until the late 1980s and more rapidly after this.

Figure 3.6: First births to mothers aged 35-39 as a percentage of all first births in Scotland

Completed family size

Figure 3.7: Completed family size by age 44 by maternal birth cohort year (as at 2004)



Completed family size (cumulative fertility by age 44) by birth cohort is shown in figure 3.7 above. A downward trend of family size begins around the 1935 birth cohort, with completed family size gradually falling from 2.63 to 1.9 in the 1960 cohort.

BMI trends

In the women aged 16-64 in the Scottish Health Surveys) age-standardised mean BMI increased from 25.7 kg/m^2 in 1995 to 26.9 kg/ m^2 in 2003 (Bromley et al. 2005; Dong et al. 1995; Shaw et al. 2008. In women aged 55-64, mean BMI increased from 27.6 kg/ m^2 in 1995 to 28.6 kg/ m^2 in 2003. The percentage of women with BMI over 25 was 47.2% in 1995 and 57.3% in 2003; for women aged 55-64, percentage rose from 68.2% to 73%.

Alcohol trends

The average weekly alcohol consumption of women aged 16-64 in the Scottish Health Surveys of 1995, 1998 and 2003 showed an increase between 1995 and 2003, from 6.4 to 7.4 units. In women aged 55 to 64, weekly consumption increased from 4.6 to 5.4. Similarly, there was a small increase in the proportion of women aged 16-64 whose drinking exceeded 14 units per week, increasing from 13% in 1995 to 15% in 1998, and again to 17% in 2003. In women aged 55-64 the increase was from 8 to 11%.

Hormone Replacement Therapy

Data were available on total numbers of prescriptions of hormone therapy in Scotland. The reduction in numbers of prescriptions has been demonstrated in other countries and almost certainly reflects a response to the published findings of the Million Women study about increased risk of breast cancer (Usher 2006).

Figure 3.8: Numbers of prescriptions of hormone replacement therapy in Scotland, 1993 to 2003

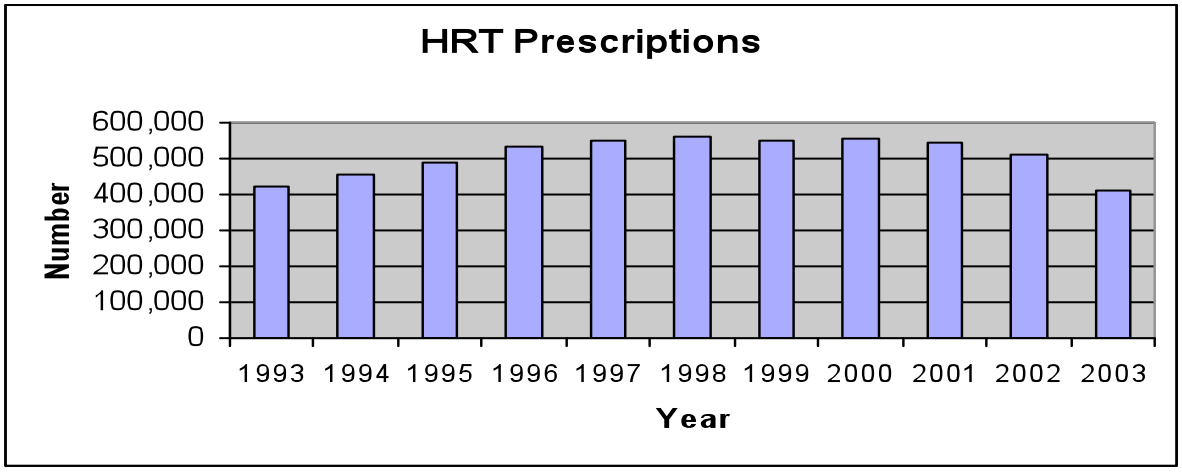


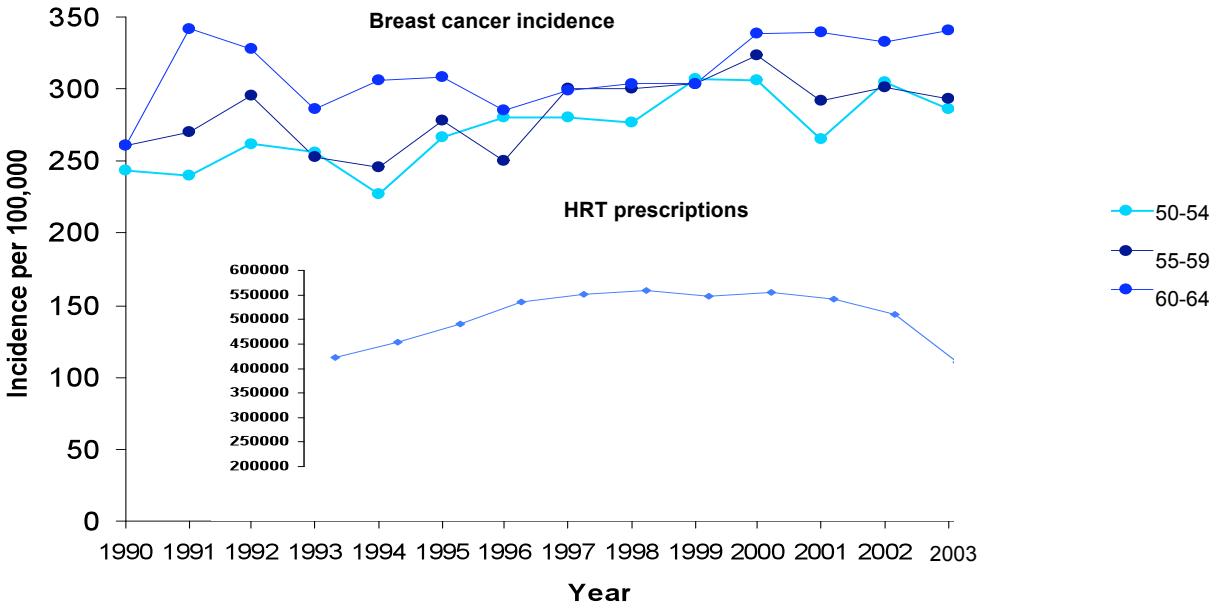
Table 3.3: Calculated HRT prevalence

Year	Women using HRT (prescriptions / 4)	Number of 40-64 year olds	Prevalence (%)
1993	105500	764246	13.8
1994	113377	769040	14.7
1995	122777	773511	15.9
1996	133668	778828	17.2
1997	137894	785338	17.6
1998	139692	795053	17.6
1999	136823	805416	17.0
2000	138851	815507	17.0
2001	135496	827346	17.0
2002	127712	838568	15.0
2003	103088	852358	12.0

Each HRT prescription is for a 3-month supply. Previous studies of HRT prevalence have suggested that up to 90% of all women using HRT will use it for at least a year (Bromley, de Vries, & Farmer 2004). If it is assumed that most women using HRT will require 4 prescriptions in a year, then the overall annual prescription rate can be divided by 4 to obtain an estimate of the number of women using HRT in any given year. The above number for women using HRT was divided by the number of women aged 40-64 in each year (as in Townsend, 1998), based on annual population estimates published by the General Register Office for Scotland to obtain an estimated prevalence level. This allowed prevalence in 40-64 year olds to be calculated, as in table 2.3. It can be seen that the calculated prevalence rates increased up to 1996 but changed little between then and 2001 and thereafter prevalence has fallen.

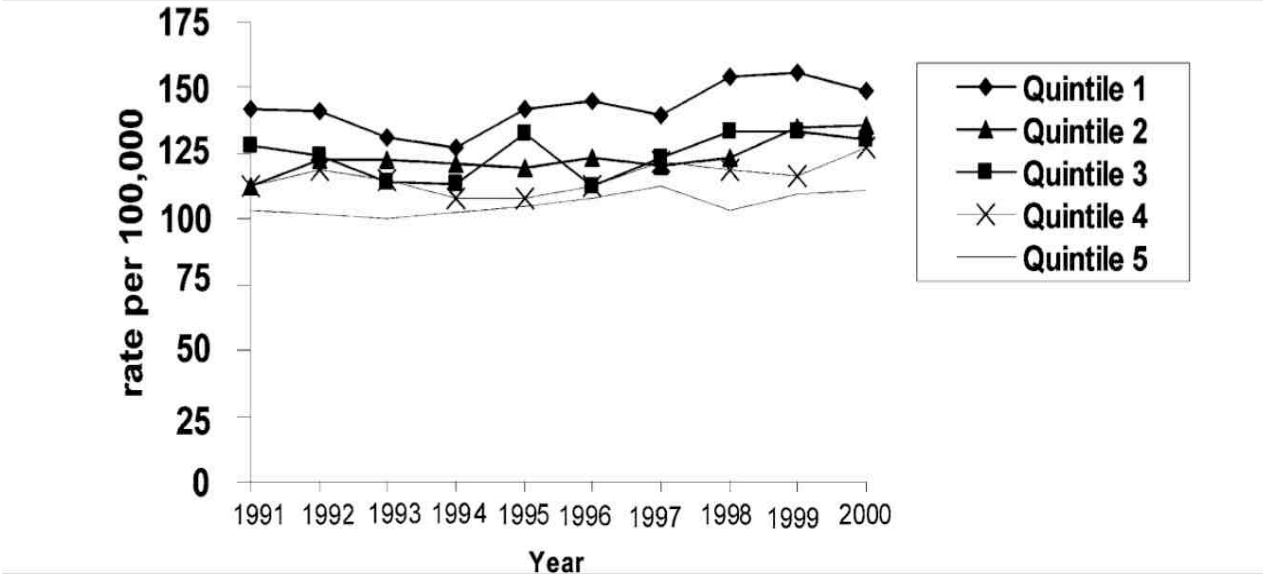
A comparison of HRT prescriptions and breast cancer incidence for women aged 50-64 is shown in figure 3.9 below.

Figure 3.9 Comparison of HRT prescriptions over time and breast cancer incidence in Scotland



3.5. Breast Cancer Incidence and Deprivation

Figure 3.10: Breast cancer incidence 1991-2000 by quintile of deprivation (1= least deprived, 5 = most deprived)



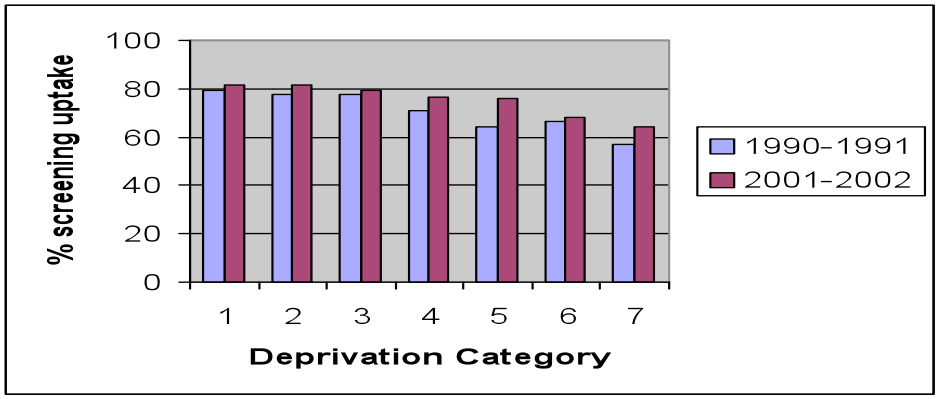
It can be seen from the figure that between 1991 and 2000, breast cancer incidence rates in Scotland continue to be lower with increasing deprivation. Linear regression of log-transformed data reveals a significant rise in incidence over this period in all quintiles except for quintile 3 and 4.

Quintile 1: p = **0.44**
Quintile 2:, p = **0.06**
Quintile 3:, p = **0.250**
Quintile 4: p **= 0.102**
Quintile 5: p = **0.016**

However, including an interaction term into the analysis revealed regression slopes to be parallel (p=0.835), confirming that incidence rates are rising to the same degree in all deprivation categories.

3.6 Breast screening uptake and deprivation

Figure 3.11 : Percentage uptake of screening invitations by deprivation category



It is well recognised that uptake of organised screening programmes is lower in areas of socio-economic deprivation, and this is true in the Scottish Breast Screening Programme. While higher levels of screening uptake do not explain the breast cancer excess in affluent populations – these trends were well recognised before screening began, it was felt possible that increased uptake of screening in affluent women with levels in deprived women staying static could explain the rising incidence in the affluent seen in the Scottish figures above.

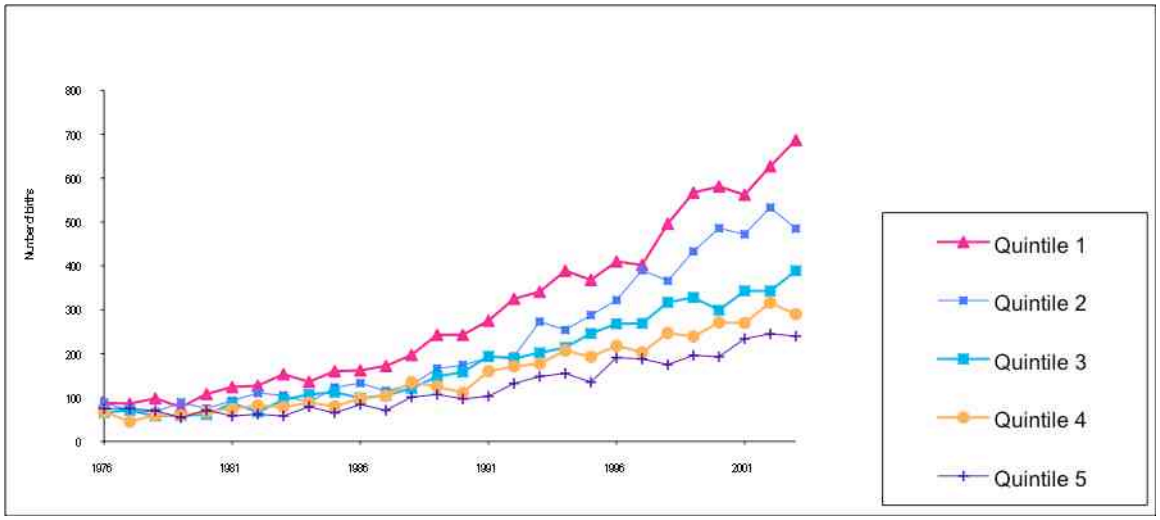
Screening uptake data for each one-year period from 1990-91 to 2001-02 for each deprivation category was analysed by linear regression. This revealed that all deprivation categories have seen a rise in screening uptake over this ten year period. (p for category 1 = 0.01 , category 2 = 0.1 , category 3 = 0.099, category 4 = 0.029, category 5 = 0.02, category 6 = 0.11, category 7 = 0.16). However p value for the interaction analysis was 0.551, indicating that uptake has increased to the same

extent in all categories, with no diverging trend to explain a widening gap in incidence.

3.7 Risk factors and socioeconomic status

Late age at first pregnancy

Figure 3.12: Numbers of first births in Scotland at maternal ages 35-39 since 1975 (Quintile 1 = least deprived, quintile 5 = most deprived)



This graph of numbers of first births at age 35-39 divided by deprivation category shows that the number of first birth to women aged 35-39 has increased in all deprivation categories but the magnitude of this rise increases with increasing affluence – that is, there is a widening gap between the deprived and affluent in terms of first pregnancy over 35.

The p value for the interaction analysis was <0.001 indicating a larger increase in numbers of first births between quintile 1 and 2 than between 2 and 3, a larger increase between 2 and 3 than between 3 and 4 and so on: i.e. confirming numbers of first births at over 35 are increasing by greater magnitude in the affluent than in the deprived.

BMI trends

As seen in table 3.4, in both 1995 and 1998, mean BMI appeared to increase slightly from social class I to V although the correlation was not statistically significant (1995 p=0.397 and 1998 p=0.084). The mean BMI in each social class had changed little between 1995 and 1998. Although a different classification of socioeconomic status was used in 2003, precluding direct statistical comparison, mean BMI in all categories had increased markedly since 1998. The correlation of higher BMI with increasing deprivation was significant ($p<0.01$) (table 3.5).

In 1995 a statistically significant inverse association of social class and obesity prevalence was seen (p=0.019). In 1998 the significant association was still present ($p<0.01$) (table 3.6). Prevalence of obesity in all classes appeared to have increased between 1995 and 1998 but grouping of social classes together in 1998 meant that direct statistical comparison was not possible. In 2003 a statistically significant inverse association between quintile of Scottish Index of Multiple Deprivation and obesity prevalence was still noted ($p<0.01$) (table 3.7). Again, in 2003 the prevalence of obesity in all quintiles appeared to have increased from the prevalence in individual categories in 1998 but a statistical comparison could not be made.

Table 3.4: Mean BMI (kg/m^2, age-standardised), women aged 16-64 by Registrar General Social Class , from Scottish Health Survey 1998 (Shaw, McMunn, & Field 2008)

	I	II	IIINM	IIIM	IV	V
1995	22.8	22.3	23.5	23.6	23.3	23.0
1998	22.8	22.9	22.9	24.0	23.8	23.6

Table 3.5: Mean BMI (ages-standardised), women aged 16 and over by quintile of Scottish Index of Multiple Deprivation: from Scottish Health Survey 2003 (Bromley, Sproston, & Shelton 2005)

	1st	2nd	3rd	4th	5th
2003	26.4	26.9	27.1	27.4	28.1

Table 3.6: Percentage prevalence of obesity (BMI >30) in women aged 16-64 (1995) and 16-74 (1998) by Registrar General Social Class, from Scottish Health Surveys 1995 (Dong & Erens 1997) and 1998 (Shaw, McMunn, & Field 2008)

	I	II	IIINM	IIIM	IV	V
1995	13.9	9.9	17.8	18.1	22.1	20.2
1998	18.2		19.7	25.5	26.0	

Table 3.7: Percentage prevalence of obesity (BMI>30) in women aged over 16 by quintile of Scottish Index of Multiple Deprivation, from Scottish Health Survey 2003 (Bromley, Sproston, & Shelton 2005)

	1st	2nd	3rd	4th	5th
2003	21.0	22.8	27.7	27.9	32.1

Results Part 2: Laboratory Project

3.8 Patient and tumour characteristics

The original sample size had been 1076 patients (423 in 1984-86 [‘cohort 1’] and 653 in 1996-97 [‘cohort 2’]). 900 tumour blocks were available (323 in cohort 1, 577 in cohort 2). Mean age of diagnosis was 56.9 in the first cohort and 58.4 in the second cohort. All tumours in the first cohort had been detected symptomatically rather than by screening, the screening programme having yet to be introduced in Scotland. 71% of tumours in the second cohort were detected symptomatically and 29% had been detected at the screening programme. There was no significant difference in the cohorts as regards socioeconomic status. In cohort 1, 12.1% of patients were affluent (deprivation category 1-2), 40.9% intermediate (category 3-5) and 47% deprived (category 6-7); in cohort 2, 16.5% of patients were affluent, 47.2% intermediate and 36.3% deprived. 59.3% of tumours in the 1984-86 cohort had been node-positive compared with 42.4% of the tumours in the 1996-97 cohort. Comparisons were performed using chi-squared analysis (Fisher’s exact test in the case of screening as the numbers were small).

Table 3.8 Patient demographics

	1984-1986	1996-1997	p for difference
Mean age at diagnosis	56.9	58.4	**0.049**
Median age at diagnosis	59	58	**0.179**
Age range	23-74	24-93	
%detected at screening	0	29	**<0.001**
% of patients in each deprivation category:			
Affluent	12	17	
Intermediate	41	47	**0.05**
Deprived	47	36	
Node positive %	59.3	42.4	**0.001**

3.9 Grade

Overall grade distribution:

862 tumours (less than the full study complement of 900) underwent standardised grading using modified Scarff-Bloom-Richardson classification. The percentage of tumours in each cohort that were of grade 1, grade 2 and grade 3 was calculated and the difference in grade distribution between cohorts was assessed using Pearson's chi-squared (χ^2) analysis, using SPSS v.14.

Cohort 1 (1984-86):

8% of tumours were grade 1 , 49.2% grade 2, and 42.9% grade 3

Cohort 2 (1996-97):

14.9% of tumours were grade 1, 48.3% grade 2 and 36.8% grade 3

Pearson's χ^2 analysis revealed there to be a significant difference in grade distribution between the two cohorts (p=0.009); this effect appears to be a result of there being fewer grade 3 tumours and more grade 1 tumours in the later cohort.

Grade distribution and relation to screening

In cohort 1 none of the tumours were screen-detected as the National Breast Screening Programme had not yet been introduced; in cohort 2 a proportion of tumours would be screening-detected at a time when the screening programme had been long established. A layered χ^2 analysis was performed to assess differences in grade distribution in symptomatically detected tumours in different cohorts, and the grade distribution of screen detected as compared to symptomatic tumours in cohort 2.

For symptomatic patients only:

Cohort 1 (1984-86)

8% of tumours were grade 1, 49.2% were grade 2 and 42.9% were grade 3

Cohort 2 (1996-97):

12.2% of tumours were grade 1, 46.8% were grade 2 and 41% were grade 3
Pearson's χ^2 showed there to be no significant distribution in grade (p=0.2) strongly suggesting that the difference in grade distribution between the older and more recent cohort is being exerted by the screen detected tumours, with the grade of symptomatically detected tumours not differing between the cohorts.

Symptomatic vs. screen-detected in Cohort 2:
Screen-detected tumours: 22.3% were grade 1, 53% were grade 2 and 24.7% were grade 3.
Symptomatic tumours: 12.2% of tumours were grade 1, 46.8% were grade 2 and 41% were grade 3.

The difference in grade distribution was significant ($p<0.001$).

The data supports the hypothesis that the difference in grade distribution between the two cohorts is being exerted by the screen-detected tumours in cohorts 2 having fewer grade 3 and more grade 1 tumours.

A further analysis was performed in order to show the tumour grade in women having a subsequent ('incident') screen; as discussed later, the difference in grade between tumours detected at incident screens and symptomatic tumours has been used to support the hypothesis of 'phenotypic drift' of breast cancer. Data on whether tumours were detected in a woman having her first screen or a subsequent screen had not been recorded; however any women aged 50 to 53 with a screen detected tumour is likely to have been having a first screen as these are the ages at which women are invited for a first screen. Therefore analysis was performed of the grade distribution within screen-detected tumours in women aged over 53 as compared with women aged 50 to 53 and with non screen-detected tumours. A potential confounding factor in the use of the age cut-off as a surrogate for screening type is that some women aged over 53 may be having a first screen having not attended screening previously. Also, some 53-year old women may have previously had a screen aged 50 and be having a repeat screen.

Symptomatic tumours in cohort 2: 12.2% of tumours were grade 1, 46.8% were grade 2 and 41% were grade 3.
Tumours presumed detected at subsequent screen in cohort 2: 21.9% of tumours were grade 1, 52.3% were grade 2 and 25.8% were grade 3.
The p value as calculated by Pearson's χ^2 was <0.001 showing that there was a significant difference in grade distribution between women likely to have been having a 'subsequent' screen and those with symptomatic tumours. The obvious possibility of misclassification of screening episodes decreases the validity of this conclusion.

Grade and socioeconomic status

In both cohort 1 and cohort 2 there was no significant difference in grade distribution of tumours between socioeconomic categories (p=0.95, p=0.822).

3.10 Oestrogen Receptor

Overall difference in percentage of tumours ER positive:

20% of the 323 tumours in the original 1984-1986 cohort and 19% of the 577 in the 1996-1997 cohort did not undergo ER staining as none of the three cores put in tissue microarrays contained a tumour sample, mainly as cores had become fragmented but also occasionally because normal tissue had been sampled. The percentage of tumours that were ER positive (weighted histoscore of 10 or over) in each cohort was calculated and the difference between the cohorts calculated using Pearson's χ^2, using SPSS v.14.

Cohort 1:
64.2% of the tumours were ER positive and 35.8% ER negative
Cohort 2:

71.5% of tumours were ER positive and 28.5% ER negative

7.3 % more of the tumours in the newer cohort were ER positive than in the older cohort. Pearson's χ^2 analysis revealed this to be a significant difference (p=0.042).

Comparison of mean ER score in each cohort:

Further analysis was performed to assess whether there was not only a change in ER positivity but a change in mean ER score for the two cohorts; as the data were normally distributed a two-tailed unpaired t-test was performed.

Cohort 1: mean ER score 97.1

Cohort 2: mean ER score 102

T-test showed that there was no difference in mean ER score between the two cohorts (significance = 0.454)

In cohort 1 the median ER score was 104.2; the interquartile range was 0-190; in cohort 2 the median ER score was 120 and the interquartile range 0-180. (p by Mann-Whitney 0.744).

ER status and pathological factors

Tumour size:

A layered Pearson's chi-squared analysis was performed to assess whether ER status was related to tumour size in either cohort. Tumour size classification was based on the TNM classification: <2cm, 2-5cm and over 5cm.

Cohort 1: within each tumour size group, there was no difference in the percentage that were ER positive and negative (p=0.234)

Cohort 2: within each tumour size group, there was no difference in the percentage that were ER positive and negative (p=0.431)

Nodal status:

59.3% of the tumours in the 1st cohort and only 42.4% of those in the second cohort were node positive (p<0.001). A layered Pearson's χ^2 analysis was performed to assess whether ER status was related to nodal status in either cohort.

Cohort 1: 68.8% of node positive patients were ER positive and of 56% of node negative patients were ER positive
Cohort 2: 70% of node positive patients were ER positive and 71% of node negative patients were ER positive
In neither cohort was there found to be a significant difference in ER status between node-positive and node-negative groups (p=0.058 and p=0.819 for cohorts 1 and 2 respectively).

ER status and screening

Having noted that there was an overall difference in ER status between groups, further χ^2 analyses was performed to establish whether, as in the case of tumour grade, this was related to the impact of screening, as cohort 1 patients were diagnosed before screening and cohort 2 when screening had been long established.
Within the tumours that successfully underwent ER analysis, 66.8% of symptomatic patients were ER positive and 78.4% of the screen-detected tumours were ER positive; this difference was statistically significant (p = 0.009).
Within symptomatic patients only, in cohort 1, 64.2% of patients were ER positive and in cohort 2 68.8% were ER positive (p=0.32).
In cohort 2, 68.8% of symptomatic patients were ER positive and 78.4% of screen-detected tumours were ER positive – again this was significant (p=0.04). These analyses could perhaps suggest that the rise in ER positivity appears to be exerted by the presence of screen-detected tumours.

ER status and deprivation

As detailed in the introduction, studies have suggested that deprived populations may have higher rates of ER negative tumours. A comparison was made of the distribution of deprivation status (affluent [‘depcat’ 1 or 2], intermediate [‘depcat’

3,4 or 5] and deprived [‘depcat’ 6 or 7]) between the different cohorts to assess whether this could have influenced the results.
Cohort 1: 12.1% of patients were affluent, 40.9% intermediate and 47% deprived.
Cohort 2: 16.5% of patients were affluent, 47.2% intermediate and 36.3% deprived.
There was a borderline significant difference in deprivation status of the patients in each cohort (p=0.05).

An assessment was therefore made of the relationship between deprivation status and ER status in the patients. Overall in the study there was no significant difference in ER status by deprivation status (p=0.979). 68.6%, 69.6% and 69% of the affluent, intermediate and deprived groups respectively were ER positive. When cohorts were separated there was still no significant relation of ER status and deprivation (p=0.926 and p=0.842 for cohorts 1 and 2 respectively).

ER Status and Age

It is notable that the upper limit of age in the second cohort is higher than in the first, although as seen in table 3.1 the influence this has had on mean and median age at diagnosis is small. A breakdown of percentage ER positivity within the different age ranges in the two cohorts is shown below. The number of cases over the age of 75 in the later cohort represents 44 cases.

Table 3.9 ER positivity by age

	1984-86 % ER positive	1996-97 % ER positive
Age: under 29	**100**	**33**
30-34	**43**	**60**
35-39	**38**	**44**
40-44	**54**	**50**
45-49	**59**	**73**
50-54	**55**	**69**
55-59	**59**	**65**
60-64	**72**	**77**
65-69	**74**	**77**
70-74	**80**	**80**
75-79		**77**
80 -84		**100**
85 and over		**100**

The percentage positivity for each age range is on the whole higher in the second cohort than in the first except for those under 29, although the small numbers reduce the validity of this observation. Interestingly an initial peak in ER status at the 45-49 age group is seen, with rates then rising again from around the 65-59 age group. The ER status of breast tumours becomes more likely to be positive with increasing age at diagnosis, as seen here (Elwood, 1980). The peak at age 45-49 was noted by Elwood and authors in their 1980 study.

3.11 Multivariate Analysis of Factors Influencing ER positivity

Multivariate analysis using binary logistic regression was performed to ascertain the influence of the following factors on ER positivity: cohort, age, screening, deprivation, in order to ascertain whether observed differences in demographics of the two cohorts could have resulted in the difference in ER positivity detected in the study. The distribution of age and ER status above suggested a non-linear relationship of age and ER positivity; plotting the B coefficients of the logistic regression equation for age category and ER status confirmed the non-linearity and hence age category was not felt appropriate to include in logistic regression. Instead 'age over or under 60' was included as a categorical variable. In the final model, cohort and deprivation were no longer significant predictors of ER status, with. screening status and age over 60 remaining significant predictors of ER status. It is notable that when only age and cohort were included in the model, correcting for age over/under 60 made the link between cohort and ER status just below the level of significance (p=0.068).

3.12 PR status

Overall difference in percentage of tumours PR positive:

14% of the 323 tumours in the original 1984-1986 cohort and 10% of the 577 in the 1996-1997 cohort did not undergo PR staining as none of the three cores put in tissue microarrays contained a tumour sample, mainly as cores had become fragmented but also occasionally because normal tissue had been sampled. The percentage of tumours that were PR positive (weighted histoscore of 10 or over) in each cohort was calculated and the difference between the cohorts calculated using Pearson's χ^2, using SPSS v.14.

Cohort 1:

44.9% of the tumours were PR positive and 55.1% PR negative

Cohort 2:

49.9% of tumours were PR positive and 50.1% PR negative

5% more of the tumours in the second cohort were PR positive than in the first cohort; this was not a significant difference (p=0.181).

Comparison of mean PR score in each cohort:

Further analysis was performed to assess whether there was not only a change in PR positivity but a change in mean PR score for the two cohorts; as the data were normally distributed a two-tailed unpaired t-test was performed.

Cohort 1: mean PR score 41.2

Cohort 2: mean PR score 37.9

The mean PR scores of the two cohorts are not significantly different (p=0.418)

The median PR score in cohort 1 was 0 and the interquartile range 0-79.6; the median PR score in cohort 2 was 8.3 and the interquartile range 0-61.3 (p by Mann-Whitney 0.181).

3.12 Combined ER/PR status

χ^2 analysis was performed to assess whether the rise in ER status is limited to ER positive tumours that are also PR positive or whether it is independent of ER status. Analysis was performed on those tumours that had successfully undergone ER and PR immunohistochemistry.

Cohort 1:

42.4% were ER+ve/PR+ve

21.8% were ER+ve/PR-ve

33.3% were ER-ve/PR-ve

2.5% were ER-ve/PR +ve

Cohort 2:

46.7% were ER+ve/PR+ve

24.8% were ER+ve/PR-ve

23.5% were ER-ve/PR-ve
5% were ER-ve/PR+ve

χ^2 analysis showed there to be a significant difference in the distributions (p=0.023). The most marked difference is a 10% decrease between the first and second cohorts of percentage of ER-ve/PR-ve tumours; there is a corresponding increase between the two groups of the prevalence of the other tumour types.

3.13 HER-2

15% of the 323 tumours in the original 1984-1986 cohort and 18% of the 577 in the 1996-1997 cohort did not undergo HER-2 staining as none of the three cores put in tissue microarrays contained a tumour sample, mainly as cores had become fragmented but also occasionally because normal tissue had been sampled. The percentage of tumours that were HER-2 positive (weighted histoscore of 90 or over) in each cohort was calculated and the difference between the cohorts calculated using Pearson's χ^2, using SPSS v.14.
Cohort 1: 21.5% of tumours that could be scored were HER-2 positive and 78.5% negative
Cohort 2: 20.6% of tumours were HER-2 positive and 79.4% negative
There was no significant difference between the cohorts in terms of percentage that were HER-2 positive (p=0.772)

Further analysis was performed to assess whether there was an increase in mean HER-2 score for the two cohorts; as the data were normally distributed a two-tailed unpaired t-test was performed.
Cohort 1: mean HER-2 score 52.2
Cohort 2: mean HER-2 score 43.1
Although mean HER-2 score appears to be lower in the second cohort there is no significant difference between the groups (p=0.170).

The median Her 2 score in cohort 1 was 0 and the interquartile range 0-50; the median HER-2 score in cohort 2 was 0 and the interquartile range 0-66.7 (p by Mann-Whitney =0.773).

3.14 Survival Data

Overall and Cancer-specific Survival

Figure 3.13 below shows the overall 5-year survival within the study group of patients by cohort. Log-rank test confirmed that 5-year survival in cohort 1 (1984-1986) is significantly lower than in cohort 2 (1996-1997) ($p<0.001$). The cumulative 5-year survival in cohort 1 was 0.58 and in cohort 2, 0.834.

Figure 3.13: Overall survival by cohort (cohort 1 = 1984-86, cohort 2 = 1996-97)

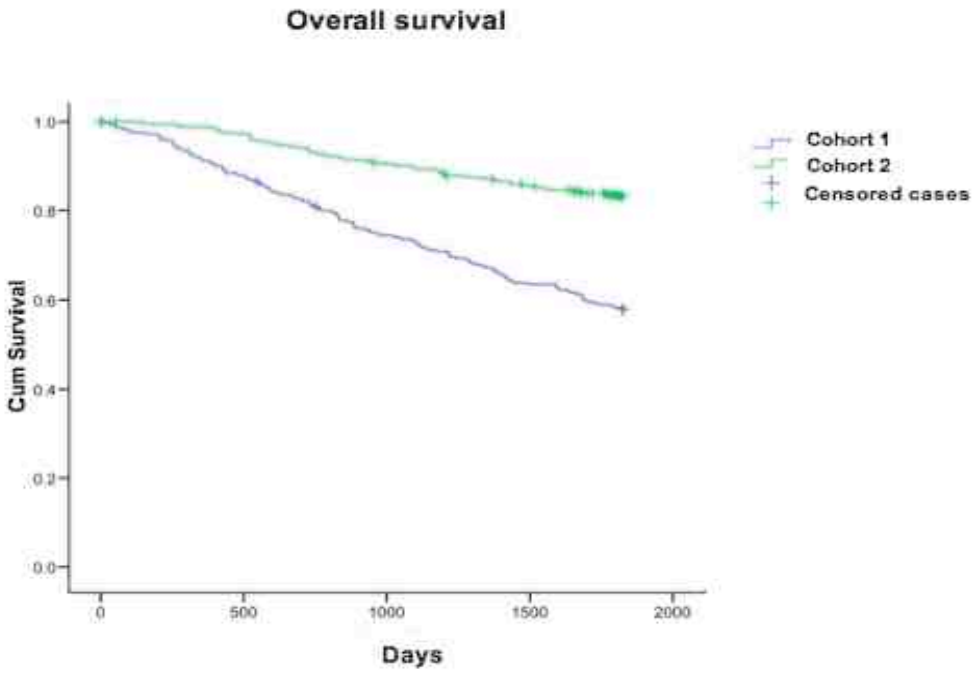
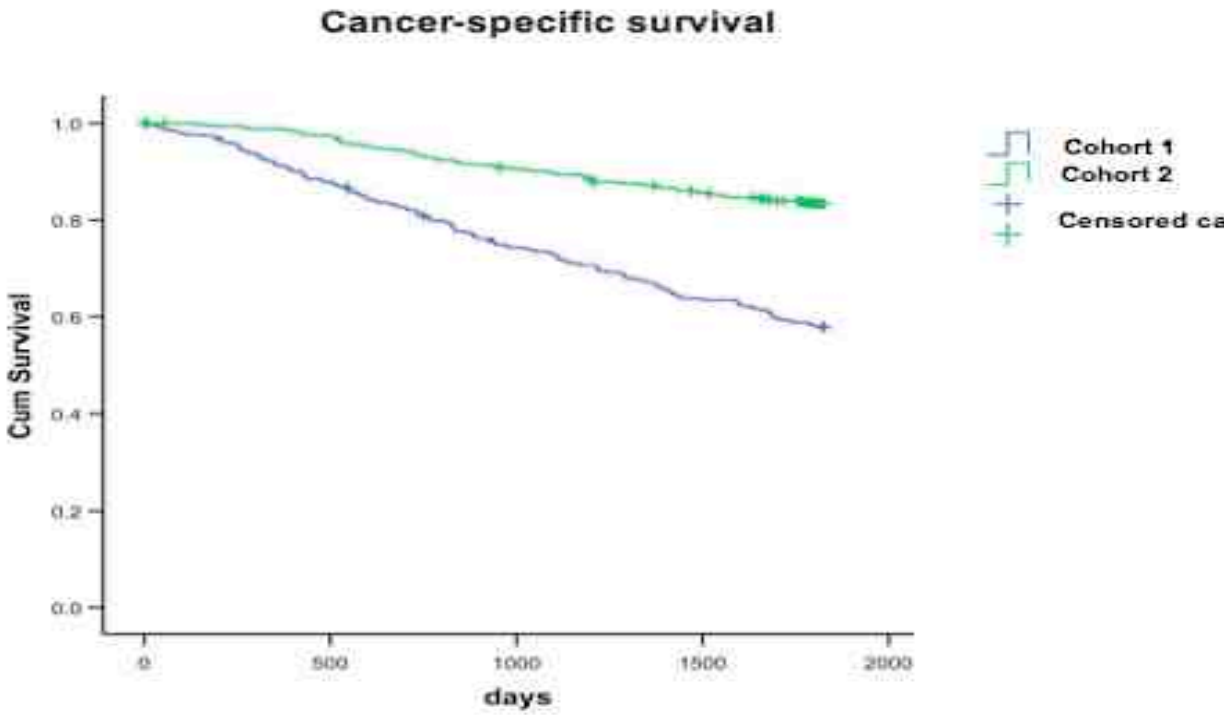


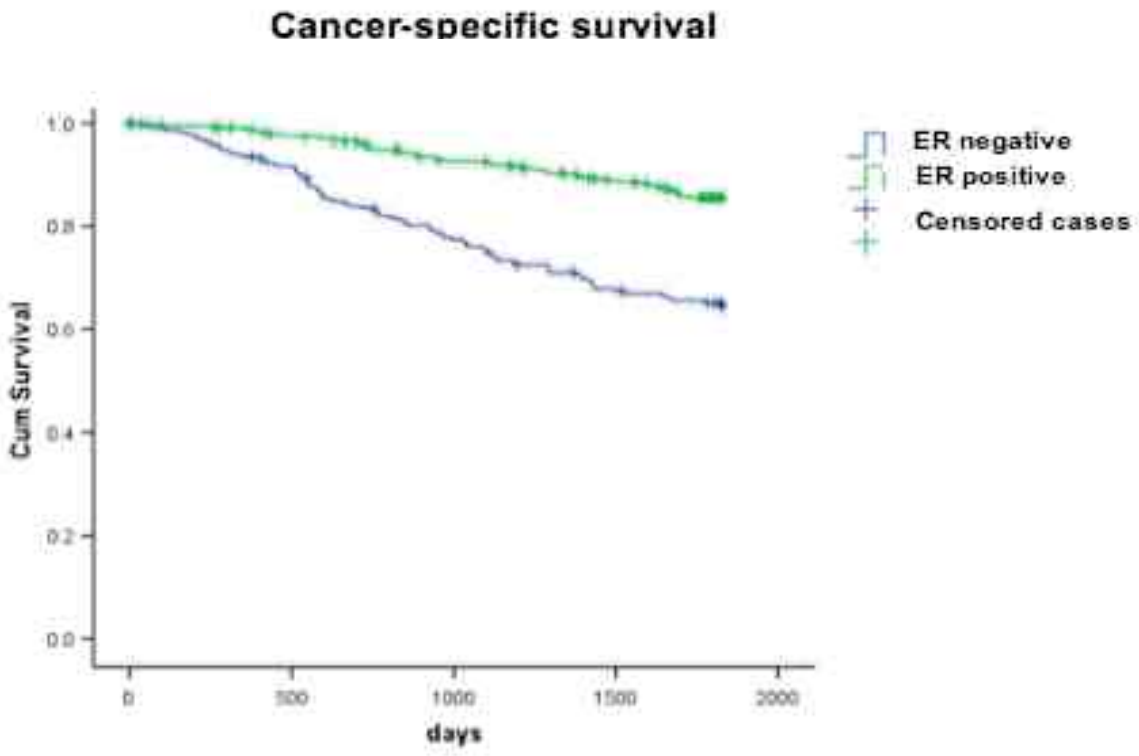
Figure 3.14: Breast cancer-specific survival by cohort



In relation to figure 3.14 above, the log-rank test again confirmed that breast cancer-specific 5-year survival in cohort 1 (1984-1986) is significantly lower than in cohort 2 (1996-1997) ($p<0.001$). 5 year survival in cohort 1 was 0.62 ; survival in cohort 2 was 0.887.

ER Status and Breast Cancer Survival

Figure 3.15 Breast cancer-specific survival by ER status



Log rank test confirms the significantly higher disease-specific survival in the ER positive patients (ER status 1) ($p<0.001$). 5 year cumulative survival in ER negative patients was 0.647 and ER positive patients 0.856.

Figure 3.16: Breast cancer survival by ER status in cohort 1, 1984-86

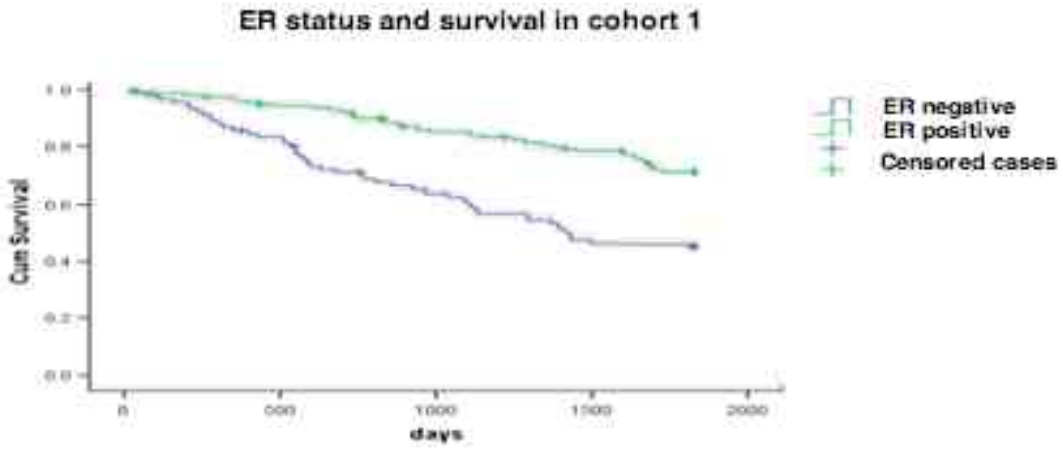
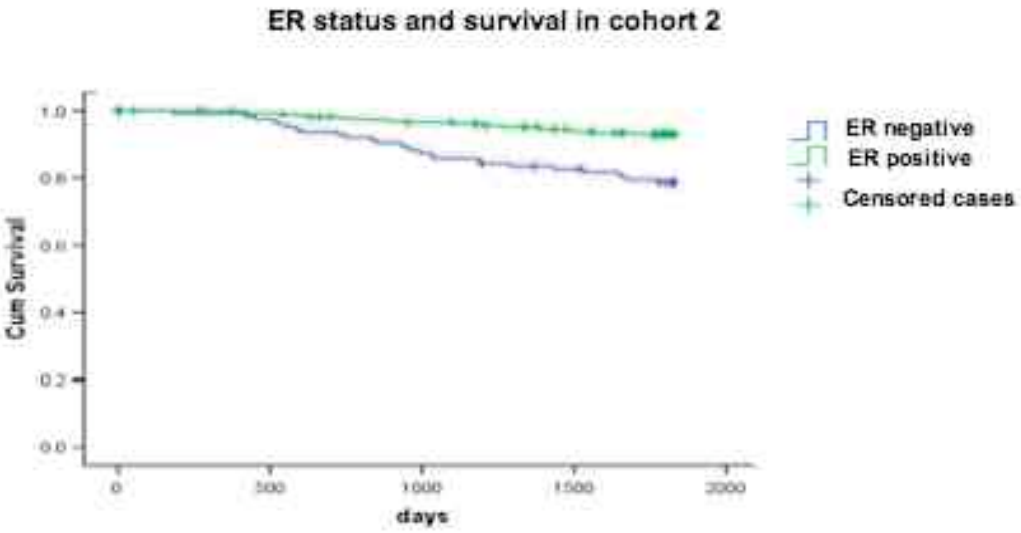


Figure 3.17: Breast cancer survival by ER status in cohort 2, 1996-97

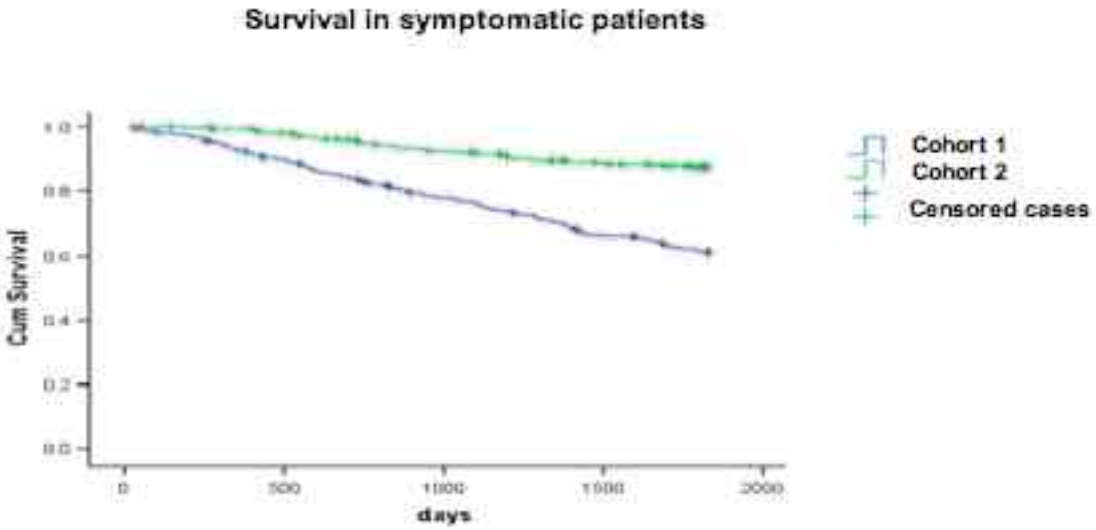


In both 1984-1986 (cohort 1) and 1996-1997 (cohort 2) there is a significantly higher disease-specific 5-year survival in the ER positive patients ($p<0.001$ for both). In cohort 1, cumulative 5-year survival in the ER negative patients was 0.453 and in the

ER positive patients 0.709. In cohort 2, 5-year survival in the ER negative patients was 0.786 and in ER positive patients 0.930. Although survival has increased over time for both ER positive and ER negative patients, the survival of ER negative patients has improved to a greater extent; possible reasons for this will be discussed in the Discussion.

Survival in patients with screen-detected and symptomatic tumours

Figure 3.18 Breast cancer survival by cohort (cohort 1=1984-86, cohort 2 = 1996-97) in patients whose tumours were symptomatic and not screen-detected



For breast cancer-specific survival there is a significant improvement in survival in symptomatic women in the second cohort compared to the first cohort (cumulative survival in cohort 1 =0.620, cohort 2= 0.874, $p<0.001$). This suggests that screening is not the only explanation for the higher survival in the later cohort of patients.

Figure 3.19 : Breast cancer survival and screening status in entire study

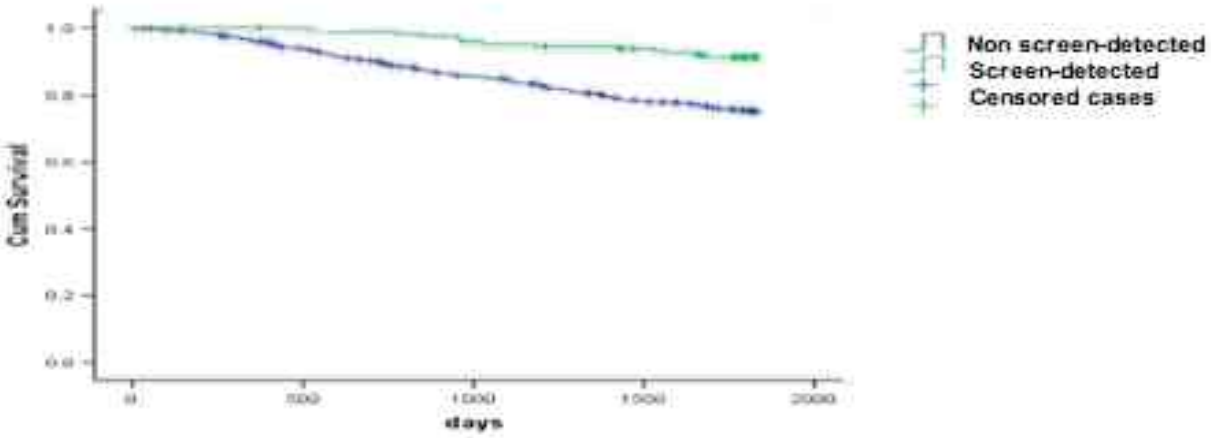
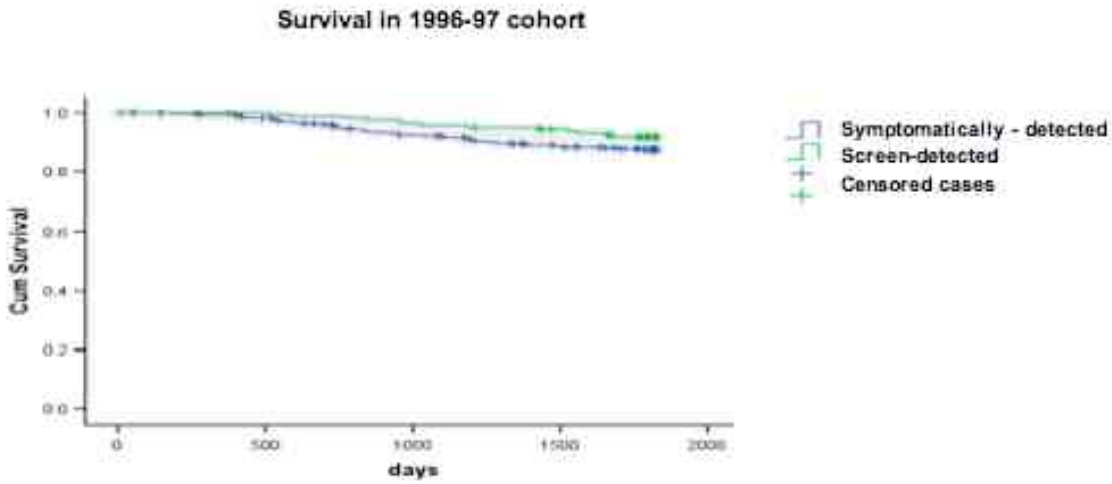


Figure 3.20: Breast cancer survival by screening status in cohort 2, 1996-97



When all the study patients are assessed, survival in those women whose tumours were screen-detected was significantly higher than those who had tumours detected symptomatically (cumulative survival for screened= 0.916; for symptomatic= 0.753, $p<0.001$). However, when the second cohort was analysed there was no significant disease-specific survival difference in between women whose tumours were screen-

detected and those detected symptomatically (cumulative survival for screened = 0.916; for symptomatic = 0.874, p=0.148). Possible explanations for this will follow in the Discussion.

Survival and Nodal Status

In both cohort 1 and cohort 2, nodal status was related to survival, with node positive patients having a lower 5-year survival than node-negative patients (0.523 vs 0.799 and 0.801 vs 0.946, with $p<0.0001$ for each). As with ER status, in the second cohort again the survival difference appeared to be narrower than in the first.

3.15 Cox's proportional hazards analysis

When the effect of cohort (1 or 2) on survival was adjusted for differences in ER status alone using Cox's proportional hazard regression, the cohort type was a significant independent factor in survival, confirming that the changes in ER status over time seen in this study were not solely responsible for differences in survival. After correcting for all the factors in the model, the effect of cohort on survival persisted; patients in cohort 1 still had a higher relative risk of death than cohort 2, with hazard ratio of breast cancer death of 3.43. Grade, screen-detected status, HER-2 status and intermediate deprivation status were not included in the final Cox model which means they were not independent predictors of survival in the study as a whole and could be explained by differences in other factors. Increasing age, increasing tumour size, node-positive status, ER negative status and deprived status correlated with increased hazard of breast cancer death after adjustment for other factors.

Table 3.10: Cox proportional hazard regression analysis of factors influencing survival in the study group

Variable	p value	Hazard ratio of death	95% CI
1984-86 cohort	**<0.001**	**3.43**	**2.2-5.2**
ER negative status	**<0.001**	**4.29**	**2.8-6.4**
Increasing age (per year)	**0.09**	**1.02**	**1-1.04**
Tumour size (per 1mm rise)	**0.01**	**1.02**	**1-1.02**
Affluent socioeconomic vs deprived	**0.08**	**0.29**	**0.11 – 0.72**
Intermediate socioeconomic vs deprived	**0.345**	**0.82**	**0.54 – 1.23**
Node positive status	**<0.001**	**2.65**	**1.66 – 4.2**
HER-2 status	**Not included in model**	**Not included in model**	

3.16 Immunohistochemistry: inter-observer correlation

For each of the three antibodies, 10% of samples were also scored by an experienced scorer; for weighted histoscores that differed by over 50 scoring was carried out again with scorers discussing and agreeing on a final score. The intra-class correlation coefficient between the two scorers was then calculated using SPSS (using the two-way mixed model method). This coefficient should be as close to 1 as possible to ensure reliability of the two observers.

ER: intra-class correlation coefficient = 0.928: $p<0.001$

PR: intra-class correlation coefficient = 0.954 : $p<0.001$

HER-2 :intra-class correlation coefficient = 0.986: $p<0.001$

Therefore the correlation of the two observers is confirmed and the validity of the scores of the remaining 90% of samples that were assessed by a single observer scores assured.

Chapter 4: Discussion

The first part of this thesis aimed to establish trends in breast cancer incidence in Scotland up to the year 2003 and how these have been could have been influenced by the NHS breast screening programme and trends in known risk factors for breast cancer. The age-adjusted rate of breast cancer in women in Scotland in 1975 adjusted to European standard population was 79.6 cases per 100,000 women and in 2001 it was 111.2 cases per 100,000 women. Incidence rates differed significantly in different age groups. Breast cancer incidence in those age groups under 49 remained relatively stable over time, and within these age groups as expected incidence was higher with successive age group. In women aged 75 and above rates of breast cancer have been increasing over time, again with incidence being higher with each successive age group, although women aged 85 and over the incidence has begun to decrease since the late 1990s. In women aged 55-69, however, there have been more complex changes in incidence over time. In women aged 50-64, those women invited for screening as part of the NHS breast screening programme, breast cancer incidence was seen to be increasing even before the screening programme was introduced in 1987, with a mean rise in incidence of 2.31 cases per 100,000 per year. After the introduction of the screening programme there was a sharp rise in incidence similar to that seen in the initial stages of mammographic screening programmes elsewhere in the world (Moller et al. 2005; Olsen et al 2003; Schouten et al. 2002; Zahl et al. 2004).

In 1987 women in a few areas in Scotland aged 50-64 were invited for their first screening mammogram; with every year after that women aged 50-64 in a larger geographical area of Scotland were invited for their first screen until by 1994 every woman in the 50-64 age group had been invited for a first screen. From 1994 onwards, women were invited for a first screen at some point between the ages of 50 and 53, with the age of invitation differing between different GP catchment areas. Screening is offered up until age 64 although certain areas in Scotland have been inviting women for screening to age 70 since 2003. In the first year of the programme a certain number of asymptomatic tumours would have been detected in

the women aged 50-64 in the areas offered screening, which would have been detected in addition to tumours detected symptomatically in unscreened women and hence added to the incidence. In the next year, the women aged 50-64 in a larger geographical area of the country will undergo their first screen, leading to an even higher incidence of asymptomatic and hence total breast cancer than in the year before. Rates of breast cancer would have increased year-on-year thereafter; however in the last few years of the prevalent round there were few geographical areas remaining in which all eligible women had not been invited for screening and hence fewer women were having a first screen, so the rates began to fall from the peak.

Once the prevalent round of a mammographic screening programme such is this is complete, the incidence rate in the screened age group is expected to remain stable. The incidence consists of symptomatic tumours in unscreened women, screen-detected tumours at first screen in women aged 50 to 53, screen-detected tumours at a subsequent screen, and a small number of 'interval' cancers detected in between screening episodes. Only the detection of hitherto asymptomatic tumours in the women aged 50 to 53 should contribute to there being a higher incidence in the 50-64 age group than before screening began, as the incidence rate of the other tumour types combined should be similar to the incidence rate of symptomatic tumours in this age group before 1987. An observed-expected analysis of 1995-1999 rate compared with the trends that had been developing before 1987 was performed to find out if this had been the case.

One notable factor of this analysis is the fact that incidence rates in women aged 50-64 had been increasing before the screening programme began, with a mean increase of 2.3 cases per 100,000 per year since 1975. In women aged 50-54, observed incidence rates of breast cancer in Scotland since the prevalent round of screening was completed were 58% higher than would have been expected, had the underlying trend before screening continued. As women are invited for a first screen in Scotland between 50 and 53 years of age, the majority of the 50-54 year-old age group were undergoing 'prevalence' screening and this could explain the increased breast cancer incidence compared with before screening began. However, the increased incidence rate in these women compared to the previous background

incidence should be a stable phenomenon but instead the incidence rate has clearly been increasing with every year since the end of the prevalent round. In women aged 55-59 and 60-64, there were 42% and 40% increases, respectively, in incidence over the underlying trend that had been developing before 1987. In women aged 65-69 incidence rates would have been expected to fall from 1995 onwards. These women should have been through the screening programme at least once and after a normal screening mammogram, it should take some time for a woman to develop a symptomatic tumour. Therefore incidence rates in these women should fall, with the incidence rate rising again in the subsequent age group; however in these women no such fall was seen. It appears then that breast cancer incidence in middle-aged woman has been rising due to factors other than the breast screening programme.

The analyses performed cover the incidence of breast cancer in Scotland up to the year 2003. Criticism could be levelled that trends developing between then and now could have given additional support or indeed contradicted the conclusions drawn here about the relationship between breast cancer, screening and risk factors. However, the process by which cancer cases are registered and the data compiled creates a lag time in which the true number of cases in a year only becomes fully known several years afterward. Hence the data here, which were correct as of 2008, can be said to be as stable as possible. The 'observed versus expected' analysis is based on estimation of what the background incidence rate would be if trends had continued from 1987; it is possible that the background incidence rate did not simply follow this trend. The use of a mean for a 5-year period helps increase the validity of the 'observed' rates, but as with all disease incidence rates the presence of random fluctuations with time (random error) can decrease the validity of the calculated trends.

A further study was made of incidence rates by birth cohort in order to see if this shed any additional light on changes in incidence. Studying cancer incidence in women born in the same birth cohort year or range of years regardless of age can be useful as these women can be expected to have a similar 'risk experience' of factors such as fertility patterns and contraceptive pill patterns around the time of their childbearing years and HRT prevalence patterns around their time of menopause that is independent of the effect of age on their risk of breast cancer. However, factors

that may influence the incidence of breast cancer that have an identifiable time period in which they extended their influence across all age groups – period effects– can easily be superimposed upon and confound cohort effects. The NHS breast screening programme is one of these potential period effects. The incidence rate in birth year cohorts in which women are aged 50 to 64 during the 'prevalent' round of screening is likely to be different from the rate in cohorts in which women were aged 50 to 64 before screening began to be offered or after the prevalent round had been completed regardless of a true difference between the cohorts. The analysis performed here attempted to analyse how birth cohort effects relate to the screening programme in Scotland.

An overall analysis of birth cohort effect in women aged 45 to 69 was initially performed; a defined age range was chosen in order to give more information on changes with more recent birth cohorts, where information is obviously not available on incidence rates in the oldest women. In addition, women aged 45 to 69 may be to be more sensitive to birth cohort effects than women who are still in their childbearing years or older women in whom the effects of ageing on breast cancer incidence rate may be stronger than birth cohort effect. An increase in incidence rate with increasing birth cohort year was seen from the 1920 birth cohort onwards up until the 1938 cohort; thereafter rates appeared to level out. However when the data were broken down into individual age groups, incidence rates in each age groups 50-54,55-59 and 60-64 increased with every year difference in birth cohort and there was no levelling off at the 1938 cohort. There is a possibility that this levelling off is artefactual, resulting from the fact that some age groups will be ceasing to have a contribution to incidence rates as other are beginning to contribute (as demonstrated in figure 3.3); this problem is inherent to any study of birth cohort incidence rates. Certainly it would appear that in women aged 50-64 the incidence rate has truly been increasing with more recent birth cohort. A further analysis was performed looking at the likely effects of screening in these women to see if the effects could have been produced by the screening programme,

In each of the screening groups, the most marked rises in incidence with rising birth cohort year were within cohorts where some or all women were offered screening during the prevalent round. This initial trend might be entirely explained

by the increasing national coverage of the Scottish population by screening. However the prevalent round does not explain the continued increase in incidence seen with successive birth cohorts in the 50-54 and 55-59 age groups after the prevalent round had been completed. Although confidence intervals for these rises are wide, it still appears that this is a true birth cohort effect. Potential contributing factors to a birth cohort specific change in breast cancer incidence rate are the reproductive factors explored below. In women aged 60-64 the relationship of incidence to birth cohort since the prevalent round ended was less clear. In women aged 45 to 49 a non-significant rise in breast cancer incidence was developing from the 1942 cohort onward; in women aged 65 to 69 there was little change in breast cancer incidence across the different birth cohorts. It may be that in women aged 65-69, the general effect of ageing on cancer incidence is of greater importance than reproductive risk factors.

There are several limitations to the approach taken here, the most important being the potential for misclassification of screening experience, particularly because the study is not based individual patient data and whether their tumours were screen-detected. In addition some women being screened during the introduction of the prevalent round may in fact have also had a subsequent screen three years after the original. The study method attempted to minimise the potential for misclassification by calculating incidence rates for individual birth cohort years instead of ranges, and dividing women into groups based on a calculation of their likely exposure to screening. but are still counted amongst 'women being screened during the prevalent round'. Another approach would have been to undertake formal age-period-cohort analysis, taking into account screening experience, as performed for England and Wales data by Waller (2007). However, using this approach Waller et al generated informative data on incidence rate ratios within different screening groups but did not generate data on incidence by birth cohort and the effects of screening on these. For the purposes of this thesis, the analysis performed here was felt to be more informative than formal age-period cohort modelling.

Reasons for an increased temporal and birth cohort incidence of breast cancer

Screening Trends

The analyses above suggest that the increased incidence of breast cancer is not accounted for by the effects of the screening programme alone. However, the expected effects of an established screening programme on cancer incidence are based on this phase of the programme screening women in a stable way, and changes such as an increased power of the programme to detect cancers or an increase in uptake of screening invitations could contribute to increased incidence rates. As described in chapter 2 the standardised detection ratio of the screening programme in Scotland (rate of invasive cancers detected divided by the number expected for the background incidence rate) for women having a first screen increased from 1.1 just after the prevalence 'round' of screening was completed to 1.5 in 2001/02. Factors that may have contributed to this include a change from one- to two-view mammography for prevalent screens, film density becoming standardised at 1.4-1.8D, implementation of quality assurance procedures and the establishment of a skill base of radiographers and radiologists (Blanks, Moss, & Patnick 2000). For second ('incident') screens the SDR increased from 1.0 to 1.2 between 1994/5 and 2001/2; this is consistent with the fact that changes in the programme's ability to detect tumours would be expected to have a greater impact on women having their first screen and with the fact that two-view mammography was introduced for first screens only. It is possible that the increasing SDR of the screening programme and the resultant increased detection of asymptomatic tumours could have contributed to a rise in breast cancer incidence in women being screened during a period when the effects of the screening programme would have been expected to have stabilised out, particularly seeing as the greatest effect was in women having a first screen. It is unclear whether this effect would have been enough to impact on population incidence rates.

A similar effect on incidence could be produced by a gradual rise in the uptake of screening invitations. This study has shown a small but significant trend towards increasing breast screening uptake in Scottish women with every year from 1991/2 to 2001/2, with the uptake rising from 70.9% of women taking up the screening invitations to 74.5%; in women being invited for their first screen (ie aged 50-53) there was also a significant increase in uptake from 70.9% to 73.8%. The increase in uptake could have contributed to an increasing incidence of breast cancer in the 50-53 year old age group. If women did not take up their first screening invitation then they were invited again three years later, and increasing uptake of these invitations this may have contributed to more asymptomatic cancers being detected in the age groups who would usually have been undergoing second and subsequent screening. It is, however, unlikely that this small rise in uptake has contributed substantially to an increase in the disease in any of these age groups. Not enough information is available on the demographics of women self-presenting for screening to assess the increasing numbers of self-referrals for screening that this has had on breast cancer incidence.

Risk Factor Trends

As detailed extensively in the Introduction, hormonal risk factors are of pivotal importance in the individual risk of breast cancer and the high incidence of the disease means that population trends in risk factors could significantly influence the population incidence of the disease. This thesis has detailed Scottish trends in the following factors: age at first pregnancy, parity, body mass index and use of HRT. Data were not available on oral contraceptive use in Scottish women; the absence of these data are unlikely to be a significant omission in the analysis of contributions to breast cancer incidence in women of screening, as the 50-64 age group that this thesis concentrates on are likely to have ceased to use the oral contraceptive at least 10 years previously, and after this point the effect on breast cancer incidence has been shown to disappear (Collaborative Group on Hormonal Factors in Breast Cancer, 1996).

Fertility

There has been a dramatic change in the ages at which women in Scotland have their first child since the mid-1970s; the number of first births to women aged 20-24 has halved over the 25 year period while the number of pregnancies to women aged 30-34 have tripled in this period and the number of pregnancies to women aged 35 to 39 have increased five-fold. Of particular interest are the trends in women aged 35-39; as detailed in the Introduction a first pregnancy over the age of 35 is a significant risk factor for breast cancer; a woman with a first pregnancy at age 35 has around a 5% higher relative risk of breast cancer than a nulliparous woman and an even higher risk compared to a woman who was parous at a younger age. First pregnancies in women aged over 39 also increase risk but the rates are small (several hundred only) and have changed little over time and it was felt more useful to concentrate on the 35-39 age group. In 1976 1.4% of all first pregnancies in Scotland were in women aged 35 to 39 but by in 2001 9% of first pregnancies were to women in this age group; the percentage was increasing slowly year-on-year up to the late 1980s with a greater increase thereafter. The magnitude of the increase in first pregnancies in women aged to 35 to 39 is such that it could have influenced population rates of breast cancer. However, women aged 35 to 39 in 1988 would be aged 45 to 49 in 1998, an age group with little in the way of a breast cancer incidence increase, and hence had not reached the 50-64 age range in which the greatest increases in breast cancer incidence have occurred. Therefore the increase in first pregnancies over age 35 does not explain the incidence trends described in this thesis. However there is clearly a possibility that these fertility trends will have an impact on breast cancer incidence rates in the 50-64 age group in the future.

Low parity is also a risk factor in breast cancer; data on cumulative fertility by age 44 was available in relation to maternal birth year. There was little change in completed family size up to the 1935 birth cohort but thereafter there was a continued drop in family size from 2.63 to 1.9 in the 1960 birth cohort. In terms of how the observed fertility patterns are linked to the observed birth cohort breast cancer incidence, the birth cohort breast cancer incidence data in chapter 2 describe data up to the 1949 birth cohort, at which point completed family size had dropped to 2.12. As displayed in figure 2.4, in women aged 50-54 a steeper increase in

incidence can be seen to be developing after the 1935 cohort, although the cohorts immediately after the 1935 cohort were within the prevalent round of screening and, as already discussed, this could have increased incidence rates with rising birth cohort. Continued incidence rate rises in cohorts not affected by the prevalent round, however, could have been contributed to by the continued fall in completed family size. In women aged 55-59 the 1935 cohort comes at around the end of the birth cohorts that would have been influenced by screening, and the continued incidence rate rises thereafter could have been affected by the completed family size fall. In women aged 60-64 the trend in incidence with birth cohort from 1935 onwards is shows rises and falls in incidence with no clear developing trend. Interestingly Chia showed decreases in overall breast cancer incidence from the 1935 cohort onwards in both Singapore and Sweden (Chia et al. 2005). In Singapore the authors found evidence of a relationship between cohort trends in late age at first pregnancy and parity but in Sweden they could not.

BMI

In the women in the Scottish Health Survey (Dong & Erens 1997; Bromley et al. 2003; Shaw, McMunn& Field 2008) mean BMI increased from 25.7 kg/ m^2 in 1995 to 26.9 kg/ m^2 in 2003. In women aged 55-64, mean BMI increased from 27.6 kg/ m^2 to 28.6 kg/ m^2. The percentage of women with BMI over 25 was 47.2% in 1995 and 57.3% in 2003; for women aged 55-64, percentage rose from 68.2% to 73%. With postmenopausal obesity being a risk factor for breast cancer the 5% rise in overweight and obesity in women of screening age could be a possible explanation for a rising background incidence rate.

Alcohol

Data from large cohort studies suggests that alcohol is a significant factor in postmenopausal breast cancer and surveys show that mean alcohol consumption has been increasing in women aged 55-64 , along with the proportion of women drinking over 14 units per week. The increases are small, however they could have been enough to have significantly contributed to an increase in postmenopausal breast cancer rates.

Hormone Replacement Therapy

Hormone replacement therapy increases risk of breast cancer, with a large meta-analysis of trial results concluding that the risk of breast cancer increase by a factor of 1.023 for every year of use and that this increased risk disappeared 5 years after cessation of use. The use of hormone replacement therapy in the UK doubled between 1973 and 1976, fell again and began to rise substantially from the late 1980s onwards; prevalence in England was estimated to have risen from 2.2% of 45-64 year olds in 1987 to 21.7% in 1994 (Townsend 1998). Between the mid-1990s and 2001 prevalence of use stabilised out (Townsend 2005). Data were available on hormone replacement therapy prescriptions from 1993 onwards. The calculated estimated prevalence showed that incidence increased from 13.6 in 1993 to 17.2 in 1996 but thereafter there was little change up to 2001. Clearly there are potential sources for error in the calculations used to estimate prevalence; the inclusion of the population denominator of ages 46 to 64 may underestimate prevalence as the youngest women are likely to have lower prevalence than the oldest, and the amount of time the HRT was used for will have varied. However, in the absence of accurate prevalence data this methodology comes as near as possible to estimating HRT prevalence.

It is unfortunate that data are not available on prevalence of HRT before 1993, but the small rise in prevalence and eventual stabilising out of HRT rates demonstrated here suggest that the rise in breast cancer incidence by birth cohort since the prevalent round of screening ended cannot be explained by changing HRT use.

The analyses of breast cancer by birth cohort above support the fact that these hormonal risk factors could be of importance. However, the observations above about the relative contribution of each of these to overall incidence of breast cancer are estimates based on literature, given that complex modelling of these risks is outwith the scope of this thesis. There are many potential sources of bias within these estimates. There is the potential that type I or type II errors have occurred: where the author has discounted a risk factor's importance if its trend does not fit with the breast cancer trends seen, or suggested an important effect if the trend in the

risk factor fits with breast cancer trends. Essentially the basis of these observations is the application of individual risk factors to population incidence rates, and while it is a common concept in epidemiology there is no foolproof statistical way of linking the two factors. The extrapolation of risk factors changes to population cancer incidence also assumes that breast cancer patients have been exposed to the changes in risk factors seen in the general population; if this is not true for some previously undetected reason, there is a real risk of misclassification bias. There is also the possibility that a previously undetected period (temporal) factor has been the major defining feature in temporal changes in breast cancer incidence rather than the risk factors above.

Affluence and deprivation

Breast cancer is commoner in the affluent than the deprived; the exact reason for this is likely to be multifactorial and involve a variety of risk factor differences. The question also remains as to whether incidence rates are rising in parallel across all social classes. As noted in the introduction. Dano et al. (2003) studied incidence rates over time in social classes in Denmark, and found that rates in the affluent were rising at a lower rate than before and rates in the deprived rising at a faster rate than before. Recent trends in those enrolled in the Longitudinal Cohort Study of 250,000 UK women supported this same narrowing incidence gap (Population Trends, 1997). It is demonstrated in this thesis that breast cancer incidence rates in Scotland between 1991 and 2000 were increasing in parallel in affluent and deprived women. The biggest factor that could affect the interpretation of these results is whether the breast cancer patients contained within these data have been correctly assigned to a particular socioeconomic category, that is, whether there has been misclassification bias. The use of quintiles of deprivation, as in the trends shown here, has become popular in recent years. However, the use of quintiles which each contain 20% of the population as opposed to the use of unequal deprivation categories risks misclassification because the top quintile could contain a mixture of very affluent households and far less affluent households. Also, the fact remains that all these measures of deprivation are based on postcode, and it has been argued that the association of a geographical area with a characteristic of the individual people

within it such as socioeconomic deprivation is an 'ecological fallacy'. However, studies have been carried out at the level of the individual that claims to support the use of area-based measures as a surrogate marker of deprivation (Sloggett, 1998), and the use of area-based assessment of deprivation is still the norm today.

The reproductive risk factors discussed at length in this thesis such as increasing age at first pregnancy, reductions in parity, use of hormone replacement therapy and oral contraceptives and changing patterns in BMI were studied in different socio-economic categories in order to assess their possible impact on breast cancer incidence rates. Indeed, studies in Marin County in California (Hwang et al. 2005; Prehn et al. 2002; Robbins et al 1997; Wrensch et al. 2003), a geographical area with an affluent population and corresponding excess of breast cancer, had suggested that differences in reproductive risk factors may explain the observation of higher breast cancer within that population. Differing uptake of screening invitations among women of different socio-economic categories is also well-known phenomenon (CDC 2005; Gatrell et al. 1998), with a higher percentage of affluent women taking up screening invitations, and an investigation of screening patterns was also performed. It is unfortunate that a breakdown on the figures for affluent and deprived women by age was not available, as trends could potentially differ between premenopauasal and postmenopausal women as a result of the 'crossover' effect of reproductive risk factors. However we have presumed that with postmenopausal breast cancer being the majority of cases, trends in postmenopausal women are likely to reflect the overall trend within each socioeconomic group.

A study of numbers of first births in women aged 35–39 revealed that numbers per year have always been highest in the most affluent and lowest in the most deprived. This may be a potential explanation for the persisting high rates of breast cancer in the affluent; a woman with a single pregnancy over age 35 can have up to double the risk of a woman with several births at a young age (Rosner, Colditz & Willett 1994). Since the late 1980s, the number of first births at late maternal age has been markedly increasing every year in all deprivation categories, albeit to a greater extent with each successive quintile. This is a potential explanation for the rise in breast cancer rates across the whole socio-economic spectrum and would be expected to produce incidence rates in the affluent that are rising higher than in the

deprived, a trend which has not developed. However as noted in the discussion of the general effect of fertility trends on breast cancer incidence, the steep increase in late first pregnancies from the late 1980s may result in an even steeper rise in breast cancer rates in the near future when these women become postmenopausal, and this rise may be especially marked in affluent women. Data on parity differences between the affluent and deprived women may have given additional information on reproductive influences on breast cancer incidence, but such data are not maintained by any agency.

Extrapolating from cross-sectional survey data, it appears that the prevalence of obesity and mean BMI in Scottish women of all socio-economic categories increased between 1995 and 2003 (Dong & Erens 1997; Bromley et al. 2003; Shaw, McMunn & Field 2008). Again, this could potentially contribute to rising breast cancer incidence across all socio-economic categories; a pooled analysis of several studies has suggested that the relative risk of breast cancer in women with a BMI over 28 is 1.26 and the RR is 1.0 for women with a BMI under 21 (Key et al. 2003). This level of risk suggests that rising levels of obesity could significantly influence patterns of breast cancer incidence. However, the surveys suggest that BMI and obesity have consistently remained higher in the lowest socio-economic categories, and thus BMI differences do not explain the observed socio-economic gap in incidence. Torgerson (1994) suggested that differences in BMI may explain the socioeconomic effects on breast cancer survival but did not study effects on incidence.

The presence of an organised screening mammography programme can strongly affect breast cancer incidence rates. Women undergoing incidence screens have incidence rates that should reflect the background incidence, but the youngest women in the programme are always undergoing ‘prevalence screening’, with the detection of a relatively large number of asymptomatic tumours which have been present for varying lengths of time An increase in the percentage uptake of screening invitations is likely to result in increased prevalence screening and therefore may cause incidence rates to rise. Figures for the Scottish Breast Screening Programme show that screening uptake over 1990–2000 increased by a few percent in all deprivation categories - this is unlikely to be enough to explain the overall breast

cancer incidence rises in all categories. The socio-economic gap in uptake has persisted over time, although it is small, with an absolute difference in uptake of 17% between highest and lowest quintile in 2001.

These data suggest that breast cancer incidence in Scotland is rising in all deprivation categories but that rates remain higher in the affluent. Reproductive trends shown here may explain the persistent socioeconomic gap, but do not appear to be an adequate explanation for rising breast cancer rates. A rising prevalence of obesity could be contributing to rises in breast cancer rates, but would not explain the deprivation - affluence incidence gap. Screening differences are of insufficient magnitude to explain either phenomenon. There was no data available to analyse in order to assess differences in hormone replacement therapy use in different socioeconomic categories in Scottish women.

Discussion of Laboratory Project

This study has demonstrated a rise over time in the percentage of breast cancers that were ER positive. The rise from 64.2% to 71.5% is statistically significant and clinically significant - in that a rise in the number of breast tumours potentially responsive to hormonal manipulation could contribute to increasing survival rates as a result of the clear prognostic advantage of hormone-treated ER positive disease over ER negative disease (Clarke 2005). There was also a significant change in combined ER/PR receptor status, most notably a marked decrease in the percentage of tumours that had the poor prognostic ER negative/PR negative status. The percentage of tumours that were PR positive and HER-2 positive did not change over time; notably there was no change in mean score over time for any of the three receptors.

A significant change in grade distribution was seen, particularly a reduction in the frequency of grade 3 tumours and an increase in the frequency of grade 1 tumours. This distribution change appeared to be exerted by the presence of screen-detected tumours in the second cohort, as there was no significant difference in the grade distribution of the symptomatically-detected tumours in both cohorts. The pathological grade of screen-detected tumours and its significance has received much attention in the literature; while it is accepted that tumours detected at a first

screening mammogram are of lower grade than symptomatically detected tumours it is uncertain as to whether this represents an interruption of phenotypic drift or whether the lower-grade tumours are preferentially detected at screening because of their longer asymptomatic preclinical phase (length bias) (Alexander 1997; Crisp 1993; Duffy 1991; Tabar 1999). An attempt was made in this thesis to estimate whether women were receiving an incident screen or not, as length bias is an unavoidable problem with tumours detected at prevalence screening; the results suggested a significant difference in grade between symptomatic patients and patients having a tumour detected at an incident screen. This would appear to support the theory of phenotypic drift but clearly misclassification of screening episode is a clear possible confounding factor.

The study has also demonstrated significant differences in the percentage of tumours that are ER positive within patients with screen-detected and symptomatic tumours, with 78.4% of screen-detected and 68.8% of symptomatic patients in the newest cohort being ER positive. Certainly on multivariate analysis screening status consistently remained a significant factor in the change in ER status over time (there was an increase in the number of ER positive tumours between the symptomatic patients in both cohorts but this did not reach statistical significance). There is evidence to suggest that screen-detected tumours are more likely to be ER positive than negative (Klemi, 1992; Ernst, 2002), probably because the ER positive tumours are more likely to be slower-growing with a significant asymptomatic phase.

ER status did not appear to relate to levels of deprivation in the patients in the study on univariate or multivariate analysis; this is in contradiction to studies such as that of Carnon et al .(1994) which would seem to suggest that ER positive disease is commoner in the affluent than the deprived. Fertility-related factors such as late age at first pregnancy noted in Part 1 of the thesis which are undoubtedly affecting breast cancer incidence would be expected to produce more ER positive disease, and are commoner in the affluent – as discussed in the Introduction this is a possible mechanism of higher levels of ER positive disease in the affluent. It may be that the small numbers of affluent patients in our study could be impairing the ability to detect a difference in the different deprivation groups.

Multivariate analysis suggested that the observed changes in ER status did not persist when adjusting for screen-detected status and age within these groups. As noted above, the increased ER positive prevalence in screened patients likely reflects the fact that tumours detected at screening are slower-growing and more likely to be ER positive than the symptomatic tumours. it may be argued that even although these tumours are preferentially detected at screening, the screening itself is not actually causing a change in the hormone receptor status. One would expect a corresponding decrease in rates of ER positivity of symptomatically detected tumours over the time period, and the fact that this had not occurred could suggest that a true change in biology has occurred. However, it is also possible that these ER positive cancers are those that are in fact being 'overdiagnosed' as discussed in the introduction – that these ER positive tumours are the tumours that would not have ordinarily been diagnosed at the time of a patient's death from other causes, and hence this apparent shift in tumour biology has been artefactual and could not have contributed to any changes in survival over time.

As for age, it is notable that the age distribution of the two patient groups did differ significantly, with the upper limit of the second cohort being 93, reflecting a total of 44 patients being operated on over the age of 75, whereas the upper limit in the first cohort was 74. This is potentially significant in view of the well-documented observation of increased ER positivity after the menopause (Elwood 1980; McCarty 1983). It was not appropriate to include age by decade in the multivariate analysis as there is not a linear relationship between age and ER status. Interestingly, a slight increase in ER positivity towards the 45-49 age group was seen, and this was also demonstrated by Elwood et al. (1980). Thereafter, rates of ER positivity did appear to increase after the age of 60, so 'age before 60' and 'age after 60' were included as a categorical dichotomous variable. When age was included as a factor in the analysis, this appeared to explain the differences in ER status between the groups. However, the use of 60 as a cut-off is an arbitrary measure. Direct comparison of percentage ER positivity within the two cohorts when divided into 5 year age groups shows that the percentage of ER positive tumours has increased in each of these age groups over time , and while the individual numbers in each group are too small to prove the individual significance of these results, it suggests that the

changes in ER status seen in the project are not artefactual, produced by the age distribution of the study population.

The results of this study are in accordance with the increase in ER positivity seen in other studies (Bradburn 1998; Glass 2007; Henley 2005; Li 2003). These studies are a heterogeneous group with carrying approaches and study populations. In most, assays and criteria used to determine ER positivity changed several times during the study periods as a result of development of new methods of ER determination. Studies of the concordance of ligand-binding assay and immunohistochemistry for oestrogen receptor have showed that the concordance can be as low as 82% (Allred 1990). The importance of the study presented here is that it used immunohistochemistry on all samples, thereby eliminating the possibility that an increase in ER positivity has been artefactual. Furthermore for each antibody, all the samples underwent immunohistochemistry together to eliminate the potential effect of changing laboratory conditions on staining.

One criticism that could be levelled at this study is that the most recent patients in this study are from 1996-97 and hence the project describes changes in ER status that had developed between twenty and ten years ago. The reason for this is that a secondary aim of the project was to explore how changes in ER status could have contributed to survival; when the laboratory project began in 2004, the cohorts were designed to end in 1997 to allow long enough follow-up. Therefore, whereas papers such as that of Glass (2007) demonstrate more recent changes in ER status of tumours they do not directly apply these to survival data. The demonstration of changes in ER status in this ten year period could be the basis for continued research into how ER status has changed in more recent years, applying a similar technique of re-analysing stored tissue. Image-detection technology could allow computer scoring of samples to reduce the time taken to carry out such a project and gain a more up-to-date view of changes in molecular epidemiology.

One explanation for a preferential increase in ER positive breast cancers could be a population-wide change in the prevalence of certain factors that have been shown to increase the frequency of ER positive breast cancer in large cohort studies. As detailed extensively in the introduction, such factors include late age at first pregnancy and postmenopausal obesity (Althuis 2004; Colditz 2004; Potter 1995);

and use of hormone replacement therapy in one study (Potter 1995). Within Part 1 of this study it has been demonstrated that profound changes in certain reproductive and endocrine factors have been occurring in Scotland, although hormone replacement therapy prevalence changed little over the study time period. Certainly, the percentage of women in the study that had used hormone replacement therapy and the relationship to hormone receptor status would have provided important epidemiological information but this was not readily available and the fact that many of the casenotes of the patients in the earlier had been destroyed would have precluded any useful comparisons.

Rates of PR positivity increased, but not significantly, between the two time periods. In cohort studies PR status has been shown to be influenced by similar risk factors to ER status; it may be that the numbers in the study have not been enough to let the increase in PR positivity become statistically significant, as the study is underpowered to detect such a difference. Interestingly, the distribution of combined ER and PR status changed significantly. Particularly noticeable was a 10% decrease in the percentage of the poor prognosis ER-/PR- tumours from 33% to 23%; this could have influenced the survival differences between the two cohorts. The fact that HER-2 distribution has remained unchanged is not unexpected; changes in epidemiological risk factors are unlikely to have influenced HER-2 status. However, again there may be a true difference that the study has been underpowered to detect. In addition the use of Herceptest alone to assess HER-2 distribution rather than FISH may have affected the validity of these findings.

This study was powered to detect a 10% difference in ER positive prevalence, and is hence slightly underpowered to detect the observed 7% difference. The inability to retrieve tumour blocks for all patients means that the calculated figures are not from the full original study population; the cohort is further reduced by the tumours which were not suitable for immunohistochemistry because of sampling error or damage to the core while being inserted into the tissue microarray, a factor commonly seen in studies using archived tissue for microarray technology (Hager et al. 2007). Those tumours that underwent analysis should be representative of the study tumours as a whole, as it is unlikely that the lost tissue or non-retrieved tissue is anything other than a random sample of the study population.

Breast cancer-specific and overall 5-year survival in cohort 1 were significantly lower than in cohort 2. 5-year breast cancer-specific survival was significantly higher in ER positive patients than ER negative patients in the study overall and in each cohort independently. In the second cohort, improved survival in ER positive patients may reflect the fact that in 1984-1986, whilst beginning to be used, hormonal therapies may have been relatively under-prescribed by today's standards due to different ER techniques and cutoffs for 'positivity' and different advice on suitability for hormonal therapy; the more marked survival improvement in ER negative patients over this time period is most likely due to improvements in chemotherapy. Unfortunately treatment data are not available for the women in this study, as in 1984-86 the Cancer Registry did not routinely record treatment received and casenotes were not available.

In the second cohort there was no disease-specific survival difference between women whose tumours were screen-detected and those whose tumours were detected symptomatically; within the symptomatic patients there was a significant survival difference between cohorts. However, this cannot be directly extrapolated to the conclusion that screening prolongs survival; the effects of screening on survival as opposed to mortality are complex because of the potential for lead-time bias and further analysis of these data are outwith the scope of the current study.

Changes in ER status may have contributed to the survival improvements observed in the study; breast cancer-specific survival in the 1984-86 cohort was significantly lower than in 1996-97. In the Cox proportional hazard analysis, the effect of cohort on survival was adjusted for ER status alone, the cohort remained a significant independent factor in survival - the difference in survival between cohorts is not fully explained by differences in ER status. This is not surprising - treatment and global management changes have undoubtedly contributed to changes in survival over time (Bradburn 1998; Thomson 2004). However a true change in ER status could also have implications for the application of data from clinical trials carried out in previous decades to the women of today, as discussed further in 'Conclusions, Implications and Future Research' section below.

Chapter 5: Conclusions, Implications and Future Research

The purpose of this thesis was to explore changes in the incidence and molecular epidemiology of breast cancer in Scottish women up to 2003 and the possible factors contributing to these changes. The study concentrated particularly on incidence in women aged 50 to 64 an age range in which a wide variety of factors could have been contributing to breast cancer incidence. The incidence of breast cancer in Scottish women aged 50-64 was shown to have increased within expected limits during the initial rounds of the NHS breast screening but then to have failed to level off as would be expected. Formal assessment of this shows levels in the late 1990s that were up to 42% higher than would have been predicted from trends that were developing before 1987. Analysis suggests the contribution of birth cohort factors to this pattern, supporting the fact that reproductive factors are likely to have been contributing. Changes in fertility patterns, BMI and use of alcohol could have contributed, although patterns of HRT use would appear not to have made as great a contribution. Completed family size is shown here to be continuing to decrease, and prevalence of first pregnancy over age 35 to increase, with the rates of the latter increasing particularly steeply. Given that these factors contribute to incidence of breast cancer later in life (that is, in the postmenopausal period), there is the potential for ongoing increases in individual risk of postmenopausal breast cancer, and hence continued increases in postmenopausal breast cancer incidence. The patterns shown here does not support a great contribution of HRT use to the population breast cancer incidence in Scotland but if in fact it has been contributing, then continuing decreases in HRT use and hence risk could mitigate any chances produced by reproductive factors. Continuing research into the impact of HRT prevalence on population breast cancer incidence would be informative.

The possibility of modifying hormonal risk factors in an attempt to reduce breast cancer incidence is controversial, as decisions about family size and age at first pregnancy are highly emotive decisions made by individuals based on many factors. Perhaps information on the contribution of hormonal factors to breast cancer

could be included in general information aimed at promoting breast health, e.g. in GP surgeries, but any stronger attempts to influence decisions on family planning are unlikely to be welcomed. Obesity is a more modifiable risk factor (although again, modification relies on decisions being made by individuals about their health). The Scottish government has begun a campaign to reduce obesity, and perhaps information about the excess cancer risk posed by obesity could be included within such advertising campaigns. When it comes to HRT prescribing, women should always be able to make an informed decision, and should be given information about the risks and benefits, including the increased risk of breast cancer.

Predicting cancer incidence trends is important for the future distribution of health service resources. While inadequate to allow accurate prediction of future trends, analyses here are an important initial step in suggesting future areas of research. These could include complex modelling of trends in hormonal factors based on their known contributions to individual risk, in an attempt to refine predictions of breast cancer incidence. Such models could also assess the potential effect of risk factors on the biology of breast cancer in terms of more ER positive disease. In addition, the author acknowledges that despite the obvious importance of hormonal factors in breast cancer risk, there remains the possibility that an as yet undetermined temporal factor is the major driving force in population breast cancer trends. Further research into the contribution of birth cohort effects (as a surrogate for reproductive risk factors) and period (temporal) effects to breast cancer incidence, and hence determining which of these will have continued relevance to ongoing breast cancer incidence, would also be useful. While formal 'age-period-cohort' modelling is one way of doing this, there is growing interest in the 'median polish' statistical method which is even better at untangling the complex interaction of birth cohort and temporal effects in cancer research and this could be applied to the data used in this thesis. Indeed this is a research area that the author intends to continue to pursue.

Changes in screening and their possible effects should continue to be studied. The introduction of two-view mammography at 'subsequent' screens, and age extension in either direction, could cause apparent increases in incidence which actually reflect lead-time bias.

This work has also shown that breast cancer incidence appears to be rising in parallel across all socioeconomic categories, although the most affluent continue to have significantly higher rates than the most deprived. Certain risk factor trends could explain this pattern – patterns in late age at first pregnancy mirror this change, with rates increasing over time across all socioeconomic categories but with a persistently higher rate with higher socioeconomic status. Again, the rate of change is increasing with every year. Hence it is important that the increasing rates of breast cancer in deprived women continue to be investigated and monitored, as there is the danger of deprived women missing out on information and advice if breast cancer is thought of as a 'disease of the affluent' and resources distributed accordingly.

The conclusions of the laboratory work were that the biology of breast cancer is changing towards more ER positive disease. As discussed above, the fact that this change was 'cancelled out' by the presence of screen-detected tumours on the Cox regression analysis does not rule out the possibility of a change in biology, as screen-detected tumours are more likely to be slower-growing, as in ER positive disease. There are several limitations to the methodology used here. Certainly the information reflects changes that would have been ongoing up to ten years ago, suggesting that it would be of critical importance to repeat the project in the future in order to assess more recent changes in molecular epidemiology. One disadvantage is that the project only assesses the proportions of ER positive to ER negative disease; an interesting area of future research would be to directly calculate trends in the prevalence of ER positive and ER negative disease to assess if the increased incidence of breast cancer is exclusively due to more ER positive disease, or if rates of ER negative disease have also increased, as this would be of greater predictive value.

The implications of rising numbers of ER positive disease could have the simple effect of increasing demand for tamoxifen and aromatase inhibitors. It could also have implications for the application of data from clinical trials carried out in previous decades to the women of today. The original clinical trials of many chemotherapeutic and hormonal therapy agents used today were carried out in the 1980s and 1990s. In certain groups, especially postmenopausal women, the survival benefit is narrow. If the molecular biology of women has truly altered towards

disease with better prognosis it is possible that such margins may be smaller now if such trials were repeated today. Rising levels of better-prognosis disease and the consequent rise in numbers of survivors of a disease will also have implications in terms of research into longterm follow-up and the prevention of late recurrences.

Survival analysis of the patients in the study showed that a change toward better-prognosis disease did not fully explain the observed improvements in survival, as expected. Significant changes in grade distribution were seen between 1984-86 and 1996-97. There was a reduction in the frequency of grade 3 tumours and an increase in the frequency of grade 1 tumours, a change that seemed to be exerted by the presence of screen-detected tumours in the second cohort, as there was no significant difference in the grade distribution of the symptomatically-detected tumours in both cohorts. An attempt was made to assess whether or not this was due to length bias (preferential detection of less aggressive tumours by screening) by removing from the analysis women who were estimated to be having their first screen, and the difference in grade distribution persisted. This would appear to support the concept of 'phenotypic drift' of breast cancer toward higher grade with time. Research into this controversial concept could have profound implications in for the benefit of screening programmes and for interpretation of survival data. As observation of the phenomenon *in vivo* is obviously not possible, continued research and audit of tumour grade within screening programmes is the best way of investigating the concept.

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Appendix 1: Lexis diagram

	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
1920	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83
	142.8	172.0	179.4	201.4	178.1	241.9	201.1	243.2	219.6	180.4	206.1	207.4	238.9														
1921	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77					82
	117.4	228.5	162.0	212.2	169.6	245.5	155.5	221.1	196.1	271.7	249.8	204.3	187.2	219.9													
1922	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76					81
	136.2	149.8	141.9	174.4	237.7	199.2	211.3	207.6	157.9	196.9	250.9	275.8	256.1	293.9	260.7												
1923	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75					80
	204.2	180.2	201.4	167.2	175.1	196.9	229.5	225.3	193.6	178.8	220.6	260.3	245.6	216.2	304.6	245.6											
1924	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74					79
	149.1	96.0	145.2	133.4	195.1	197.2	219.4	151.4	214.6	207.3	227.9	248.9	237.9	253.4	269.5	267.4	226.2										
1925	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73					78
	166.7	181.0	149.9	203.9	195.1	146.6	188.2	193.4	219.3	163.0	189.4	248.5	272.5	280.3	236.7	271.7	288.9	212.6									
1926	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72					77
	157.2	136.1	162.5	144.6	166.8	203.8	166.5	187.7	163.0	222.0	254.8	206.3	215.7	250.4	254.3	233.1	237.3	250.0	294.3								
1927	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71					76
	137.9	125.8	94.1	173.4	177.4	208.4	196.9	181.7	183.3	250.6	194.0	199.5	292.8	285.3	347.3	253.7	183.5	236.7	249.5	278.9							
1928	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70					75
	195.2	163.5	151.4	165.6	159.8	150.8	202.4	193.9	198.9	204.2	153.5	144.4	180.8	261.7	352.2	371.7	257.7	231.8	228.7	313.3	231.3						
1929	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70				74
	176.1	170.5	118.7	159.1	199.3	144.7	172.3	170.1	184.8	166.2	194.9	217.4	208.3	262.6	347.0	333.5	338.0	265.2	213.1	262.6	216.9	339.7					
1930	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71		73
	169.5	180.1	200.5	159.3	180.9	139.8	153.8	138.3	179.0	186.9	269.1	149.4	235.4	237.3	368.0	330.2	277.2	363.4	179.1	252.5	200.2	257.0	312.5				
1931	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
	148.3	168.5	146.4	153.6	156.5	127.9	184.6	175.6	163.2	184.2	168.4	213.7	191.0	251.0	294.7	308.0	307.9	254.1	347.5	202.0	190.1	299.1	250.5	227.6			
1932	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71
	137.6	161.4	185.1	159.2	156.3	190.0	177.9	179.0	176.8	191.4	152.2	217.6	225.2	299.1	260.0	296.6	246.3	259.2	294.1	246.7	242.6	242.1	268.2	288.2	277.6		
1933		45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70
		176.7	170.5	143.7	129.4	211.9	123.7	165.8	131.9	181.6	175.6	205.2	227.4	311.4	328.2	344.9	256.3	320.4	263.8	352.9	300.0	257.7	222.3	264.9	236.5	249.3	
1934			45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
			124.1	152.2	181.6	196.0	172.8	159.7	153.4	147.3	162.2	226.8	223.6	224.8	290.6	245.3	279.4	332.4	294.5	263.6	322.8	310.5	237.5	228.5	285.9	222.5	242.7
1935				45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68
				154.2	178.8	135.5	187.1	153.7	188.6	179.3	155.9	187.9	181.2	217.0	253.3	332.2	216.6	200.1	330.6	278.8	281.0	331.4	271.4	285.6	299.7	331.4	343.9

1936					45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67
					187.0	181.1	121.4	176.0	169.7	156.9	157.7	172.2	196.9	277.5	226.6	234.8	253.1	261.3	283.9	282.5	281.5	261.5	267.7	303.0	276.3	200.3	325.0
1937						45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
						172.8	177.0	130.8	155.0	159.2	193.9	174.4	188.3	178.6	255.5	318.8	268.4	294.3	267.7	230.7	307.3	295.5	359.6	319.3	362.9	250.9	280.4
1938							45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65
							164.3	178.1	158.3	189.5	118.9	160.4	167.5	308.2	226.9	241.4	238.8	226.1	290.5	267.8	293.9	313.8	319.8	366.3	387.5	317.6	261.0
1939								45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
								201.3	124.5	148.5	193.2	170.1	218.0	232.1	260.2	274.6	275.5	241.8	281.1	237.4	274.1	321.4	298.4	332.7	320.6	313.0	308.5
1940									45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63
									155.5	145.9	166.8	164.1	181.5	222.9	268.4	245.0	276.8	246.5	261.3	237.9	316.6	321.2	298.0	366.9	333.7	361.5	317.2
1941										45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62
										141.6	160.0	178.4	189.2	178.8	186.7	266.3	284.6	177.1	286.2	272.3	291.9	285.8	330.7	369.5	289.5	306.7	335.2
1942											45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61
											146.2	204.8	119.5	147.1	185.3	281.6	226.9	213.6	262.7	250.4	318.0	270.2	264.2	240.7	362.2	357.8	359.7
1943												45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
												197.5	142.6	168.7	211.2	179.1	218.3	231.4	202.6	229.5	240.1	300.3	327.8	369.0	247.3	318.8	356.9
1944													45	46	47	48	49	50	51	52	53	54	55	56	57	58	59
													138.4	164.5	196.9	158.2	190.6	258.6	298.1	334.8	231.5	196.1	298.4	342.2	273.8	334.4	362.2
1945														45	46	47	48	49	50	51	52	53	54	55	56	57	58
														130.7	184.6	154.5	208.3	211.4	279.1	303.3	297.1	307.5	250.7	295.6	285.7	259.6	301.9
1946															45	46	47	48	49	50	51	52	53	54	55	56	57
															135.9	207.2	165.4	220.7	194.9	283.2	326.6	278.3	268.8	279.4	299.8	327.5	285.4
1947																45	46	47	48	49	50	51	52	53	54	55	56
																175.2	152.7	182.8	175.5	221.5	300.4	316.3	304.5	254.3	203.8	268.2	240.6
1948																	45	46	47	48	49	50	51	52	53	54	55
																	152.5	158.1	224.1	211	189.6	275.7	337.4	351.9	227.6	253.3	278.9
1949																		45	46	47	48	49	50	51	52	53	54
																		102.4	205.1	148.3	203	217.8	358.9	356.9	349.1	263.2	289.2

Appendix 2: Published Papers

Breast Cancer Res Treat
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ORIGINAL PAPER

Breast cancer incidence trends in deprived and affluent Scottish women

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Abstract
Objective Breast cancer is commoner in the affluent and breast cancer rates in many countries are rising; it remains unclear whether this incidence rise is consistent across the different socio-economic groups. The rising incidence of breast cancer may be related to changes in population risk factor profiles. This study aimed to determine breast cancer incidence trends in women of different socio-economic categories and whether these trends were related to breast cancer risk factor trends.
Design Data on breast cancer incidence rates by deprivation quintile in Scotland 1991–2000 were analysed using linear regression. Data on first births at late maternal age, BMI trends (based on the Scottish Health Surveys) and breast screening uptake trends in the different categories were also analysed and their relation to breast cancer incidence trends explored.
Population and setting Breast cancer incidence data was based on all women in Scotland. BMI data was based on representative cross-sectional survey data from the Scottish Health Surveys—women in the 1995, 1998 and 2003 surveys were 16–64, 16–74 and aged 16 and over, respectively. First birth data was based on all women aged 35–39 in Scotland. Breast screening uptake data was studied in women of screening age, that is, aged 50–64.
Results Breast cancer incidence rates in Scottish women are rising in parallel across all socio-economic categories and the incidence gap between deprived and affluent still remains. Since the late 1980s, numbers of first birth in Scottish women aged 35–39 have risen dramatically, especially in the affluent, but numbers were stable before this. The prevalence of obesity and mean BMI has increased over time in all socio-economic classes but BMI continues to be higher in the deprived. Uptake of screening invitations has increased in all socio-economic groups.
Conclusions Breast cancer is rising in women of all socio-economic status in Scotland and the deprived–affluent gap remains. Trends in late age at first pregnancy, prevalence of obesity and screening uptake do not fully explain the observed trends.

Keywords Epidemiology · Breast · Cancer · Incidence · Deprivation · Socio-economic · Reproductive

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Introduction

An excess risk of breast cancer in affluent women has long been recognised [1–6]. The reasons for this are indeterminate, but established risk factors for breast cancer such as first pregnancy over 35, nulliparity, use of hormone replacement therapy and oral contraceptives and increased BMI could differ between socio-economic groups [7–9] and result in a social gradient of incidence. Social inequalities in uptake of screening [10] could also potentially contribute to incidence differences. Incidence of breast cancer in the UK, as in many countries worldwide, is rising. To ascertain whether incidence of breast cancer is rising to the same

extent in all socio-economic categories, a study of incidence trends in deprived and affluent women in Scotland was performed. The population of Scotland is well-suited to such analyses due to its stability and wide socio-economic spectrum.

Recent breast cancer incidence trends could be influenced by mammographic screening programmes and reproductive risk factors. A study of risk factor and screening trends in affluent and deprived women was therefore performed to establish if changes over time in these risk factors reflect breast cancer incidence trends in the different socio-economic groups.

Methods

Incidence

Incidence data for breast cancer incidence per 100,000 women (age-standardised to the European Standard Population) from 1991 to 2000 in each deprivation quintile of the Scottish population was obtained from the Information and Statistics Division of the Scottish Executive (ISD) [11]. Deprivation quintiles divide the Carstairs and Morris index—an index calculated for individual postcodes based on factors such as occupation of head of household—into five equal categories. Data on age and menopausal status of the patients in each of these categories was not available. Linear regression of trends in incidence was performed for each quintile with an interaction term added to the analysis to assess whether regression slopes for each deprivation quintile were significantly different.

Risk factors/screening

Late age at first pregnancy

Information and Statistics Division of the Scottish Executive [11] provided data on numbers of first pregnancies at different maternal ages every year from 1976 to 2001. Data on births to mothers aged 35–39 at first pregnancy was chosen for analysis, due to the association of late age at first pregnancy and breast cancer risk. These data were divided by deprivation quintile. Linear regression was performed for each quintile to establish the significance and the magnitude of these trends, with an interaction term added into the analysis to assess whether trends differed significantly between quintiles. Parity data by deprivation category is not maintained by any agency.

BMI

The Scottish Health Surveys of 1995, 1998 and 2003, cross-sectional studies each of around 5,000 subjects [12–14] have been shown to be representative of the Scottish population. Part of these surveys involved the measurement of BMI. For 1995 and 1998, data was available on obesity prevalence and mean BMI in women by Registrar General Social Class, and for 2003, data was available for obesity prevalence and mean BMI in women by Scottish Index of Multiple Deprivation (another classification based on Carstairs and Morris index, in turn based on social class). The association of BMI and social class in each study year was analysed using Spearman's correlation.

Screening

The Scottish Breast Screening Programme is part of the UK-wide NHS Breast Screening Programme. Up until the recent introduction of an age-extension project, the Programme invited women aged 50–64 for 3-yearly mammography. Data on percentage uptake of screening invitations for every year from 1990/1991 to 2001/2002 was provided by ISD [15]; the older classification method of deprivation category, which divides Carstairs and Morris index into seven unequal categories, was used in this data. Linear regression of trend in screening uptake by year was performed for each category, including an interaction term.

Results

Incidence and socio-economic gradient

It is clear that between 1991 and 2000, breast cancer incidence rates in Scotland continue to be lower with increasing deprivation. Linear regression reveals a significant rise in incidence over this period in all quintiles except for quintile 2. In the most affluent the rise in incidence was of the magnitude of 1.9 cases per 100,000 per year and in the most deprived 1.0 cases per 100,000 per year. Interaction analysis revealed regression slopes to be parallel ($P = 0.778$), confirming that incidence rates are rising to the same degree in all deprivation categories (Fig. 1).

Risk factors

Late age at first pregnancy

Figure 2 demonstrates that the overall numbers of first births of mothers aged 35–39 in Scotland were fairly

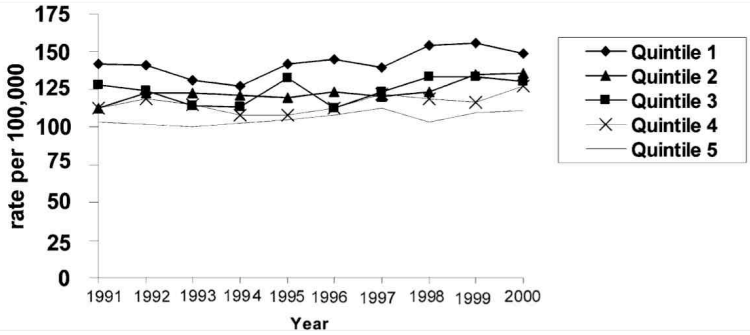

Fig. 1 Incidence of breast cancer in Scotland by deprivation quintile

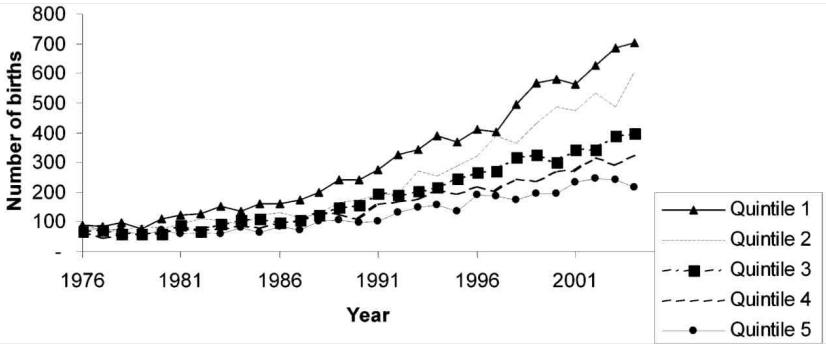

Fig. 2 First births at maternal age 35–39 (Scotland) by deprivation quintile

stable throughout the late 1970s and 1980s and were always higher in the affluent than the deprived. From the late 1980s onwards, however, numbers have been increasing markedly in all socio-economic groups, with the rate of increase clearly highest in the most affluent and lowest in the most deprived. Regression analysis reveals the increase in all groups to be statistically significant, and the presence of a widening gap between the groups is confirmed by interaction analysis ($P < 0.01$).

BMI trends

As seen in Table 1, in both 1995 and 1998, mean BMI appeared to increase slightly from social class I to V although the correlation was not statistically significant (1995, $P = 0.397$, and 1998, $P = 0.084$). The mean BMI in each social class had changed little between 1995 and 1998. Although a different classification of socio-economic status was used in 2003, precluding direct statistical comparison, mean BMI in all categories had increased markedly since 1998. The correlation of higher BMI with increasing deprivation was significant ($P < 0.01$) (Table 2).

In 1995 a statistically significant inverse association of social class and obesity prevalence was seen ($P = 0.019$). In 1998 the significant association was still present ($P < 0.01$). Prevalence of obesity in all classes appeared to have increased between 1995 and 1998 but grouping of social classes together in 1998 meant that direct statistical comparison was not possible. In 2003 a statistically significant inverse association between quintile of Scottish Index of Multiple Deprivation and obesity prevalence was still noted ($P < 0.01$). Again, in 2003 the prevalence of obesity in all quintiles appeared to have increased from the prevalences in individual categories in 1998 but a statistical comparison could not be made (Tables 3, 4).

Uptake of screening invitations

A socio-economic gap in uptake of screening invitations has persisted from 1990 onwards, with an absolute difference in uptake of 22% between category 1

Table 1 Mean BMI (kg/m^2, age-standardised), women aged 16–64 by Registrar General Social Class, from Scottish Health Survey 1998 [14]

	I	II	IIINM	IIIM	IV	V
1995	22.8	22.3	23.5	23.6	23.3	23.0
1998	22.8	22.9	22.9	24.0	23.8	23.6

Table 2 Mean BMI (age-standardised), women aged 16 and over by quintile of Scottish Index of Multiple Deprivation, from Scottish Health Survey 2003 [13]

	First	Second	Third	Fourth	Fifth
2003	26.4	26.9	27.1	27.4	28.1

Table 3 Percentage prevalence of obesity (BMI > 30) in women aged 16–64 (1995) and 16–74 (1998) by Registrar General Social Class, from Scottish Health Surveys 1995 [12] and 1998 [14]

	I	II	IIINM	IIIM	IV	V
1995	13.9	9.9	17.8	18.1	22.1	20.2
1998	18.2		19.7	25.5	26.0	

Table 4 Percentage prevalence of obesity (BMI > 30) in women aged over 16 by quintile of Scottish Index of Multiple Deprivation, from Scottish Health Survey 2003 [13]

	First	Second	Third	Fourth	Fifth
2003	21.0	22.8	27.7	27.9	32.1

and category 7 in 1990/1991, and a difference of 17% between category 1 and category 7 in 2001/2002. Linear regression of uptake rates for each year revealed that screening uptake has risen slightly but significantly in all seven deprivation categories between 1990/1991 and 2001/2002. The magnitude of the rise was seen to be 2.7% in category 1 and 7.3% in category 7, but interaction analysis revealed that the uptake is not significantly different between categories (P for interaction term = 0.438) (Fig. 3).

Discussion

Breast cancer incidence is rising in the UK, in common with many other countries throughout the world. Breast cancer is commoner in the affluent than the deprived. The question remains as to whether incidence rates are rising in parallel across all social classes. Dano et al. [1] studied incidence rates over time in social classes in over one million women in Denmark and found that rates in the affluent were rising at a lower rate than before and rates in the deprived rising at a faster rate than before. Recent trends in those enrolled in the Longitudinal Cohort Study of 250,000 UK women support this same narrowing incidence gap [16]. It is demonstrated here that breast cancer incidence rates in Scotland between 1991 and 2000 were increasing in parallel in affluent and deprived women. Age breakdown of the figures was unfortunately not available.

Reasons that have been postulated for the increasing incidence of breast cancer include the influence of the UK-wide Breast Screening Programme and changing trends in known risk factors for breast cancer—for example increasing age at first pregnancy, reductions in parity, use of hormone replacement therapy and oral contraceptives and changing patterns in BMI. Such risk factors could differ between socio-economic categories [7–9], and indeed work carried out in Marin County in California, a geographical area with an affluent population and corresponding excess of breast cancer suggests that differences in reproductive risk factors may explain the observation of higher breast cancer rates in the affluent [8, 9, 17]. Differing uptake of screening invitations among women of different socio-economic categories is also well-known phenomenon, with a higher percentage of affluent women taking up screening invitations [10, 18].

The fact that incidence rates are increasing to the same extent in all deprivation categories prompted an investigation of whether reproductive and screening trends are changing to a similar extent in all the socio-economic categories. These factors are positively associated with breast cancer risk in postmenopausal women; for premenopausal breast cancer the associations are less clear and may have opposite effects on breast cancer risk [19–21]. Indeed, it is a limitation of the epidemiological data that there is no age breakdown within the categories of deprivation. However, rates of premenopausal breast cancer are relatively low and it is reasonable to assume that rates in postmenopausal women in this study will account for a large part of the incidence rates. Similarly, changes in screening could be reasonably expected to make a contribution to overall breast cancer incidence.

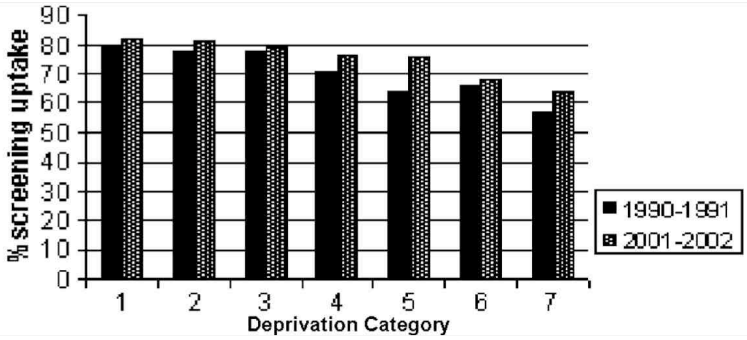

Fig. 3 Comparison of screening invitation uptake rates 1990/1991 and 2001/2002 by deprivation category

A study of numbers of first births in women aged 35–39 revealed that numbers per year have always been highest in the most affluent and lowest in the most deprived. This may be a potential explanation for the persisting high rates of breast cancer in the affluent; a woman with a single pregnancy over age 35 can have up to double the risk of a woman with several births at a young age [21]. Since the late 1980s, the number of first births at late maternal age has been markedly increasing every year in all deprivation categories, albeit to a greater extent with each successive quintile. This is a potential explanation for the rise in breast cancer rates across the whole socio-economic spectrum. However, those women who had late first pregnancies from the late 1980s onwards will only now be becoming perimenopausal and it is possible that the contribution of rising age at first pregnancy to rising breast cancer rates has in fact been relatively minor so far. Indeed, the steep increase in late first pregnancies from the late 1980s may result in an even steeper rise in breast cancer rates in the near future when these women become postmenopausal, and the rise may be more marked in affluent women. Data on parity differences between the affluent and deprived women may have given additional information on reproductive influences on breast cancer incidence, but such data is not maintained by any agency.

Extrapolating from cross-sectional survey data, it appears that the prevalence of obesity and mean BMI in Scottish women of all socio-economic categories increased between 1995 and 2003. Again, this could potentially contribute to rising breast cancer incidence across all socio-economic categories; a pooled analysis of several studies has suggested that the relative risk of breast cancer in women with a BMI over 28 is 1.26 [22] (RR 1.0 for women with a BMI under 21). This level of risk suggests that rising levels of obesity could influence patterns of breast cancer incidence. However, the surveys suggest that BMI and obesity have consistently remained higher in the lowest socio-economic categories, and thus BMI differences do not explain the observed socio-economic gap in incidence.

The presence of an organised screening mammography programme can strongly affect breast cancer incidence rates. Women undergoing incidence screens have incidence rates that should reflect the background incidence, but the youngest women in the programme are always undergoing 'prevalence screening', with the detection of a relatively large number of asymptomatic tumours which have been present for varying lengths of time [23]. An increase in the percentage uptake of screening invitations is likely to result in increased prevalence screening and therefore may cause incidence rates to rise. Figures for the Scottish Breast Screening Programme show that screening uptake over 1990–2000 increased by a few percent in all deprivation categories—this is unlikely to be enough to explain the overall breast cancer incidence rises in all categories. The socio-economic gap in uptake has persisted over time, although it is small, with an absolute difference in uptake of 17% between highest and lowest quintile in 2001.

These data suggest that breast cancer incidence in Scotland is rising in all deprivation categories but that rates remain higher in the affluent. Reproductive trends shown here may explain the persistent socio-economic gap, but do not appear to be an adequate explanation for rising breast cancer rates. A rising prevalence of obesity could be contribute to rises in breast cancer rates, but would not explain the deprivation–affluence incidence gap. Screening differences are of insufficient magnitude to explain either phenomenon.

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EPIDEMIOLOGY

Increasing incidence of breast cancer: distinguishing between the effects of birth cohort and a national breast screening programme

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Abstract The incidence of breast cancer in post-menopausal women has been affected by the introduction of national breast screening programmes. The study describes the incidence of breast cancer in Scottish women aged 50–64 by year of birth before, during, and after the prevalent round of screening. Breast cancer registrations in Scotland for women aged 45–69 years from 1977 to 2003 were obtained. Birth cohort incidence rates were calculated and interpreted in the light of screening patterns at particular calendar time points. In the years before screening, there was a small rise in breast cancer incidence by birth cohort in women aged 50–54 which was not seen in other ages. During the prevalent screening round, incidence increased significantly with increasing birth cohort and thereafter continued rises in incidence by birth cohort occurred. The observed rise in breast cancer incidence among post-menopausal women is likely to be due to both screening effects and a true increase in incidence.

Keywords Breast · Cancer · Epidemiology · Incidence · Screening · Cohort

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Introduction

The incidence of breast cancer in Scotland is rising in common with many other countries, with the increase being greatest among post-menopausal women [1–3]. The increase may be artefactual or real. The most likely explanation for an artefactual increase is the introduction of a national breast screening programme, which would be expected to detect earlier, asymptomatic breast cancers and thus result in a transient rise in incidence during its initial "prevalent" round [4, 5]. In Scotland, the prevalent round of screening began in 1987 and was gradually extended throughout the country to all eligible women aged 50–64 years by 1994. An age extension to 70 years was introduced in certain areas of Scotland in 2003.

A true increase in incidence of breast cancer might be due to changing reproductive patterns [2, 6] as nulliparity and late age at first pregnancy are significant risk factors for the development of the disease [7–10]. Population-based changes in other factors such as alcohol consumption may also be contributing [11]. Changing fertility patterns are postulated to have a distinct cohort effect on breast cancer incidence rates [2, 3, 9]. Women born in the same year or year range (birth cohort) can be expected to have similar incidence rates at any age as a result of similar population 'risk exposure' during their reproductive years. However, these birth cohort effects are altered by the introduction of a national breast screening programme as it selectively increases cancer detection in some birth cohorts in particular calendar years.

We have not identified published analyses of breast cancer trends that have attempted to disentangle these artefactual and real components of rising incidence rates. Our aim was to describe patterns of breast cancer incidence in Scotland over a period when the first round of a national

breast cancer screening programme was introduced and completed and to distinguish between birth cohort effects (which might indicate true changes in incidence) and the effects of screening.

Methods

We obtained data on all new diagnoses of female breast cancer in Scotland between the calendar years 1977 and 2003 inclusive from the Scottish Cancer Registry, which operates within the Information Services Division of the NHS in Scotland. The Registry collects all incident cases of cancer nationwide via a compulsory notification system. Age and year specific breast cancer incidence rates per 100,000 were supplied to us for women aged 45–69 years. We calculated 5 calendar year mean incidence rates for ages 45–49, 50–54, 55–59, 60–64 and 65–69 for each birth year cohort from 1920 to 1949. We identified the point—if there was one—at which each birth cohort became eligible for screening in the initial round between 1987 and 1994. Breast cancer incidence rates were then plotted against birth year for each age range in a Lexis diagram, with the period indicated at which eligibility for screening began. Ninety five percent confidence intervals for the differences in breast cancer incidence rates between years were calculated using the method described by Altman and others for differences in proportions for paired samples [12]. Data on the NHS Breast Screening Programme in Scotland itself were also requested from the Information Services Division in order to aid the interpretation of the findings.

Results

In Scottish women the age-adjusted (to European population) incidence rate of breast cancer has increased by 46% from 80.7/10^5 women in 1977 to 118.0/10^5 in 2003. In women aged 45–69 the crude incidence rate increased from 178.2/10^5 women in 1977 to 293.9/10^5 in 2003, an increase of 65% (an increase of 115.7/10^5, CI 73.2–158.2). Figure 1 shows breast cancer incidence rates in women aged 50–64 by birth year cohort, with incidence rates grouped by 5-year age-group. Each point represents the age-group mean incidence rate of women born in the same year. The data for each point are therefore necessarily drawn from 5 calendar years and can be interpreted as rolling averages. For example, women born in 1933 were aged 50–54 between 1983 and 1987. Some of the 50–54-year old age-group who were born in 1933 were thus eligible for screening when the round began in 1987, albeit only those aged 54. In the 1934 birth cohort, only 53 and 54-year olds were eligible for screening. The 1937 birth cohort was thus the first in which all 50–54-year olds were eligible for screening in the prevalent round. Similarly, as women in the oldest ages of any age-group reach the end of the prevalent round in 1994 they will then go on to be screened during the established ("incident") round of screening. Thus the 1940 cohort is the last in which all 50–54-year olds might be part of the prevalent screening round.

Figure 1 shows that the incidence of breast cancer was higher with increasing age between 50 and 64 years. This was consistently found in all birth year cohorts. In 50–54-year olds and 55–59-year olds there were small increases in incidence with birth cohort year before the screening programme began. In all age-groups there were significant rises in incidence throughout the duration of the prevalent round. The fall in incidence that was expected after the majority of Scottish women had been screened at least once [4] was not observed. Large rises in breast cancer incidence with increasing birth cohort continued after the screening programme had been fully established (although as described below, the confidence intervals were wide).

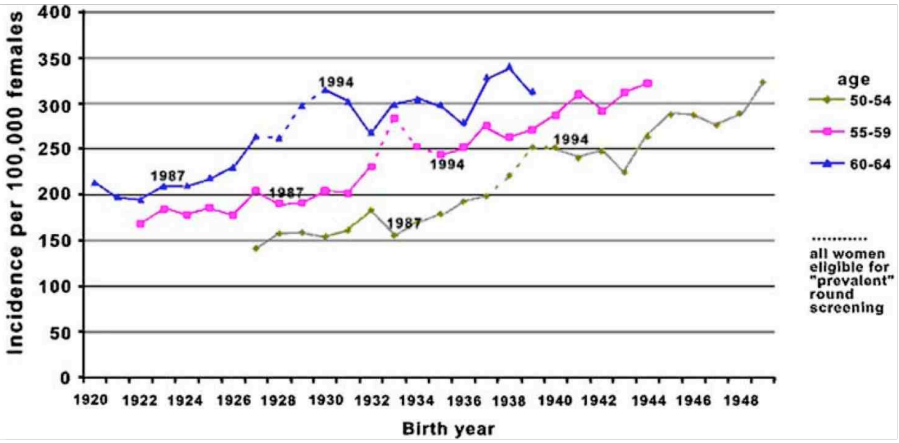

Fig. 1 Breast cancer incidence in women aged 50–64 years, by year of birth and age, 1977–2003. For each age-group, "1987" and "1994" mark the points when the oldest individuals began and ceased, respectively, to be eligible for the prevalent screening round

Rising incidence with increasing birth cohort occurred in parallel across all three age categories.

In 50–54-year olds, cohort incidence changed from 141.7 to 183.0/10^5 in the years before screening (a rise of 41.3/10^5, 95% CI 6.1–76.5); from 155.7 to 263.8/10^5 (a rise of 108.1/10^5, 95% CI 68–148.2) in cohorts where some or all women were eligible for screening in the prevalent round; and from 287.5 to 323.5/10^5 (a rise of 35.9/10^5, 95% CI −12.45 to 82.9) in cohorts where women were entering an established screening programme. In 55–59-year olds, cohort incidence changed from 168.0 to 204.2/10^5 in the years before screening (a rise of 36.2/10^5, 95% CI −1.5 to 73.9); from 190.6 to 271.17/10^5 (a rise of 80.5/10^5, 95% CI 38.5–122.7) in cohorts where some or all women were eligible for the prevalent round; and from 287 to 322.2/10^5 (a rise of 35.2/10^5, 95% CI −13.1 to 83.5) in cohorts who were entering screening during the 'incident round'. In 60–64-year olds, cohort incidence changed from 213.1 to 194.6/10^5 in the years before screening (a decrease of 18.5/10^5, 95% CI −17.4 to 54.4); from 209.53 to 304.8/10^5 (a rise of 95.2, 95% CI 50.8–146) in cohorts where some or all women were eligible for screening in the prevalent round; and from 298.7 to 314.7/10^5 in the cohorts beginning screening after the prevalent round was complete (an increase of 16/10^5, 95% CI −32.4 to 64.4).

Figure 2 shows breast cancer incidence in the same birth cohorts presented in Fig. 1 but for women in the 5-year age-groups below and above the screening ages. There was no appreciable change in incidence in 65–69-year olds from the 1922 to the 1948 birth cohort. Among 45–49-year olds, there was little change in incidence until the 1942 birth cohort and a small but non-significant rise thereafter from 160.6 to 175.3 (a rise of 14.7 cases/10^5 female population, 95% CI −21.1 to 50.5) in the 1948 cohort.

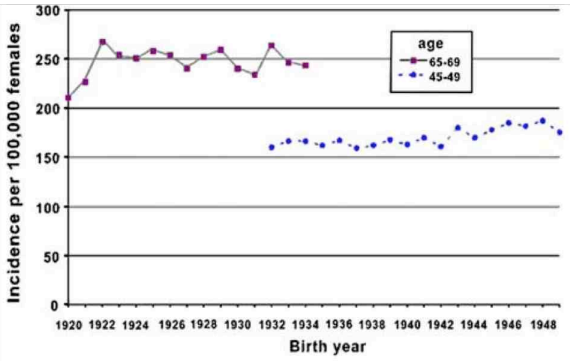

Fig. 2 Breast cancer incidence in women aged 45–49 and 65–69 years, by year of birth and age, 1977–2003

Discussion

Our analysis confirms that significant increases in breast cancer incidence in the United Kingdom have occurred among women who were eligible for screening. The anticipated fall in incidence after the prevalent round of screening had been completed was not observed. Instead, there was a suggestion that continued increases in incidence among 50–64-year old women occurred. Increases in incidence had also been developing before the screening programme began (as demonstrated by the incidence rise with birth cohort in women aged 50–54). True increases in incidence—possibly due to changing reproductive patterns—rather than screening may explain these patterns. Such an explanation is supported by the fact that breast cancer incidence has been shown in many countries worldwide to differ between birth cohorts [3, 6, 13]; women born in different years may have different population risk exposures as a result of trends in fertility while they were in the reproductive years of life and hence have a different risk of breast cancer at all ages. This could impact on population incidence. Several studies have shown links with trends birth cohort incidence of breast cancer and the corresponding population fertility trends related to each birth cohort year [3, 6, 13]. In Scotland, family size has gradually fallen since the 1935 maternal birth cohort year [14] and this may have contributed to a rise in breast cancer incidence.

Hormone replacement therapy increases risk of breast cancer, with a large meta-analysis of trial results concluding that the risk of breast cancer increases by a factor of 1.023 for every year of use and that this increased risk disappeared 5 years after cessation of use. Changes in the population use of HRT could affect population breast cancer risk and hence population breast cancer incidence. Evidence from Australia and the US [15, 16] suggests that breast cancer incidence could reflect population HRT prevalence. The use of hormone replacement therapy in the UK doubled between 1973 and 1976, fell again and began to rise substantially from the late 1980s onwards; prevalence in England was estimated to have risen from 2.2% of 45–64-year olds in 1987 to 21.7% in 1994 [17]. Between the mid-1990 and 2001 prevalence of use did not change [18]. Data obtained by the authors from the Information and Statistics Division suggests a similar pattern occurred in Scotland. This would therefore not support changes in HRT prevalence being responsible for the breast cancer incidence changes seen here.

Birth cohort incidence of breast cancer in Scotland since the advent of the organised mammographic screening programme has not been reported previously. Variation in the activity of the breast screening programme since its inception may have produced apparent birth cohort

incidence effects that are in fact late temporal observational biases. Entry into the "prevalent" screening programme is transitional in at least three ways. First, the programme was only introduced to women aged 50–64 over a period of 7 years. In the early years, only a minority of the eligible population would have been screened. As coverage of the population gradually increased, an artefactual increase in the incidence of breast cancer would be expected as more asymptomatic cancers were detected than before [4, 5]. Data on uptake of screening are available from 1990 onwards. These show that about a fifth of all women aged 50–64 participated in prevalent screening each year until it was complete in 1994/1995. The number of incident screens rose through the early 1990s so that by 1992/1993 the number of women screened annually in Scotland reached a plateau of about 100,000, or about 70% of those who were invited. Most of the artificial increase in incidence would therefore have ceased after 1994/1995 and some reduction in incidence due to lead-time bias would be expected for a year or more thereafter. Thus the screening programme would not explain the observed sustained increases in breast cancer incidence.

Second, prevalent and follow-up rounds occur simultaneously after several years of a national screening programme. Women in some birth cohorts will have been offered screening during the prevalent round, others will have not been offered screening at all, and some will have been offered screening when the screening programme had long been established. Third, in order to show age and birth cohort specific rates, we have shown 5-year rolling averages. Thus within any given age-group, full eligibility for screening was only achieved after 5 years. In each of the screening groups, the most marked rises in incidence with rising birth cohort year were within the group of women offered screening during the prevalent round. This initial trend might be entirely explained by the increasing national coverage of the Scottish population by screening. Incidence increased with each birth cohort year. However, this does not explain the continued increase in incidence seen with successive birth cohorts in the 50–54 and 55–59 age-groups after the prevalent round had been completed. There were no major technical changes in the screening programme [19] after this time to account for this continued rise. Although confidence intervals for these rises are wide it still appears that this is a true birth cohort effect. In women aged 60–64 during established screening the relationship of incidence to birth cohort was less clear. In women aged 45–49 a non-significant rise in breast cancer incidence was developing from the 1942 cohort onward; in women aged 65–69 there was little change in breast cancer incidence across the different birth cohorts. It may be that in women aged 65–69, the general effect of ageing on cancer incidence is of greater importance than reproductive risk factors.

There are several limitations to this population-based study. One is the potential for misclassification of screening experience. For example, not all women of eligible age were offered or took-up breast screening between 1987 and 1994. However, uptake of breast screening invitations in the NHS breast screening programme in the UK has changed little since the start of the programme [19] and thus variations in screening uptake should not be a significant source of error. The study method attempted to minimise the potential for misclassification by calculating incidence rates for individual birth cohort years instead of ranges, and dividing women into groups based on a calculation of their likely exposure to screening. Many women being screened during the introduction of the prevalent round may in fact have also had a subsequent screen but are still counted amongst 'women being screened during the prevalent round' as it is the overall effect of the prevalent round on the group that is of interest.

In conclusion, while some of the increases in breast cancer incidence in Scotland in women aged 45–64 can be explained by the prevalent round of screening, continued rises in incidence with later birth cohorts are not readily explained by the screening programme. The reasons for the true increase in birth cohort incidence are uncertain. Possible contributing factors are changes in reproductive and hormonal factors such as changing fertility patterns [3, 6, 13, 14] or changes in HRT prevalence.

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Short Communication

Is the biology of breast cancer changing? A study of hormone receptor status 1984–1986 and 1996–1997

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Using archived tumours, those from 1984–1986 and 1996–1997 underwent immunohistochemistry for hormone receptors and grade analysis. A significant shift towards more ER-positive and low-grade disease was found; this appears to reflect screening practices, but could still influence survival.
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Keywords: breast; epidemiology; hormone receptor status; survival

Breast cancer is the commonest cancer in women in the United Kingdom, with over 40 000 cases diagnosed annually, and the incidence is increasing. Survival rates are also increasing partly due to advancements in hormonal and chemotherapeutic management, in addition to a trend towards multidisciplinary management and specialist surgeons. The UK nationwide mammographic screening programme was also designed to reduce mortality. It has been suggested that the epidemiology of breast cancer may have changed over time, with more oestrogen receptor (ER)-positive tumour than in the past (Pujol *et al*, 1994; Bradburn *et al*, 1998; Li *et al*, 2003; Glass *et al*, 2007). However, studying retrospective data on ER status has the disadvantage that the assays used to establish ER status, and hence their sensitivity and specificity have changed over time. We, therefore, examined trends in the molecular biology of breast cancers in patients from two large centres in Glasgow by performing immunohistochemistry on archived tumour samples, thereby avoiding artefactual changes in receptor status over time. We also examined the survival of study patients.

MATERIALS AND METHODS

Patient selection

This study aimed to compare the molecular phenotype of stored tissue samples from two separate cohorts of patients, defined by the period in which they had their surgery. All female patients who had surgery for operable breast cancer at two teaching units in Glasgow during 1984–1986 and 1996–1997 were identified. The study had the approval from local ethics committee. Full pathological, demographic, screening and 5-year survival data were either available from the Scottish Cancer Registry or obtained from the patient's case record or pathology records. Deprivation status was ascertained using established postcode Carstairs deprivation categories (1–7) derived from 1981 or 1991 census data. For each patient, an archived paraffin-embedded tumour block was searched for within the relevant pathology department. Following sample size determination, there were originally 1076 patients (423 in 1984–1986 (cohort 1) and 653 in 1996–1997 (cohort 2)) from which 900 tumour blocks were available for analysis (323 and 577 in cohorts 1 and 2, respectively).

Tumour sections were prepared according to routine pathological techniques, and then sent to a pathologist for determination of tumour grade using the modified Scarff–Bloom–Richardson scale and marking of suitable tumour areas. Three 0.6-mm circular cores were then taken from the marked areas in each tumour block and placed into paraffin blocks in tissue microarray format. Sections from each block were taken for ER immunohistochemistry, Her-2 receptor and progesterone receptor (PR) to be performed; each full set of sections underwent ER, PR or HER-2 immunohistochemistry at the same time. In all, 862 of the 900 samples (95%) underwent grade analysis, and 20% of the tumours in cohort 1 and 19% of tumours in cohort 2 did not undergo ER immunohistochemistry due to fragmented cores or absence of tumour in the core. For the same reasons, 14% of tumours in cohort 1 and 10% in cohort 2 did not undergo PR immunohistochemistry, and 15% of tumours in cohort 1 and 18% of tumours in cohort 2 did not undergo Her-2 staining.

Oestrogen receptor immunohistochemistry was carried out using Novocastra 6F11 mouse antihuman ER (Novocastra, Newcastle-Upon-Tyne, UK) with a manual protocol at a dilution of 1 : 50, with epitope retrieval carried out using EDTA at pH 8.0 with a microwave pressure cooker technique for 5 min. After the primary antibody step, slides were refrigerated overnight, with the rest of the steps carried out at room temperature. Progesterone receptor immunohistochemistry was carried out using Dako 636 mouse antihuman PR (Dako, Ely, UK), using a dilution of 1 : 50 and epitope retrieval using citrate pH 6.0 and a microwave pressure cooker technique for 5 min, with the final protocol being carried out at room temperature using a Dako Autostainer. Her-2 immunohistochemistry was carried out in a Dako Autostainer at room temperature using the standard Dako Herceptest protocol.

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Analysis

Once immunohistochemistry had been carried out, each core was assessed by light microscopy and scored by an experienced scorer using a weighted histoscore ((% tumour cells scoring at intensity 1) + (2 × % scoring at intensity 2) + (3 × % scoring at intensity 3)). As each tumour had been cored in triplicate, a mean histoscore for each core was calculated. For ER and PR, 'positive' was taken as a histoscore of 10 or over, and for Her-2, positivity was taken as a histoscore of 90 or over. A second experienced scorer examined 10% of the cores (Kirkegaard *et al*, 2006). Statistical analysis was performed using SPSS statistics software (SPSS, Chicago, IL, USA), version 14.0. Comparison of the demographics of the two groups was carried out using χ^2-analysis (Fisher's exact test in one case where numbers were small). Comparison of the molecular profiles in the groups was carried out using χ^2-analysis for hormone receptor or grade status, *t*-test to compare mean receptor levels and Mann–Whitney test to compare median receptor levels. A multivariate analysis was performed using binary logistic regression to assess whether age, screening and deprivation affected the percentage of ER-positive tumours in the groups. Initial tests suggested that there was not a direct linear relationship between ER and age, but ER-positivity rates increased from 60 years of age, and hence 'age over or under 60' as a categorical variable was included in the regression. Kaplan–Meier survival analysis (censored to 5 years) was carried out, using the log-rank test to test for difference between the groups. Stepwise Cox's proportional hazard regression was carried out to compare factors influencing survival.

Table 1 Patient demographics

	1984–1986	1996–1997	*P* for difference
Mean age at diagnosis	56.9	58.4	0.049
Median age at diagnosis	59	58	0.179
Percentage detected at screening	0	29	<0.001
Percentage of patients in each deprivation category			
Affluent	12	17	
Intermediate	41	47	0.05
Deprived	47	36	
Node positive	59.3	42.4	0.001

RESULTS

The demographics of the patients whose tumour blocks were available are seen in Table 1. All tumours in cohort 1 had been detected symptomatically rather than by screening, the screening programme having yet to be introduced in Scotland.

A comparison of the results of immunohistochemistry based on cohort (and screening status within the 1996–1997 cohort) is presented in Table 2. In a multivariate analysis, the significance of the impact of cohort period (1984–1986 or 1996–1997) on ER status did not persist after correction for percentage of patients aged over 60 and screening status, in combination with other factors or on their own. Oestrogen receptor positivity within each 10-year-age range increased from 1984–1986 to 1996–1997 (except in those under 30 years), although the numbers involved meant that this did not approach statistical significance. Table 3 shows Cox's proportional hazard regression analysis of survival;

Table 2 Results

	Cohort 1 1984–1986	Cohort 2 1996–1997	Cohort 2 Symptomatic	Cohort 2 Screened
Grade distribution: percentage grades 1, 2 and 3	8, 49.2 and 42.9	14.9, 48.3 and 36.8 *P* = 0.009 for *vs* cohort 1	12.2, 46.8 and 41 *P* = 0.2 for *vs* cohort 1	22.3, 53 and 24.7 *P* < 0.001 for *vs* cohort 2 symptomatic
ER-positive tumours	64.2%	71.5% (*P* = 0.042)	68.8% *P* = 0.325 for *vs* cohort 1	78.4% *P* = 0.024 for *vs* cohort 2 symptomatic
Mean ER score	97.1	102 (*P* = 0.454)		
Median ER score	104.2 (IQR 0–190)	120 (IQR 0–180) (*P* = 0.774)		
PR-positive percentage	44.9	49.9 (*P* = 0.181)		
Mean PR score	41.2	37.9 (*P* = 0.418)		
Median PR score	0 (IQR 0–80)	8.3 (IQR 0–61) (*P* = 0.181)		
Her-2-positive percentage (*P* = 0.170)	21.5%	20.6%		
Mean Her-2 score	52.2	43.1 (*P* = 0.772)		
Median Her-2 score	0 (IQR 0–50)	0 (IQR 0–67) (*P* = 0.773)		
ER+/PR+ percentage	42.4	46.7		
ER+/PR− percentage	21.8	24.8		
ER−/PR− percentage	33.3	23.5		
ER−/PR+ percentage	2.5	5 (*P* = 0.023)		
5-year breast cancer cumulative survival	0.620	0.887 (*P* < 0.001)	0.874 *P* < 0.001 for *vs* cohort 1	0.976 *P* = 0.148 for *vs* cohort 2 symptomatic

Epidemiology

Table 3 Cox's proportional hazard analysis

Variable	*P*-value	Hazard ratio of death	95% confidence interval
1984–1986 cohort	<0.001	3.43	3.87–4.71
ER-negative status	<0.001	4.29	1.01–1.04
Increasing age (per year)	0.09	1.02	1.01–1.03
Tumour size (per 1 mm rise)	0.01	1.02	−0.63–1.21
Affluent socioeconomic *vs* deprived	0.08	0.29	0.41–1.23
Intermediate socioeconomic *vs* deprived	0.345	0.82	2.18–3.11
Node-positive status	<0.001	2.65	2.99–3.87
Her-2 status in model	Not included in model	Not included	

when the effect of the period of diagnosis on survival was adjusted for differences in ER status of the patients alone, the period of diagnosis (i.e., 1984–1986 or 1996–1997) remained as a significant independent factor in survival. After correcting, for all the factors in the model, the effect of the period of diagnosis on survival persisted, with survival being higher in 1996–1997.

DISCUSSION

A significant change in grade distribution over time was seen in this study, particularly a reduction in the frequency of grade 3 and an increase in the frequency of grade 1 tumours, a change that appeared to be due to screen-detected tumours in cohort 2. The pathological grade of screen-detected tumours has received much attention in the literature, these being of lower grade than symptomatically detected tumours; it is uncertain, however, whether this represents an interruption of 'phenotypic drift' or simply a longer asymptomatic preclinical phase (Tabar *et al*, 1999).

This study also showed an increase over time in the percentage of breast cancers that were ER positive. The increase from 64.2 to 71.5% was significant on χ^2-analysis. The increase did not persist on logistic regression after adjusting for the prevalence of patients over 60 in the groups. However, an increase in the percentage of ER positivity within each 10-year-age group (except those under 30 years) in the study period, although not reaching statistical significance because of the numbers in each subgroup, suggests a trend towards the overall more ER-positive disease in cohort 2. The ER-positive rise also did not persist after adjustment for the screening status of the patients in the groups. This almost certainly reflects the fact that screen-detected tumours are slower growing, and hence more likely to be ER positive than negative. As there is unlikely to be any phenotypic drift from ER-positive to ER-negative status within breast cancers, it is possible that the screening programme has merely detected an excess of ER-positive breast cancers, which have developed as a result of a true change in biology.

There was a significant change in combined ER/PR receptor status over time, most notably a marked decrease in the percentage of tumours that had the poor prognostic ER-negative/PR-negative status. The percentage of tumours that were PR positive and Her-2 positive did not change over time; notably, there was no change in mean score over time for any of the three receptors.

A study of incidence rates of ER-negative and ER-positive breast cancers in a US health plan found that the incidence rate for ER-negative disease had remained relatively constant with a decline from 1999 onwards, whereas for ER-positive disease, there was a significant increase in incidence throughout the study period (Glass *et al*, 2007). Other studies have also suggested an increase in percentage of ER positivity over time (Pujol *et al*, 1994; Bradburn *et al*, 1998; Li *et al*, 2003). In most of the studies of trends in ER status over time, the assays and criteria used to determine ER positivity changed several times during the study periods. Critically, in this study, we used immunohistochemistry on all samples, thereby ruling out an artefactual increase. Furthermore, all samples underwent immunohistochemistry together to eliminate the potential effect of changing laboratory conditions on staining. The study was powered to detect a 10% difference in ER-positive prevalence, and is hence slightly underpowered to detect the observed 7% difference. The inability to retrieve tumour block for all patients and fragmented samples (factors common to studies involving tissue microarrays) reduced the number of samples in each cohort that were analysed, but the tumours that underwent analysis should be representative of the whole cohort.

One explanation for a preferential increase in ER-positive tumours could be a population-wide change in the prevalence of factors that increase the frequency of these tumours, such as late age at first pregnancy, postmenopausal obesity (Potter *et al*, 1995; Colditz *et al*, 2004) and use of hormone replacement therapy (HRT) (Potter *et al*, 1995). There is evidence that the percentage of all children being born to mothers aged 35 years and over is increasing in Scotland, and that mean BMI and prevalence of obesity are increasing (Brown *et al*, 2007). Data on HRT use by the patients in this study were not available.

A change in ER positivity could influence the survival. In this study, breast cancer-specific survival in the 1984–1986 cohort was significantly lower than in 1996–1997. When the effect of period of diagnosis (i.e., 1984–1986 or 1996–1997) on survival was adjusted for the ER status of the patients alone, the period of diagnosis remained a significant independent factor in survival (with survival being higher in 1996–1997). As expected, the difference in survival between cohorts is not fully explained by differences in ER status; treatment and global management changes have undoubtedly contributed to changes in survival over time (Bradburn *et al*, 1998; Thomson *et al*, 2004). However, a true change in ER status could also have implications for the application of data from clinical trials carried out in previous decades to the women of today, as a change in the prevalence of ER-positive disease could alter the overall survival benefit seen from chemotherapy and different hormonal therapies.

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