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Breast cancer incidence and survival trends by molecular subtypes in Scotland

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Doctor of Philosophy

Declaration

I declare that this thesis has been composed solely by myself and that it has not been submitted, in whole or in part, for any other degree or professional qualification. The work presented is entirely my own except where explicitly stated otherwise.

Part of the work presented in Chapter 3 of this thesis was previously published in the British Journal of Cancer as "Distinct temporal trends in breast cancer incidence from 1997 to 2016 by molecular subtypes: a population-based study of Scottish cancer registry data" and the paper has been included in Appendix D.

Signature: Date: 21/08/2020

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Abstract

Background:

Breast cancer (BC) is the most common cancer among women and leading contributor to cancer mortality, hence constitutes a major public health issue worldwide. In Scotland, over 4,000 women are diagnosed with BC every year and around a 1,000 die from this disease. Monitoring incidence, mortality and survival trends is key for surveillance of disease progression. BC is heterogeneous, with multiple subtypes defined by molecular markers, such as the oestrogen receptor (ER), that have different aetiology, targeted treatments and prognosis, yet standard reporting of incidence and mortality rates is not usually done using tumour marker data. The Scottish Cancer Registry was the first registry in the UK to collect molecular marker data and therefore, constitutes an excellent opportunity to explore incidence and survival trends over time by molecular subtypes. This PhD aims to describe temporal trends in BC incidence and survival by molecular subtypes in Scotland to inform public health prevention programmes, diagnostic and therapeutic services.

Methods:

A systematic review was conducted to determine the extent of available data on BC incidence trends by ER in population-based studies of women of European ancestry. In addition, the Scottish Cancer registry data on over 72,000 women diagnosed with incident primary BC from 1997 to 2016 (the focus of most analyses for this dissertation) was used to describe trends in incidence and survival in Scotland. Age-standardised incidence rates (ASiR) and age-specific incidence were estimated by BC subtype after imputation of molecular marker data. Joinpoint regression and age-period-cohort (APC) models were used to assess whether significant differences were observed in incidence trends by ER, the human epidermal growth factor receptor 2 (HER2) and the immunohistochemistry (IHC) defined molecular subtypes. Kaplan-Meier (KM) estimates and traditional and extended Cox proportional hazards models were computed to assess breast cancer specific survival (BCSS) by BC subtypes. Sensitivity analysis was carried out to compare results for the Cox models from complete case analysis (CCA) and multiple imputation analysis (MIA). The effect of

individual, tumour characteristics and treatments on BCSS for each subtype was also investigated. Trends in 5-year survival by age, grade and stage characteristics for the different subtypes (ER+ and ER-) were investigated to identify the characteristics of women showing greatest and lowest improvements over time. Other causes of death were also explored and cumulative incidence functions (CIF) were investigated.

Results:

The systematic review showed that ER+ BC incidence increased and ER- BC incidence decreased in the last four decades (EAPCs ranging from 0.8% to 3% for ER+ tumours and -2.1% to -3.4% for ER- tumours) and that the rise in overall incidence trends is mainly driven by increases of ER+ tumours in women of screening age. In Scotland, BC incidence rates showed the same divergent pattern between ER+ and ER- tumours observed in other countries. ER+ tumour incidence increased by 0.4% per year from 1997 to 2011 and increases were mainly among routinely screened women aged 50 to 69 years. In contrast, ER- tumour incidence decreased among all ages by -2.5% per year over the study period. Apart from the period effects observed, APC models showed that older cohorts of women born in 1912-1940 had lower incidence rate ratios (IRR) for ER+ tumours, and younger cohort of women born in 1960-1986 had lower IRR for ER- tumours, compared to women from the 1941-1959 birth cohorts. Results for the IHC defined subtypes showed that luminal A tumours, that account for more than half of all tumours, had similar patterns to those observed for ER+ tumours, with increases until 2011. In contrast, luminal B tumours declined over time, particularly in women over 50 years of age. There was no clear trend for HER2-enriched or triple negative breast cancers (TNBC) overall but TNBC tumours seemed to increase in younger women aged 20 to 49 years.

BCSS also differed between subtypes with ER+ tumours having better survival than ER- tumours, luminal A tumours having the best survival of all IHC defined subtypes and TNBC having the worst survival. Age, grade, stage, screening and surgery were the most important prognostic factors irrespective of tumour subtype, with women who had older age, higher grade, stages III-IV, tumours not screen detected and who did not have surgery having worse survival. Deprivation was also associated with lower BCSS, with women living in the most deprived areas of Scotland having increased

BC-specific mortality when compared to women in the least deprived areas and this relationship was observed for all subtypes with slightly higher HR for HER2-enriched subtypes (but wider CI). Five-year BCSS trends showed improvements in the last two decades, especially for women aged 50 to 69 years. The greatest gains in survival were seeing in women with advanced tumours (high grade or stage III-IV tumours) and ER- tumours seemed to have greatest improvements than ER+ tumours, although their survival remained lower than for ER+ tumours. The improvements observed for women with high grade and stage III-IV tumours were observed in both screen and not screen detected tumours but the rise was sharper amongst women with screen detected tumours. Women younger than 50 years showed similar improvements than those observed in women aged 50 to 69 years. Older women aged 70 years or more showed no consistent survival improvements over time and over 50% of women in that age had a primary cause of death other than BC with cardiovascular diseases (CVD) being a major contributor (22% of all deaths).

Conclusions:

This project is the first in the UK to describe incidence and survival trends by molecular subtypes of BC using population-based data. Divergent incidence trends found in Scotland are similar to those observed in other countries and confirm different aetiology of BC molecular subtypes. Increases in the incidence of hormone sensitive tumours are likely to be driven by the implementation of mammographic screening programmes, population aging and changes in risk factors (RFs) that have differential effects on the subtypes, such as, reproductive factors and obesity. Survival improvements in Scotland are likely due to multiple contributors with two major factors such as screening and the improvement and development of new treatments likely playing a role. This PhD has allowed us to further understand disease progression of the different subtypes in Scotland and has identified groups of women (those with advanced tumour characteristics, living in the most deprived regions of Scotland or women aged 70 years or older) with lower survival and/or lower improvements in survival trends that could benefit from further prevention and treatment programmes. This PhD also highlights the importance of monitoring future incidence and survival by molecular subtypes to inform clinical planning and cancer control programmes.

Lay summary

BC is the most common cancer in women worldwide and a major cause of mortality. In Scotland, almost 4,000 new BC cases are diagnosed every year and around 1,000 women die from this disease. BC is not a single disease but rather multiple diseases, with BC subtypes defined depending on the presence of tumour markers. Oestrogen and progesterone receptors (ER and PR) and other substances such as the human epidermal growth factor 2 (HER2) are such tumour markers that have been the target of different therapies. The Scottish Cancer Registry has been collecting data on ER status since 1997 and on PR and HER2 from 2009. However, national official statistics do not report the number of new cases or deaths by these markers but overall. This PhD aims to describe trends of new cases and survival by BC subtypes in Scotland for the last two decades. Monitoring these trends can improve our understanding of the different subtypes and identify groups of women that might benefit from more treatment options or programmes aimed to reduce the individual risk of having BC.

Previous research from other countries have shown that tumours that are ER+ have been increasing in recent decades, especially amongst women of screening age, while ER- tumours are declining. In Scotland, we observed similar trends likely due to multiple factors. For example, the adoption of a national screening programme for women aged 50 to 69 years may have contributed to the increases observed in ER+ tumours as these tumours are more likely to be diagnosed through screening than ER- tumours.

Survival after a BC diagnosis also differed by subtype and women with ER+ tumours had better survival than those diagnosed with ER- tumours. Age, tumour grade, tumour stage, method of detection and surgery were the most important factors in survival regardless of tumour subtype. Women aged 70 years or more, women with higher grade tumours, stages III-IV tumours and non-screen detected tumours and who did not have surgery had worse survival. Deprivation was also an important factor for survival in all subtypes with women in the most deprived areas having worse survival than women living in the least deprived areas of Scotland. BC survival improved over time, especially for women younger than 70 years with more advanced diseases (high

grade tumours and stage III-IV tumours) and the improvements were greatest amongst women with ER- tumours, which is a very positive outcome as those subtypes have less treatment options available. The improvements observed for advanced tumours were greater amongst screen detected tumours which might indicate the positive effect of screening on survival. Older women aged 70 years or more showed no consistent improvements in survival over time and over 50% of them died from causes other than BC, with CVDs being the most common cause.

This project is the first in the UK to describe trends at the population level in new BC cases and survival by molecular subtypes of BC. The trends found in Scotland highlight that BCs are different diseases and that those women having worst outcomes (older age, living in most deprived areas or with more advanced tumours) will further benefit from new treatments personalised to their disease or from prevention programmes. This PhD also highlights the importance of future monitoring of number of new cases, number of deaths and survival by subtype.

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Abbreviations

AACR	American Association for Cancer Research
AAPC	Average annual percentage change
ADH	Atypical ductal hyperplasia
AI	Aromatase inhibitor
AJCC	American Joint Committee on Cancer
APC	Age-Period-Cohort
ASiR	Age-standardised incidence rate
ATAC	Arimidex, Tamoxifen, Alone or in Combination
BBD	Benign breast diseases
BC	Breast Cancer
BCD	Breast Cancer death
BCSS	Breast cancer specific survival
BIC	Bayesian Information Criterion
BMI	Body mass index
BRCA	Breast and ovarian cancer susceptibility protein
CCA	Complete Case Analysis
CHI	Community Health Index
CI	Confidence Interval
CIF	Cumulative Incidence Function
CRR	Cohort Rate Ratio
CVD	Cardiovascular disease
COPD	Chronic Obstructive Pulmonary Disease
DBCG	Danish Breast Cancer Group

DCC	Dextran-coated charcoal assay
DCIS	Ductal carcinoma in situ
DFS	Disease free survival
DMFS	Distant metastasis free survival
EAPC	Estimated annual percentage change
EIA	Enzyme immunoassay
ENCR	European network of cancer registries
ER	Oestrogen receptor
ERICA	Oestrogen receptor immunocytochemical assay
FISH	Fluorescence in-situ hybridisation
GP	General Practitioner
GWAS	Genome-wide association studies
HER2	Human epidermal growth factor receptor 2
HIC	High-income countries
HR	Hazard Ratio
HT	Hormone therapy
IARC	International Agency for Research on Cancer
IBM	Incidence-based mortality
ICD	International Classification of Diseases
IHC	Immunohistochemistry
IQR	Interquartile Range
IRR	Incidence Rate Ratio
ISD	Information Services Division
KM	Kaplan-Meier
KPNW	Kaiser Permanente Northwest registry

LCIS	Lobular carcinoma in situ
LMIC	Low-and-middle income countries
MAR	Missing At Random
MCAR	Missing completely at random
MHT	Menopausal Hormone Therapy
MI	Multiple Imputation
MIA	Multiple Imputation Analysis
MICE	Multiple Imputation using Chained Equations
MNAR	Missing not at random
MR	Mendelian randomisation
NCI	National Cancer Institute
NCRI	National Cancer Registry of Ireland
NBCSP	Norwegian Breast Cancer Screening Programme
NHS	National Health Service
NR	Not reported
NSS	National Services Scotland
NST	Invasive carcinoma of “no special type”
OC	Oral contraceptives
OS	Overall survival
PBPP	Public Benefit and Privacy Panel
PH	Proportional Hazards
PR	Progesterone receptor
PRR	Period Rate Ratio
RCT	Randomised control trial
RER	Relative excess risk

RF	Risk factor
RNA	Ribonucleic acid
RR	Relative risk ratio
SD	Standard Deviation
SEER	Surveillance, Epidemiology and End Results
SERM	Selective ER modulator
SES	Socioeconomic status
SIMD	Scottish Index of Multiple Deprivation
SMR	Scottish Morbidity Records
SNP	single-nucleotide polymorphism
SSE	Sum of squared errors
TDLU	Terminal duct lobular unit
TNBC	Triple negative breast cancer
TNM	Tumour size, Nodes, Metastases
TVE	Time-Varying Effect
UKIACR	United Kingdom and Ireland Association of Cancer Registries
WHI	Women's Health Initiative study
WHO	World Health Organization
WCRF	World Cancer Research Fund

Thesis outline

During this thesis I aim to investigate BC incidence and survival trends by molecular subtypes defined using routinely collected immunohistochemistry (IHC) molecular marker data in Scotland to identify subgroups of women with increasing incidence and/or worse outcomes to inform clinical planning and cancer control programmes.

Chapter 1 of this thesis gives an overview of BC epidemiology and how the use of molecular subtypes has evolved over time and informed BC treatments. In Chapter 2, contemporary incidence trends of BC in European ancestry populations are described by oestrogen receptor (ER), the first targeted receptor discovered for BC. Results from the systematic review show divergent trends by ER status in most countries with available data and provides an important context to compare and contrast with the data from Scotland. Further, if differences between countries are observed, it provides potential hypotheses on which factors might cause incidence or survival differences.

In Chapter 3, BC incidence trends in Scotland by ER and by IHC defined molecular subtypes are presented. Joinpoint regression analysis, which gives an overall estimate of the direction of the trend and defines probable time points at which there is a change in trend and APC models, used to determine whether the observed trends are due to age, period or cohort effects, were performed. This chapter presents, for the first time in the UK, population-based BC incidence by molecular subtypes and highlights the importance of using individual and molecular tumour markers to assess incidence trends for cancer surveillance.

Chapter 4 presents survival analysis by different molecular subtypes of BC and shows clear differences in prognosis and the importance of other characteristics such as age, grade and stage in survival. This chapter also presents survival trends by method of detection, for which data is not collected in most cancer registries, and by deprivation which is an important risk factor for worse prognosis in Scotland. Survival trends for combinations of the most important prognostic factors are presented along with trends for treatment use within the Scottish population of women diagnosed with BC.

Chapter 5 provides a general discussion of the PhD summarizing the key findings and its contribution to the literature, strengths and limitations of the PhD and future research implications for clinical practice and cancer surveillance.

Chapter 1 Background

1.1 Natural history of breast cancer

1.1.1 Anatomy of the breast

The human breast consists of a combination of stromal and epithelial elements (Figure 1.1). The stroma is responsible for the structure of the breast, surrounds the mammary gland and provides important growth factor signalling for breast development [1]. It is composed of adipose and connective tissue, blood and lymphatic vessels. The epithelial elements and functional units of the breast are the terminal duct lobular units (TDLU). TDLUs are also the predominant source of breast cancers. TDLUs are composed of a terminal duct and its lobules [2]. The lobules or milk glands are responsible for the production of milk. The ducts are also called milk conduits and their main purpose is to carry the milk from the lobules to the nipple for discharge [3]. The ducts are small conduits that grow following a tree branching pattern during puberty and end in oval shaped glands called lobes [4]. The human breast has 12 to 20 lobes and each lobe is composed of lobules.

Figure 1.1 Anatomy of the female breast

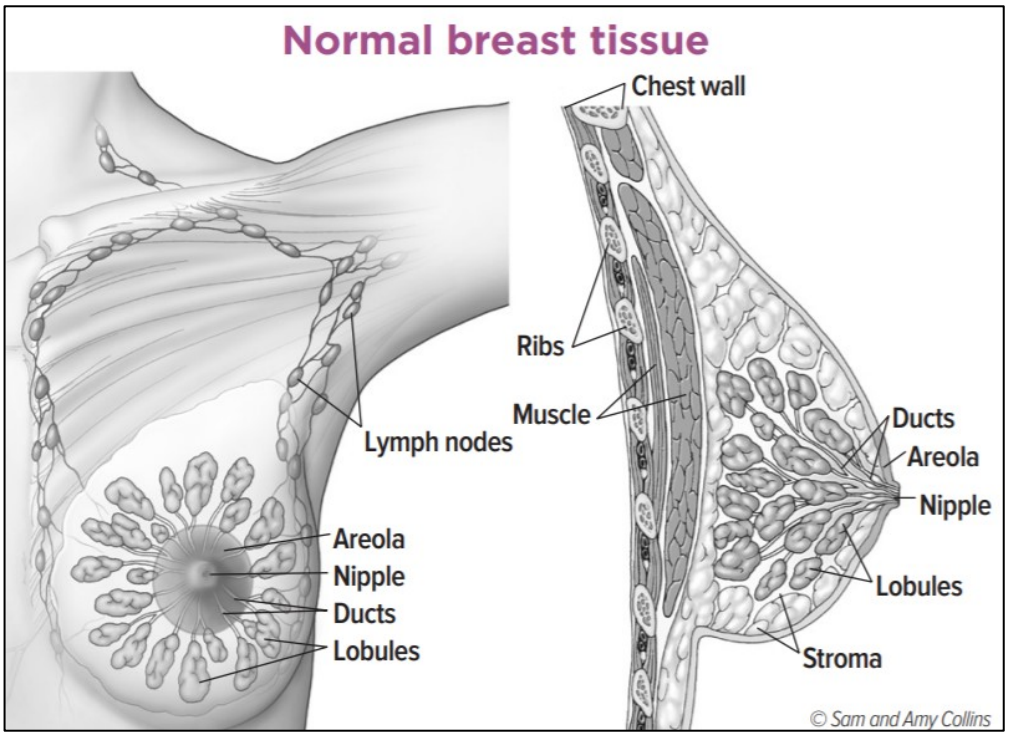


Figure taken from American Cancer Society webpage: What is breast cancer? [5]

The breast undergoes various developmental stages over the course of a woman's life. At birth, men and women's breasts are similar consisting of a primitive mammary gland, but with puberty the breast of a woman starts to differentiate and the primitive terminals grow into secondary branches due to the influence of ovarian hormones. At this stage, the fatty tissue and areola also grow leading to an increase in size of the breast. In an adult woman, breast changes occur with every menstrual cycle. The ovarian hormones (oestrogen and progesterone) stimulate the growth of the TDLUs and the breast changes in texture and size in preparation for pregnancy. If pregnancy takes place, the TDLUs continue to grow and more lobules are formed to allow for lactation. On the contrary, if there is no pregnancy, the breast will recover its size and the process will start again with the next cycle. After pregnancy, once the woman stops breastfeeding, apoptosis of the epithelial cells cause regression of the TDLUs to its pre-pregnancy state in a process called involution [6]. During menopause, circulating hormone levels decrease dramatically producing changes in the breasts. The drop of oestrogen leads to loss of elasticity of the connective tissue that shrinks and loses its shape.

The breast also contains ligaments, nerves, lymph vessels, lymph nodes and blood vessels. Lymph nodes are collections of immune cells that play a major role in the spread of the disease to other parts of the body. Lymph nodes are connected by lymph vessels throughout the body forming a network called the lymphatic system. Cancerous cells from the breast infiltrate the lymph vessels and travel to the nearest lymph nodes where they start to grow [3]. When lymph nodes around the breast contain tumour cells, it is more likely that the disease may affect other parts of the body. Patients diagnosed with BC undergo a sentinel lymph node biopsy to test if the closest lymph node to the tumour contains cancerous cells.

1.1.2 Precursor lesions

Most BCs originate in the epithelial cells of the breast, specifically in the TDLUs [7], and are classified as carcinomas. Rarely (<1%) they can have their origin in the bone tissue near the breast (sarcomas). Carcinomas are divided in two major subtypes based on its histology. The first of those subtypes are the in situ or non-invasive carcinomas.

In situ or non-invasive BCs are pre-invasive lesions similar to the invasive BCs and they account for approximately 15-25% of all cases diagnosed [8, 9]. In non-invasive breast tumours the malignant cells are confined and do not invade the stroma. They are classified as ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS). This distinction does not depend on the tissue of origin, as most carcinomas in the breast arise from the TDLU [7], but on the difference in structure, function and chemical composition of the cells [10]. DCIS is the most common type of non-invasive precursor lesion of invasive BC and presence of DCIS is associated with a 2 to 8-fold increased risk of invasive BC [11, 12], especially within the 5 to 10 years after diagnosis [13]. In contrast to DCIS, whether LCIS is a precursor lesion is inconclusive: LCIS carries a smaller risk of recurrence and is usually considered a marker of risk of invasive BC and not a precursor lesion [14]. However, LCIS has been associated with risk of bilateral involvement and a population-based study by Li et al. on 4490 LCIS patients suggested that it might be a precursor lesion of invasive lobular cancer [12]

Precursor lesions are considered to increase the risk of progression to invasive BC. Apart from DCIS, benign breast diseases (BBD) are also considered precursor lesions. Atypical ductal hyperplasia (ADH) is the most important of BBD as it is associated with a 4- fold increase in risk of invasive BC [15]. In the natural history of BC, TDLUs can transform into premalignant lesions (ADH and DCIS) and invasive BC (Figure 1.2).

Figure 1.2 Breast cancer progression from normal breast tissue to malignant breast cancer

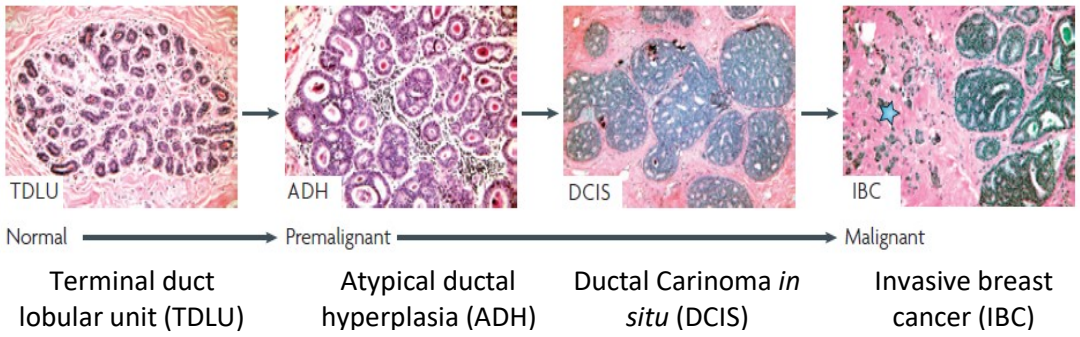


Figure adapted from [16]

1.1.3 Invasive breast cancer

BC originates in the breast tissue when cells in this region start to grow abnormally forming a tumour. The abnormal growth of the cells is consequence of mutations and other molecular abnormalities responsible for cell growth. Healthy cells undergo a process of cell regeneration in which they replace themselves with new cells. In cancer cells, the process of cell division and growth is altered from normal process of cell division (homeostasis), and cancerous cells keep dividing without control forming a tumour. The tumour is considered a cancer or malignant when the abnormal breast cells start to invade other surrounding tissues in the gland and when it reaches even more distant areas of the body is called metastasis.

Precursor lesions (ADH and DCIS) may infiltrate the surrounding stroma of the breast and become malignant tumours (Figure 1.2), termed invasive BC. Invasive BC is a heterogeneous group of tumours of which the most common is the "invasive carcinoma of no special type" (NST) that accounts for 75 to 80% of all invasive carcinomas [4]. NST tumours were previously known as invasive ductal carcinomas [6]. The second most common invasive carcinoma is the invasive lobular carcinoma that accounts for 5 to 15% of all the cases and is considered a "special type" because of its distinct morphology and clinical behaviour. Other "special types" are mucinous carcinoma, adenoid cystic carcinoma, medullary carcinoma, tubular carcinoma, papillary carcinoma and metaplastic carcinomas [4]. These 'special types' tumours usually have a good prognosis.

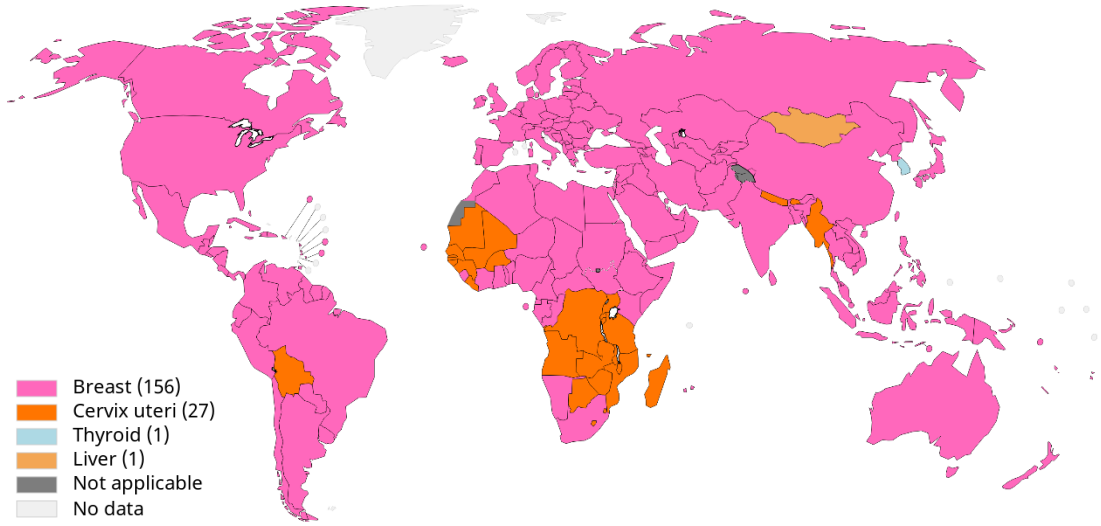
1.2 Epidemiology of breast cancer

1.2.1 Incidence rates of BC

BC is the most common cancer diagnosed in women (Figure 1.3) in 156 countries (75%) [17]. In 2017, 1.9 million women were estimated to have been diagnosed with BC (24% of all cancer cases) [18]. BC incidence has increased considerably in recent decades worldwide. The implementation and improvement of enhanced screening regimens in high-income countries (HIC) and changes in risk factors (RF) experienced

in recent decades likely contributed to the increased incidence. Since cancer is a disease associated with advanced age, with increased longevity, incidence rates are expected to continue increasing. By 2030 it is estimated that incidence rates of cancer will increase by 68% worldwide and BC incidence by 2% annually in HIC [19].

Figure 1.3 Most common cancer per country in women of all ages, estimated age-standardised incidence rate (World) in 2018



Data source: GLOBOCAN 2018
Graph production: IARC (http://gco.iarc.fr/today)
World Health Organization

BC incidence rates differ considerably between countries, with Western and Northern European countries, North America and Australia/New Zealand having the highest incidence rates in the world [age-standardised incidence rate (ASiR) =113 women per 100,000 in Belgium (1st ranked country), ASiR =109 in Luxembourg (2nd), ASiR =95 in Australia (7th), ASiR =94 in the UK (8th) and ASiR =85 in the USA (22nd)] and Shout-East Asia (ASiR =27) and Africa (ASiR =35) having the lowest incidence [17, 18].

The higher incidence of BC in HIC is associated with the introduction of screening programmes and with increased prevalence of BC RFs, such as, obesity and changes in reproductive factors [4]. However, in the last two decades, the increase in BC

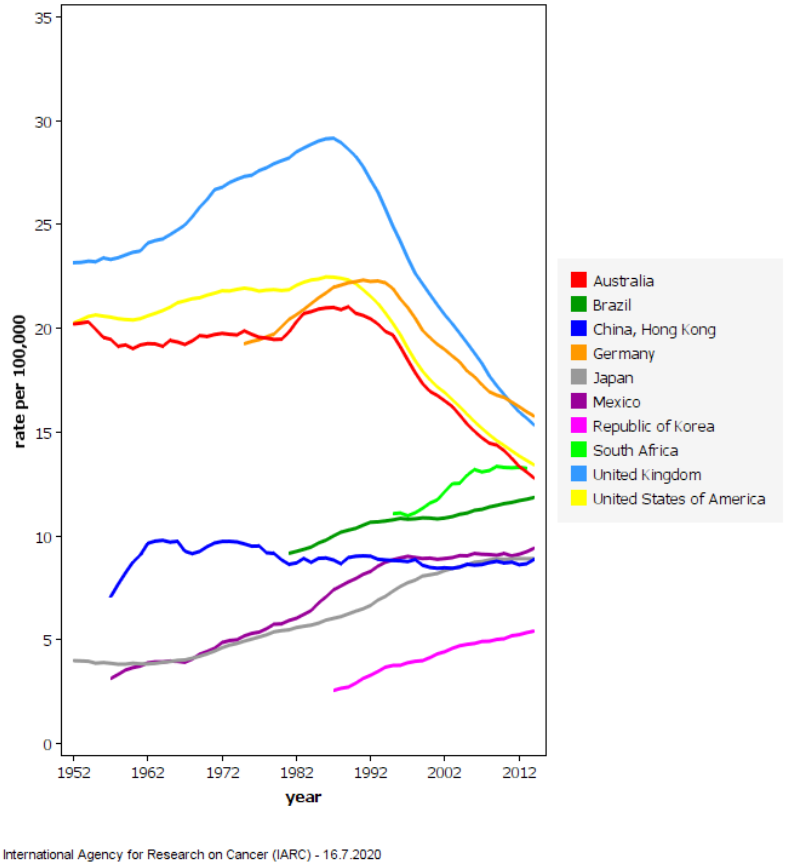
incidence in some HICs has started to decrease or has remained stable due to plateaus in screening [20] and incidence increases have been larger in low-and-middle-income countries (LMIC) with approximately a 5% rise each year [10]. For example, rural areas of China have seen their ASiR increase by 8% each year [21] and BC in urban areas of India has overtaken cervical cancer as the most common cancer in women [22]. Cancer registry data from Uganda and Algeria, report doubling of incidence rates in the last 20 years, and BC is becoming more prevalent in Africa [23]. It is estimated that, in the next decade, 19.7 million BC cases will be diagnosed worldwide, of which 10.6 million (54%) will be in LMICs [17, 24].

All these examples support that demographic changes (including improved longevity, prevention and diagnostic tools and survival from infectious diseases) and the adoption of lifestyle factors associated with BC, such as delayed childbearing, obesity and increased smoking and alcohol consumption may contribute to the increases in LMICs [25]. Hence, BC is a major global public health problem that needs new strategies for prevention to combat the increasing risk in incidence and improved access to targeted treatments to improve survival [26].

1.2.2 Mortality rates of BC

BC mortality rates have been decreasing since the 1990s particularly in HICs (Figure 1.4) due to improved surveillance, the introduction of screening programmes and the development of targeted treatments [27]. However, BC mortality is still the leading cause of mortality amongst women in 104 (50%) countries worldwide and over 600,000 women were thought to have died from the disease in 2017 [18]. Further, BC mortality is increasing in many LMICs (Figure 1.4) in Asia, Africa and South America.

Figure 1.4 Female breast cancer mortality trends for selected countries from 1952 to 2012



1.3 Risk factors associated with breast cancer

BC is associated with multiple RFs [28, 29]. While BC can affect men and women, it is very rare for men (only <1% of all BCs in the UK [30]. Hence, below I summarise the established RFs for female BC identified by reviewing breast cancer reports convened from expert panels from the World Cancer Research Fund (WCRF), the International Agency for Research on Cancer (IARC) and the American Association for Cancer Research (AACR).

1.3.1 Age

The risk of BC rises with increasing age. Evaluation of age-specific incidence rates show a log linear relationship until the age of menopause (approximately 50 years)

when incidence rates slow down. In 1983, Pike et al. suggested that the relationship between BC incidence and age was closely related to the rate of breast tissue aging, which was not chronological but biological –related to the breast tissue changes in response to different hormone exposures during the lifecourse of a woman including reproductive factors (e.g. menarche, parity and menopause) during the reproductive life of a woman. Hence, these data supported a relationship to hormone exposures [31]. Pike et al also suggested that the role of hormones in the breast tissue was an important factor in the development of BC, which has been supported with further evidence evaluating circulating hormones [32].

1.3.2 Height

Higher adult height has been associated with an increased risk of BC in many systematic reviews and meta-analysis of epidemiological studies [33-36] with most reviews reporting a linear dose-response relationship. A recent study reporting results from a meta-analysis of 26 prospective studies found that for every 10 cm increase in height, the risk of breast cancer increased by 17% (95% CI from 15% to 19%) for all the combination of all studies [37]. This study also conducted a mendelian randomisation (MR) analysis in two consortiums aimed to determine the causal relationship between height and breast cancer risk using an instrument with 168 height associated variants. They found an OR of BC of 1.22 (95% CI = 1.13 to 1.32) in the first consortium and 1.21 (95% CI = 1.05 to 1.39) in the second consortium per 10 cm increase in genetically predicted height.

1.3.3 Breast density

Breast density measures the macroscopic composition of the breast tissue, which can be visualised radiographically and assessed routinely through mammographic screening. Women with dense breasts have a greater ratio of fibroglandular tissue (stromal and epithelial) to fatty tissue. Breast cancers arise predominantly from epithelial cells, but a challenge to breast cancer early detection has been noted especially for women with dense breast tissue. This is because cancerous tissue and dense tissue both appear as a solid white area, it makes reading of the mammograms more difficult which can lead to a delayed diagnosis of BC especially for younger women where density is higher. However, breast density is not only associated with

delayed diagnosis but also with an increased risk of BC [38]. A systematic review and meta-analysis with over 14,000 cases and over 200,000 controls from 42 studies found that women with a percentage density of 5% to 24%, 25% to 49%, 50% to 74%, and ≥75% had a pooled RR of 1.8 (95% CI: 1.5 to 2.2), 2.1 (1.7 to 2.6), 2.9 (2.5 to 3.4), and 4.6 (3.6 to 5.9) respectively compared to women with a breast density of less than 5% [39]. Therefore, breast density has been established as one of the strongest risk factors of BC and research is now focusing on whether breast density should be considered for screening stratification via a risk prediction model.

1.3.4 Family history and genetic factors

A meta-analysis of 74 studies, found that family history of BC is associated with a 2-fold increased risk of the disease [RR = 1.9 (95% CI, 1.7 to 2.0)], and this risk is higher if BC occurs in a first-degree relative compared to a second-degree relative [40]. It is estimated that 5 to 10% of BC cases have a strong genetic predisposition with 4-5% caused by high penetrance genes [41]. Historically, high risk variants in BRCA1 and BRCA2 were the most studied, and a meta-analysis of the penetrance of mutations carriers for BC estimated that a woman at 70 years of age with a mutation in BRCA1 has a cumulative risk of BC of 57% (95% CI, 47% to 66%), and for mutations in BRCA2, of 49% (95% CI, 40% to 57%) [42]. Other moderate penetrance genes, including TP53, CHD1, NF1, PALB2, ATM, CHECK2, PTEN and NBN that contribute to the heritability of BC have also been identified [43].

In more recent years, genome-wide association studies (GWAS) have identified a substantial number of single-nucleotide polymorphisms (SNPs) associated with BC risk, which along with other known variants explain about 41% of the familial relative risk [44], hence much of the genetic contribution to breast cancer risk still remains unknown.

In 2017, utilising data from 68 individual studies included in the Breast Cancer Association Consortium (BCAC) and the Discovery, Biology and Risk of Inherited Variants in Breast Cancer Consortium (DRIVE), Michailidou et al. published a GWAS study in which they identified 65 new loci associated with BC risk, more predominantly with ER+ subtype [44]. This study constituted a major development for BC susceptibility as the new identified loci accounted for 44% of the known genetic

susceptibility from all SNPs identified to that date. More recently, Milne et al. identified a further 10 variants and replicated the associations observed in previous studies for ER- tumours confirming 125 variants associated with ER- BC risk, which account for 16% of the familial relative risk for this subtype [45]. The number of loci identified is likely to increase in the following years, as new insights into the genetic susceptibility of BC would be useful to implement risks scores for personalised screening and prevention.

1.3.5 Reproductive factors

1.3.5.1 Age at menarche and age at menopause

Early age at menarche and late age at menopause have been associated with an increased risk of BC. A meta-analysis of 117 epidemiological studies in over 100,000 women diagnosed with BC showed that women with younger age at menarche had their risk of BC increased by 1.05 (95% CI=1.04 to 1.06) for every year younger [46]. This study also found that a delay in menopause was also associated with an increased BC risk, with a pooled estimate of 1.029 (95% CI; 1.025 to 1.032) for every year older at menopause. They also reported that menopausal status had a differential effect in risk, with premenopausal women having an increased risk of 1.4 (95% CI: 1.3 to 1.5) at age 45-54 years compared to postmenopausal women of the same age. Both RFs have been related to a longer exposure to hormones (especially oestrogen) and to a higher number of menstrual cycles during a woman's life.

1.3.5.2 Age at first birth and number of live births

Older age at first live birth has been associated with an increased risk of BC. Data from several studies (including a meta-analysis of 8 studies from Nordic countries) showed an increased risk for women aged 35 years or older at the time of their first birth compared to women aged 20 years or younger [28, 47]. This relationship may have to do with the changes that occur in the breast tissue during pregnancy, time at which the breast reaches its full maturity. Colditz et al. created a multiple birth model based on Pike's model [31] that shows that pregnancy has a dual effect on the risk of BC- the first live birth has an adverse effect when it occurs but it decreases the risk later in life, as it is associated with a decline of tissue aging [48]. Further, tissue aging decreases with each consecutive live birth and the shorter the time between births the lower the

rate of tissue aging and risk of BC. Therefore, the number of births, the age at each birth and the time between births also have an effect on risk. The model by Colditz et al showed that nulliparous women have an increased risk of BC compared to women that have multiple children at a younger age. In contrast, having one birth at the age of 35 years or older carries a higher risk of developing BC in the future than not having children [48].

1.3.5.3 Breastfeeding

Breastfeeding is a protective factor for BC. A recent study from the Collaborative Group on Hormonal Factors in Breast Cancer using data from 47 epidemiological studies in 30 countries, reported a 4.3% (95% CI: 2.9 to 5.8) decrease in the relative risk (RR) of BC for every 12 months of breastfeeding for women that breastfed compared to women who never breastfed [49].

1.3.6 Oral contraceptives

Oral contraceptives (OC) have been used for decades and their use has been associated with a small increased risk of BC. Data from 54 studies, showed that women using OC had a small increased risk of BC at the time of use and up to 10 years after cessation compared to non-users (RR ranging from 1.24 (95% CI: 1.15 to 1.33) for current users to 1.07 (95% CI: 1.02 to 1.13) for 5-9 years after cessation) [50]. A more recent population-based study in Denmark found an increased RR of 1.2 (95% CI: 1.14 to 1.26) among current users compared to non-users and this increase was higher with a prolonged use of OC (ranging from 1.09 (95% CI: 0.96 to 1.23) for less than a year of use to 1.38 (95% CI: 1.26 to 1.51) for more than 10 years of use) [51]. However, the association between BC risk and OC depends on the concentration of hormones used. A population-based case-control study by Althuis et al. found a higher RR of BC for users taking OC with high concentrations of oestradiol [RR=2.0 (95% CI: 1.2 to 3.2)] than users taking OC with a low dose [RR=1.3 (95% CI: 0.9 to 1.7)] compared to non-users [52]. A report from the Nurses' Health Study II in over 110,000 nurses showed that current OC users risk of BC was as high as 3.1 times (95% CI: 2.0 to 4.7) that in non-users if they were in the estradiol and levonorgestrel combination [53].

1.3.7 Postmenopausal hormones

Postmenopausal hormone therapy use, also known as hormone replacement therapy or menopausal hormone therapy (MHT) was introduced in the UK in 1965 and has been associated with an increased risk of BC. The Women's Health Initiative (WHI) study was a randomised controlled trial (RCT) in the US that aimed to determine the relationship between MHT use and risk of multiple diseases. The results from the WHI study were published in 2002 concluding that MHT, especially oestrogen plus progestin use, increased the risk of heart disease and BC [54]. In the UK, the Million Women study confirmed the results from the WHI study when they found that current MHT users had a higher risk of BC than never users (RR=1.7, 95% CI: 1.6 to 1.8) [55]. The increase differed depending on the hormone combination, with women in the oestrogen-progestogen group having a higher increase in incidence than women in the oestrogen only group. The publication of these studies had an impact on MHT use that started to decrease shortly after and, as a consequence, incidence of BC declined in most Western countries [56-62]. The Collaborative Group on Hormonal Factors in Breast Cancer reviewed all the evidence from prospective cohort and randomised studies on the type and timing of MHT use. This study confirmed the results from WHI and the Million Women study and estimated that a causal relationship would result in 1 in 50 women using daily oestrogen-progestogen preparations from the age of 50 and for 5 years would develop BC and 1 in 70 for those using oestrogen and intermittent progestogen and 1 in 200 for those using oestrogen only [63].

1.3.8 Obesity, BMI and diet

Epidemiological studies have established that obesity is a RF for BC, however, this relationship seems to differ according to menopausal status. Results from the Million Women study showed that a 10 units increase in body mass index (BMI) was associated with an increased RR of 1.40 (95%CI: 1.31 to 1.49) for BC, but only among postmenopausal women [64]. A more recent meta-analysis of 31 studies found a weaker but statistically significant association (RR=1.12, 95% CI: 1.08 to 1.16) between a 5 kg/m2 increase in BMI and postmenopausal BC but an inverse association with premenopausal BC (RR=0.92, 95% CI: 0.88 to 0.97) [65, 66]. Apart from BMI, other measures of obesity, such as central obesity and weight gain during adulthood

have also been associated with BC risk. For example, a cohort study and a case-control study in the US reported that weight gain after the age of 18 years was associated with increased BC incidence after menopause [67, 68]. A more recent study using data from the Nurses's Health Study, found increased risk of BC after menopause amongst women with long-term increased weight after the age of 18 years both pre and post menopause, but there was no association with premenopausal BC [69].

The role of diet in BC risk has also been investigated but evidence is less conclusive than for BMI and other obesity measures. A recent review of 32 studies found that the Western dietary pattern was associated with an increased risk (RR=1.20, 95% CI: 1.06 to 1.35) of BC in postmenopausal women but not in premenopausal women. In contrast, premenopausal women with healthy dietary patterns had a decreased risk of BC (RR=0.77, 95% CI: 0.61 to 0.98) [70]. Individual dietary items have also been identified: dietary fibre, fruit, vegetables and whole grains [71-73] are associated with a decreased risk of BC whereas processed meats are associated with an increased risk [74]. However, associations of BC and diet are still limited and more evidence is needed, especially as diet is one of the only modifiable RFs associated with BC and hence, could be a target for prevention.

1.3.9 Physical activity

The positive effect of physical activity in reducing the risk of BC and helping women diagnosed with BC to recover quickly has been widely established. A continuous project from the WCRF with data from 126 observational cohort studies in over 22,000 premenopausal and over 100,000 postmenopausal women diagnosed with BC, recently reported that vigorous physical activity was inversely associated with pre and postmenopausal BC risk [RR=0.79 (95% CI: 0.69 to 0.71) for premenopausal and RR=0.90 (95% CI: 0.85 to 0.95) for postmenopausal women] [75]. This systematic review and meta-analysis also found that increasing sitting time in postmenopausal women was associate with a 20% (95% CI: 0 to 44%) higher BC risk and that walking did not showed a positive effect for either premenopausal or postmenopausal women in terms of reduced risk of BC.

1.3.10 Alcohol and smoking

A causal relationship between alcohol consumption and BC has been established [76]. A recent meta-analysis of 22 prospective cohort studies found a dose-response relationship between total alcohol consumption and wine consumption and BC risk, with an increase of 10g per day of total alcohol or wine associated with increases in BC risk by 11% (95% CI: 8% to 13%) and 9% (95% CI: 4% to 14%) respectively [77]. This association has been established in both pre and postmenopausal women and data from two cohort studies conducted in nurses in the US supports an association between alcohol consumption in early life (teenager and young adult years) and risk of BC later in life [78, 79].

Alcohol consumption and smoking are highly correlated factors and determining the causal relationship of smoking and BC risk has been difficult due to the effects of time of exposure, the role of alcohol as a confounder or effect modifier and the possible effect of menopausal status [80]. A study with pooled data from 14 cohort studies in 36,000 invasive BCs found that smoking was associated with BC risk, particularly if it was initiated before a first full-term pregnancy and regardless of alcohol intake [81].

1.3.11 Socioeconomic status

BC has been labelled as a "welfare disease" since incidence increases with higher socioeconomic status (SES), especially education [82]. A recent meta-analysis of 25 European studies found a 25% (95% CI: 17% to 32%) increase in incidence of BC in women with higher SES [83]. However, this association was no longer significant after adjustment for other RFs, supporting the idea that women in different socioeconomic classes may have distinct reproductive and lifestyle factors and a different uptake of screening programmes that influence their risk of BC. Apart from education, other socioeconomic measures, such as, individual measures of occupation, education or income, or area-based indices of deprivation have also been investigated in relation to BC incidence. For example, using an area-based measure of deprivation, the Scottish Index of Multiple Deprivation (SIMD), Brown et al found higher incidence rates in women in the least deprived areas of Scotland compared to women in the most deprived and they postulated that reproductive factors, especially age at first birth, may be responsible for the higher incidence seen in affluent women [84]. In contrast to the

increased risk of BC in women with high SES, BC mortality rates are higher amongst women with low SES, known as the BC paradox which has been hypothesised to be related to screening uptake. A recent review of 13 studies from 7 European countries reported that women in most deprived areas were less likely to attend BC screening than women in the least deprived areas [85].

1.4 Breast cancer subtypes

BC is heterogeneous, with different subtypes that can be considered as biologically different diseases. While there are many different ways to try to classify homogenous subtypes of BC, for the purposes of this dissertation, I will primarily focus on the key endocrine hormone receptor for oestrogen and progesterone, and on the human epidermal growth factor receptor 2 (HER2).

1.4.1 Oestrogen receptor and progesterone receptor

Ovarian hormones, particularly oestrogen and progesterone have been historically associated with BC. In 1895, Beatson performed the first oophorectomy in a woman with BC and discovered that the tumour completely remitted when the ovaries of the woman were removed [86]. The procedure stopped the production of oestrogen in the ovaries and led to the idea that BC was associated with the circulation of hormones, especially oestrogens.

Oestrogens are hormones produced in the ovaries involved in the regulation of the reproductive system of women. They are present in the breast and can enter the cells and bind to the oestrogen receptor (ER). This binding activates cell proliferation and growth. Therefore, when the binding takes place in a BC cell, it can lead to the formation of a tumour. Breast tumours that express oestrogen are called oestrogen receptor positive (ER+) and they account for three out of every four invasive tumours, and those that don't express oestrogen are called oestrogen receptor negative (ER-).

Another important hormone associated with BC is progesterone. Progesterone receptor (PR) status is highly correlated with ER status. PR synthesis is regulated by oestrogen and therefore, the presence of PR indicates the presence of ER in breast tumours [87]. For that reason, measuring both gives a better indication of whether patients may respond to hormone therapy (HT). The majority of ER+ tumours are also PR+ and

survival is higher when the tumour expresses both hormone receptors than when the tumour expresses only one or neither receptor [87, 88].

ER and PR status are usually investigated when a woman is diagnosed with BC, as they are important markers for treatment decision and prognosis. In the early 70s, the US and some European countries started measuring ER and PR status using immunohistochemistry (IHC).

1.4.2 Human epidermal growth factor receptor 2

In the 1990s, with advances in molecular technologies, HER2 was discovered and it was observed that women with tumours that overexpress HER2 had worse overall and relapse-free survival than women who did not overexpress this marker [6, 89, 90]. HER2 positivity prevalence ranges from 12-30% [91-93] depending on the characteristics of the population and since its discovery, targeted treatments have been developed, such as, Trastuzumab significantly improving survival for both early stage and metastatic patients [94-96].

1.4.3 Molecular subtypes of breast cancer

Perou et al.[97] published in 2000 the seminal paper that defined the intrinsic molecular subtypes of BC that classified tumours in four subtypes based on their gene expression patterns, ER+/luminal-like, basal-like, HER2+ and normal breast. This study also showed for the first time that ER- tumours, as defined clinically, comprise at least two biologically different subtypes: basal-like and HER2+ tumours. Since the development of the molecular subtype classification, BC subtypes have been used for treatment guidance, especially for adjuvant therapy, and for risk stratification of patients [98]. However, genetic expression profiling is a costly technique and hence, not available for most tumours diagnosed in clinical practice. For that reason, in recent years research efforts have been focused in the use of IHC clinically available markers (ER, PR, HER2 and the tumour proliferation marker Ki-67) as surrogates for the intrinsic subtypes of BC. In 2011, the St. Gallen International Expert Consensus [99] highlighted the need of a simplified classification of BC subtypes based on clinicopathological markers that could be adopted in clinical practice to aid treatment management of patients. Based on Cheang et al. [100] classification they proposed the following surrogate definitions (Table 1.1).

Table 1.1 Surrogate definitions of intrinsic subtypes of breast cancer using IHC markers as defined by St Gallen 2011 consensus

Intrinsic subtype	Clinico-pathologic surrogate definition				Agreement IHC and intrinsic*
	Surrogate subtype	ER/PR expression	HER2 expression	Ki-67	
Luminal A	Luminal A-like	ER and/or PR positive	Negative	"low" <14%	73-100%
Luminal B	Luminal B-like (HER2-)	ER and/or PR positive	Negative	"high" >=14%	73-100%
	Luminal B-like (HER2+)	ER and/or PR positive	Positive	Any	
HER2-overexpression	HER2-enriched	ER and PR negative	Positive	Any	41-69%
Basal-like	Triple Negative	ER and PR negative	Negative	Any	80%

ER=oestrogen receptor, PR= progesterone receptor, HER2= human epidermal growth factor 2, Ki-67=marker of proliferation Ki-67, IHC= immunohistochemistry. Table modified from St.Gallen Consensus 2011 [99] *Column from [101]

In 2013, the panel voted to change the Ki-67 threshold to >=20% indicating "high" proliferation. Based on the study by Prat et al. [102] the panel also suggested that PR had the ability to distinguish between luminal A and luminal B-like tumours, and that a PR threshold of >=20% could be used to differentiate luminal A-like of luminal B-like (HER2-) tumours [103]. In the proposed classification Ki-67 had an important role in distinguishing between luminal A- like and luminal B-like tumours, but as this marker is not routinely collected, they suggested that other measures of proliferation such as grade could be used instead [99, 104]. One year later, a study in over 9,000 women by Maissonneuve et al. [105] proposed an updated definition of the intrinsic molecular subtypes based on an intermediate cut-off for Ki-67 and the use of PR as a prognostic factor only for tumours with intermediate Ki-67. This classification maximises the number of tumours classified as luminal A-like for which chemotherapy can be omitted. This study also found that women with high grade luminal A-like tumours had similar prognosis than women with luminal B-like tumours, suggesting

that grade might be a useful factor to differentiate between luminal A- like and luminal B-like tumours. A study by Ehinger et al. [106] investigating the role of grade in the subtype classification showed that patients with ER+/HER2- low grade tumours had similar prognosis to luminal A-like tumours whereas prognosis in patients with ER+/HER2- high grade tumours was more similar to that in patients with luminal -B like tumours. Further, the study by Lundgren et al. [107] looking at the agreement between the intrinsic molecular subtypes and their surrogate classification showed that using grade to further identify luminal A and luminal B- like tumours improved agreement to 80% in comparison with the original St.Gallen classification or that proposed for Maissonneuve et al. with agreement rates of 62 and 66% respectively. The agreement between the molecular subtypes and its surrogates using IHC markers have been seen to differ depending on the subtype (last column, Table 1.1). TNBC have been shown to be a good surrogate for basal-like tumours with 80% of all basal-like tumours found to be TNBC [101, 108]. However, tumours classified as HER2-enriched by IHC markers show a lower agreement rate with the corresponding molecular subtype with only 41 to 69% estimated to match [109, 110].

1.4.4 Risk factors differences by molecular subtypes

The RFs noted above (section 1.3), have been more consistently associated with ER+/luminal tumours than with ER-/HER2-enriched or TNBC, for which fewer RFs have been identified. ER+ tumours have been associated with reproductive factors (age at menarche, age at menopause, age at first birth, number of births and breastfeeding) and MHT use [111-114]. In contrast, ER- tumours have been more consistently associated with genetic RFs. For example, BRCA1 mutation carrier status is significantly associated with risk of ER- tumours compared to ER+ tumours [115]. Data support aetiologic heterogeneity by molecular subtypes with differential patterns for some RFs.

This section 1.4.4 highlights some of the RFs associated with ER+/luminal and ER-/HER2-enriched or TNBC. A more detailed summary of the established RFs can be found in a recent edition of cancer epidemiology and prevention, 4th edition [6] and Table 1.2 from [116].

Table 1.2 Summary of established BC risk factors by molecular subtypes

Risk factor	ER+		ER-	
	Luminal A	Luminal B	HER2-overexpressing	Triple Negative
Younger age at menarche	+ +	+	unk	+ + +
Greater parity	− − −	unk	unk	+ +
Older age at first birth	+ +	unk	unk	unk
Breastfeeding	− −	− −	unk	− − −
Older age at menopause	+ +	unk	unk	+
Greater BMI (premenopausal)	−	unk	unk	+
Greater BMI (postmenopausal)	unk	unk	unk	unk
Family history	+ + +	+	+ + +	+ + +
Alcohol use	+	unk	+	unk
Use of oral contraceptives	−	unk	unk	+
MHT use	+ +	unk	unk	unk

+++ Consistent evidence of a positive association, ++ probable positive association, + possible positive association. Minuses indicate similar consistency of negative associations. ER=oestrogen receptor, HER2= human epidermal growth factor 2, BMI= body mass index MHT=menopausal hormone therapy, unk=unknown. Table from [116]

1.4.5 Breast cancer treatment by subtype

Apart from tumour characteristics, such as size, grade and stage, hormone receptor status and HER2 status are essential for determining treatment plans, as the different subtypes of BC respond differently to the available treatments. Most primary breast tumours are treated with surgery, chemotherapy and radiotherapy. However, BC treatment usually involves multiple treatment methods and women may also receive therapy according to their hormone receptor status and/or HER2 status.

ER+ breast tumours are usually treated with anti-oestrogen therapy, also known as endocrine therapy or hormone therapy (HT). Anti-oestrogen therapies for the treatment and prevention of BC have been used for decades, and have considerably improved prognosis and reduced the likelihood of recurrence [117].

Table 1.3 presents a summary of the most important treatments developed to target ER and HER2 receptors.

Table 1.3 Therapies targeted to ER and HER2 receptors most commonly used for pre and/or postmenopausal women and date of approval

Treatment	Targeted receptor	Indicated for pre and/or postmenopausal women	Time on market
SERMs			
Tamoxifen	ER	Both (pre and post)	1977
Raloxifene	ER	Postmenopausal women	1997
Fulvestrant	ER	Postmenopausal women	2002
Aromatase Inhibitors			
Formestane	ER	Postmenopausal women	Mid-1980s
Anastrozole	ER	Postmenopausal women	1995
Letrozole	ER	Postmenopausal women	1997
Exemestane	ER	Postmenopausal women	1999
Trastuzumab (Herceptin)	HER2	Both (pre and post)	1998

ER=oestrogen receptor, HER2= human epidermal growth factor 2, SERM= selective oestrogen receptor modulator

Tamoxifen, a selective ER modulator (SERM), was the first anti-oestrogen therapy. Developed in 1977, tamoxifen acts by blocking the binding of oestrogen to the ER of the BC cells and therefore, preventing growth and proliferation of ER+ breast tumours [118]. Tamoxifen was first approved for the treatment of advanced BC but has been since used as adjuvant therapy for the treatment of primary BC. Treatment with tamoxifen is sometimes combined with chemotherapy, especially in women with higher risk of recurrence, as combined treatment is more effective than tamoxifen alone [119]. This treatment usually lasts for many years since the benefit is greater in women treated for 5 years compared to women treated for 1 or 2 years. A RCT from the early breast cancer trialists' collaborative group found a reduction in recurrence and mortality of 47% (SD 3) and 26% (SD 4) respectively for women treated for 5 years, almost double the reduction found for women treated for 1 or 2 years [117]. However, the long periods of tamoxifen use in women with BC may lead to the development of drug resistance. Further, tamoxifen has been found to be associated with an increased risk of endometrial cancer in postmenopausal women and thromboembolism [120]. In contrast, tamoxifen use for the treatment of BC in

premenopausal women is not associated with adverse risk. Although the benefits of this drug in postmenopausal women are considered to outweigh its harms, other hormone therapies with lower side effects have been developed over time. For example, raloxifene, another SERM produced to treat osteoporosis, has been seen to reduce the risk of BC by 50% (95% CI: 29% to 85%) and, in contrast to tamoxifen, it may be associated with a decreased risk of endometrial cancer [121]. Fulvestrant, an ER antagonist to treat metastatic postmenopausal women with ER+ breast tumours, was also developed in the early 2000s. Compare to tamoxifen, fulvestrant decreases PR expression while maintaining the same levels of efficacy and tolerance as tamoxifen [122].

In the last 20 years, aromatase inhibitors (AI) are increasingly being used to treat BC. AI indirectly target the ER by inactivating the aromatase enzyme responsible for the conversion of androgen to oestrogen and, therefore, decreasing the levels of circulating oestrogen. Formestane was the first AI tested in clinical trials in women who had relapsed after being treated with tamoxifen or other available treatments. AI are as effective as tamoxifen and the third generation (anastrozole, letrozole and exemestane) have been found to be better than tamoxifen as first line treatment for advanced cancers. A trial comparing letrozole and tamoxifen found that letrozole was associated with longer time to disease progression, longer time to treatment failure and better overall response rate than tamoxifen in postmenopausal women with advance disease [123]. Several studies have also reported the efficacy of AI for treatment of early BC in postmenopausal women with ER+/PR+ cancers. The Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial reported that adjuvant treatment with anastrozole was better than treatment with tamoxifen or combined treatments for hormone receptor positive women, and that anastrozole treatment had a longer effect in tumour reduction than tamoxifen [124]. Another trial reported that treatment with exemestane after two to three years of tamoxifen use, reduced the risk of disease compared to five years of treatment with tamoxifen [125].

In addition, AI are well tolerated, with fewer side effects than tamoxifen. Results from the ATAC trial suggests that women treated with anastrozole had lower incidence of vaginal bleeding and discharge, thromboembolism, hot flashes and endometrial cancer but higher incidence of fractures and musculoskeletal disorders than women treated

with tamoxifen [124]. For all these reasons, AI are now challenging the use of tamoxifen as the current adjuvant endocrine therapy standard for ER+ breast tumours. However, AI are not indicated for premenopausal women. Further, women with ER-negative or PR-negative tumours are unresponsive to both AI and tamoxifen.

HER2 status has also been an important indicator for treatment in the last years. Approximately 12-30% of breast tumours overexpress HER2 [91-93], these tumours are generally more aggressive and sometimes they cannot be treated with chemotherapy [90]. For that reason, anti-HER2 therapy specifically targeted to these tumours has been an important treatment development in the last years. Trastuzumab (Herceptin), the first anti-HER2 drug, was approved in 1998 and launched in 2006 in the UK. Trastuzumab is the primary anti-HER2 therapy that improves overall survival (OS) in women with early and advanced disease that overexpresses HER2.

HT is used for ER+ tumours, however, only 5% of ER- tumours respond to anti-oestrogen therapy [126] explaining why they are not usually treated with this kind of therapy. ER-negative tumours have limited treatment options, usually surgery and chemotherapy. Research is now focused on developing new treatments for advanced BCs and the more aggressive subtypes and using genetic molecular profiling to develop personalised therapies [127].

1.5 Breast cancer screening

Early detection of BC allows earlier treatment and therefore, it is associated with a decreased risk of mortality regardless of lead time bias. In a recent meta-analysis of 11 RCTs, participants in the screening programme showed a 20% (95% CI: 11% to 27%) reduction in mortality compared to those in the control group [128]. Mammography is the most widely used method for early detection of BC and most countries have implemented mammographic screening programs. In Scotland, a national mammographic screening programme was established in 1988 and women aged 50 to 70 years old are invited to have a routine screen every three years. Women over 70 years of age are able to make appointments for continued screening.

Although mammographic screening and early detection programs for BC have substantial benefits, they are also associated with some harms. A recent independent review by Marmot et al investigated the potential benefits and harms of BC screening

[129]. Marmot et al. reported that the main harm of BC screening was overdiagnosis, which consists of the detection of a tumour that would not have been detected otherwise during a woman's lifetime. Based on three RCTs, the review estimated that the frequency of overdiagnosis was 19% (95% CI: 15% to 23%) for a woman invited to screening during the period of the screening programme. Evidence from observational studies estimated overdiagnosis ranging from 0 to 37% [129]. The main consequences of overdiagnosis are that women become patients; they may receive unnecessary treatment, suffer from physical and psychological distress and have poorer quality of life due to the diagnosis. Further, women may have a false-positive mammogram result. A systematic review by Bond et al. [130] found that women with a false-positive result may have psychological distress for a long period of time (up to three years after the mammogram). Besides, a false-positive result may also have a negative effect in the likelihood of a woman returning for screening in the next round [130].

BC screening has an effect on incidence and therefore it should be considered when looking at incidence rates of BC. A UK study looking at the effect of mammographic screening on BC incidence reported a long-term increase in incidence of BC for women who attended screening compared to women who did not attend [131]. In the US, screening mammography has also been associated with a 2-fold increased incidence of early-stage breast tumours but not with the incidence of advanced tumours that decreased by 8% [132].

A reduction of advanced BC incidence in screened populations compared to non-screened populations is an indicator of the effectiveness of a mammographic screening programme. Autier et al, reported that, in general, trends in advanced BC incidence in areas with sustained mammographic screening (7 to 15 years of screening) did not change over time [133]. New evidence suggests that the decrease in mortality in countries with BC screening programmes, is mostly due to an improvement in patient treatments and not to mammographic screening [134]. Autier et al concluded that new methods for BC screening should be implemented in order to decrease mortality and minimise the harms of mammographic screening [134].

1.6 Justification for this PhD project and aims

BC incidence has been shown to differ by ER status in limited studies in the last three decades. ER represents an important target for responsiveness to anti-oestrogen therapy and aetiologic differences by RFs. Few countries collect data on ER status and other molecular markers routinely. In Scotland, ER data were collected on BCs from 1997-present, representing the longest duration of data collection in the UK, since Wales does not hold any data on ER, Northern Ireland holds data from around 2008, and England holds data from about 2009. For that reason, the Cancer Registry data from Scotland provides a unique resource for the analysis of longer-term temporal trends of BC by ER status and molecular subtypes in the UK. Only one article has looked at the incidence of BC by ER status in Scotland from 1997 to 2007 and therefore, current trends by ER and the molecular subtypes of BC are unknown.

The literature review will describe the temporal trends of BC incidence by ER status observed in countries with European ancestry populations and will help identify the gaps in the literature and provide comparison data for the trends in Scotland. Further, the analysis of the temporal trends in Scotland will help us understand if there are differences in incidence and survival by molecular subtypes and investigate the underlying RFs associated with the observed trends. It may also shed some light about the specific characteristics of the women who are at greater risk of developing aggressive tumours and have worse prognosis therefore, more likely to benefit from prevention, screening or treatment interventions.

ER and HER2 status in BC is essential for treatment decisions and therefore, knowing the evolution over time of the different BC subtypes and predicting future incidence patterns will help to allocate resources for treatment and prevention programmes, as well as inform policy.

Therefore, the overall aims for this project are:

- To systematically identify population-based studies in incidence trends by ER status that can be used as a comparison for the trends observed in Scotland
- To describe temporal incidence and survival trends of BC by molecular subtypes in Scotland

- To identify subgroups of women with increased incidence risk and worse outcomes that could further benefit from targeted prevention or intervention programmes

Chapter 2 Literature Review

2.1 Background

This chapter relates to the first aim of the PhD: to perform a systematic search of the literature on trends of incidence of BC by ER status in Europe, US, Canada, Australia and New Zealand.

In order to know how many high-income countries were collecting ER data in their cancer registries, I contacted the cancer registries (national or regional) in 47 countries: 43 countries with European ancestry majority populations in the WHO European region, Canada, the US, Australia and New Zealand. The full list of countries can be found Appendix A.1.

Of the 47 cancer registries contacted only registries from 16 countries (34%) collected ER status data (Figure 2.1). Six countries (Bulgaria, Denmark, Ireland, Iceland, New Zealand and Norway) were collecting ER status in their national cancer registries and ten countries (Australia, Canada, France, Germany, Italy, Spain, Sweden, Switzerland, the UK and the US) in some of their regional registries. Twenty-one countries (45%) were not collecting ER data and 10 countries (21%) did not answer my query.

Figure 2.1 Cancer registries in high-income countries collecting ER status

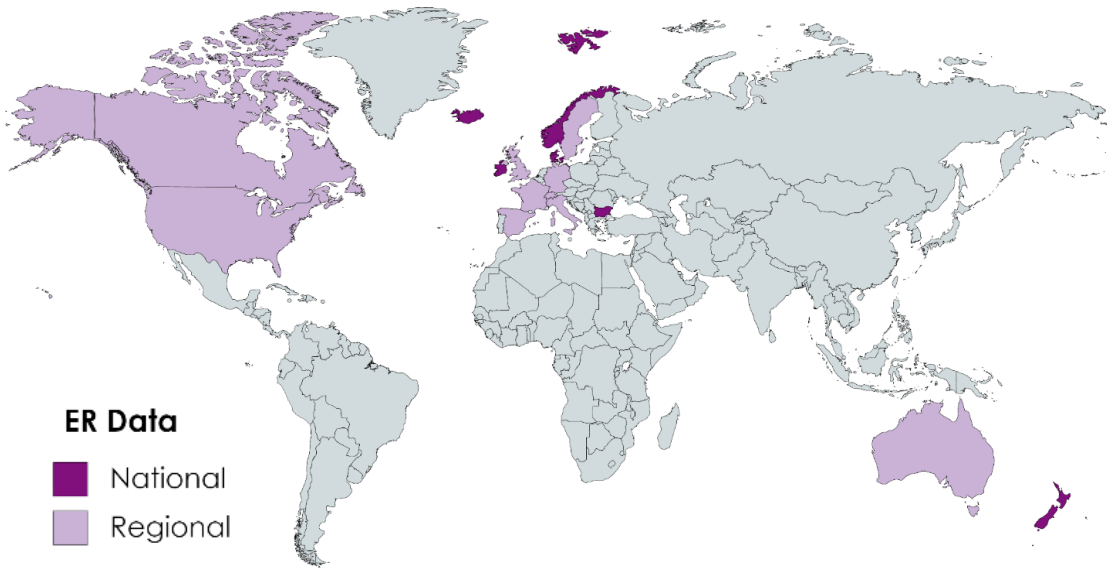


Figure created with mapchart.net

Apart from the limited information about ER status in the cancer registries of HIC, to my knowledge this is the first systematic review on incidence of BC by ER status. For those reasons, the objective of this systematic review is to describe temporal trends of BC incidence by ER status in HIC and to determine if data support the hypothesis that incidence trends by ER status have changed over time.

2.2 Methods

2.2.1 Search strategy

An electronic literature review search on MEDLINE, EMBASE and Web of Science core collection was conducted, using a combination of MeSH and keywords for 'breast cancer', 'incidence' and 'oestrogen receptor'. The search strategy was devised with advice from librarians at the University of Edinburgh and is available in Appendix A.2. The search was restricted to English articles in humans published up to January 2018. In addition to the online search, cancer registries for all the WHO European area countries with European ancestry majority populations (43 countries), the US, Canada, Australia and New Zealand were contacted, when possible, to request information on ER status and published articles looking at BC incidence by ER status. The publication sections and library resources from individual cancer registries webpages, IARC and major cancer association webpages were also searched for publicly available reports. The bibliographies of the selected articles were reviewed and relevant studies included in the final selection.

2.2.2 Inclusion criteria

Studies were selected if they reported incidence of invasive female BC stratified by ER status. Additionally, study data had to come from population-based or cancer registries of countries with a majority of European ancestry populations. Reviews, editorial comments with no additional data reported, conference or meeting abstracts, or studies with duplicate populations were excluded. Studies reporting only incidence rates in men or reporting incidence rates for a subgroup (other than ER status) or by age at diagnosis instead of year of diagnosis were also excluded. Additional inclusion criteria are reported in Appendix A.3.

2.2.3 Screening, selection and data extraction

Record screening was based on title and/or abstract review. A second pass screening was performed on abstracts and/or full texts where eligibility was uncertain. During the data extraction phase, information on country, data source, study start and end year, number of cases, number (%) of cases with ER status, women's age, categories of BC subtypes reported in the study, year at which collection of ER, PR and HER2 status started and information on screening was extracted (Table 2.1). The outcomes for each study were also extracted and are presented in Figure 2.3 and Table 2.2 (overall incidence rates) and Table 2.3 (age-specific incidence rates). Results were summarised using narrative synthesis.

2.3 Results

The initial search identified 5413 articles, 1976 from Medline, 1795 from Embase, 1609 from Web of Science, 31 from individual cancer registries and IARC webpages and two from bibliographic references of the selected articles. After 1572 duplicate articles were excluded, 3841 articles remained for title and/or abstract screening. During the screening phase, 3648 articles were excluded because they were not relevant. Therefore, a total of 193 articles were retrieved for full-text assessment. Of these, 179 were excluded as they did not fulfil the inclusion criteria (Figure 2.2) and 14 articles were selected [135-148]. These 14 studies presented data from eight countries: Denmark (n=2), France (n=1), Germany (n=1), Ireland (n=1), Norway (n=1), Scotland (n=1), Sweden (n=1) and the US (n=6). The studies were published between 2007 and 2017 and reported incidence trends from 1980 to 2013. Study sample sizes ranged from more than half million women for the US articles using Surveillance, Epidemiology and End Results (SEER) data to 3,792 cases for the French study from Fontenoy et al [139]. The percentage of total BC cases that had available ER status ranged from 71% to 94% and there was variability between years in some studies. Nine studies reported incident BC cases in women of all ages and five studies looked only at women aged 50 years or older. There was also variability in the hormonal status reported by studies: 11 studies reported incidence by ER status, five reported incidence by joint ER/PR status and one reported incidence by ER/HER2 combination. Seven countries had a national or regional screening programme

implemented (Appendix A.4), with women aged 50 years or older screened every two to three years. In the US, no national or regional screening programme has been implemented but women aged 40 to more than 75 years may still be screened every one to two years. Study characteristics are summarised in Table 2.1.

Figure 2.2 Study selection flow diagram for breast cancer incidence by ER status

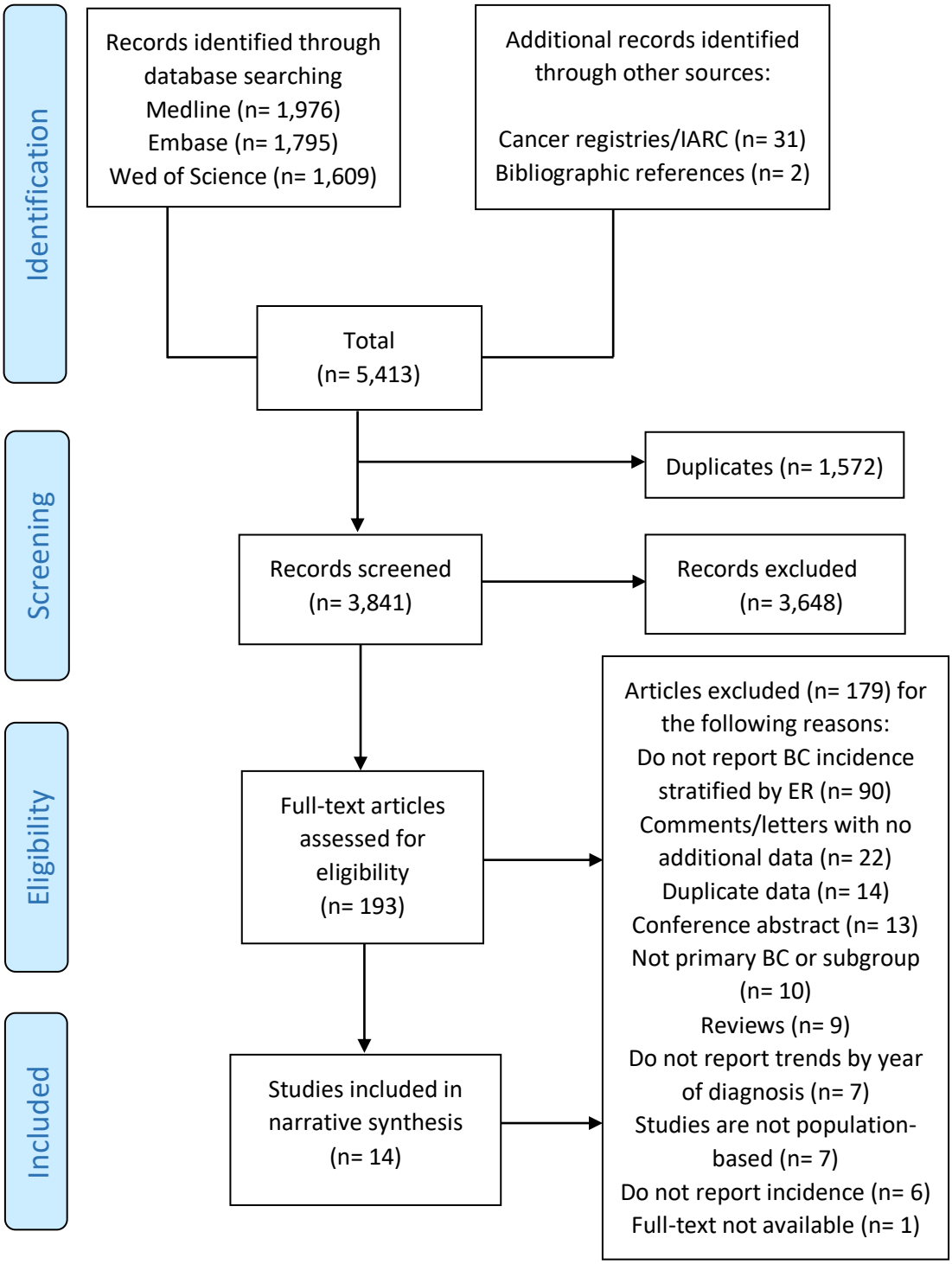


Table 2.1 Characteristics of the studies included in the review of breast cancer incidence by ER status organised by study period start year (page 1 of 3)

Study	Country	Data source	Start year	End year	Number of cases	Number of cases with ER status (%)	Women's age	Breast cancer subtypes categories reported	Year that collection of ER, PR, HER2 started	Screening programme? (Year of implementation)	Age of women invited for screening and time interval
Glass et al. (2007)	USA	Population-based tumour registry of Kaiser Permanente and medical records	1980	2006	7,386	81% in 1989 and 99% in 2006.	<45 to 60+	ER status: ER+, ER- and unknown.	ER status since mid-1970s.	No	40 to 75+ years, every 1-2 years
Jemal et al (2007)	USA	SEER 9	1990	2003	394,891	NR	40 years or older	ER status: ER+, ER- and unknown. ER/PR status also reported.	ER/PR status since 1990.	No	40 to 75+ years, every 1-2 years
Keegan et al. (2007)	USA	California Cancer registry	1990	2004	NR	83% in San Francisco Bay Area and 71% in the rest of California.	<40 to 70+ years	ER status: ER+, ER- and unknown. ER/PR status also reported.	ER/PR status since 1990.	No	40 to 75+ years, every 1-2 years
Anderson et al. (2011)	USA	SEER 13	1992	2008	429,757	358,624 (83%)	30 to 84 years	ER status: ER+, ER- and unknown.	ER status since 1990.	No	40 to 75+ years, every 1-2 years
Anderson et al (2013)	Denmark	Danish breast cancer group (DBCG) and Danish cancer registry	1993	2010	62,549	57,587 (92%)	30 to 84 years	ER status: ER+, ER- and unknown.	ER status since 1977.	Yes (1991)	50 to 69 years, every 2 years

Table 2.1 (continued) Characteristics of the studies included in the review of breast cancer incidence by ER status organised by study period start year (page 2 of 3)

Study	Country	Data source	Start year	End year	Number of cases	Number of cases with ER status (%)	Women's age	Breast cancer subtypes categories reported	Year that collection of ER, PR, HER2 started	Screening programme ? (Year of implementation)	Age of women invited for screening and time interval
Bigaard et al (2012)	Denmark	DBCG and Danish cancer registry	1996	2007	37,544	35,195 (93%)	Younger than 80 years old.	ER status: ER+, ER- and unknown.	ER status since 1977.	Yes (1991)	50 to 69 years, every 2 years
Hofvind et al. (2012)	Norway	NBCSP and Norwegian Cancer registry	1996	2009	NR	NR	50 to 70 years	ER/PR status: ER+/PR+ and ER-/PR-	ER/PR status since 1996.	Yes (1996)	50 to 69 years, every 2 years
Sharpe et al. (2010)	Scotland	Scottish Cancer Registry	1997	2005	NR	NR	50 to 74 years	ER status: ER+, ER- and unknown.	ER status since 1997.	Yes (1988)	50 to 70, every 3 years
Lambe et al. (2010)	Sweden	Cancer registries of Stockholm-Gotland, Vastra Gotaland, and Uppsala-Orebro	1997	2007	NR	89%	50-59 years	ER status: ER+, ER- and unknown.	In Stockholm-Gotland since 1976, in Vastra Gotaland unknown date, and in Uppsala-Orebro since 1992.	Yes (1986)	40 to 74 years, every 18 months (age 40 to 49) and every 2 years (age 50+)
Rusner et al. (2012)	Germany	Population-based cancer registries of Brandenburg, Munich and Saarland.	1998	2007	50,378	85% -93%	50 to 70+ years	ER/PR status: ER+/PR+, ER-/PR-and mixed: ER+/PR- and ER-/PR+.	Depends on regional registry.	Yes (2002)	50 to 69 years, every 2 years

Table 2.1 (continued) Characteristics of the studies included in the review of breast cancer incidence by ER status organised by study period start year (page 3 of 3)

Study	Country	Data source	Start year	End year	Number of cases	Number of cases with ER status (%)	Women's age	Breast cancer subtypes categories reported	Year that collection of ER, PR, HER2 started	Screening programme ? (Year of implementation)	Age of women invited for screening and time interval
Hou et al. (2013)	USA	SEER 18	2000	2009	677,774	538,716 (79%)	20 years or older	ER status: positive (ER+), negative (ER-) and unknown.	ER status since 1990.	No	40 to 75+ years, every 1-2 years
DeSantis et al. (2011)	USA	SEER 12	2000	2007	NR	Ranging from 82% in 2000 to 93% in 2007.	30 to 70+ years	ER status: positive (ER+), negative (ER-) and unknown.	ER status since 1990.	No	40 to 75+ years, every 1-2 years
Fontenoy et al. (2010)	France	Population- based Loire-Atlantique and Vendee Cancer Registry	2003	2007	3,792	3,555 (94%)	50 to 64 years	ER/PR status: positive (ER+/PR+), negative (ER-/PR-), mixed (ER+/PR- and ER-/PR+) and unknown.	ER/PR status since 2003.	Yes (1989)	50 to 74 years, every 2 years
Mullooly et al. (2017)	Ireland	NCRI	2004	2013	24,845	23,425 (94%)	20 to 84 years	ER status: positive (ER+), negative (ER-) and unknown. ER/HER2 status also reported.	ER status since 2004. No information about HER2 status.	Yes (2000)	50 to 64 years, every 2 years

Abbreviations: DBCG= Danish Breast Cancer Cooperative Group, ER= Oestrogen receptor, HER2= human epidermal growth factor receptor 2, NBCSP= Norwegian Breast Cancer Screening Programme, NCRI= National Cancer Registry of Ireland, NR= Not reported, PR= Progesterone receptor, SEER= Surveillance, Epidemiology and End Results, USA=United States of America.

2.3.1 Overall incidence trends by ER

The outcome data for the seven studies reporting overall rates is summarised in Figure 2.3 and Table 2.2. Outcome measures reported among the studies were age-standardised incidence rates (ASiR) and/or estimated annual percentage change (EAPC). ASiR are the gold standard to report incidence rates that account for the age structure of the population, therefore allowing for comparison between populations. The EAPC is a popular method of trend analysis and estimates the annual percentage change for ASiR assuming a constant rate of change over time (linearity) [149].

Seven studies from three countries (US, Denmark and Ireland) reported overall incidence trends by ER status [135, 136, 138, 140, 143, 144, 146] for women of all ages. These studies show that ER+ BC incidence increased and ER- BC incidence decreased overall between 1980 and 2013 (Figure 2.3). However, incidence rates by ER status fluctuated between time periods.

2.3.1.1 Studies with trends estimated using joinpoint regression analysis

Four of the seven studies [138, 140, 143, 144] (all from the US) used joinpoint regression analysis to investigate whether changes in BC incidence were observed at any time point and EAPC for periods when linear trends were observed. Joinpoint regression analysis is used when the overall trend in incidence is not constant over the entire period of time (nonlinearity). Glass et al, using data from the Kaiser Permanente Northwest (KPNW) registry, reported an increase in incidence of BC from 1980 to 2001, and a subsequent annual decrease of 2.7% until 2006. In contrast, ER- tumours incidence rates decreased from 1980 to 2006 and the decrease was especially sharp from 1999 to 2006 (9.8% annual decrease) [140]. That same year another two studies were published in the US with similar results but for different populations. Jemal et al explored further the temporal incidence trends in the nine oldest SEER cancer registry areas looking particularly at age and ER status [143]. Their findings were consistent with those reported by Glass et al. ER+ rates increased 3% annually (95% CI: 2.0% to 3.9%) from 1990 to 2000. and decreased by 9.1% from 2002 to 2003. ER- rates decreased 1.1% (95% CI: 0.6% to 1.7%) per year from 1990 to 2003. Keegan et al looked at changes in BC incidence in the San Francisco bay area, known for having one of the highest rates of BC in the world, in comparison with the rest of women in

California [144]. Incidence rates of ER+ and ER- breast tumours followed the same pattern than those observed in the SEER and KPNW populations, with ER+ increasing and ER- decreasing. A decrease in ER+ incidence was also observed in the San Francisco bay area and the rest of California after 2001-2002. The most recent study by DeSantis et al. also found a decrease in the incidence of ER+ tumours after 2000 but incidence rates started to slightly increase from 2003 to 2007 (0.8% annually, p value=0.18). ER- tumour incidence decreased for the whole study period [138].

2.3.1.2 Studies with trends estimated using APC models

The other three studies [135, 136, 146] reported the annual percentage change for the whole study period for three countries: the US, Denmark and Ireland. ER+ BC incidence increased over time in all countries, with an annual percentage change ranging from 0.1% in the US [135] to 3% in Denmark [136], and ER- incidence decreased (EAPC range: -2% in the US [135] to -3.4% in Ireland [146]). The incidence of ER+ tumours fluctuated, especially in the US where, in general, ER+ incidence increased from 1980 until the early 2000s when it fell sharply [135, 140, 143, 144]. In contrast with the decrease observed in the US, in Denmark, ER+ incidence rates remained constant from 2002 to 2007 and increased again after that time.

Figure 2.3 Summary of age standardised incidence rates of BC by ER status for all the studies reporting overall rates

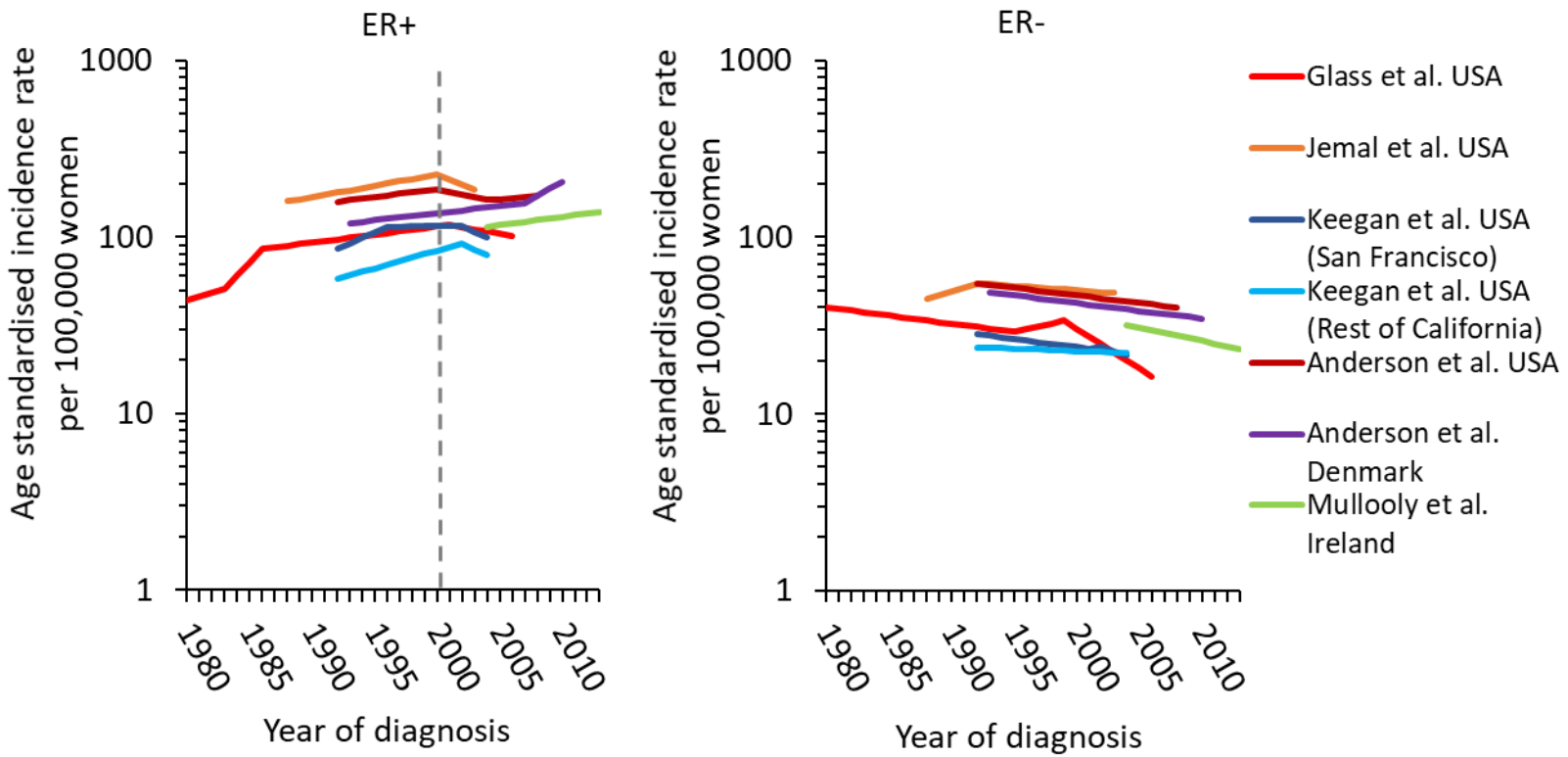


Figure has been calculated using ASiR and EAPCs reported in the individual studies and found in **Error! Not a valid bookmark self-reference.** and is presented in the log scale. ASiR=Age standardised incidence rate, EAPC=estimated annual percentage change, ER= oestrogen receptor, USA=United States of America. Dotted line represents year 2000, around the time of the publication of the WHI study. Rates from DeSantis et al. not presented as they did not report overall incidence rates by ER status only EAPCs.

Table 2.2 Age-standardised incidence and estimated annual percentage change in incidence of BC by ER status for seven studies reporting overall rates organised by study date and time periods (page 1 of 2)

Study and country	Overall estimates by time period				
	1980-1989	**1990-1998**	**1999-2002**	**2003-2007**	**2008-2013**
Glass et al. (2007), USA	EAPC: ER+= **5.0% (3.7, 14.4)** from 1980 to 1983, **18.9% (0.1, 41.2)** from 1983 to 1986. ER-= **-2.1% (-3.2, -1.0)** from 1980 to 1995	EAPC: ER+= **2.1% (1.2, 2.9)** from 1986 to 2001. ER-= 3.7% (-9.0, 18.1) from 1995 to 1999.	ASiR: ER-= 24.0 in 2002-2003. EAPC: ER+= -2.7% (-6.4, 1.1) from 2001 to 2006 ER-= **-9.8% (-12.8, -6.6)** from 1999 to 2006.	ASiR: ER-= 15.9 in 2004-2005 and 16.6 in 2005-2006	
Jemal et al (2007), USA		EAPC: ER+=**3% (2.0, 3.9)** from 1990 to 2000. ER-=**-1.1% (-0.6, -1.7)** from 1990 to 2003,	EAPC: ER+=-9.1% from 2002 to 2003. ER-=-4.8% from 2002 to 2003.		
Keegan et al. (2007), USA		EAPC: *San Francisco Bay area:* ER+=**6.9% (3.0, 10.8)** from 1992 to 1996, 0.2% (-2.3, 2.9) from 1996 to 2002 ER-=**-2.1% (-2.9, -1.4)** from 1992 to 2004. *The rest of California:* ER+=**4.5% (3.7, 5.4)** from 1992 to 2001 ER-=-0.6% (-1.3, 0.2) from 1992 to 2004.	ASiR: *San Francisco Bay area*: ER+/PR+=96.0 from 2001 to 2002. ER+/PR-=19.5 from 2001 to 2002. ER-/PR+=2.3 from 2001 to 2002. ER-/PR-=21.6 from 2001 to 2002. *The rest of California:* ER+/PR+=76.0 from 2001 to 2002. ER+/PR-=15.9 from 2001 to 2002. ER-/PR+=1.9 from 2001 to 2002. ER-/PR-=20.5 from 2001 to 2002 EAPC: *San Francisco Bay area:* ER+= -10.4% (-20.2, 0.7) from 2002 to 2004.ER-=**-2.1% (-2.9, -1.4)** from 1992 to 2004. *The rest of California:* ER+= **-7.3% (-11.2, 3.2)** from 2001 to 2004. ER-=-0.6% (-1.3, 0.2) from 1992 to 2004	ASiR: *San Francisco Bay area*: ER+/PR+= 83.7 from 2003 to 2004. ER+/PR-= 15.3 from 2003 to 2004. ER-/PR+= 1.0 from 2003 to 2004. ER-/PR-= 20.3 from 2003 to 2004/ *The rest of California:* ER+/PR+= 65.9 from 2003 to 2004. ER+/PR-= 14.1 from 2003 to 2004. ER-/PR+= 1.3 from 2003 to 2004. ER-/PR-= 19.6 from 2003 to 2004.	

Table 2.2 (continued) Age-standardised incidence and estimated annual percentage change in incidence of BC by ER status for seven studies reporting overall rates organised by study date and time periods (page 2 of 2)

Study and country	Overall estimates by time period				
	1980 -1989	1990-1998	1999-2002	2003-2007	2008-2013
Anderson et al. (2011), USA		ASiR: in figure. EAPC: ER-=**-1.95% (-2.12, -1.79)**			
Anderson et al (2013), Denmark		ASiR: ER+=rose from 155 to 206 in 2007. ER-=fell from 48 to 37 during the study period (1993 to 2010). EAPC: ER+= **3.0% (2.8, 3.3)**, ER-=**-2.1% (-2.5, -1.6)**.			
DeSantis et al. (2011), USA				ASiR: ER+= constant. ER-= decreased EAPC: ER+=0.8%. ER-=-3.4%.	
Mullooly et al. (2017), Ireland				ASiR: in figure. EAPC: **ER+=2.2% (1.0, 3.5), ER-=-3.4% (-5.1, -1.8), ER+/HER2-=2.9% (1.3, 4.4)**, ER+/HER2+=-1.6% (-4.3, 1.3), **ER-/HER2+=-4.6% (-6.5, -2.6), ER-/HER2-=-3.0% (-4.9, -1.1).**	

Abbreviations: ASiR= age-standardised incidence rate, EAPC= estimated annual percentage change, ER= Oestrogen receptor, PR= Progesterone receptor, USA=United States of America. Significant results in bold.

2.3.2 Incidence trends by ER between pre and postmenopausal women

Nine studies reported incidence trends for women in all age groups and six studies for women aged 50 years or older (Table 2.1). One study defined postmenopausal status as not having menstrual bleeding for the past year [137]. In the rest of studies reporting menopausal status, age was used as a proxy, with 50 years being the cut-off point for menopausal status, i.e. women age<50 years are considered premenopausal and women aged 50 years or older are considered postmenopausal.

2.3.2.1 Results from studies reporting pre and postmenopausal incidence rates by ER.

Age specific estimates of incidence by ER status for pre and postmenopausal women are summarised in Table 2.3. Of the 11 studies presenting age-specific incidence rates, five reported incidence for pre and postmenopausal women [135, 137, 138, 142, 146] and their results are summarised below by country.

US

Anderson et al. showed that the increase in incidence of ER+ tumours from 1992 to 2008 in the US, was higher in women of 50+ years of age than in women of 30 to 49 years of age [135]. In contrast, annual declines in ER- incidence were more marked in the younger than the older age group (2.4% vs 1.4% annually). SEER data from 18 registries for the years 2000 to 2009, found an increasing trend of ER+ incidence for the premenopausal age groups (20-39 and 40- 49 years) [142]. For women >=50 years of age ER+ incidence decreased from 2000 to 2004 and started to increase again in 2005. ER- incidence decreased for all age groups, irrespective of menopausal status but the decrease was most marked in women aged 50 to 69 years (4.5% per year). DeSantis et al. reported that ER+ breast tumours in US women significantly increased 2.7% per year from 2003 to 2007 for women aged 40 to 49 years and there was a trend towards an increase for women aged 30 to 39 years [138]. For postmenopausal women, there was a slight decrease of 0.3% per year for the age groups from 50 to 59 and 70 or more, and an increase of 1.6% for women aged 60 to 69, none of these time trends were statistically significant. The ER- incidence trend was consistent with previous studies showing a decrease for all age groups. The decrease was higher among women aged 40 to 49 years and 60 to 69 years.

Denmark

A study in Denmark by Bigaard et al.[137] found that postmenopausal women had a similar increase in incidence of ER+ tumours (2% per year, 95% CI: 1.1 to 2.8), than women younger than 35 years (2.2%, 95% CI:-0.4 to 4.8). They also found a significant increase in ER+ incidence for premenopausal women aged more than 35 years from 1996 to 2002 that levelled off after that time up to 2007. ER- incidence decreased for the whole study period for women aged 35 to 49 years and for postmenopausal women by 4.5% (95 % CI =-6.5 to -2.5) and 3% (95 % CI =-4.3 to -1.7) per year respectively, but increased for women younger than 35 years (1.4%, 95% CI: -2.8 to 5.7), however it was not statistically significant.

Ireland

In Ireland, ER+ incidence increased and ER- incidence decreased for all age groups from 2004 to 2013 [146]. However, the drop in incidence of ER- tumours was higher for premenopausal women aged 30 to 49 years (-3.1%, 95% CI: -4.5 to -1.7) and for postmenopausal women aged more than 65 years (-4.2%, 95% CI: -5.8 to -2.5).

2.3.2.2 Results from studies reporting only postmenopausal incidence rates by ER.

The other six studies presented incidence trends for post-menopausal women only [139, 141, 143, 145, 147, 148] and their results are summarised below by country.

Norway

A study from Norway by Hofvind et al. [141] showed that the incidence of hormone receptor positive tumours (ER+/PR+) increased for all age groups during the study period, incidence in women aged 55 to 59 years peaked in 2002 and this trend was not observed for the rest of age groups.

Scotland

In Scotland, Sharpe et al investigated trends in BC incidence by ER status using Scottish cancer registry data [148]. They found that ER+ incidence increased for postmenopausal women from 1997 to 2000, after which a sharp decrease in incidence took place and this was most marked in women aged 50 to 64 years (11.2% decrease from 2000 to 2005). This decrease was also observed in women aged 65 to 74 years but it was smaller and only lasted until 2002 when the incidence of ER+ tumours

started to increase again. ER- incidence decreased by 44.3% from 1997 to 2005 in women aged 50 to 64 years and remained constant in women aged 65 to 74 years.

Sweden

Another study using data from three regional population-based cancer registries in Sweden [145] reported similar results to those seen in Norway and Scotland. In postmenopausal women aged 50 to 59 years, ER+ incidence increased from 1997 to 2003 and decreased from 2003 to 2007, while ER- incidence slightly decreased. The time at which ER+ incidence trend changed was later than in Scotland but more similar to Norway.

Germany

In Germany, Rusner et al [147] EAPC in BC incidence by ER status for data from 3 regional registries. The results for this German study were different to those observed in the rest of the countries with no clear evidence of an ER+/PR+ incidence increase from 1998 to 2007, except for women aged 70 years or more in the Munich area for whom ER+/PR+ incidence rose 2.4% per year (95% CI: 1.7 to 3.2). ER-/PR-, ER+/PR- and ER-/PR+ incidence remained constant.

France

The last study reporting incidence trends by ER status for postmenopausal women used data from the Loire-Atlantique region in France and reported a sharp decrease in incidence of ER+/PR+ tumours from 2003 to 2006 (EAPC=-12.2% , 95% CI: -17.2 to -6.8) [139]. ER-/PR- tumours were also observed to decrease for this time period but not statistically significant.

Overall, the literature supports higher annual increase in incidence of ER+ tumours among postmenopausal women than among premenopausal women, however postmenopausal women also experienced a more marked decrease around the early 2000s. ER- incidence fell in most studies and the decrease was not consistently associated with age.

Table 2.3 Age-specific incidence and annual percentage change in incidence of breast cancer for studies reporting age specific rates (n=11) (page 1 of 2)

Study and country	Age-specific estimates	
	Pre-menopausal	*Post-menopausal*
Jemal et al (2007) , USA	NR	50 to 69 years: Much larger decrease from 2002 to 2003 in ER+/PR+ tumours than in ER-/PR- tumours. 65 to 69 years: ER+=-20% from 2002 to 2003. ER-=2% from 2002 to 2003.
Anderson et al. (2011) , USA	30 to 49 years: ER+=1.2% (1.0, 1.3), ER-= **-2.4% (-2.66, -2.18%)**.	50 to 84 years: ER+=high (driving the overall pattern), ER-=-**1.35% (-1.52, -1.19).**
Bigaard et al (2012), Denmark	Younger than 35 years: ER+= 2.2% (-0.4, 4.8). ER-=1.4% (-2.8, 5.7). Premenopausal, >35 years: ER+= **10.4% (7.3, 13.6)** from 1996 to 2002 and **-3.4% (-6.6, -0.1)** from 2003 to 2007. ER-=**-4.5% (-6.5, -2.5).**	Postmenopausal: ER+= **2.0% (1.1, 2.8)**. ER-=**-3.0% (-4.3, -1.7).**
Hofvind et al. (2012), Norway	NR	50 to 54 years: ER+/PR+=increased slowly (no peak) from 1996 to 2009. 55 to 59 years: ER+/PR+=increased. Peaked in 2002 with 280 women per 100,000 cases. Decreased after 2002. 60 to 64 years: ER+/PR+=increased from 1996 to 2009.
Sharpe et al. (2010), Scotland	NR	Overall percentage change: 50 to 64 years: ER+=31.5% from 1997 to 2000 and -11.2% from 2000 to 2005. ER-=-44.3% from 1997 to 2005. 65 to 74 years: ER+=30.4% from 1997 to 2000, -4.1% from 2000 to 2002 and 41.3% from 2002 to 2005. ER-=constant.
Lambe et al. (2010), Sweden	NR	50 to 59 years: ER+=increase from 1997 to 2003 and decrease from 2003 to 2007. ER-=slight decrease

Table 2.3 (continued) Age-specific incidence and annual percentage change in incidence of breast cancer for studies reporting age specific rates (n=11) (page 2 of 2)

Study and country	Age-specific estimates	
	Pre-menopausal	*Post-menopausal*
Rusner et al. (2012), Germany	NR	50 to 69 years: Brandenburg: ER+/PR+=constant, Munich: ER+/PR+=constant, Saarland: ER+/PR+=constant. 70 years or older: Brandenburg: ER+/PR+=constant, Munich: ER+/PR+=**2.4% (1.7, 3.2)**, Saarland: ER+/PR+=constant. ER-/PR- and mixed tumours did not reveal obvious pattern neither.
Hou et al.(2013) , USA	20-39 years: ER+=1.8%. ER-=-1.8%. 40-49 years: ER+=1.5%. ER-=-3.0%.	50-69 years: ER+=-4.8% from 2000 to 2004 and 1.4% from 2004 to 2009. ER-=-4.5%. 70 years or older: ER+=-3.3% from 2000 to 2004 and 1.6% from 2004 to 2009. ER-=-2.1%.
DeSantis et al. (2011) , USA	30 to 39 years: ER+=1.5%. ER-=-3.1%. 40 to 49 years: **ER+=2.7%. ER-=-5.7%.**	50 to 59 years: ER+=-0.3%. ER-=-4.9%. 60 to 69 years: ER+=1.6%. **ER-=-2.3%.** 70+ years: ER+=-0.3%. ER-=-0.9%.
Fontenoy et al. (2010), France	NR	50 to 64 years: ER+/PR+=**-12.2% (-17.2, -6.8)**, ER-/PR-=-6.9% (-17.2, 4.7), ER+/PR-=0.1% (-10.5, 12.0), ER-/PR+ =25.5% (-14.5, 84.3).
Mullooly et al. (2017), Ireland	30 to 49 years: ER+=1.2% (-1.4, 3.9) **ER-=-3.1% (-4.5,-1.7)**	50 to 64 years: ER+=3.0% (-1.4, 7.6), ER-=-3.4% (-7.1, 0.6) 65 years or older: **ER+=2.0% (0.0, 4.0), ER-=-4.2% (-5.8, -2.5)**

Estimates are estimated annual percentage change (EAPC) unless stated otherwise. Significant results in bold. ER= Oestrogen receptor, NR= Not reported, PR= Progesterone receptor, USA= United States of America.

2.3.3 Incidence trends by ER before and after WHI

As explained in section 1.3, MHT use has been associated with an increased risk of BC, especially for ER+ tumours. Whether incidence trends by ER changed after the publication of the Women's Health Initiative study (WHI) in 2002 was evaluated in 12 studies. These studies provide evidence that ER+ incidence decreased after the results of the WHI study and this was observed in different countries (Table 2.2).

In the US, all studies reported decreases in ER+ incidence in the early 2000s [135, 138, 140, 142-144]. Some studies also showed that this drop was higher in postmenopausal women [136, 138, 142]. There was variability between studies in the year at which the incidence of ER+ tumours started to decline and some estimates did not reach significance. Studies also suggest that the decrease stopped around 2006 and ER+ incidence started increasing again after that time [138, 142].

In other countries, a drop in ER+ breast tumours around the time of the publication of the results of the WHI study has also been reported. The highest decrease was observed in France where ER+/PR+ incidence drop by 12.2% (95% CI: -17.2 to -6.8) annually from 2003 to 2007 [139]. In Norway, the decrease was only observed for the incidence of ER+/PR+ tumours in women aged 55 to 59 but not for women aged 50 to 54 or older than 60 years of age [141]. The study by Sharpe et al. reported a decrease in ER+ incidence in Scotland for all postmenopausal women that was especially sharp (11.2% per year) for women aged 50 to 64 years [148]. However, this drop in incidence in Scotland started in 2000, before the publication of the WHI results. In Sweden, ER+ incidence also decreased from 2003 to 2007 for women aged 50 to 59 years [145].

In contrast with these results, Bigaard et al found that in Denmark the decrease in incidence of ER+ breast tumours after 2002 was only observed for premenopausal women aged 35 years or older, whereas in postmenopausal women the incidence of ER+ tumours increased significantly after 2002 [137]. A more recent study in Denmark confirmed that ER+ incidence rates did not decrease after 2002 but remained constant [136].

2.4 Discussion

2.4.1 Summary of key findings

This review of 14 studies in 8 countries showed that overall ER+ BC incidence have increased and ER- BC incidence have decreased in the last four decades, with EAPCs ranging from 0.8% to 3% for ER+ tumours and -2.1% to -3.4% for ER- tumours. The results also show that the increasing overall trend is mainly driven by the increase of ER+ cancer incidence since they account for around 75% of the total BC cases and ER- cancer incidence is decreasing. It is reassuring to note declining incidence of ER- tumours, as these tumours are usually more aggressive and have worse prognosis than ER+ subtypes.

A possible explanation for the divergent patterns observed between subtypes may be changes in RFs that have different effects on risk of ER+ and ER- tumours. HR+ tumours have been associated with reproductive factors and postmenopausal obesity [150]. Reproductive patterns have considerably changed over time, especially in HIC where women are having less children and at an older age than in the past, both factors associated with an increased risk of ER+ tumours. Obesity has also been associated with an increased risk of ER+ tumours in postmenopausal women but not in premenopausal women. Obesity prevalence is increasing worldwide with the latest estimates reporting an increase in obesity prevalence from 6 to 15% in women between 1975 and 2014 [151]. If obesity rates continue to rise this could result in an increased incidence of ER+ tumours. Age could also contribute to the increase in incidence since people are living longer and most breast tumours are diagnosed at an older age.

Another factor likely important for the increase in incidence of ER+ tumours, is mammographic screening, implemented in all the countries from which the papers for the review were identified during the 80s, 90s and 2000s. Improvement in screening such as the change from analogue to digital mammography, and the extension of mammographic screening invitation to older women may have also contributed to the increases observed.

The literature supports that, in general, postmenopausal women had a higher increase in incidence of ER+ tumours than premenopausal women. Declines in ER- incidence

were similar across all age groups. The greater annual increase in incidence observed for postmenopausal women compared to pre-menopausal women has been hypothesised to be in part related to MHT use. In fact, the reviewed studies showed that although overall ER+ incidence increased over time, around the early 2000s most countries experienced a decrease in ER+ tumour incidence that coincides with the publication of the results of the WHI study that linked MHT use to an increased risk of BC. Most countries reported a decrease in postmenopausal ER+ tumours that was especially high in France (12.2% annual decrease from 2003 to 2006) and in the US. Studies with more recent data suggest that ER+ incidence rates increased again after 2006.

This review also indicates that there is a gap in the literature in the incidence trends by ER for the last decade, with only one study showing incidence trends by ER up to 2013 [146] this is possibly due to the introduction of PR and HER2 as molecular markers and the use of the intrinsic subtypes or its surrogates IHC markers to estimate recent BC incidence trends. In addition, cancer registries from at least 16 HIC with European ancestry populations are collecting ER status routinely but only eight countries have actually published the observed incidence rates by ER status. For the remaining eight countries, incidence trends by ER status remain unknown or unpublished. Although the studies included in this literature review are only from HIC with majority European ancestry populations, the divergent pattern by ER may be observed worldwide and for other ethnicities since overall BC incidence is also on the rise in LMIC. In fact, this divergent pattern has been observed for other ethnic groups in the US [142].

2.4.2 Limitations of the systematic review

2.4.2.1 Limitations related to the studies included in the review.

The studies included are mainly descriptive and with high quality data but bias or confounding may be present. Ascertainment bias could occur as BC diagnosis criteria have changed over time, along with ER/PR measurement techniques. For example, in the US, the cut-off value for classifying a tumour as ER+ changed from 10% to 1% which may have reduced the number of tumours classified as ER- and therefore may have had an effect on the trends observed [152]. Missing ER status was a limitation in all studies and ranged between 29% and 6%. Three studies [135, 136, 146] used

imputation techniques to adjust for missing ER and HER2 status and reported that ignoring missing molecular marker data could result on biased rates especially in the early years for which missing molecular marker data was higher. The definition of a BC and the selection of BC cases used for computing incidence trends are likely to differ between countries. Population estimates, usually based on Census figures, are prone to bias and may underestimate the number of people in each age group and, as a result, overestimate BC incidence rates. The use of number of tumours instead of number of persons as the numerator of the incidence rates might overestimate BC incidence rates too. Furthermore, interpretation of these results is limited since the studies included in the review use population aggregated data and there is restricted or no information on mammographic screening, MHT use and other RFs that could contribute to the incidence trends observed. Future studies should examine the possible reasons for the divergent pattern of ER+ and ER- tumours and look for RFs associated with each of these subtypes.

2.4.2.2 Limitations related to the methodology used to conduct the systematic review

The systematic review consisted on a narrative synthesis of the BC incidence trends by ER status and meta-analysis to estimate the pooled incidence rate per 100,000 women by ER was not conducted as this was not the main purpose of the review. The main aim was to find how many countries in the world were collecting BC molecular marker data routinely on their cancer registries and to compare the rates by ER status between countries. Further, only seven studies were found to report overall BC incidence trends by ER status and heterogeneity between studies was high, including: different methodology to calculate the standardised rates, different time periods presented and different age groups of women; for that reason, some of the time periods had very limited information and summarising the results with a pooled estimate was not deem to be adequate. Another reason was the fact that most studies did not report ASiR for all years and to estimate the ASIR I had to rely on visually inspecting the graphs of the trends to estimate approximate rates- given that some graphs were on the log scale this was difficult to do. Lastly, five of the seven studies that reported overall rates were from the US and given the large sample size of these studies (over 400,000 women) in comparison with the European studies the pooled estimate would be reflecting the trends in the US and not for all countries.

Another limitation of the systematic review was that no standardised quality assessment method, such as the commonly used Newcastle-Ottawa scale (NOS), was used to review the quality of the studies included in the review. Data from all studies, except for that in Glass et al. [140], which comes from KPNW (a large prepaid US health plan), came from regional or national cancer registries of HIC with European ancestry populations and was deem to have a good quality both in terms of completeness and accuracy.

Further, another limitation was the focus on studies that were written or translated into English. However, I did a thorough search for any reports published in the websites of the cancer registries for all European ancestry countries and used google translate to translate the original reports that were not in English and did not find any report presenting incidence by ER status so this limitation is not likely to have excluded any additional articles. Finally, the systematic review was conducted by one reviewer (single screening) due to time constraints instead of the international standard of two reviewers (double screening).

2.5 Conclusion

This systematic review showed that BC incidence differed by ER status in most countries with available data and that increases in recent decades are driven by increases of ER+ tumours which constitute the majority of BCs. The review suggests that molecular marker data is still limited in many countries and that future reporting of trends should be done by ER given the heterogeneity of BCs. Further, it describes international BC trends by ER status that will be compared during this PhD to the observed trends in Scotland, allowing to further generate hypothesis about the factors that might be driving the trends.

Chapter 3 Breast cancer incidence trends by molecular subtypes

3.1 Background

Numbers of incident breast tumours continue to increase due to population aging and the implementation of mammographic screening programmes in the UK [153] and other HIC [154]. The increase in obesity prevalence and changes in major RFs for BC, such as, changes in reproductive factors, may have also contributed to the increases in incidence observed in recent decades [155].

Recent changes in the prevalence of RFs that may have differential effects in the incidence trends, such as, obesity, alcohol consumption and reproductive factors may have had different effects on different subtypes of BC. Further, the literature conducted in Chapter 2 provides evidence that incidence patterns differ by BC subtypes with countries like the US, Denmark and Ireland reporting divergent incidence trends by ER status, with incidence increasing for ER+ tumours and decreasing for ER- tumours. I hypothesise that similar incidence trends may be observed in Scotland.

This chapter aims to describe temporal trends of BC in Scotland by ER status, HER2 status and the combination of ER, PR and HER2 used as a proxy for the intrinsic molecular subtypes derived from genetic profiling. I will also investigate the role of mammographic screening as a possible driver that may be affecting the observed trends and contrast the results with those already observed in other countries.

3.2 Hypotheses

The hypotheses that will be explored in this chapter of the PhD are:

- Incidence trends may differ between molecular subtypes of BC and similar trends to those observed in other countries are also likely to be seen in Scotland due to similar RFs patterns.
- Incidence trends may have changed over time. Increases are expected due to period effects, as the introduction of mammographic screening, and cohort effects, such as, changes in reproductive factors patterns, especially for hormone sensitive breast tumours.

- Increasing age is strongly associated with an increased risk of BC. However, age might have a different effect in incidence depending on the BC subtype.
- Screening is associated with increasing incidence of BC but it might have a differential effect in incidence for the different molecular subtypes.

3.3 Methods

3.3.1 Scottish cancer registry data

The data used for this PhD project have been obtained from Information Services Division (ISD) of the NHS National Services Scotland, the national organisation in Scotland for health information and intelligence that provides statistical services. ISD holds the Scottish Cancer Registry (also known as SMR06) that contains person and tumour based records that are created by linkage of hospital administration records, screening datasets, death registration data, private hospitals data and community prescribing records [156].

The Scottish Cancer Registry was established in 1958, with electronic data available from 1981 and it holds over 1.8 million records, covering all Scottish residents that have ever been diagnosed with cancer. In Scotland, the use of the patient Community Health Index (CHI), that uniquely identifies all Scottish residents registered with a general practitioner (GP), increases the ability to link the cancer data to hospital admissions and death registration data. For that reason, coverage of the Scottish cancer registry is high with an overall estimate of ascertainment of BC cases that exceeds the 98% and that is independent of age [157].

An anonymized dataset derived from SMR06 containing all primary invasive BCs from 1997 (the most recent year for which ER data is available) to 2016 was requested. The governance process consisted of an application to access the data submitted to the Public Benefit and Privacy Panel (PBPP) that contained a thorough explanation of the research project and how the data would be used. Data access was granted by PBPP and available from April 2018 through the National Services Scotland (NSS) National Safe Haven secure environment. The SMR06 variables requested and included in the dataset are shown in Table 3.1 along with a description of data completeness.

Table 3.1 Variables included in the requested SMR06 dataset of primary breast cancer among women in Scotland for 1997-2016 with completeness rate

Variable included in the dataset requested to ISD	Completeness
Individual characteristics	
Age at diagnosis	100%
Scottish health board	100%
NHS Scottish region	100%
SIMD	100%
Tumour characteristics	
Date of diagnosis	100%
Date of registration	100%
Tumour morphology	100%
Tumour grade	83.5%
Clinical TNM classification	
T stage (clinical)	72.1%
N stage (clinical)	64.8%
M stage(clinical)	45.5%
Pathological TNM classification^	
T stage (pathological)	79.9%
N stage (pathological)	79.4%
M stage(pathological)	19.4%
Tumour size	77.1%
Nodal Status	80.8%
Number of positive nodes	99.8%
Method of first detection	97.8%
ER status	92.6%
PR status*	73.8%
HER2 status*	88.9%
Treatment	
Surgery	99.1%
Radiotherapy	95.4%
Chemotherapy	97.5%
Hormone therapy	94.0%
Other therapy	96.4%
Mortality	
Vital status	99.8%
Date of death	100%
Primary cause of death	99%

*Variables registered since 2009 for which no data are available for previous years. ^Pathological TNM stage available from 2005. Percentage based on available years. ER=Oestrogen receptor, HER2= human Epidermal Growth Factor 2, PR= Progesterone receptor, SIMD= Scottish Index of Multiple Deprivation, TNM= tumour, nodes and metastases.

3.3.1.1 Individual characteristics

Age at diagnosis was recorded for all women and age at death was extracted from age at diagnosis and date of death. Age at diagnosis was further stratified in three categories based on the age at which women are routinely invited for mammography screening in Scotland: women aged less than 50 years, women aged 50 to 69 years (approximate ages at which women are routinely screened) and women aged 70 years or older (usually not screened unless they request it). Throughout this thesis the term age is related to age at diagnosis of BC.

The **Scottish health board** in which a woman resided at the time of diagnosis was recorded for all women. There are 14 NHS health boards in Scotland (NHS Ayrshire and Arran, NHS Borders, NHS Dumfries and Galloway, NHS Western Isles, NHS Fife, NHS Forth Valley, NHS Grampian, NHS Greater Glasgow and Clyde, NHS Highland, NHS Lanarkshire, NHS Lothian, NHS Orkney, NHS Shetland, NHS Tayside). The NHS health boards were further classified in larger **Scottish regions**: North region comprising NHS Western Isles, NHS Grampian, NHS Highland, NHS Orkney, NHS Shetland and NHS Tayside; South-East region comprising NHS Borders, NHS Dumfries and Galloway, NHS Fife and NHS Lothian; and West region comprising NHS Ayrshire and Arran, NHS Forth Valley, NHS Greater Glasgow and Clyde and NHS Lanarkshire.

The Scottish Index of multiple Deprivation (SIMD) is an area-based measure of deprivation based on 7 domains: income, employment, health, education, crime, access to services and housing. SIMD ranks the 6,976 data zones in Scotland from the most deprived to the least deprived area and decile and quintiles of all areas are derived from them. SIMD is often expressed in quintiles or deciles and I will use SIMD quintile as measure of deprivation throughout the PhD thesis and compare women in the 20% most deprived areas (quintile 1) with women in the 20% least deprived areas (quintile 5). SIMD was available for all women within the cancer registry with a Scottish postcode. Several SIMD versions (SIMD 2004, 2006, 2009, 2012 and 2016) were available for our study period from 1997 to 2016. The most appropriate SIMD version for each year of diagnosis was selected as recommended in the deprivation guidance for analysts [158] and a unique quintile was used for each woman throughout the PhD.

The **Charlson Comorbidity Index** is a measure used to classify people with respect to their comorbid conditions that might influence mortality risk. The measure consists on a weighted index that takes into account the number of comorbidities and the severity of each comorbidity [159]. The original index developed by Charlson et al. defined 30 clinically important comorbidities but has been adapted for the different versions of the ICD codes. In 2004, an Australian version of the Charlson index adapted the score to include 17 comorbidities weighted from (1=least severe to 6=most severe) using ICD10 codes. The 17 scores (weight) are: acute myocardial infarction (1), congestive heart failure (1), peripheral vascular disease (1), cerebral vascular accident (1), dementia (1), pulmonary disease (1), connective tissue disorder (1), peptic ulcer (1), liver disease (1), diabetes (1), diabetes with complications (2), paraplegia (2), renal disease (2), cancer (2), metastatic cancer (3), severe liver disease (3) and HIV (6) [160]. The final score is then calculated by adding each of the individual comorbidities (with their weights) for an individual patient. In Scotland, the score is included within the SMR01- General/acute inpatient and day case records to establish a prior morbidity weighting and used as a proxy for co-morbidity. The score is calculated by looking back at 1 and 5 years before the patient's most recent admission [161].

3.3.1.2 Molecular markers

Oestrogen receptor (ER) status is available since 1997 for all invasive tumours diagnosed histologically, through biopsy, surgical excision or histology of nodes or metastases. The method used to assign ER status (positive or negative) to a tumour was the Allred score system. The scores are summed to give a maximum of eight depending on the combination of scores assigned following immunohistochemical staining for the proportion of cells that stain positively and the intensity of staining [162]. A score of 0-1 indicates a negative result and a score of 2-8 indicates a positive result, with higher score indicating a stronger positive result. ER status can also be recorded as a value from which the status is derived depending on the assay method. The three most common assay methods used and how they classify the tumours as ER positive and ER negative are given in Table 3.2.

Table 3.2 Assay methods for the classification of tumours depending on their ER status

Assay method	ER positive	ER negative
DCC	>=20fmols ER/mg protein	<20 fmols ER/mg protein
EIA	>=20fmols ER/mg protein	<20 fmols ER/mg protein
ERICA	>=10% positive staining	<10% positive staining

DCC= dextran-coated charcoal assay, EIA= Enzyme immunoassay, ER=oestrogen receptor, ERICA= oestrogen receptor immunocytochemical assay, fmols=femtomoles, mg= milligram

Progesterone receptor status was measured using the same method as the ER and data are available from 2009. **HER2 status**, or the over-expression of HER2 receptors in a tumour cell, was measured from 2009 using IHC HER2 receptor test. This test shows how much of the HER2 protein is present in a tumour cell. When the result is borderline, the fluorescence in-situ hybridisation (FISH) test is carried out to confirm the result.

3.3.1.3 Breast cancer subtype definition

ER, PR and HER2 combinations were used as a proxy for the classification of molecular BCs through genetic profiling known as intrinsic molecular subtypes of BC [97]. ER and PR were combined as hormone receptor status and defined as hormone receptor positive (HR+) if either ER or PR were positive, hormone receptor negative (HR-) if ER and PR were negative or one of them was negative and the other had unknown status, and hormone receptor unknown if ER and PR were unknown. HER2 was defined as HER2+, HER2- or HER2 unknown.

The combinations of ER/PR (hormone receptors) and HER2 status were used as surrogates for the molecular classification as defined according to the St Gallen 2011 consensus (Table 1.1 in Chapter 1) with a full description in Appendix B.1. HR+/HER2- tumours will be defined as luminal A, HR+/HER2+ as luminal B, HR-/HER2+ as HER2-enriched and HR-/HER2- as triple negative breast cancers (TNBC) throughout the dissertation.

Ki-67 a marker for tumour proliferation is not currently recorded in the Scottish cancer registry, which is why grade was used to further differentiate luminal A and luminal B tumours. Luminal A tumours with high grade were reclassified as luminal B tumours and sensitivity analysis is presented.

3.3.1.4 Other tumour characteristics definitions

For each woman with a BC diagnosis, the registry collects tumour information on **tumour grade** (low grade or well differentiated, medium grade or moderately well differentiated, high grade or poorly differentiated and unknown grade), **tumour size** in centimetres (cm) (categorised into <1cm, 1-2cm, more than 2 cm and unknown), **nodal status** (positive, negative or unknown), the number of positive nodes detected and the **method of first detection** (screen-detected, not screened detected and unknown).

Clinical and pathological **TNM stage** is also recorded as individual T, N and M stage variables. Clinical TNM stage was available for all study period and pathological TNM stage was only available from 2005. As a general principle, pathological TNM stage was prioritised over clinical stage as it tends to be more accurate. However, there were some exceptions:

- M stage which is based on clinical examination and imaging was prioritised over pathological except when clinical stage was unknown or when pathological stage indicated metastasis (stage IV) and clinical stage did not. M status was often unknown or not recorded and, in this case, I assumed that no metastasis was present.
- If the woman had neoadjuvant therapy (radiotherapy, chemotherapy or HT) at least 4 weeks before surgery, the clinical stage was prioritised.
- Stage T4 which indicates primary tumour involvement of chest wall or skin is often obvious at clinical examination, for that reason if clinical T stage was T4 was prioritised over pathological T stage.

Following the rules above and using pathological tumour size and the number of positive nodes variables to complete missing pathological T and N variables a final TNM stage variable was derived. The full algorithm followed to derive TNM stage from clinical and pathological TNM stage records is presented in Appendix B.2. and categorised as I, IIA, IIB, IIIA, IIIB, IIIC and IV following the American Joint Committee on Cancer (AJCC) 8th Edition Cancer Staging manual [163].

3.3.2 Selection of population

The original SMR06 dataset obtained from ISD contained all tumour registrations for individual people with a primary BC (including in situ tumours in the breast and in other organs, and malignant cancers in other organs). The dataset contained 91,735 registered cancers in 74,324 people diagnosed between 1997 and 2016. Some of these tumours were not relevant to the primary analysis for this PhD (calculating incidence rates of female invasive BC) and therefore, the original dataset was modified to create the study cohort following the steps described below and illustrated in Figure 3.1 for numbers of tumours and in Figure 3.2 for numbers of people:

1. Exclusion of men
2. Exclusion of women with other primary malignant cancer prior to BC diagnosis and exclusion of all other primary malignant entries
3. Exclusion of records of in situ or benign tumours in other organs
4. Exclusion of records of in situ or unknown behaviour tumours of the breast

Figure 3.1 Flowchart of the selection of female invasive breast cancers based on number of tumours

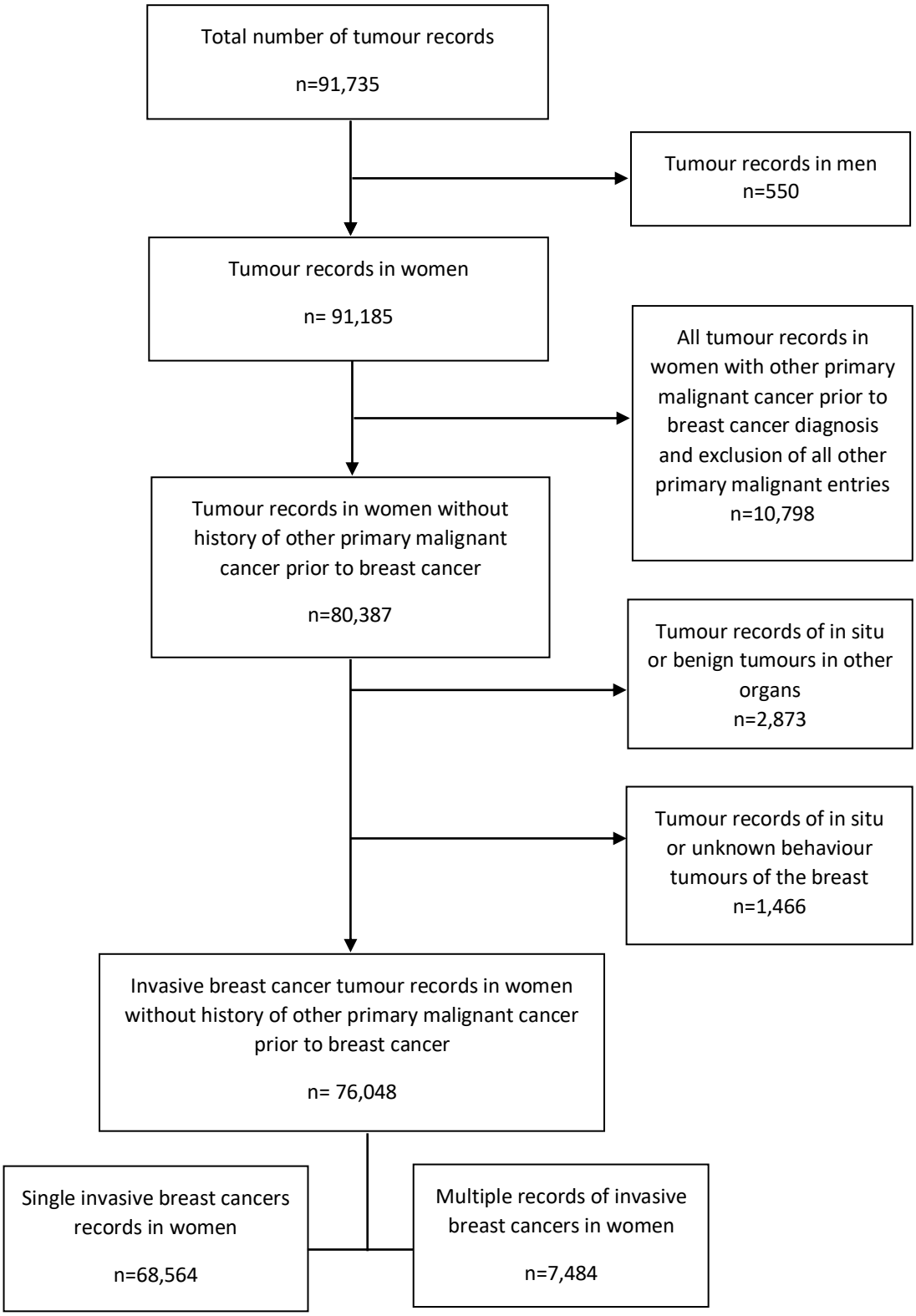
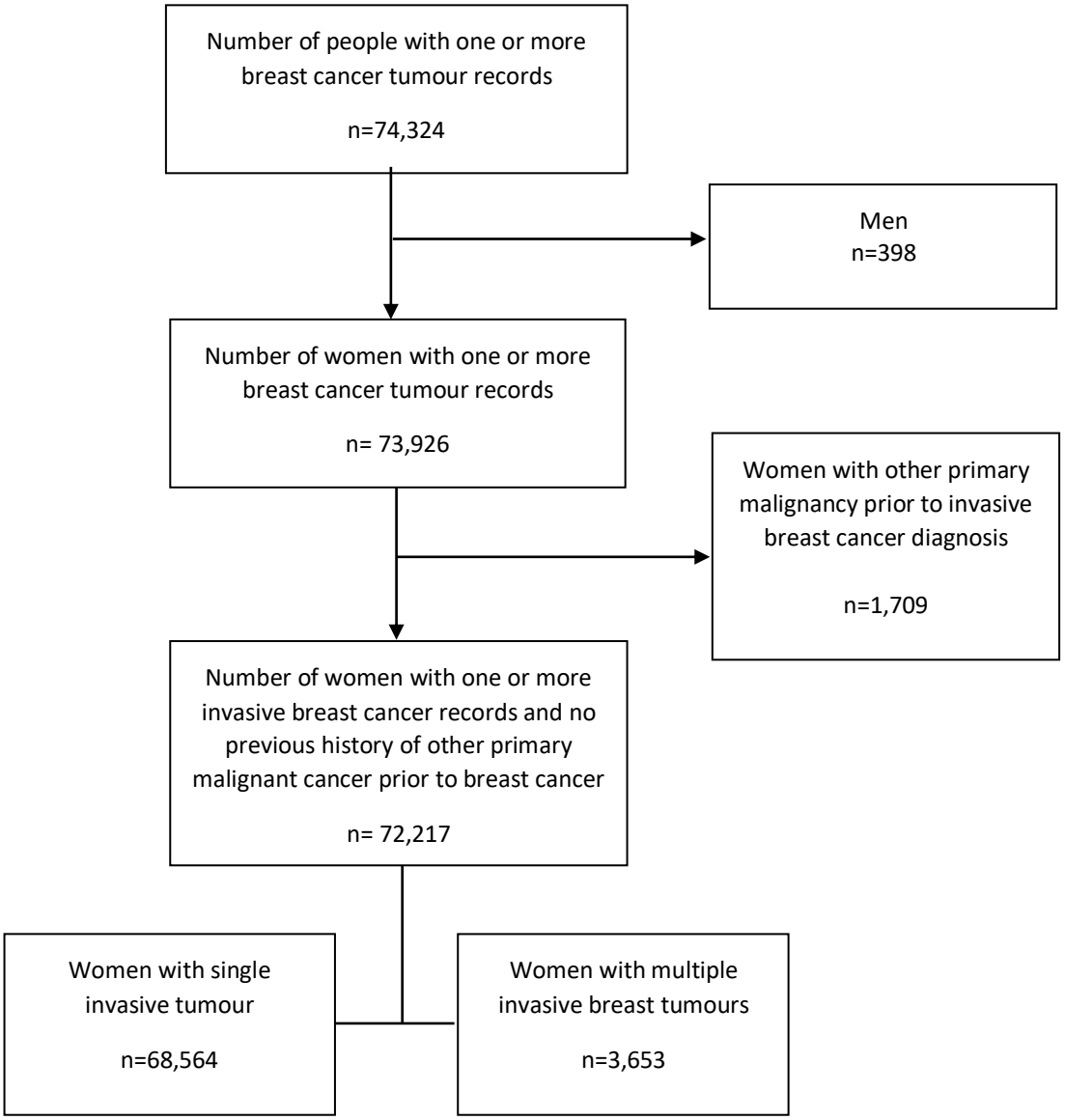


Figure 3.2 Flowchart of the selection of the study cohort based on number of people



After the exclusion of tumours that were not relevant for our primary analysis, 76,048 invasive BCs in 72,217 women remained in the dataset (Figure 3.1 and Figure 3.2).

By convention incidence rates computed by ISD-NHS Scotland, the Office for National Statistics for the UK and international agencies are tumour based rather than on an individual subject basis. I compared incidence rates reported by ISD (tumour based) with incidence rates based on a single incident case of BC per woman, the approach I used for my analysis. The final selection procedure to select only one

invasive BC per woman was established as follows: when a woman was diagnosed with multiple invasive BCs and the time of diagnosis between cancers was more than 6 months, the first primary invasive BC per woman was selected. If the diagnosis of the multiple invasive BCs was within 6 months, the more advanced invasive cancer was selected as the incident cancer using criteria based on grade and nodal status. A scoring system that established an individual score for each invasive lesion was created (Table 3.3).

Table 3.3 Scoring system to select the more advanced invasive breast cancer based on grade and nodal status

Score	Grade	Nodal Status
1	I-Well differentiated	Positive
2	I-Well differentiated	Negative
3	II- Moderately differentiated	Positive
4	II- Moderately differentiated	Negative
5	III-Poorly differentiated	Positive
6	III-Poorly differentiated	Negative

The score was only computed when both measurements (grade and nodal status) were available. The invasive BC with the highest score was selected for each woman. If the scores were the same, ER status was further investigated. If one or more of the scores could not be computed due to missing data, the record with a valid score was selected. In the case that all scores were missing the individual variables were investigated following the same order (grade, nodal status and ER). A full flowchart of the selection of the primary invasive BC for women with two invasive lesions is included in Appendix B.3. For those women with more than two invasive BCs recorded, the selection was performed manually using the same procedure. All stages of the selection procedure were performed in Stata 15 [164].

3.3.3 Statistical Analysis

3.3.3.1 Incidence rates

ASiR of BC were computed for all women by ER, HER2 and IHC defined molecular subtypes using ER/PR/HER2 combinations. Counts of BCs based on a single incident case for each woman (as described in the previous section 3.3.2) for each age and year of diagnosis were calculated and used as the numerator in the ASiR. The population estimates used as the denominator to compute the incidence rates were mid-year population estimates for each age and year of diagnosis obtained from the National Records of Scotland [165]. These estimates are derived from decennial census data with adjustment for population changes in intervening years and for under-numeration (estimated coverage was 94% in the 2011 Census) [166]. Incidence rates were standardised using the direct method to the European standard population (2013) in 5-year age groups.

Age-specific rates for each age group (<50, 50 to 69 and 70 years or older) by ER, HER2 and IHC defined subtypes were also calculated with age stratification based on the age at which women are routinely invited for screening. Age at menopause is not recorded in the cancer registry, but as the mean age of menopause in the UK is 51 years [167] women aged less than 50 years can be considered as premenopausal and women aged 50 or older can be considered as postmenopausal.

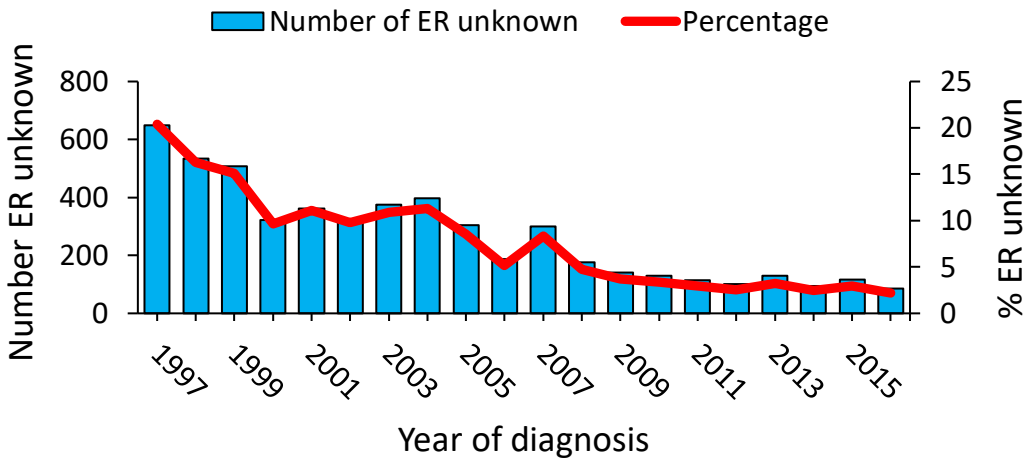
To describe differences between the incidence rates in this study (computed on the basis of one tumour per woman) and those estimated by ISD (computed on a tumour basis), I also calculated incidence rates for the study on a tumour basis. I used the total number of invasive breast tumours diagnosed from 1997 to 2016, without excluding women who had another primary tumour before BC diagnosis for this calculation.

3.3.3.2 Dealing with missing tumour marker status

Although, completeness of ER status data in the cancer registry was relatively good (missingness= 8% for ER status from 1997 to 2016) missing ER status varied by age and by calendar year which, if not taken into account, could lead to biased estimates of the rates. The number and percentage of BC cases with missing ER status declined

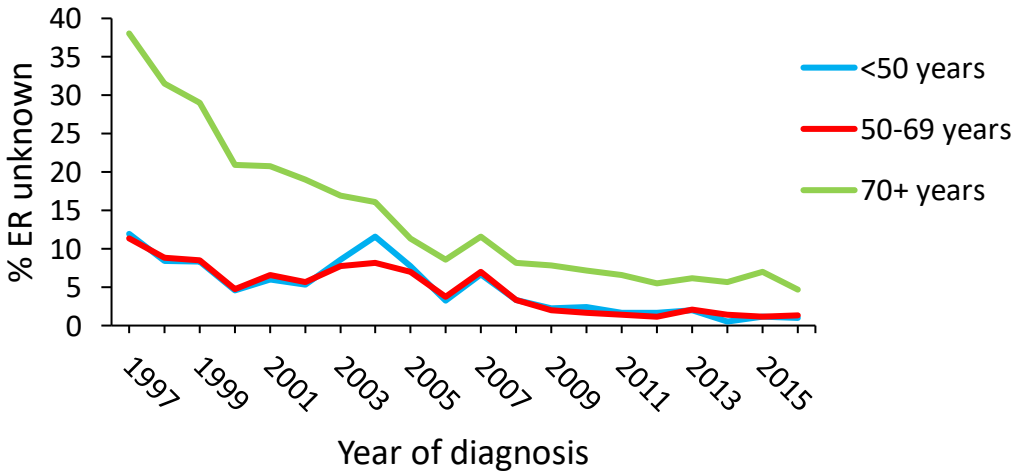
over time from over 600 cases (20%) in 1997 to less than 100 (2%) in 2016 (Figure 3.3).

Figure 3.3 Number and percentage of breast cancer cases with ER unknown status in Scotland from 1997 to 2016



There was a clear relationship between missing ER status and age at diagnosis, with older women being more likely to have an unknown ER status. Fourteen percent of women aged 70 years or older had missing ER status compared to 5% of women aged less than 70 years with missing ER status from 1997 to 2016. The percentage of women having missing ER status declined over time and was more pronounced for women aged 70 years or older (Figure 3.4).

Figure 3.4 Percentage of women diagnosed with BC in Scotland from 1997 to 2016 with missing ER status by age group



To correct ER+ and ER- counts for missing ER status, a simple method of multiple imputation (MI) developed by Anderson et al [135] was used. For each age a and calendar year y:

$$T_{ay} = P_{ay} + N_{ay} + M_{ay}$$

with T_{ay} being the total number of incident cases for each age a and calendar year y, $P_{ay}$the observed number of ER positive counts, N_{ay} the observed number of ER negative counts and M_{ay} the observed number of missing ER counts.

The model assumes that ER status is missing at random (MAR) within a single age and year of diagnosis and that the observed probability of ER+ counts is an unbiased estimator of the true probability of ER positive counts in the population. Using the observed probability of ER+ counts,

$$\hat{\pi}_{ay} = P_{ay}/(P_{ay} + N_{ay}) ,$$

the corrected ER+, $\hat{P}_{ay}$, and ER-, $\hat{N}_{ay}$ counts were calculated as follows:

$$\hat{P}_{ay} = \hat{\pi}_{ay} T_{ay}$$

$$\hat{N}_{ay} = T_{ay} - \hat{P}_{ay}$$

If the assumption of MAR is correct, these corrected ER+ and ER- estimates proved unbiased estimators of the true ER+ and ER- counts in the population that can be used to calculate the age-specific and ASiR over time. An equivalent imputation method was used to correct for missing data for HER2 status and for HR/HER2 combinations.

Other studies have used multiple imputation by chained equations (MICE) models to impute missing ER status for BC in cancer registries with both the assumptions of MAR [135] and missing not at random (MNAR) [168]. Both studies showed similar results irrespective of the assumption but different from a complete case analysis (CCA) that would give biased estimates of the rates. For that reason, I have assumed that the assumption of MAR is valid and that imputation is preferable to CCA.

3.3.3.3 Joinpoint Regression Analysis

Overall linear trends for a fixed period of time are frequently summarised using the EAPC of the ASiR. EAPC is calculated using a log-linear model that assumes a Poisson distribution. Under this model a constant change assumption is presumed, i.e. linearity of the rates on the log scale over time [169]. However, incidence rates often do not present a linear trend over time and, therefore, it is not reasonable to assume that a single EAPC accurately describes time trends. Fay et al. proposed two alternative measures to estimate the overall trend: the two point estimator and the adaptive estimator [149] when the linear assumption does not hold.

Kim et al [170] proposed the use of joinpoint regression models, also known as piecewise regression, to estimate the points at which there is a change in the incidence rates and calculate the EAPC for each identified segment of time. They also proposed the use of the average annual percentage change (AAPC) as a better estimator of the overall trend over a fixed period of time when there is not a linear trend [171]. The AAPC is appropriate even when the model indicates that there are points in time at which changes in incidence trends are observed for the specified time interval.

Joinpoint regression models were fitted for the overall BC incidence rates, for each ER and HER2 status and for the IHC defined molecular subtypes. Joinpoint analysis was also performed for each marker and combination of markers for three age groups (<50, 50-69 and 70+ years at diagnosis of BC). A maximum of three joinpoints (time points at which there is a change in incidence) were investigated. The simplest joinpoint regression model that provided the best fit to the data was selected using the permutation test method [170]. This Monte Carlo iterative procedure start by testing the null hypothesis of a model with 0 joinpoints versus an alternative hypothesis of a model with 3 joinpoints (maximum number of joinpoints previously specified). If the null hypothesis is selected the procedure continues by testing it against the alternative with 1 less joinpoint and if the alternative hypothesis is selected then it is tested against the null with 1 more joinpoint. The procedure continues until all possible hypothesis (0 to 3 joinpoints) have been tested sequentially. A total of 4,499 permutations are performed and the p value test is adjusted for multiple testing using Bonferroni correction [172].

The location of the joinpoints within the study period and the final model fitting was investigated using Lerman's grid search method [173] assuming constant variance and uncorrelated errors. This method fits a model for each possible position of the joinpoint(s) and selects the position(s) that minimises the sum of squared errors (SSE), hence, identifying time periods with changes in estimated rates. EAPCs with 95% confidence intervals (CI) were calculated and reported for each time period identified by the final model, along with AAPC for the whole study period (from 1997 to 2016). The parametric method was used to estimate the CI for AAPC and EAPCs.

Sensitivity analysis was performed for ER+ tumour trends using different model selection methods (the Permutation test, the Bayesian Information Criterion (BIC), BIC3, and modified BIC) and different errors options (uncorrelated vs autocorrelated). Joinpoint regression Software was used for all the analysis [174].

3.3.3.4 Age-period-cohort models

Apart from the classical descriptive approaches described above, age- period and age-cohort models have frequently been used for surveillance and analysis of disease rates. In recent decades, Age-Period-Cohort (APC) models have proved useful to generate and test hypothesis for aetiology and prognosis and to estimate age, period and cohorts effects, particularly in cancer rates. These three factors are all time-related and can serve as estimates of disease risk. Period and cohort effects are usually indicative of changes in external exposures, such as implementation of screening programme or changes in reproductive factors.

APC models are based on generalised linear models and describe rates as a product of these three factors: age, period and cohort. However, due to the linear relationship between the factors,

$$Cohort = Period - Age$$

also known as the "identifiability problem", the same fitted rates are predicted by many different sets of parameters. Hence, the log-linear trends in rates cannot be attributed to the influence of age, period or cohort parameters.

The first approaches used to deal with the identifiability issue consisted on adding some constraints to the full APC model [175, 176]. However, these constraints are

usually hard to prove and they must be based on biological hypothesis. Many studies have tried to address the identifiability issue and methods are summarised in a 2016 review by Smith and Wakefield [177]. However, Rosenberg et al [178], suggest that the identifiability issue has slowed down the use of APC models in epidemiological studies although the issue is intrinsic to any time to event analysis of cohort studies. They propose a new model [179] that provides a set of estimable functions that are closely related to the classical approaches used in cancer surveillance and effective in estimating patterns in cancer rates. This model has two key innovations: 1) the cohort deviations are weighted to allow cohorts to be followed-up for variable periods of time without imposing additional constraints to the model; 2) the age, period and cohort deviations that identify the non-linear trend of each effect are estimated using decomposition of quadratic components (orthogonal to intercept and the linear trend of the effect) and higher-order terms. The quadratic components of the model, or "global curvatures" parameters, represent how fast on average the trends in the rates are changing and are the main components to identify rate patterns and signals. The new method also allows for the estimation of improved functions and hypotheses tests that are summarised in Table 3.4 below.

Table 3.4 Parameters and estimable functions from APC models and hypothesis test for each estimate (page 1 of 2)

Type of effect	Estimable function	Interpretation	Hypothesis test (null)	Implications if null hypothesis is accepted
Global trend	Net Drift	Annual percentage change of the expected age-standardised rates over time. Analogue of the EAPC but adjusted for cohort effects. Log-linear component of the fitted rates. The net drift represents the sum of the linear trend from the period and cohort effects.	Net Drift=0	Fitted temporal trends are stable over time.
Age effects	Cross – sectional age curve	Fitted age-specific rates in reference period p0 adjusted for cohort effects.	Age deviations=0 (age deviations are the non-linear age effects)	Fitted cross-sectional curves are log-linear.
	Longitudinal age curve	Fitted age-specific rates in reference cohort c0 adjusted for period effects		Fitted longitudinal curves are log-linear.
	Ratio of Longitudinal vs Cross-sectional age curve	Quantifies the influence of the Net Drift on age-associated natural history		The ratio of Longitudinal vs Cross-sectional curves is constant.

Table 3.4 (continued) Parameters and estimable functions from APC models and hypothesis test for each estimate (page 2 of 2)

Type of effect	Estimable function	Interpretation	Hypothesis test (null)	Implications if null hypothesis is accepted
Period effects	Fitted temporal trends	Fitted rates over time in reference age group a0 adjusted for cohort effects. Analogue to ASR.	Period deviations=0	Fitted temporal trends and period rate ratios are log-linear.
	PRR	Ratio of the age-specific rates in a period relative to the reference period (p0)	All PRR=1	Net Drift is 0 and fitted temporal trends are constant.
Cohort effects	Local drifts	Annual percentage change of the expected age-specific rates over time. Analogue of the EAPC but for each age group.	Local drifts= Net drift	Temporal trends are the same in every age group. If the local drifts are different to the net drift there is evidence of cohort effects.
	CRR	Ratio of the age-specific rates in a cohort relative to the reference cohort (c0)	All CRR=1	Net Drift is 0 and all local drifts are 0. Temporal trends are the same in every age group.

CRR= Cohort Rate Ratio, EAPC= Estimated annual percentage change, PRR= Period Rate Ratio. Table modified from [179]

The new model has two main improvements in comparison with the traditional model:

1) all parameters are estimable since they are characteristic of the underlying Lexis diagram and an age, period and cohort reference groups are selected,

2) the new hypotheses tests are more robust and they correct for multiple testing.

Apart from the traditional Wald Chi squared test used in APC models, the new model uses Tippett's method [180] to derive a combination test (testing both quadratic and higher order terms) for period and cohort effects. This test combines the Wald test for the global curvature and the Wald test for the higher order components into a single p value test for the period or cohort effects. The new combination test is more robust and useful to capture period or cohort effects that are influencing the observed rates above the log-linear trend identified by the net drift. Model fit was checked using the deviance residuals of the APC model of each subtype with no systematic patterns indicating a good fit and are presented in Appendix Figure B.6, Appendix Figure B.9 and Appendix Figure B.22 for ER+, ER- and the IHC defined subtypes respectively.

APC models presented in this chapter are based on the new model developed by Rosenberg et al. [179] and further statistical methodology and description of how the parameters are derived can be found in the paper. APC models were fitted for the ER+ and ER- ASiR calculated after imputation of missing data. I restricted the models to women aged 30 to 85 years for consistency with similar estimates from other counties and due to small counts in women younger than 30 and older than 85 years. In order to obtain stable estimates, single year data was grouped into two-by-two age year and time period groups. There were 28 two-year age groups (from 30-31 to 84-85) and ten 2-year periods (from 1997-1998 to 2015-2016), which covered birth cohorts from 1912 to 1986. The reference years for the fitted models were identified from the mid-points of the available data and were 57 years for age, 2006 for period, and 1949 birth year for cohort.

APC models for HER2 status and the IHC defined molecular subtypes were also computed. As data for these markers were only available from 2009 to 2016, count data were not grouped as described above but used by individual year for age and time period. As for ER models counts were restricted to women aged 30 to 85 years. There were 56 one year age groups (from 30 to 85) and eight 1-year periods (from 2009 to

2016), which covered birth cohorts from 1924 to 1986. The reference years were 57 years for age, mid-2012 (2012.5) for period, and 1955 birth year for cohort. All models and statistical tests were fitted using R [181] code available from the APC web tool developed by the National Cancer Institute (NCI) [182]. P values were deemed statistically significant at the 5% level.

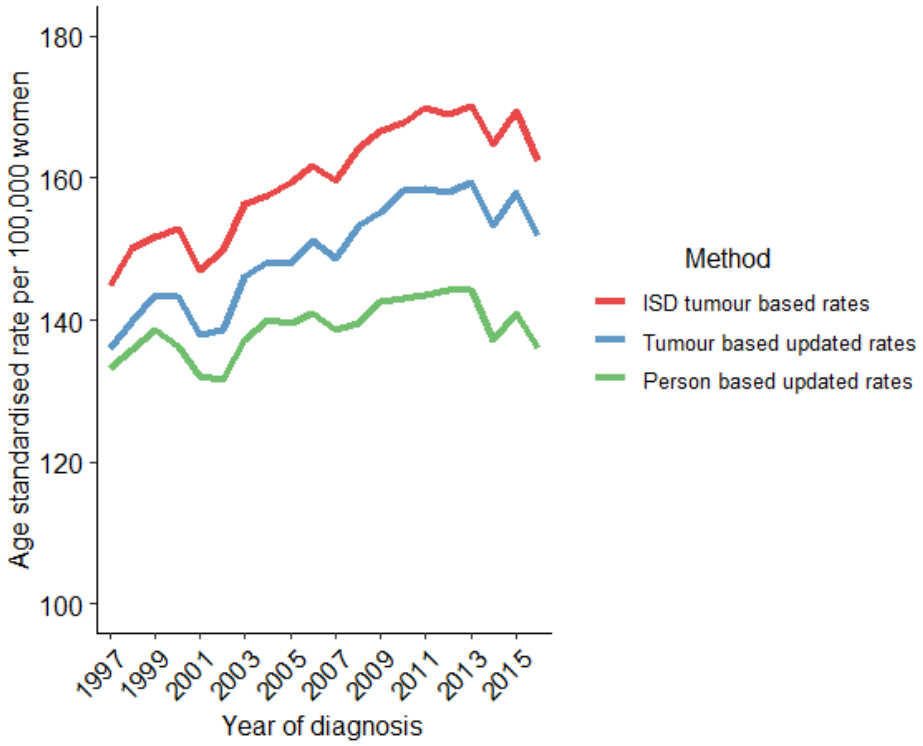
3.4 Results

3.4.1 Overall incidence trends

A total of 72,217 women were diagnosed with at least one invasive BC between 1997 and 2016 in Scotland, with approximately 3,000 to 4,000 women registered as having an incident BC per year. The overall BC incidence for the final selection of women (Figure 3.5, green line) increased over time from 1997 to 2016 but fluctuated during those years. From 1997 to 2012, there was a 0.5% annual increase in incidence (95% CI: 0.3 to 0.7%). There was a slight decrease observed between 1999 and 2002. After 2002, BC incidence increased again until 2012. In the last four years, from 2012 to 2016, a downward trend in incidence (1.3% annual decrease, 95% CI:-2.8 to 0.4%) has been observed.

Overall BC incidence based on a single tumour per woman (Figure 3.5, green line) is lower than that based on tumour incidence and there is a less striking increase in BC incidence based on data for individual women than that reported by ISD based on multiple invasive tumours per woman (Figure 3.5, red line). However, the number of invasive tumours recorded per woman did not seem to be responsible for these differences, as 6% of women in 1997 and 3% of women in 2016 had secondary invasive tumours recorded. Overall BC incidence trends using multiple invasive tumours per woman (Figure 3.5, blue line) were also calculated using the same method as ISD and the trend line is proportional to ISD overall trend.

Figure 3.5 Overall age-standardised incidence rates in Scotland from 1997 to 2016 reported by ISD (based on tumour numerators) and rates calculated for the dataset in this study using two methods: tumour based (n=77,841) and person based (n=72,217)



Possible differences observed between the rates reported here and those reported by ISD reflect both the different calculation procedures (red and blue lines are based on tumours and green line in women) and initial definition of the cohort of women (red and blue lines include women with other primary tumours and green line exclude them) used to compute the incidence rates. The difference between ISD rates (in red) and the rates computed for the cohort of women in this dissertation (in blue) using the same method (tumours as the denominator for the rates) can be due to the fact that we excluded women with previous malignancies of other type. Over 3,500 women had a previous malignancy in the cohort (approximately 175 per year), taking into account that the difference in the number of women diagnosed every year between the ISD cohort and our cohort was between 200-400 women per year, excluding women with a previous cancer diagnosis will partially account for the differences observed. Further,

cancer registration is a dynamic process and the trends presented by ISD are likely to differ from those in other publications even for the same period of time.

Table 3.5 indicates that both methods (multiple or a single tumour per woman) used to estimate incidence rates yielded a population of women with tumours of similar characteristics. The differences in tumour characteristics observed between included and excluded tumours reflect the selection criteria that aimed to retain the most advanced invasive tumour for each woman.

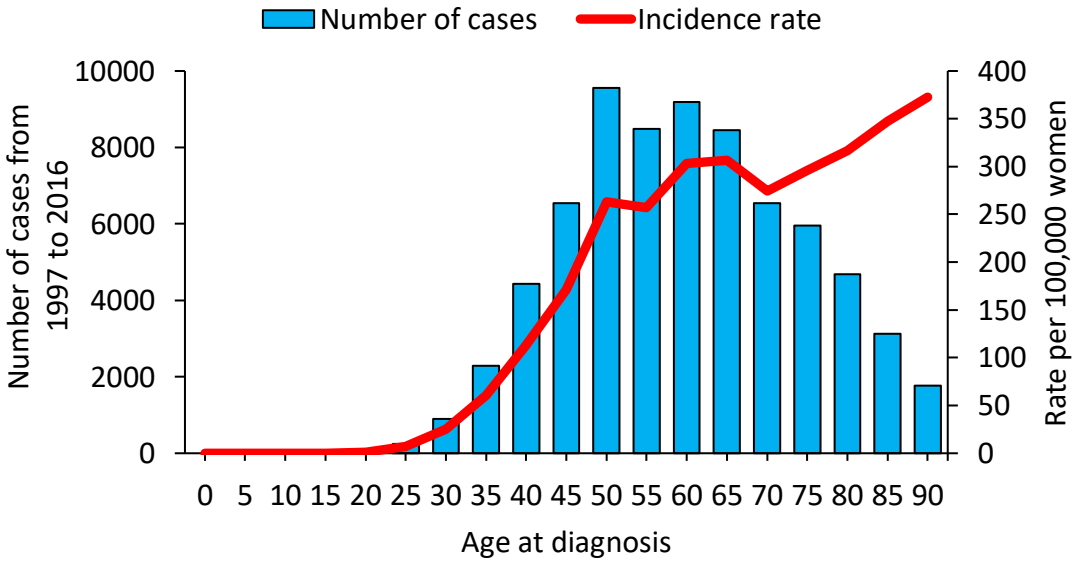
Table 3.5 Tumour characteristics of included and excluded tumours for estimates of breast cancer incidence among women in Scotland diagnosed between 1997 and 2016

Tumour characteristic		Tumours excluded n=5,624	(%)	Tumours included n=72,217	(%)	All tumours n=77,841	(%)
TNM Stage	I	2,260	(40)	23,554	(33)	25,814	(33)
	II	1,576	(28)]	25,143	(35)	26,719	(34)
	III	562	(10)	10,365	(14)	10,927	(14)
	IV	289	(5)	3,627	(5)	3,916	(5)
	Unknown	937	(17)	9,528	(13)	10,465	(13)
Grade	Well differentiated	1,075	(19)	8,715	(12)	9,790	(13)
	Moderately differentiated	2,313	(41)	28,050	(39)	30,363	(39)
	Poorly differentiated	1,312	(23)	23,525	(33)	24,837	(32)
	Unknown	924	(17)	11,927	(16)	12,851	(16)
Nodal status	No	2,901	(52)	36,463	(51)	39,364	(50)
	Yes	1,317	(23)	21,894	(30)	23,211	(30)
	Unknown	1,406	(25)	13,860	(19)	15,266	(20)
Tumour size	Less than 10mm	1,063	(19)	7,689	(10)	8,752	(11)
	10-20 mm	1,924	(34)	24,355	(34)	26,279	(34)
	more than 20mm	1,310	(23)	23,640	(33)	24,950	(32)
	Unknown	1,327	(24)	16,533	(23)	17,860	(23)
Screen detected	No	4,259	(76)	51,069	(71)	55,328	(71)
	Yes	1,271	(22)	19,552	(27)	20,823	(27)
	Unknown	94	(2)	1,596	(2)	1,690	(2)
ER status	Positive	825	(15)	11,726	(16)	12,551	(16)
	Negative	4,504	(80)	55,144	(76)	59,648	(77)
	Unknown	295	(5)	5,347	(8)	5,642	(7)

ER= oestrogen receptor, TNM= tumour, nodes, metastases.

Age is one of the most important factors influencing BC incidence rates. In Scotland, BC incidence increased with age, with older women having the highest incidence rates and over 30% of cases diagnosed in women aged 70 years or older. Figure 3.6 shows that incidence rates of BC increase rapidly with age until the approximate age of menopause at 50 years. The increase continues but it is moderate from 50 to 70 years and rises sharply again after 70 years.

Figure 3.6 Number of breast cancer cases diagnosed and age-specific incidence rates per 100,000 women in Scotland, 1997-2016



3.4.2 Incidence trends by oestrogen receptor status

3.4.2.1 Women and tumour characteristics by ER status

Of the 72,217 incident invasive tumours selected that were diagnosed in Scotland from 1997 to 2016, 76% of them were classified as ER+, 16% as ER- and 8% had unknown ER status. Table 3.6 shows how women and tumour characteristics differ by ER.

Table 3.6 Descriptive characteristics by ER status for all women with an invasive breast cancer diagnosed between 1997 and 2016 in Scotland

Characteristic		ER-		ER+		ER missing	
		n	%	n	%	n	%
		11,726	[16]	55,144	[76]	5,347	[8]
Age at diagnosis	<50 years	3,196	(27)	10,550	(19)	695	(13)
	50 69 years	5,668	(48)	28,441	(52)	1,580	(30)
	70 years or older	2,862	(24)	16,153	(29)	3,072	(57)
NHS regions	North	3,280	(28)	14,124	(26)	1,307	(24)
	South East	2,782	(24)	15,949	(29)	1,236	(23)
	West	5,663	(48)	25,071	(45)	2,804	(52)
Deprivation quintile	5-Least deprived	2,126	(18)	11,208	(20)	961	(18)
	4	2,348	(20)	11,012	(20)	1,050	(20)
	3	2,380	(20)	11,571	(21)	1,037	(19)
	2	2,431	(21)	11,258	(20)	1,139	(21)
	1-Most deprived	2,440	(21)	10,095	(18)	1,159	(22)
TNM stage	I	2,863	(24)	20,058	(36)	633	(12)
	II	4,795	(41)	19,470	(35)	878	(16)
	III	2,200	(19)	7,678	(14)	487	(9)
	IV	648	(6)	2,492	(5)	487	(9)
	Unknown	1,220	(10)	5,446	(10)	2,862	(54)
Grade	I-Well differentiated	195	(2)	8,288	(15)	232	(4)
	II- Moderately differentiated	1,714	(15)	25,734	(47)	602	(11)
	III- Poorly differentiated	8,308	(71)	14,586	(26)	642	(12)
	Unknown	1,509	(13)	6,536	(12)	3,871	(72)
Nodal Status	No	6,194	(53)	29,400	(53)	869	(16)
	Yes	4,110	(35)	17,369	(31)	415	(8)
	Unknown	1,422	(12)	8,375	(15)	4,063	(76)
Tumour size	Less than 10mm	1,017	(9)	6,470	(12)	202	(4)
	10 to 20 mm	3,428	(29)	20,449	(37)	478	(9)
	More than 20mm	4,960	(42)	18,168	(33)	512	(10)
	Unknown	2,321	(20)	10,057	(18)	4,155	(78)
Screen detected	No	9,622	(82)	37,400	(68)	4,047	(76)
	Yes	1,943	(17)	17,119	(31)	490	(9)
	Unknown	161	(1)	625	(1)	810	(15)
HER2 status*	Negative	3,050	(69)	20,329	(88)	-	-
	Positive	1,357	(31)	2,844	(12)	-	-
PR status*	Negative	3,803	(94)	3,036	(16)	-	-
	Positive	226	(6)	15,869	(84)	-	-

*Markers only recorded from 2009 to 2016, percentages for those with recorded status. Chi square p value for comparison of ER+ and ER- tumours <0.001 for all characteristics. Percentages are given for columns except for the total for which row percentages are shown. ER=oestrogen receptor, HER2= human epidermal growth factor 2, PR= progesterone receptor, TNM= tumour, nodes, metastases.

Individual characteristics of women in the cohort showed that over half of breast tumours were diagnosed in women aged 50 to 69 years, with a slightly higher percentage amongst ER+ tumours than ER- tumours (52% vs 48%). On the contrary, the percentage of women <50 years of age who had an ER- tumour was higher than the percentage who had an ER+ tumour (27% vs 19%). Tumour characteristics differed by ER status, with ER- tumours having characteristics associated with more advanced/aggressive disease. ER- tumours had higher grade, higher TNM stage (II and III) were more likely to be larger in size and have lymph nodes affected and were less likely to be screen detected than ER+ tumours.

The patterns of other molecular markers also differed by ER status, with ER- tumours more likely to be PR negative and HER2 positive than ER+ tumours. In contrast, ER+ tumours were more likely to be PR+ and HER2- than ER- tumours. Almost a third of ER+ tumours were screen detected compared to only 17% of ER- tumours. In terms of social gradient, women with ER- tumours were more likely to be in the most deprived quintile than women with ER+ tumours (21% vs 18%).

As ER status was missing for 8% of the tumours I corrected for missing ER status and final numbers of ER+ and ER- tumours are given in Table 3.7 below showing that a larger proportion of tumours with missing ER status were imputed as ER+ tumours.

Table 3.7 Number and percentage of breast cancer cases by ER status before and after correcting for missing ER status in Scotland, 1997-2016

ER status	Before correction		After correction	
	n	%	n	%
ER positive	55,144	76	59,553	82
ER negative	11,726	16	12,664	18
ER missing	5347	8		
Total	**72,217**		**72,217**	

ER=oestrogen receptor.

3.4.2.2 Incidence rates by ER status and age

BC incidence increases with age irrespective of ER status, however the pattern observed for ER+ and ER- rates is not the same. In Scotland, ER+ rates increase rapidly until 50 years of age when the increase by age slows to 70 years of age before increasing again at older age (Figure 3.7, red line). Rates of ER- tumours also suffer a rapid increase up to 50 years of age but they remain constant after that time (Figure 3.7, blue line).

Figure 3.7 Age-specific incidence rates by ER status from 1997 to 2016 in Scotland from estimated counts

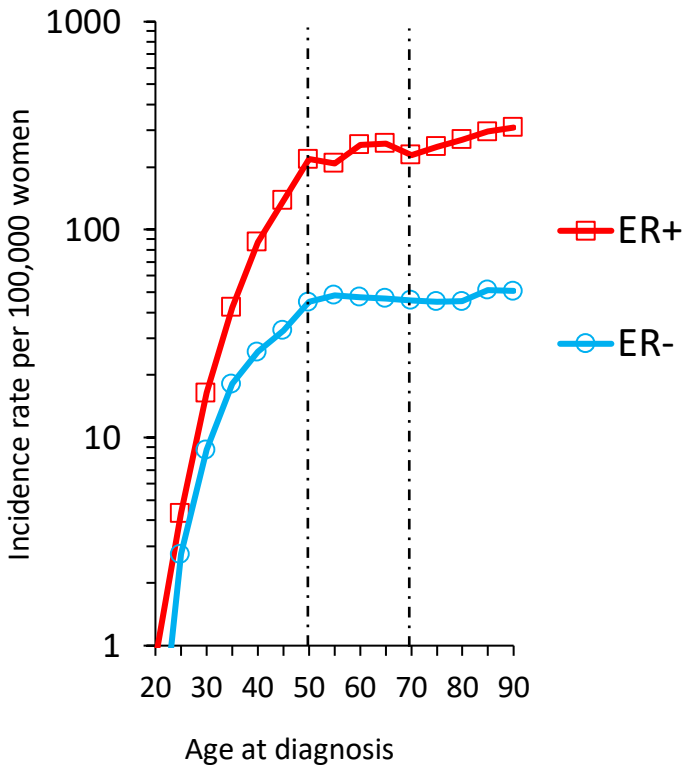
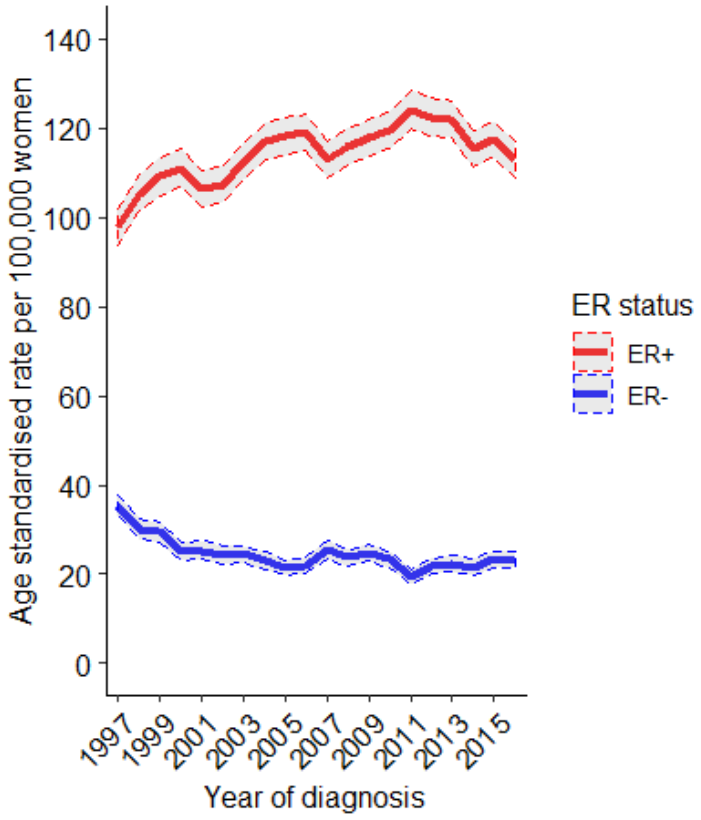


*Figure is on the natural log scale

3.4.2.3 Age-standardised incidence rates by ER status and analysis of trends over time using joinpoint regression analysis

In Scotland, ASiR of ER+ tumours after imputation of missing data increased from 98 per 100,000 women in 1997 to 113 in 2016 (Figure 3.8, red line). The AAPC estimated with joinpoint regression was 0.4% (95% CI: -0.1 to 1%). The increase was approximately constant (1.2% increase, 95% CI: 0.8 to 1.5%) from 1997 to 2012 after which incidence rates of ER+ tumours decreased by 2.2% annually (95% CI:-4.7 to 0.4%) (Table 3.8). In contrast, ASiR of ER- tumours decreased consistently over the study period (Figure 3.8, blue line), on average by 2.5% per year (95% CI: -3.9 to -1.1%), and by 11.3% per year (95% CI: -18.9 to -3%) between 1997 and 2000. From 2000 to 2016, incidence of ER- tumours decreased at 0.7% each year (95% CI: -1.5 to 0) (Table 3.8).

Figure 3.8 Age-standardised incidence rates by ER status in Scotland from 1997 to 2016 with 95% CI after correcting for missing ER status

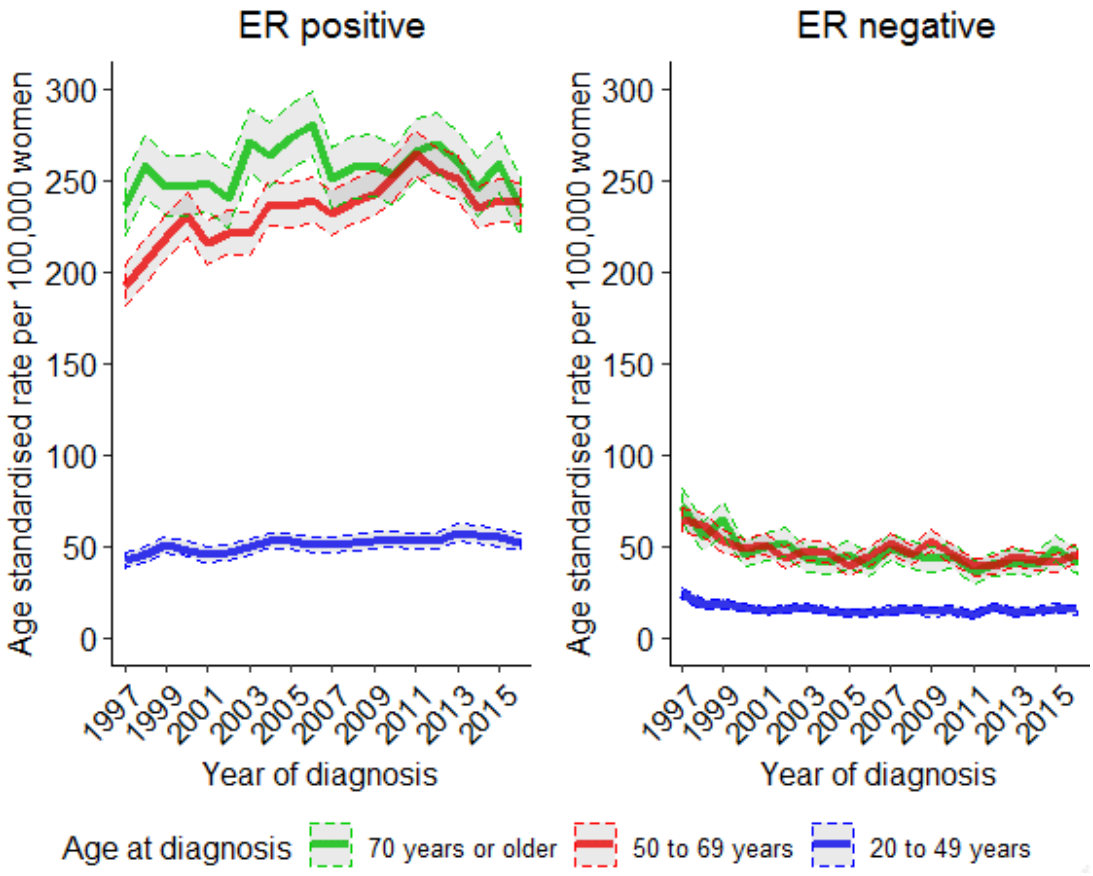


The effect of using imputation for missing ER status can be observed in Appendix B.4 were imputed rates of ER+ tumours show more moderate increases than those

observed without imputing ER. The effect on ER- tumours is the opposite, showing bigger declines in observed compared to imputed analyses, especially for the earlier years of the study period.

Incidence rates by ER status for the three age groups (Figure 3.9) show that the increases in incidence observed for ER+ tumours were mainly due to increasing incidence in women aged 50 to 69 years. This age group showed consistent increases by 0.7% (0.2, 1.3) per year between 1997 and 2011, followed by a decrease of 1.6% (-1.2, -2.1) per year from 2011 to 2016 (Table 3.8). Younger women aged less than 50 years also showed increases of 1.1% (0.7, 1.5) per year in ER+ tumours. The decreases observed for ER- tumours were consistent for the three age groups but seemed to be mainly in early years of the study period for younger women aged less than 50 and women older than 70 years (Table 3.8).

Figure 3.9 Incidence rates of breast cancer in Scotland 1997-2016 by ER status for 3 age groups (<50 years, 50-69 years and more than 70 years)



Further joinpoint regression graphs and results obtained using the joinpoint programme developed by NCI are presented in Appendix B.5. Sensitivity analysis for ER+ tumours can be found in Appendix B.6. Sensitivity analysis showed that using an uncorrelated or autocorrelated errors models (Appendix Table B.5) did not considerably changed the results, a joinpoint was still found at year 2011 or 2012 (depending on the error model selection) with very similar EAPC estimates for both periods (before and after the joinpoint) estimated. The number of joinpoints and the trends estimated differed by model selection method (Appendix Table B.6, Appendix Table B.7) with permutation method and BIC3 estimating increases in incidence of ER+ tumours by 1.2% annually from 1997-2012. BIC method found a significant increase between 1999 and 2012 and the modified BIC method an increasing trend for the whole study period.

Table 3.8 Joinpoint regression results for breast cancer incidence rates for women of all ages and for three age groups (20 to 49 years, 50 to 69 years and 70 years or more) by ER status in Scotland from 1997 to 2016

ER status	Age groups	Rate in 1997 per 100,000 women	Rate in 2016 per 100,000 women	Change in rate from 1997 to 2016 N per 100,000 (%)	AAPC (95% CI)	Years before joinpoint	EAPC (95% CI) for period before joinpoint	Years after joinpoint	EAPC (95% CI) for period after joinpoint
Positive	All ages	97.7	112.8	15.1 (13%)	0.4% (-0.1, 1.0)	1997-2012	**1.2% (0.8, 1.5)**	2012-2016	-2.2 (-4.7, 0.4)
	20-49 years	41.9	52.1	10.2 (20%)	**1.1% (0.7, 1.5)**	No significant change point identified from 1997-2016			
	50-69 years	192.3	237.4	45.1 (19%)	**0.7% (0.2, 1.3)**	1997-2011	**1.6% (1.2, 2.1)**	2011-2016	-1.8 (-3.7, 0.1)
	70+ years	235.9	234.5	-1.4 (0.6%)	0.1% (-0.3, 0.5)	No significant change point identified from 1997-2016			
Negative	All ages	35.5	23.1	-12.4 (35%)	**-2.5% (-3.9, -1.1)**	1997-2000	**-11.3% (-18.9, -3.0)**	2000-2016	-0.7% (-1.5, 0)
	20-49 years	23.8	15.2	-8.6 (36%)	**-2.2% (-3.9, -0.6)**	1997-2001	**-10.3% (-16.8, -3.3)**	2001-2016	0% (-1.1, 1.2)
	50-69 years	64.1	45.5	-18.6 (29%)	**-1.6% (-2.5, -0.8)**	No significant change point identified from 1997-2016			
	70+ years	71.8	41.2	-30.6 (43%)	**-2.4% (-4.2, -0.7)**	1997-2003	**-7% (-11.4, -2.3)**	2003-2016	-0.3% (-1.9, 1.5)

Bold results are significantly different from 0 ($p<0.05$). AAPC= average annual percent change, EAPC= estimated annual percentage change, ER=oestrogen receptor. Incidence rates are standardised to the European population. Rates for the age groups are age-specific rates.

3.4.2.4 Age-period-cohort analysis for incidence rates by ER

APC models by ER status are restricted to women aged 30-85 years (n=67,804) and, hence Table 3.9 presents the descriptive characteristics for the population used in APC.

Table 3.9 Descriptive characteristics of women aged 30 to 85 years diagnosed with invasive breast cancer in Scotland and analysed using APC models

Factor	Values	ER+ counts	(%)	ER- counts	(%)	ER-missing counts	(%)
Age	30-49 years	10,379	(20)	3,102	(28)	678	(16)
	50-69 years	28,441	(54)	5,668	(50)	1,580	(38)
	70-85 years	13,575	(26)	2,463	(22)	1,918	(46)
NHS Scottish region	North	13,374	(26)	3,142	(28)	966	(23)
	South East	15,104	(29)	2,644	(24)	954	(23)
	West	23,917	(46)	5,446	(48)	2,256	(54)
Year of diagnosis	1997-2001	10,615	(20)	2,928	(26)	1,847	(44)
	2002-2006	12,506	(24)	2,568	(23)	1,307	(31)
	2007-2011	14,103	(27)	2,854	(25)	667	(16)
	2012-2016	15,171	(29)	2,883	(26)	355	(9)
TNM stage	I	19,684	(38)	2,813	(25)	578	(14)
	II	18,667	(36)	4,647	(41)	725	(17)
	III	7,215	(14)	2,083	(19)	391	(9)
	IV	2,345	(4)	607	(5)	411	(10)
	Unknown	4,484	(9)	1,083	(10)	2,071	(50)
Grade	I-Well differentiated	8,068	(15)	191	(2)	218	(5)
	II- Moderately differentiated	24,774	(47)	1,638	(15)	559	(13)
	III- Poorly differentiated	14,124	(27)	8,031	(71)	597	(14)
	Unknown	5,429	(10)	1,373	(12)	2,802	(67)
Nodal Status	No	29,012	(55)	6,078	(54)	855	(20)
	Yes	17,059	(33)	4,005	(36)	407	(10)
	Unknown	6,324	(12)	1,150	(10)	2,914	(70)
Screen detected	No	34,729	(66)	9,145	(81)	3,033	(73)
	Yes	17,084	(33)	1,935	(17)	489	(12)
	Unknown	582	(1)	153	(1)	654	(16)
HER2 status*	Negative	19,286	(80)	2,903	(64)	36	(6)
	Positive	2,732	(12)	1,304	(28)	12	(2)
	Unknown	1,995	(8)	362	(8)	584	(92)
PR status*	Negative	2,868	(12)	3,623	(79)	<10	(0)
	Positive	15,047	(63)	213	(5)	<10	(0)
	Unknown	6,098	(25)	733	(16)	624	(99)
Tumour size	Less than 10mm	6,424	(12)	1,003	(9)	197	(5)
	10 to 20 mm	20,184	(39)	3,361	(30)	473	(11)
	More than 20mm	17,759	(34)	4,797	(43)	487	(12)
	Unknown	8,028	(15)	2,072	(18)	3,019	(72)

*Markers collected from 2009. Percentages are by column. ER= oestrogen receptor, HER2= human epidermal growth factor 2, NHS= National Health Service, PR=progesterone receptor, TNM= tumour, nodes, metastases.

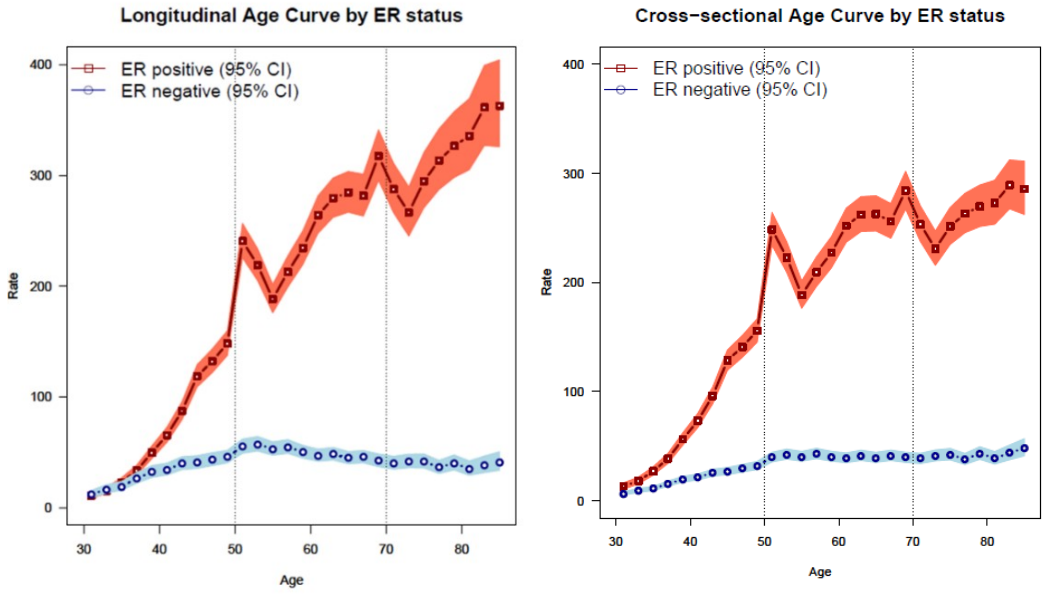
Global effect

Net drifts from the final APC model shows that the overall incidence of ER+ tumours increased by 0.8% per year (95% CI: 0.6 to 1.0%/year) from 1997 to 2016, whereas the incidence of ER- tumours decreased by 1.4% (95% CI: -1.8 to -1.1%/year). Net drifts were highly statistically significant for both ER+ (chi square=56.7 with 1df, p value<0.0001) and ER- tumours (chi square=71.8 with 1df, p value<0.0001).

Age effects

Figure 3.10 presents the fitted longitudinal and cross-sectional age curves from APC models that show a similar pattern to the observed for the age-specific incidence rates of ER+ and ER- cancers. The longitudinal curve provides a summary measure of the age-specific incidence in the reference cohort, while adjusting for period effects. In contrast, the cross-sectional age curve is a measure of the age-specific incidence in the reference period adjusted for cohort effects.

Figure 3.10 Longitudinal and cross-sectional age curves of incidence of breast cancer in Scotland 1997-2016 by ER status from APC models



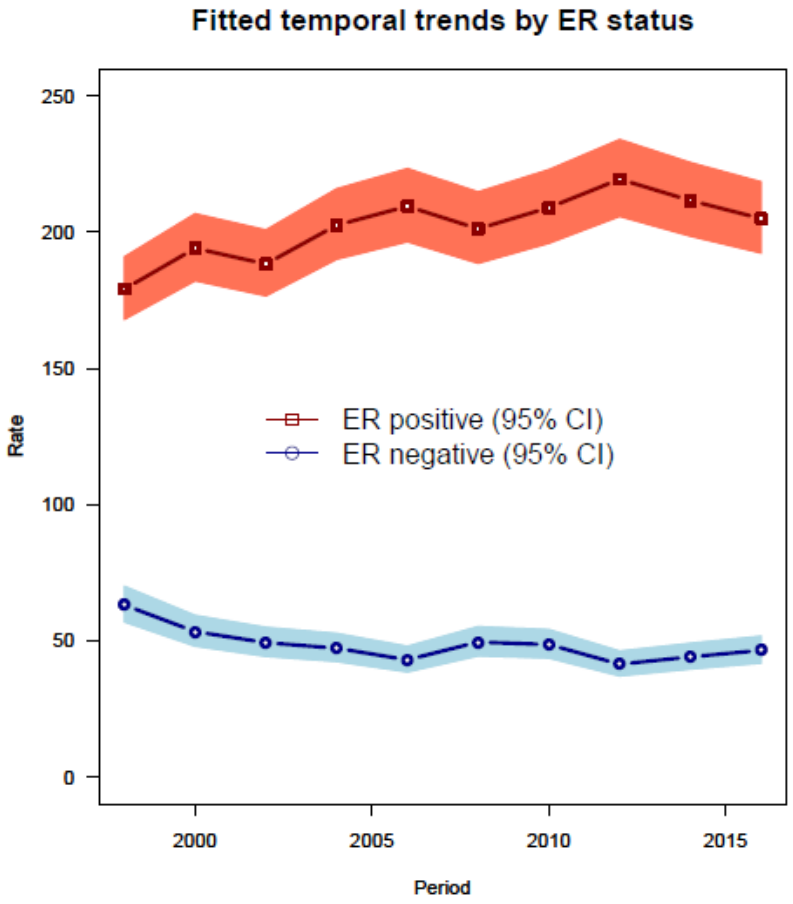
Both curves show different patterns for ER+ and ER- tumours. ER+ age specific rates sharply increased with age until the approximate age of menopause when there was a slight decline, rates increased again until older years. The further increases observed after 50 years of age were highest longitudinally (in women born in the same year).

ER- age specific incidence showed increases until 50 years of age and then remained stable in older ages at diagnosis (for the 2006 reference period) or slightly declined (for the 1949 reference cohort). The differences observed between the longitudinal and cross-sectional curves is also an indication of the existence of the drift. All hypothesis tests for the age deviations (curvature, higher order terms and the combination of both) were highly statistically significant for both ER+ (Appendix Table B.9) and ER- tumours (Appendix Table B.12) so there is a clear age effect.

Period effects

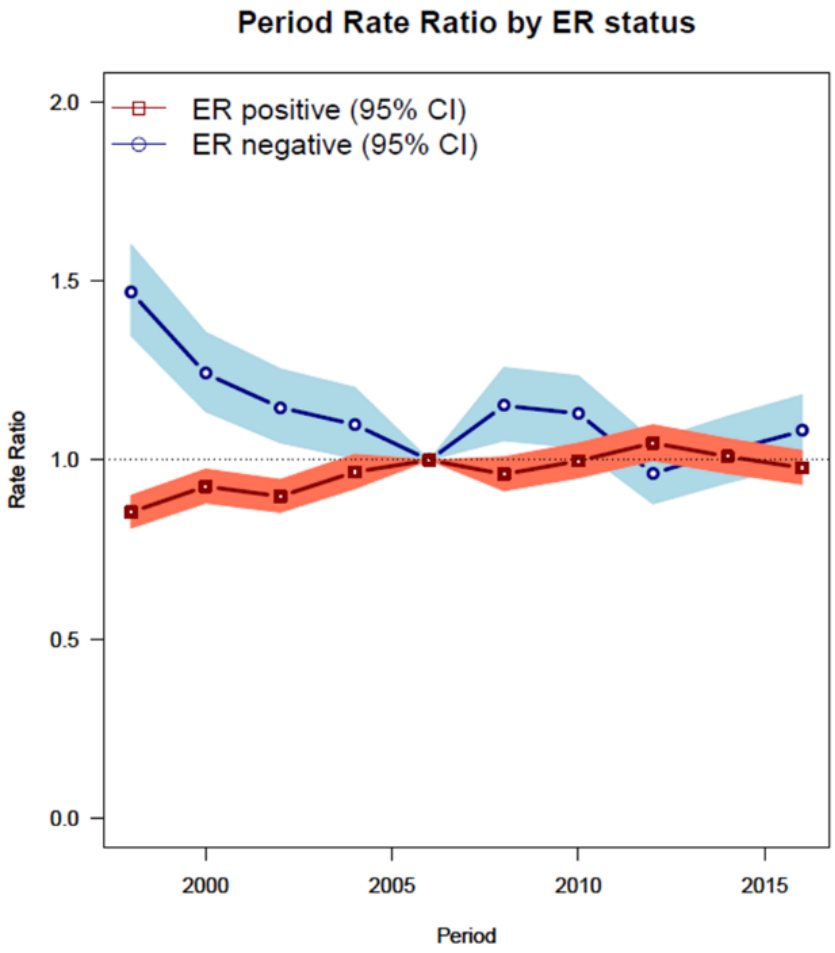
Figure 3.11 shows that temporal trends fitted by the model coincide with the trends observed and with the results from joinpoint, with ER+ tumours increasing up to 2012 and ER- tumours decreasing over time, particularly in earlier years.

Figure 3.11 Fitted temporal trends by ER status from APC model



Period rate ratios (PRRs) confirm that incidence of ER+ tumours rose and declined for ER- tumours (Figure 3.12). Compared to the reference calendar year (2006), there was a lower incidence of ER+ and a higher incidence of ER- rates in earlier years. The trends after 2006 do not show a consistent trend for either of the subtypes. The period curvature parameters for both ER+ (Appendix Table B.9) and ER- (Appendix Table B.12) tumours were highly statistically significant ($p<0.001$) indicating the existence of period effects. The combination test for period effect was also consistent with those results for both ER+ (Appendix Table B.10) and ER- (Appendix Table B.13) tumours.

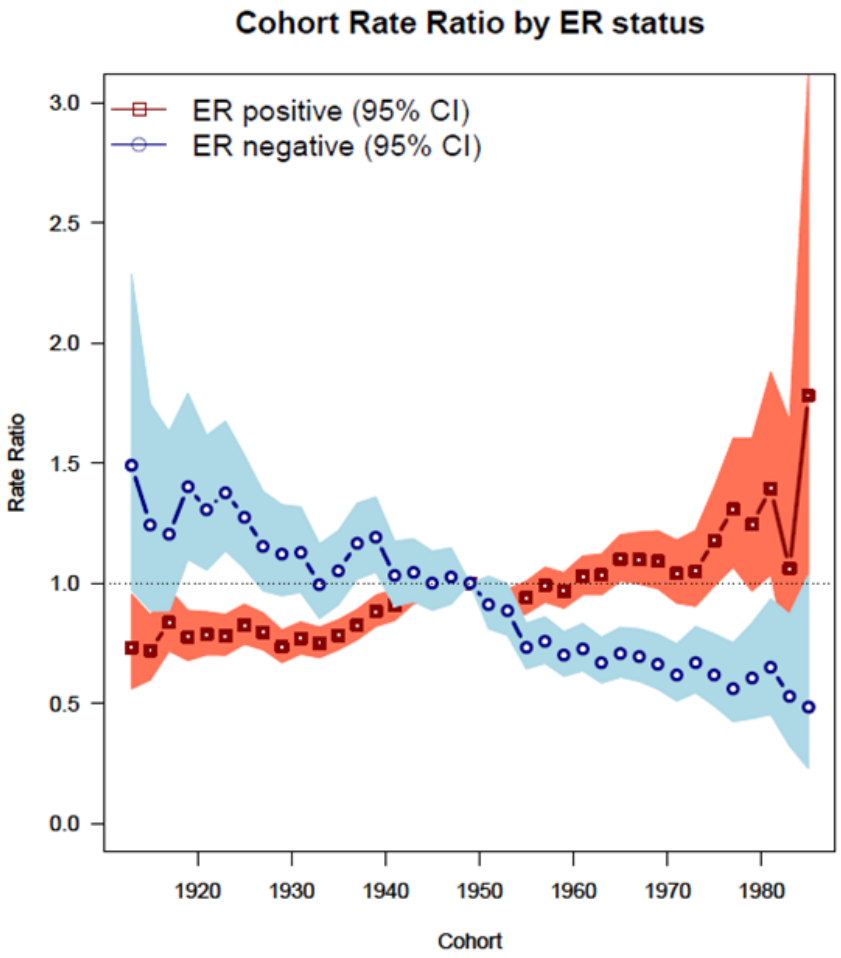
Figure 3.12 Period rate ratios for the incidence rates by ER status in each year of diagnosis (period) compared to the reference year 2006



Cohort effects

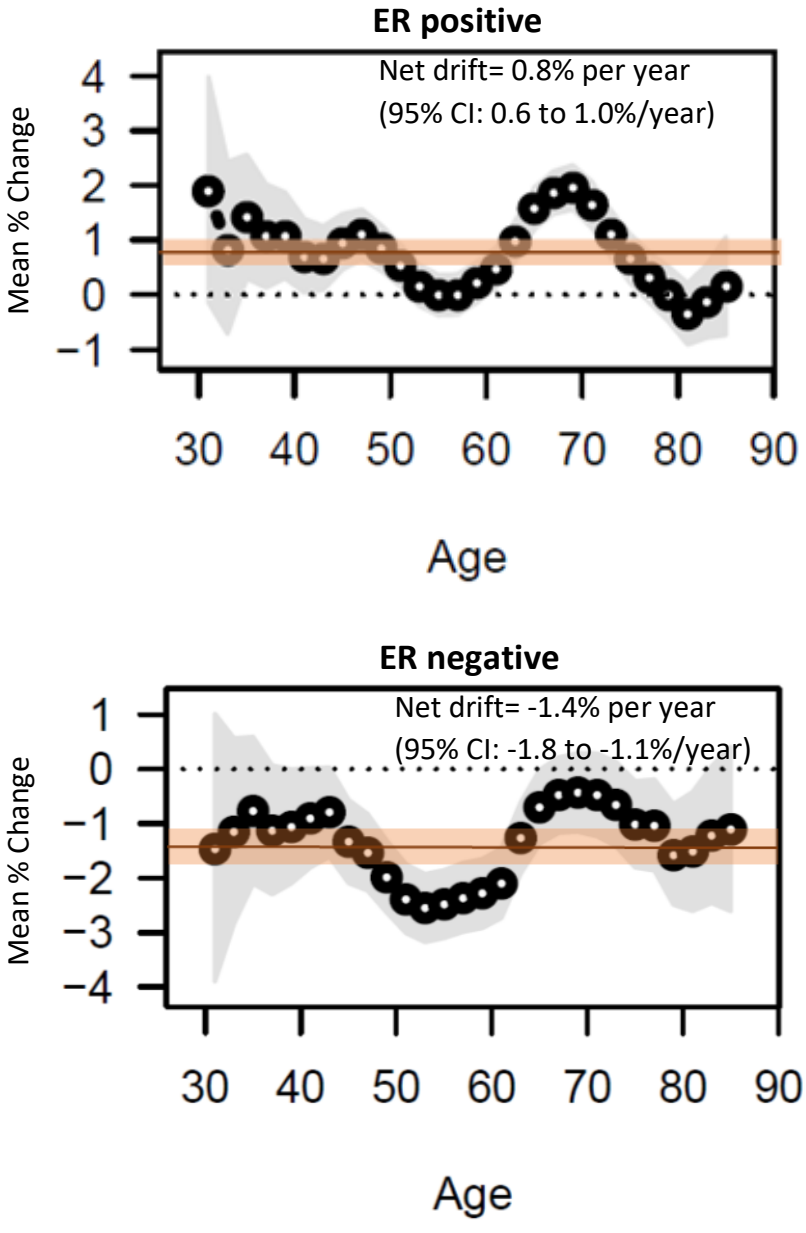
CRR curve showed a striking differential pattern between ER+ and ER- tumours (Figure 3.13). CRRs ranged from 0.7 (95% CI: 0.6 to 0.9%) for women born in 1913 to 1.8 (95% CI: 1.0 to 3.1%) for women born in 1985 compared to women born in the reference cohort of 1949 for ER+ tumours and from 1.5 (95% CI: 1.0 to 2.3%) for women born in 1913 to 0.5 (95% CI: 0.2 to 1.0%) for women born in 1985 for ER- tumours.

Figure 3.13 Cohort rate ratios for the incidence rates for each birth cohort compared to the reference cohort (women born in 1949) for ER+ tumours (red) and ER- tumours (blue)



Cohort effects were also observed in the local drifts patterns (Figure 3.14). The largest increases in incidence of ER+ tumours over time was observed in women around 68 years of age (2% per year, 95% CI: 1.6 to 2.4%], and the largest decline in incidence of ER- tumours occurred in women of 52 years (-2.6% per year, 95% CI:--3.2 to -1.9). Local drifts were different from the net drift for ER+ tumours (p<0.0001, Appendix Table B.9) but did not reach statistical significance for ER- tumours (p=0.062, Appendix Table B.12). The combination tests for the cohort effects, although more powerful than the Wald tests, showed similar results that were statistically significant for ER+ tumours (p<0.0001, Appendix Table B.10) and not significant for ER- tumours (p=0.1398, Appendix Table B.13).

Figure 3.14 ER positive and ER negative age-specific local drifts with net drift



Additional graphs and all the parameters and hypothesis test results from the APC models can be found in Appendix B.7 for ER+ tumours and Appendix B.8 for ER- tumours.

3.4.3 Incidence trends by HER2 status

Time trend analysis by HER2 and the combination of this marker with other molecular markers is limited to years 2009 to 2016 during which 31,099 women were diagnosed with invasive BC.

Table 3.10 presents the distribution of HER2- and HER2+ tumours in Scotland before and after the imputation of HER2 status. Eleven percent of all tumours diagnosed between 2009 and 2016 had missing HER2 status, with the majority of tumours (three in four) being imputed as HER2-. After correcting for HER2 status 85% of women had a tumour classified as HER2- and 15% as HER2+.

Table 3.10 Number and percentage of breast cancer cases by HER2 status before and after correcting for missing marker status in Scotland, 2009-2016

HER2 status	Before correction		After correction	
	n	%	n	%
HER2 negative	23,420	75	26,357	85
HER2 positive	4,214	14	4,742	15
HER2 missing	3465	11		
Total	**31,099**		**31,099**	

HER2= human epidermal growth factor 2

3.4.3.1 Incidence rates by HER2 status and age

Age- specific trends by HER2 status showed a similar pattern to those observed for ER status (Figure 3.15). Incidence of HER2- tumours rapidly increased until 50 years of age, when the increase slows down up to the age of 70 years when incidence increases again but less sharp than what was observed in ER+ tumours. The incidence of HER2+ tumours increased until 50 years of age when it flattened out as observed for ER- tumours.

Figure 3.15 Age-specific incidence rates by HER2 status in Scotland from 2009 to 2016

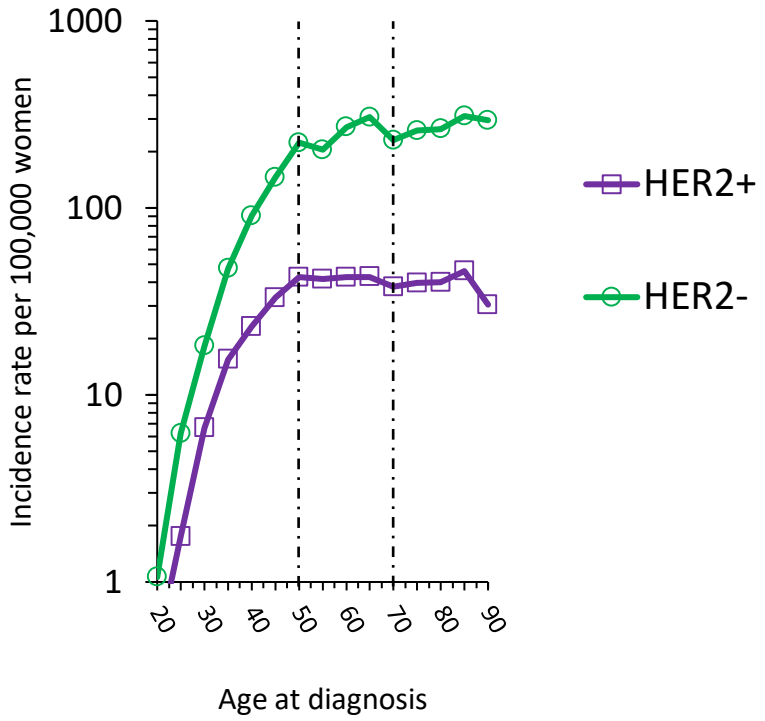


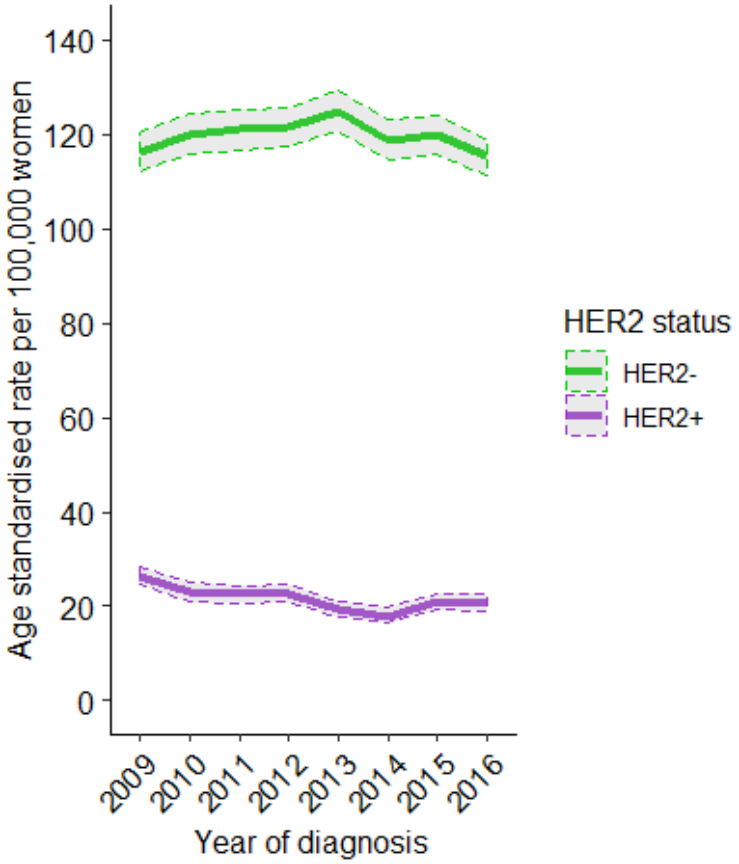
*Figure is on the natural log scale

3.4.3.2 Age-standardised incidence rates by HER2 status and analysis of trends over time using joinpoint regression analysis

BC incidence trends in Scotland from 2009 to 2016 by HER2 status resembled those observed by ER status, with HER2- trends behaving similarly to those observed in ER+ and HER2+ as ER- (Figure 3.16). Incidence of HER2- tumours increased by 1.4% per year (95% CI: -0.6 to 3.5%/year) from 2009 to 2013, followed by a 2.3% decrease per year (95% CI: -5.4 to 0.9%/year) in the most recent years (Table 3.11). However, none of those two trends identified reached significance and the most recent declines left the rates at levels lower than in 2009. In fact, the AAPC was almost constant for HER2- tumours, AAPC=-0.2%/year (-95% CI: 1.3 to 0.9). Incidence rates of HER2+ tumours showed a statistically significant decreasing trend over time, with incidence decreasing by 6.4% annually (95% CI: -11.3 to -1.2%/year) from 2009 to 2014, after

which rates remained constant. AAPC from 2009 to 2016 showed a decreasing trend by 2.7%/year but did not reach statistical significance (95%CI: -7.4 to 2.2). A graph for the differences in rates for imputed and not imputed counts can be found in Appendix B.9.

Figure 3.16 Incidence rates by HER2 status in Scotland from 2009 to 2016



Incidence rates by HER2 status were similar across age groups. Descriptively, HER2- rates in the 50 to 69 years old group showed a similar pattern to the observed for all women (Figure 3.17). Joinpoint regression results (Table 3.11) did not suggest any significant joinpoint or AAPC estimates for any of the age groups, hence, there were no consistent increasing or decreasing patterns observed.

The declines observed for HER2+ tumours were mainly driven by declines in the 50 to 69 year age group [-6.4%/year (95% CI: -11.3 to-1.2) from 2009 to 2014] and the 70 years and older [AAPC=-5.3%/year (95% CI: -9.1 to -1.3)] groups. Incidence rates in women aged 20 to 49 years were fairly constant within the study period with

AAPC=-0.9%/year (95% CI: -3.3 to 1.6). Further joinpoint results are presented in Table 3.11 below.

Figure 3.17 Incidence rates of breast cancer in Scotland 1997-2016 by HER2 status for 3 age groups (<50 years, 50-69 years and more than 70 years)

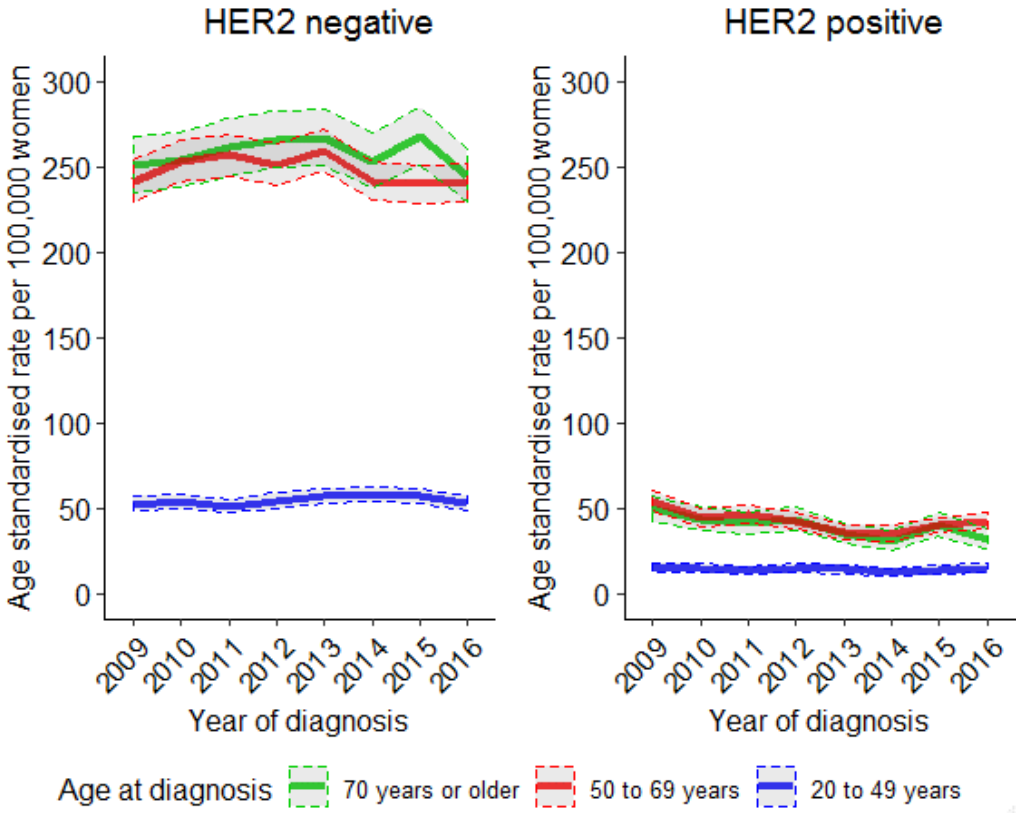


Table 3.11 Joinpoint regression analysis results for breast cancer incidence rates for women of all ages and for three age groups (20 to 49 years, 50 to 69 years and 70 years or more) by HER2 status in Scotland from 2009 to 2016

HER2 status	Age groups	Rate in 1997 per 100,000 women	Rate in 2016 per 100,000 women	Change in rate from 1997 to 2016 N per 100,000 (%)	AAPC (95% CI)	Years before joinpoint	EAPC (95% CI) for period before joinpoint	Years after joinpoint	EAPC (95% CI) for period after joinpoint
Negative	All ages	116.0	115.2	-0.8 (0.7%)	-0.2% (-1.3, 0.9)	2009-2013	1.4% (-0.6, 3.5)	2013-2016	-2.3% (-5.4, 0.9)
	20-49 years	52.2	52.6	0.4 (0.8%)	0.9% (-0.8, 2.7)	No significant change point identified from 2009-2016			
	50-69 years	241.3	240.6	-0.7 (0.3%)	-0.5% (-1.8, 0.7)	No significant change point identified from 2009-2016			
	70+ years	250.4	244.7	-5.7 (2.3%)	0.0% (-1.3, 1.4)	No significant change point identified from 2009-2016			
Positive	All ages	26.4	20.8	-5.6 (21%)	-2.7% (-7.4, 2.2)	2009-2014	**-6.4%(-11.3, -1.2)**	2014-2016	7.2% (-16.4, 37.3)
	20-49 years	14.9	14.7	-0.2 (1.3%)	-0.9% (-3.3, 1.6)	No significant change point identified from 2009-2016			
	50-69 years	54.4	42.3	-12.1 (22%)	-2.8% (-9.0, 3.8)	2009-2014	**-7.9% (-14.5, -0.9)**	2014-2016	11.2% (-19.9, 54.2)
	70+ years	49.6	31.5	-18.1 (36%)	**-5.3% (-9.1, -1.3)**	No significant change point identified from 2009-2016			

Bold results are significantly different from 0 ($p<0.05$). AAPC estimated average annual percent change, EAPC estimated annual percentage change, HER2= human epidermal growth factor 2. Incidence rates are standardised to the European population. Rates for the age groups are truncated rates for each specific age group.

3.4.3.3 Age-period-cohort analysis for incidence by HER2 status

APC models by HER2 status were restricted to rates in women aged 30 to 85 years diagnosed in Scotland from 2009 to 2016 (n=29,214).

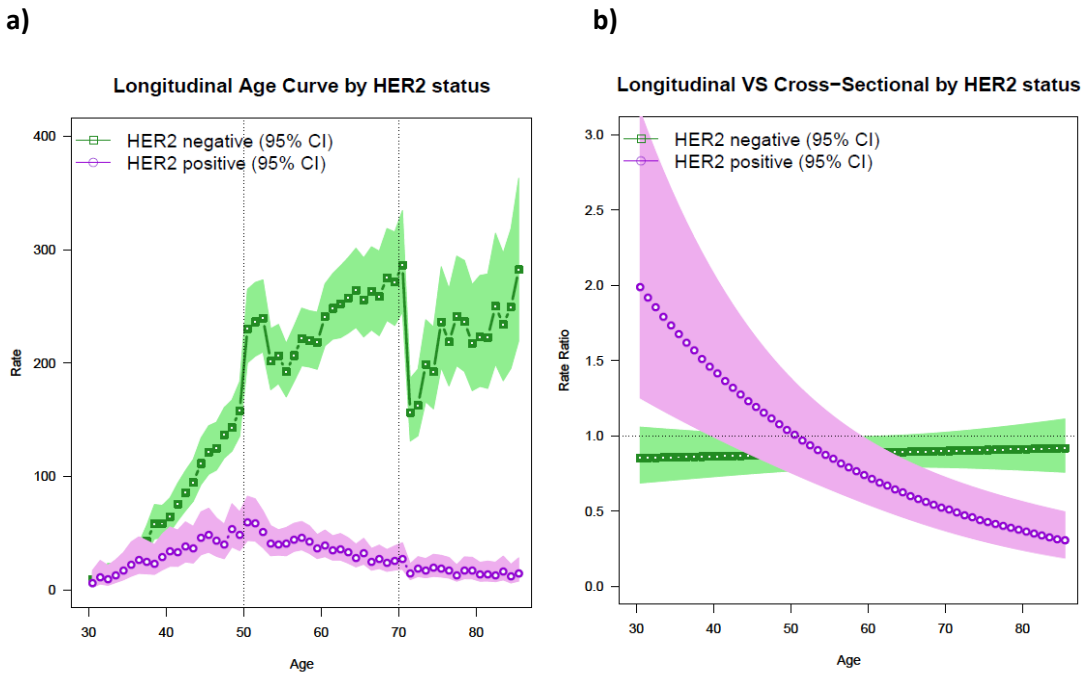
Global effect

Net drifts show that the overall incidence of HER2- tumours remained almost constant 0.1% per year (95% CI: -0.5 to 0.7%/year) from 2009 to 2016, whereas the incidence of HER2+ tumours decreased by 3.3%/year (95% CI: -4.7 to -2.0%/year). These results are consistent with those from joinpoint regression and the Wald tests for the net drift for HER2- (p value=0.663, Appendix Table B.15) and HER2+ tumours (p value<0.00001, Appendix Table B.18).

Age effects

Figure 3.18a presents the fitted longitudinal age curves from APC models that show a similar pattern to the longitudinal curve by ER status.

Figure 3.18 Longitudinal age curve (a) and ratio between longitudinal and cross-sectional curves (b) by HER2 status



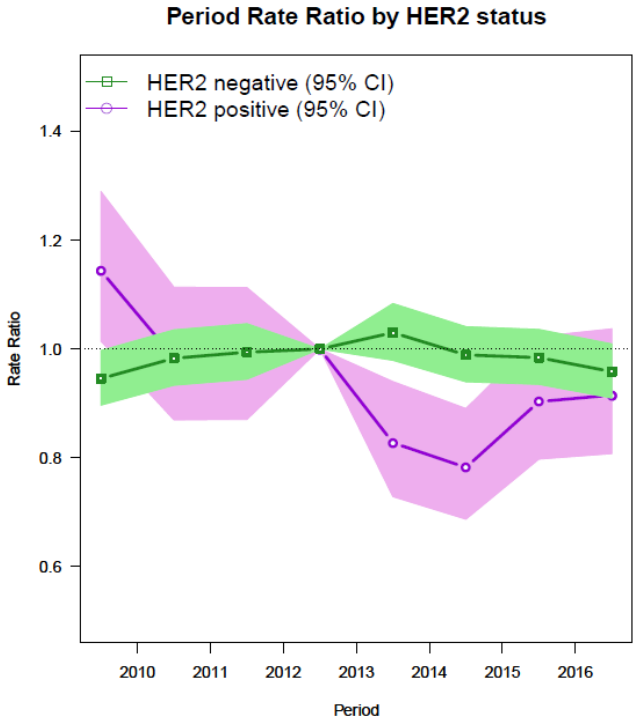
Incidence increases with age until the approximate age of menopause for HER2- tumours. Rates continue to increase after that age at a slower pace and there is an important drop in rates after 70 years of age. For HER2+ tumours, longitudinal curve is inverse v-shaped with increases until the age of 50 and decreases after.

The second figure (Figure 3.18b) shows the ratio between the longitudinal and cross-sectional age curves fitted by APC. The ratio for HER2- tumours show a constant trend that is close to 0 and therefore, the longitudinal and cross sectional curves are very similar which is also an indication of no net drift. In contrast, the ratio for HER2+ tumours ranges from 2.0 (95% CI: 1.3 to 3.2) at 30 years to 0.3 (95% CI: 0.2 to 0.5) at 85 years.

Period effects

PRRs (Figure 3.19) show no clear period differences with the reference period in mid-2012 for HER2- tumours (p=0.062) and a decreasing trend for HER2+ tumours, with rates in years 2013-2015 significantly lower than rates in the reference period in mid-2012.

Figure 3.19 Period rate ratios for the incidence rates in each year of diagnosis (period) compared to the reference year 2006

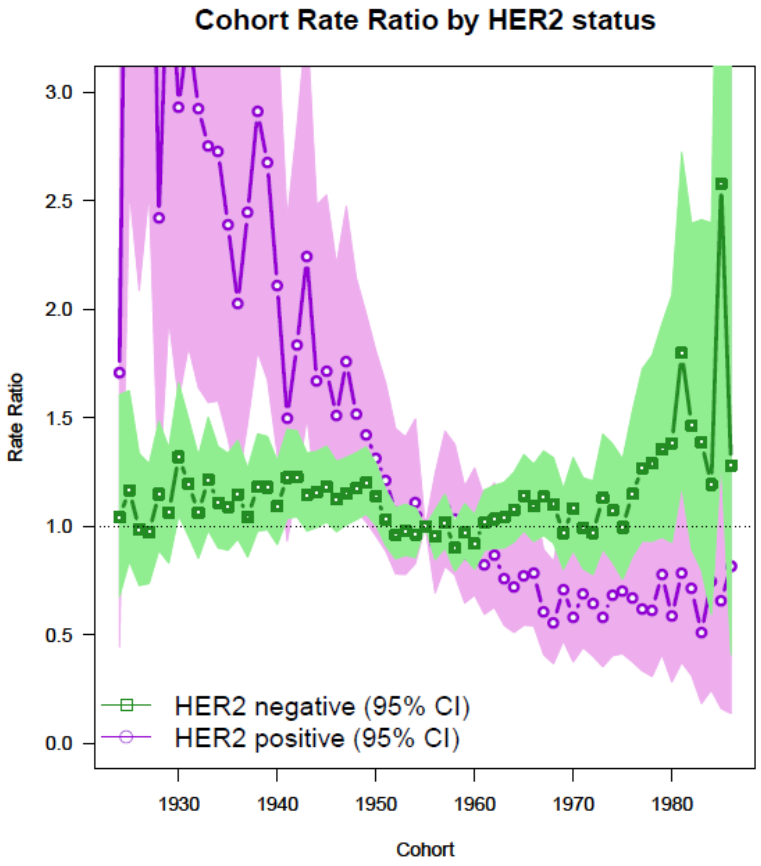


Although there is high uncertainty around the estimates, particularly for HER2+ tumours, the Wald tests for period deviations were significant for both subtypes. The combination test confirmed that period effects were observed for both ER+ and ER- tumours (Appendix B.10 and Appendix B.11).

Cohort effects

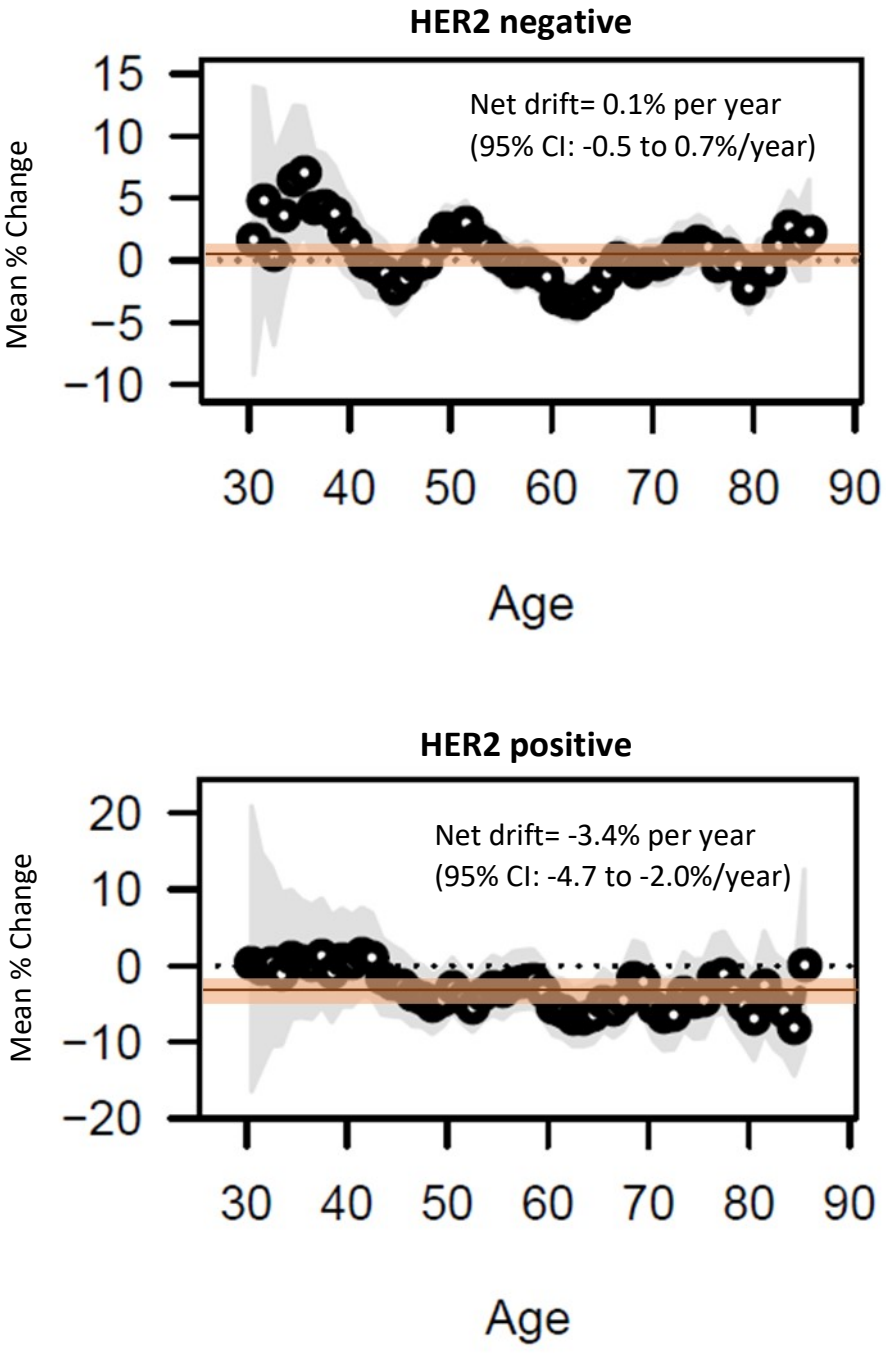
Figure 3.20 for the CRRs by HER2 status show similar results to those observed for ER. However, no consistent effects were observed for HER2-tumours. HER2+ rates seemed to be higher in women born in early 30s through the 40s and lower in younger women born in the 70s and 80s compare to the referent cohort of women born in 1955. Although descriptively the rates did not seem to have clear cohort effects for the HER2- tumours, the hypothesis tests for the cohort deviations showed significant results for both Wald test (p value=0.004) and the combination test (p value=0.008). In contrast, there were no significant cohort effects for HER2+ tumours (Wald test p value =0.830, combination test p value=0.131).

Figure 3.20 Cohort rate ratios for the incidence rates for each birth cohort compared to the reference cohort (women born in 1955) for HER2- and HER2+ tumours



The cohort effects observed for HER2- tumours were observed in the local drifts signals (Figure 3.21). Local drifts for the HER2- tumours showed the highest increases observed in younger women aged 30-40 years and the highest decreases in women aged 60 to 70 years. The pattern for HER2+ tumours did not show any of the age groups to be different from the net drift. Hypothesis test for the local drifts was significant for HER2- tumours (p value=0.001) but not for HER2+ (p value=0.769).

Figure 3.21 HER2 negative and HER2 positive age-specific local drifts with net drift



Additional graphs and all the parameters and hypothesis tests results from the APC models can be found in Appendix B.10 for HER2- tumours and Appendix B.11 for HER2+ tumours.

3.4.4 Incidence trends by IHC defined molecular subtypes

3.4.4.1 Women and tumour characteristics by molecular subtypes

Among 31,099 incident invasive tumours selected that were diagnosed in Scotland from 2009 to 2016, three in four were luminal tumours with luminal A being the majority of them (66% of all tumours) and a further 9% being luminal B. The percentage of TNBC was similar to that for luminal B tumours and the least common subtype was the HER2-enriched with only 4% of all cases. A further 11% of cases could not be classified as any of the subtypes due to missing data.

Subtypes differed by women and tumour characteristics (Table 3.12). Luminal A, the most common subtype, was more common in older women and less common in younger than the rest of subtypes. It also had tumour characteristics associated with less advanced disease, such as, smaller tumour size, lower grade or fewer tumours with affected nodal status. These differences were particularly marked in comparison to TNBC that seemed to be the more advanced ones with 79% being poorly differentiated and 43% bigger than 20mm. However, the % of women with nodes affected was bigger for luminal B and HER2-enriched tumours than for TNBC. Screening also seemed to differ by subtype with luminal A being the most screened detected (36%) and TNBC the least (17%).

Table 3.12 Descriptive characteristics by IHC defined molecular subtype for all women with an invasive breast cancer diagnosed between 2009 and 2016 in Scotland (n=31,099)

Characteristic		Luminal A (HR+HER2-)		Luminal B (HR+HER2+)		HER2-enriched (HR-HER2+)		Triple Negative (HR-HER2-)		Missing subtype		Total	
		n	%	n	%	n	%	n	%	n	%	n	%
		20,484	**[66]**	**2,915**	**[9]**	**1,288**	**[4]**	**2,899**	**[9]**	**3,513**	**[11]**	**31,099**	**100**
Age at diagnosis	<50 years	3,669	(18)	831	(28)	312	(24)	803	(28)	443	(13)	6,058	(19)
	50 69 years	10,946	(53)	1,426	(49)	654	(51)	1,390	(48)	1,566	(44)	15,982	(51)
	70 years or older	5,869	(29)	658	(23)	322	(25)	706	(24)	1,504	(43)	9,059	(29)
NHS Scottish region	North	5,410	(26)	790	(27)	333	(26)	772	(27)	790	(23)	8,095	(26)
	South East	5,867	(29)	751	(26)	325	(25)	766	(26)	784	(22)	8,493	(27)
	West	9,207	(45)	1,374	(47)	630	(49)	1,360	(47)	1,939	(55)	14,510	(47)
Tumour size	Less than 10mm	2,874	(14)	304	(10)	169	(13)	283	(10)	363	(10)	3,993	(13)
	10 to 20 mm	7,734	(38)	883	(30)	301	(23)	879	(30)	872	(25)	10,669	(34)
	More than 20mm	6,706	(33)	1,128	(39)	458	(36)	1,248	(43)	712	(43)	10,252	(33)
	Unknown	3,170	(15)	600	(21)	360	(28)	489	(17)	1,566	(17)	6,185	(20)
Grade	I-Well differentiated	3,063	(15)	97	(3)	<10	(<1)	<40	(1)	345	(10)	3,541	(11)
	II- Moderately differentiated	10,858	(53)	975	(34)	238	(18)	401	(14)	1,151	(33)	13,623	(44)
	III- Poorly differentiated	5,027	(25)	1,554	(53)	886	(69)	2,277	(79)	815	(23)	10,559	(34)
	Unknown	1,536	(7)	289	(10)	<200	(12)	189	(6)	1,202	(34)	3,376	(11)
TNM stage	I	8,705	(42)	821	(28)	296	(23)	830	(29)	1,260	(36)	11,912	(38)
	II	7,307	(36)	1,140	(39)	506	(39)	1,307	(45)	854	(24)	11,114	(36)
	III	2,241	(11)	487	(17)	259	(20)	424	(15)	318	(9)	3,729	(12)
	IV	959	(5)	221	(8)	118	(9)	147	(5)	366	(10)	1,811	(6)
	Unknown	1,272	(6)	246	(8)	109	(8)	191	(7)	715	(20)	2,533	(8)
Nodal Status	No	11,667	(57)	1,464	(50)	626	(49)	1,721	(59)	1,388	(40)	16,866	(54)
	Yes	5,946	(29)	1,083	(37)	496	(38)	907	(31)	610	(17)	9,042	(29)
	Unknown	2,871	(14)	368	(13)	166	(13)	271	(9)	1,515	(43)	5,191	(17)
Screen detected	No	13,069	(64)	2,199	(75)	1,041	(81)	2,394	(83)	2,355	(67)	21,058	(68)
	Yes	7,403	(36)	715	(25)	247	(19)	505	(17)	1,092	(31)	9,962	(32)

Brackets are row percentages and parenthesis are column percentages. HR= hormone receptor, HER2= human epidermal growth factor 2, NHS= National Health Service, PR=progesterone receptor, TNM= tumour, nodes, metastases.

After correcting for missing HR/HER2 status (Table 3.13), three in four tumours were re-classified as luminal A and a further 11% as luminal B. TNBC constituted another 11% of all tumours and HER2-enriched was the least likely subtype.

Table 3.13 Number and percentage of breast cancer cases by IHC defined molecular subtype before and after correcting for missing marker status in Scotland, 2009-2016

Molecular subtype	Before correction		After correction	
	n	%	n	%
Luminal A	20,484	66	23,093	74
Luminal B	2,915	9	3,286	11
HER2-enriched	1,288	4	1,452	5
Triple Negative	2,899	9	3,268	11
Missing subtype	3,513	11		
Total	**31,099**		**31,099**	

HER2=human epidermal growth factor 2

3.4.4.2 Incidence rates by molecular subtypes and age

Age- specific trends in BC incidence by molecular subtypes (Figure 3.22) showed a different pattern for luminal A tumours than for the rest of molecular subtypes. Luminal A age-specific incidence rates had similar patterns those observed for ER+ tumours with rapid increases until 50 years of age when rates slightly decline and increase again peaking at around 65 and 85 years. The rest of the subtypes showed slightly different patterns with incidence increasing until 50 years and remaining almost constant after that age.

Figure 3.22 Age-specific rates by IHC defined molecular subtype

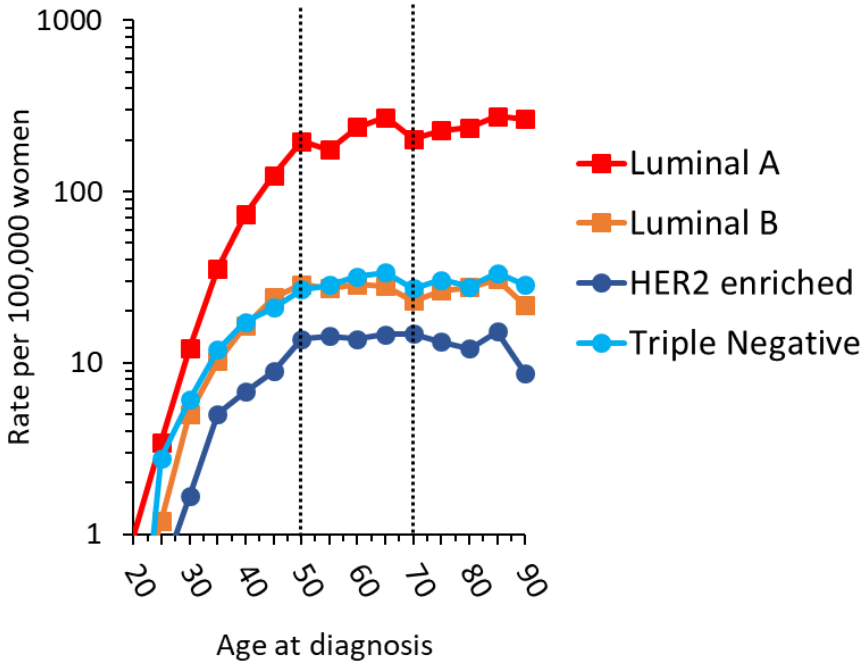
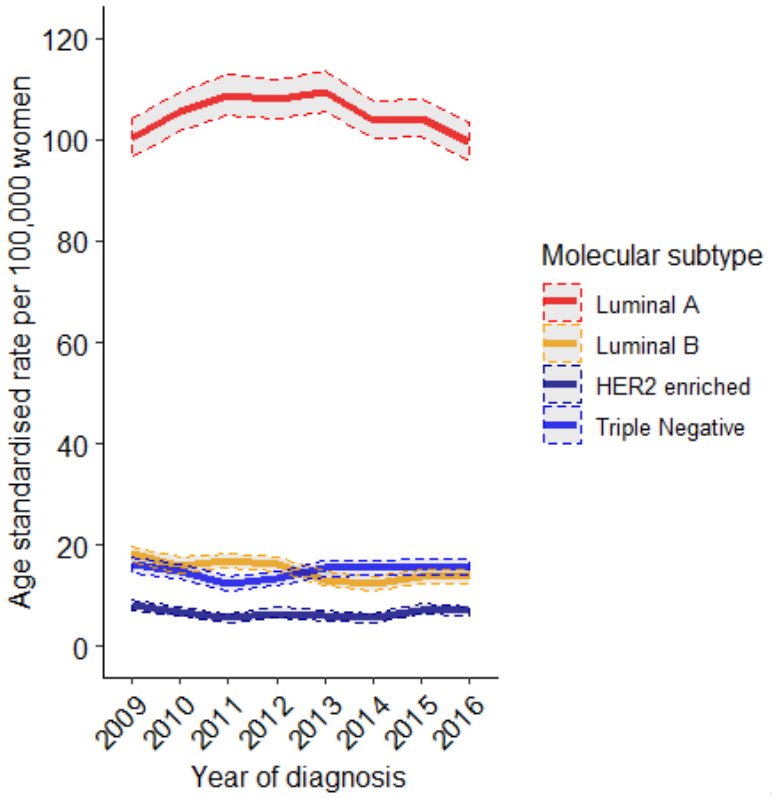


*Figure is on the natural log scale

3.4.4.3 Age-standardised incidence rates by molecular subtypes and analysis of trends over time using joinpoint regression analysis

Incidence rates for the four molecular subtypes (Figure 3.23) showed that luminal A tumours had the highest incidence rates with a similar pattern to the observed for ER+ tumours. Incidence of luminal A tumours increased by 4.9% per year (95% CI: -3.9 to 14.5%/year) from 2009 to 2011, and decreased by 1.7% per year after (95% CI: -3.5 to 0.2%/year). However, none of those two trends was statistically significant and the AAPC= 0.1%/year (95% CI: -1.6 to 1.9) suggested no change in rates.

Figure 3.23 Age-standardised incidence by molecular subtype in Scotland from 1997 to 2016 with 95% CI after correcting for missing subtype



The other subtypes had considerably lower incidence rates and different patterns than the luminal A tumours. No significant change point in incidence was identified for luminal B or HER2-enriched tumours. HER2-enriched tumours showed a slightly decreasing trend overall AAPC=-1.3%/year (95% CI: -6.1 to 3.8) and for each age group that did not reach significance. Incidence of luminal B tumours declined overall [AAPC=-4.5%/year (95% CI:-7.3 to -1.6)] with declines mainly observed in women aged 50-69 years and 70 years or older (Figure 3.24, Table 3.14). TNBC incidence decreased in the early years, 2009-2011, but increased after (from 2011 to 2016) with and EAPC= 4.5%/year (95% CI: 0.1 to 9). Those increases were mainly observed in younger women aged 20-49 years for which AAPC was 4.6%/year (95% CI: 1.2 to 8.2). Additional scaled graphs in Appendix B.12.

Figure 3.24 Incidence rates by molecular subtype and age groups (20-49 years, 50-69 years and 70 years or older)

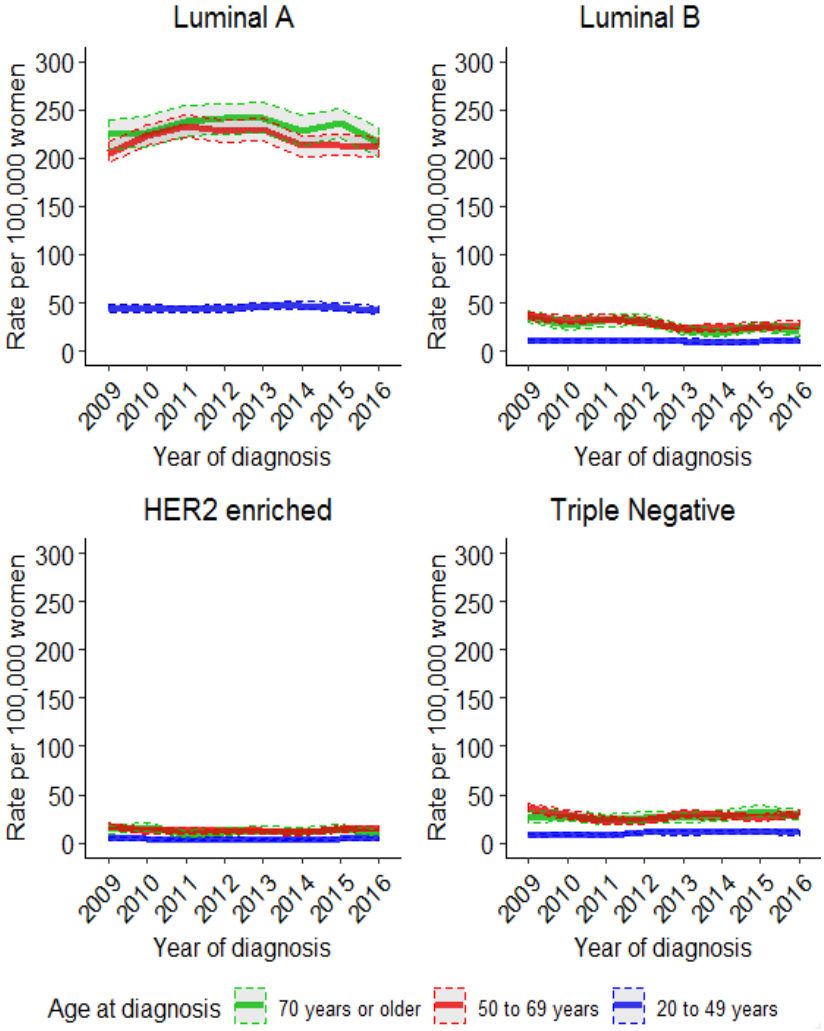


Table 3.14 Joinpoint regression analysis results for breast cancer incidence rates for women of all ages and for three age groups (20 to 49 years, 50 to 69 years and 70 years or more) by molecular subtypes in Scotland from 2009 to 2016 (page 1 of 2)

Molecular subtype	Age groups	Rate in 2009 per 100,000 women	Rate in 2016 per 100,000 women	Change in rate from 2009 to 2016 N per 100,000 (%)	AAPC (95% CI)	Years before joinpoint	EAPC (95% CI) for period before joinpoint	Years after joinpoint	EAPC (95% CI) for period after joinpoint
Luminal A (HR+HER2-)	All ages	100.1	99.6	-0.5 (0.5%)	0.1% (-1.6, 1.9)	2009-2011	4.9% (-3.9, 14.5)	2011-2016	-1.7% (-3.5, 0.2)
	20-49 years	44.1	42.1	-2 (4.5%)	0.1% (-1.5, 1.7)	No significant change point identified from 2009-2016			
	50-69 years	204.9	210.9	6 (2.9%)	0.2% (-1.9, 2.2)	2009-2011	6.6% (-3.9, 18.2)	2011-2016	**-2.3% (-4.4, -0.1)**
	70+ years	224.3	215.8	-8.5 (3.8%)	-0.1% (-2.4, 2.2)	2009-2012	3% (-3.8, 10.2)	2012-2016	-2.4% (-6.4, 1.8)
Luminal B (HR+HER2+)	All ages	18.9	13.8	-5.1 (27%)	**-4.5% (-7.3, -1.6)**	No significant change point identified from 2009-2016			
	20-49 years	10.6	10.4	0.2 (1.9%)	-0.9% (-2.8, 1.0)	No significant change point identified from 2009-2016			
	50-69 years	36.1	27.6	-8.5 (24%)	**-5% (-8.5, -1.3)**	No significant change point identified from 2009-2016			
	70+ years	35.7	20.1	-15.6 (44%)	**-7.3% (-12.6, -1.7)**	No significant change point identified from 2009-2016			

Table 3.14 (continued) Joinpoint regression analysis results for breast cancer incidence rates for women of all ages and for three age groups (20 to 49 years, 50 to 69 years and 70 years or more) by molecular subtypes in Scotland from 2009 to 2016 (page 2 of 2)

Molecular subtype	Age groups	Rate in 2009 per 100,000 women	Rate in 2016 per 100,000 women	Change in rate from 2009 to 2016 N per 100,000 (%)	AAPC (95% CI)	Years before joinpoint	EAPC (95% CI) for period before joinpoint	Years after joinpoint	EAPC (95% CI) for period after joinpoint
HER2-enriched (HR-HER2+)	All ages	8.2	7.0	-1.2 (15%)	-1.3% (-6.1, 3.8)	No significant change point identified from 2009-2016			
	20-49 years	4.3	4.4	0.1 (2.3%)	-0.8% (-7.3, 6.2)	No significant change point identified from 2009-2016			
	50-69 years	18.2	14.7	-3.5 (19%)	-1.6% (-7.8, 5.0)	No significant change point identified from 2009-2016			
	70+ years	13.9	11.3	-2.6 (19%)	-1.3% (-7.8, 5.7)	No significant change point identified from 2009-2016			
Triple Negative (HR-HER2-)	All ages	16.1	15.7	-0.4 (2.5%)	0.2% (-3.6, 4.1)	2009-2011	-9.9% (-25.5, 8.9)	2011-2016	**4.5% (0.1, 9.0)**
	20-49 years	8.1	10.4	2.3 (28%)	**4.6% (1.2, 8.2)**	No significant change point identified from 2009-2016			
	50-69 years	36.5	29.8	-6.7 (18%)	-1.7% (-7.1, 4.1)	No significant change point identified from 2009-2016			
	70+ years	26.8	29.3	2.5 (9.3%)	2.4% (-0.7, 5.6)	No significant change point identified from 2009-2016			

Bold results are significantly different from 0 ($p<0.05$). AAPC= average annual percent change, EAPC= estimated annual percentage change, HR= hormone receptor, HER2= human epidermal growth factor 2. Incidence rates are standardised to the European population. Rates for the age groups are truncated rates for each specific age group.

3.4.4.4 Age-period-cohort analysis for incidence by molecular subtypes

APC models for the molecular subtypes were restricted to women aged 30 to 85 years with invasive BC diagnosed between 2009 and 2016 (n=29,214).

Global effect

Net drifts for each of the subtypes (Table 3.15) showed very similar results to those previously seen using joinpoint regression with only luminal B tumours showing a significant declining trend by 4% per year from 2009 to 2016.

Table 3.15 Net drifts estimated from APC models for each molecular subtype

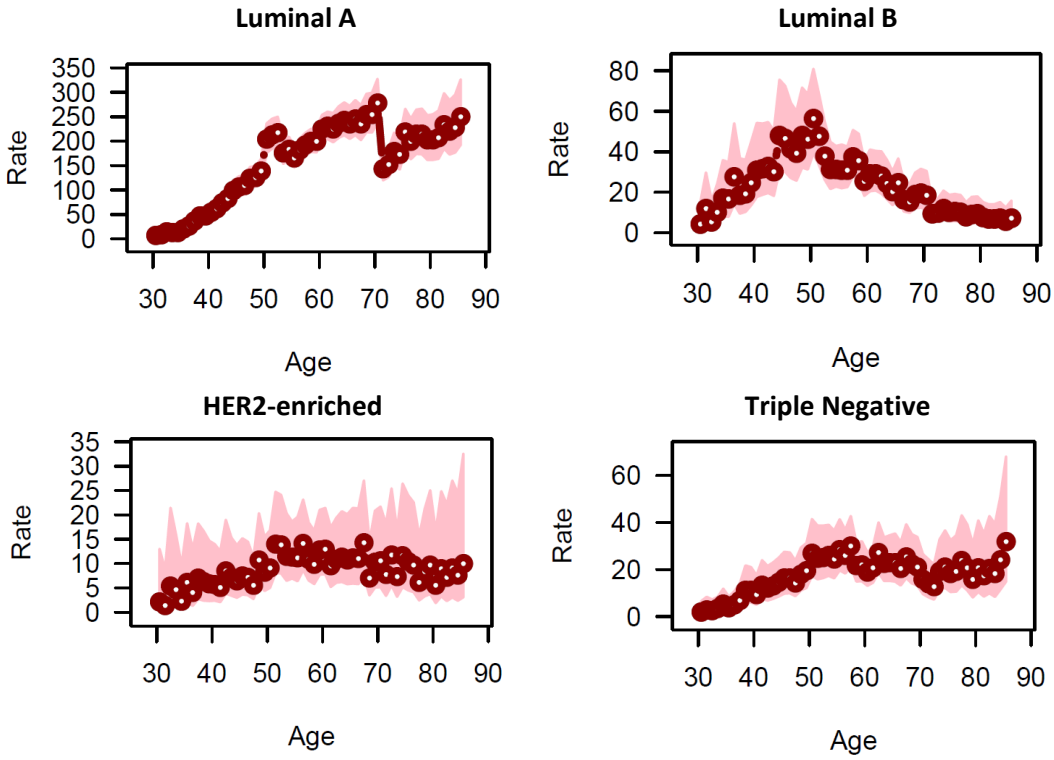
Subtype	Net Drift	Lower CI	Upper CI	P value
Luminal A	-0.04	-0.69	0.62	0.906
Luminal B	**-4.19**	-5.72	-2.63	<0.0001
HER2-enriched	-1.32	-3.63	1.04	0.272
Triple Negative	0.20	-1.45	1.88	0.813

CI= confidence interval, HER2=human epidermal growth factor 2.

Age effects

Fitted longitudinal age curves (Figure 3.25) were similar to age-specific trends in Figure 3.22. Further, longitudinal (Figure 3.25) and cross-sectional curves (Appendix B.13) for luminal A, HER2-enriched and TNBC had similar patterns suggesting no net drift. Luminal B was the only subtype with a clear difference between longitudinal and cross sectional curve indicating a significant net drift effect. Age effects were observed for all subtypes (Appendix B.13).

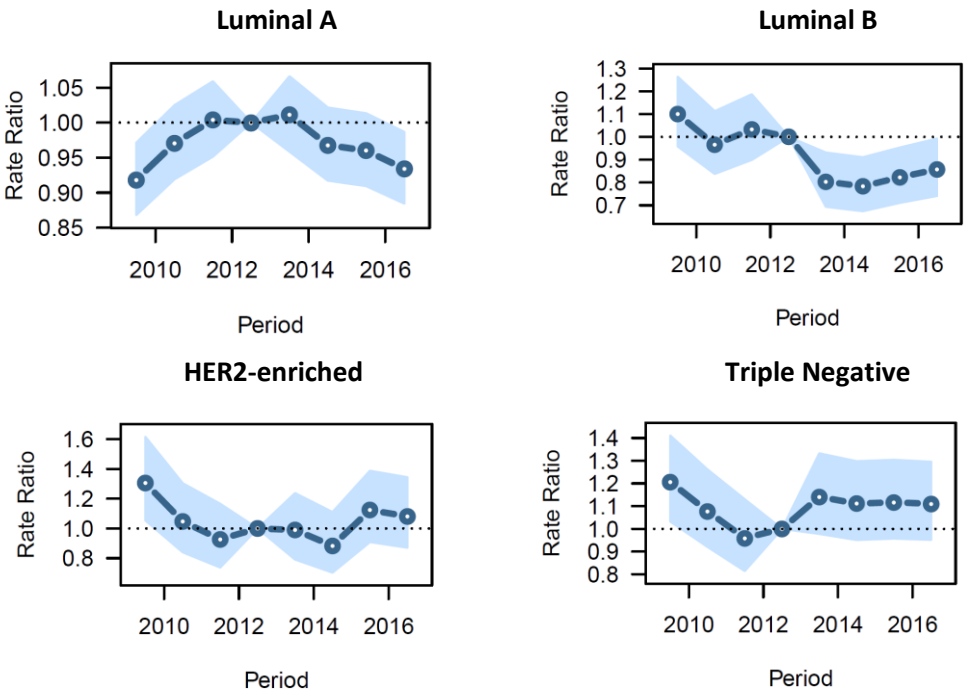
Figure 3.25 Longitudinal age curve for each molecular subtype



Period effects

The only significant PRRs compared to the reference period in mid-2012 were those observed for luminal B tumours between 2012 and 2016 that showed PRRs of around 0.8 (Figure 3.26). However, period effects were statistically significant only for luminal A and HER2-enriched tumours (Appendix B.13).

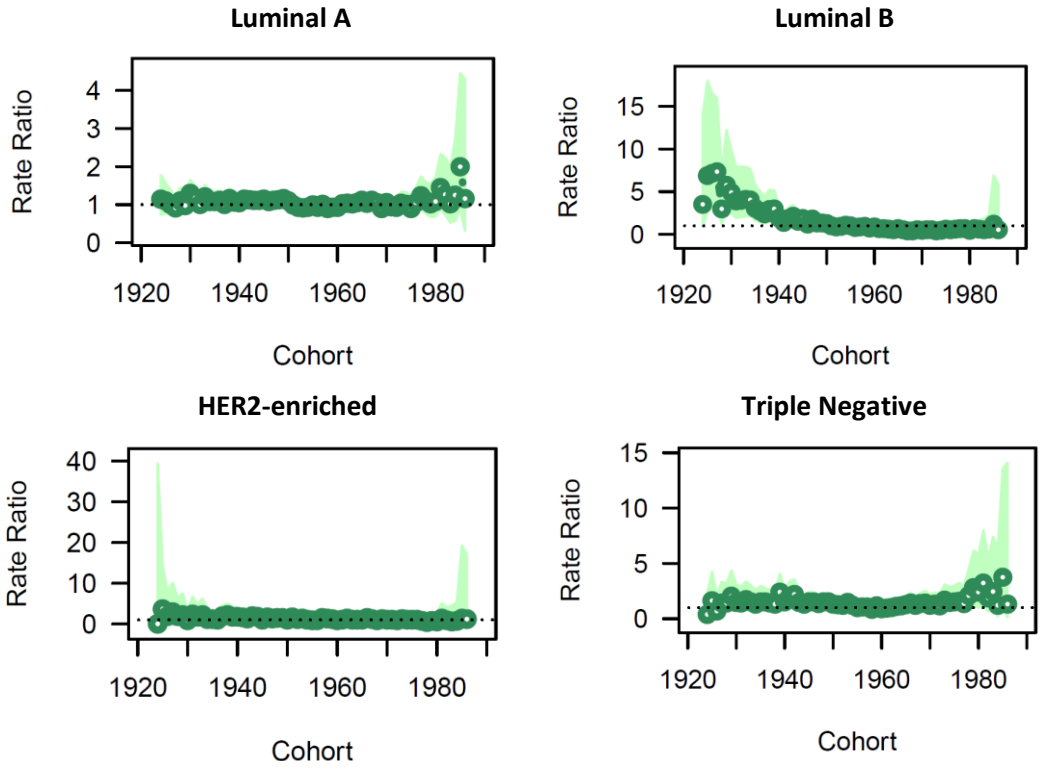
Figure 3.26 Period rate ratio for each molecular subtype



Cohort effects

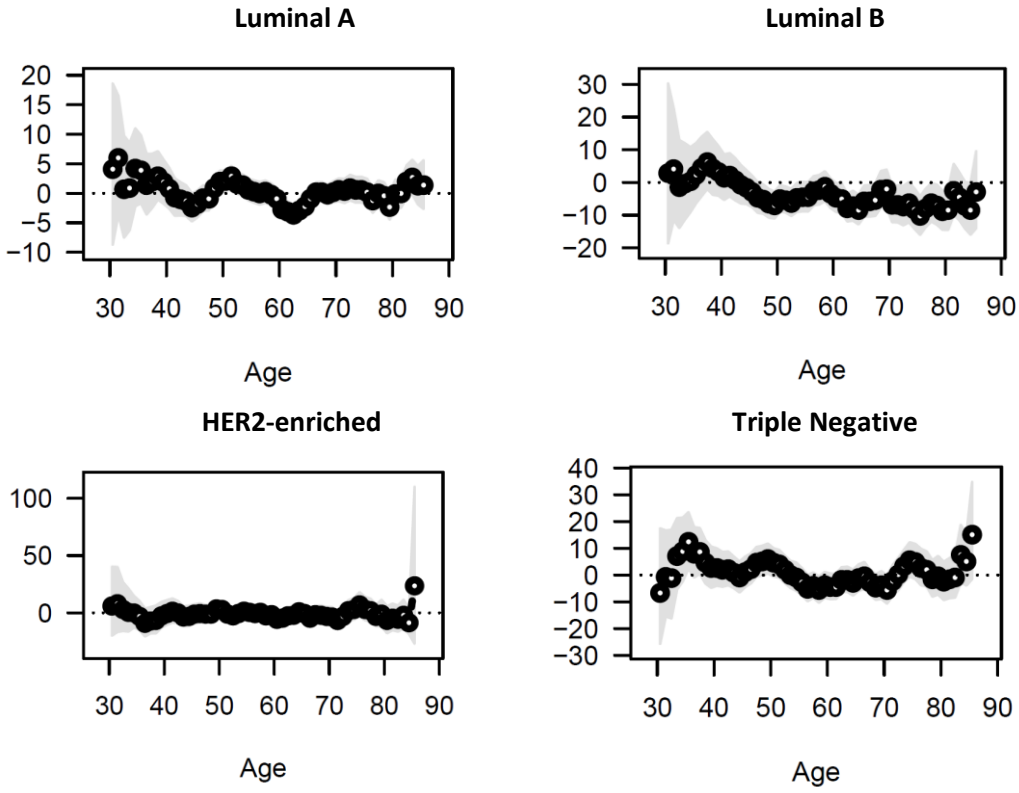
CRRs (Figure 3.27) for the molecular subtypes show that luminal B was the only subtype with a significant cohort effect with women born in the 1920s and 1930s having higher incidence of luminal B tumours in comparison to women born in the referent cohort in 1955.

Figure 3.27 Cohort rate ratio for each molecular subtype



Local drifts (Figure 3.28) suggested that the cohort effects observed in the luminal B tumours were driven by declines observed in older women aged 60 years or older. Local drifts and cohort effects for the rest of the subtypes were not as clear and did not show any significant results (Appendix B.13).

Figure 3.28 Local drifts for each molecular subtype



All additional graphs, parameters and hypothesis test for the APC models for each molecular subtype are presented in Appendix B.13.

3.4.5 Sensitivity analysis for luminal tumours

Luminal tumours are further differentiated using a cell proliferation marker known as Ki67. However, routinely collected data from most cancer registry does not include this marker. Grade is often used as a marker of cell proliferation, and luminal tumours have been further classified using grade. Luminal A tumours with high grade have been seen to be more similar in behaviour to luminal B tumours than to luminal A tumours of low or middle grade. In this part of the analysis, grade was used as a marker for cell proliferation and sensitivity analysis was performed to compare incidence rates in luminal A and luminal B tumours with and without the use of grade.

Table 3.16 presents the distribution of grade between luminal A and luminal B tumours. The new classification combined luminal A tumours of high grade (n=5027) with the original luminal B tumours of any grade.

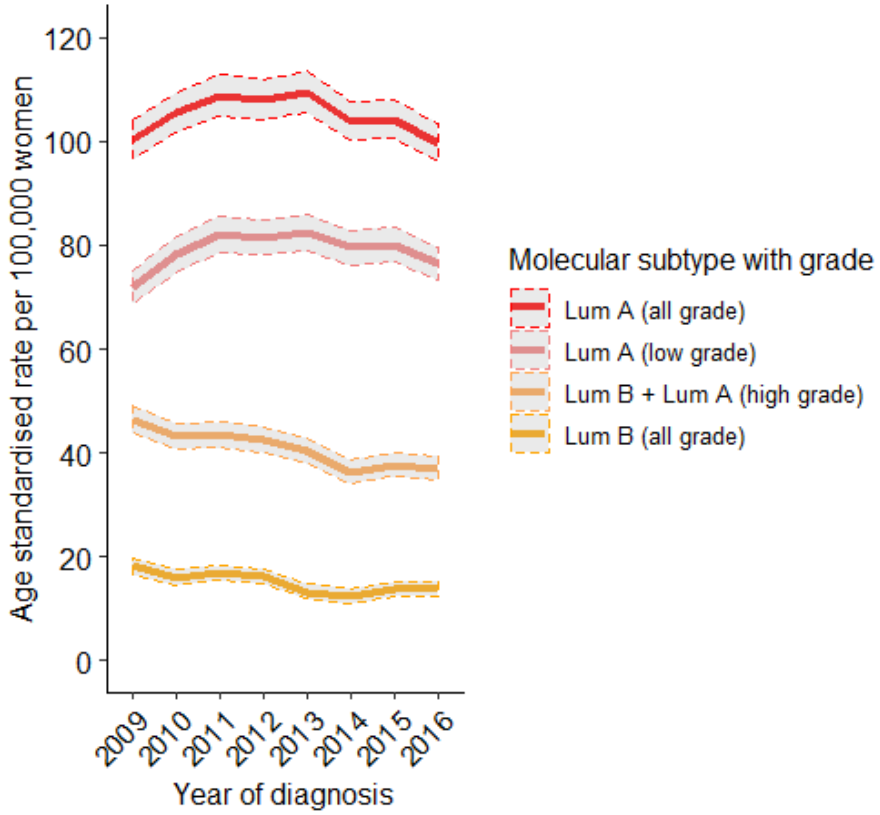
Table 3.16 Prevalence of tumour grade for each luminal subtype

Subtype	Grade		
	I-Well differentiated	II-moderately differentiated	III- poorly differentiated
Luminal A	3063 (16%)	10858 (57%)	5027 (27%)
Luminal B	97 (4%)	975 (37%)	1554 (59%)

Cells represent counts (%) among those with known subtype and grade.

Figure 3.29 shows that luminal A tumours of low/middle grade followed a similar pattern in incidence over time to that observed for all luminal A tumours, as they form the majority of tumours.

Figure 3.29 Age-standardised incidence rates of luminal tumours classified with and without the use of grade



When combining luminal A tumours of high grade with luminal B tumours, the declines in incidence over time originally observed for luminal B tumours of all grade are still observed but with a slightly more marked decline. Joinpoint analysis (Table 3.17) also shows similar results irrespective of the use of grade to further classify the luminal tumours. However, CIs considerably narrow for luminal B tumours as a result of larger numerators. Further, increases in incidence trends for luminal A tumours (low/middle) grade are now statistically significant amongst women of screening age, 50 to 69 years and they resemble the trends for ER+ tumours with recent declines from 2011.

Table 3.17 Joinpoint regression analysis results for breast cancer incidence rates for women of all ages and for three age groups (20 to 49 years, 50 to 69 years and 70 years or more) for luminal A and luminal B with and without grade in Scotland from 2009 to 2016 (page 1 of 2)

Molecular subtype	Age groups	Incidence in 2009 per 100,000 women	Incidence in 2016 per 100,000 women	Change in Incidence from 2009 to 2016 N per 100,000 (%)	AAPC (95% CI)	Years before joinpoint	EAPC (95% CI) for period before joinpoint	Years after joinpoint	EAPC (95% CI) for period after joinpoint
Luminal A (all grade)	All ages	100.1	99.6	-0.5 (0.5%)	0.1% (-1.6, 1.9)	2009-2011	4.9% (-3.9, 14.5)	2011-2016	-1.7% (-3.5, 0.2)
	20-49 years	44.1	42.1	-2 (4.5%)	0.1% (-1.5, 1.7)	No significant change point identified from 2009-2016			
	50-69 years	204.9	210.9	6 (2.9%)	0.2% (-1.9, 2.2)	2009-2011	6.6% (-3.9, 18.2)	2011-2016	**-2.3% (-4.4, -0.1)**
	70+ years	224.3	215.8	-8.5 (3.8%)	-0.1% (-2.4, 2.2)	2009-2012	3% (-3.8, 10.2)	2012-2016	-2.4% (-6.4, 1.8)
Luminal A (low/medium grade)	All ages	71.9	76.4	4.5 (0.6%)	1.1% (-0.6, 2.8)	2009-2011	7.2% (-1.4, 16.6)	2011-2016	-1.3% (-3, 0.5)
	20-49 years	27.3	28	0.7 (2.6%)	1.5% (-0.6, 3.5)	No significant change point identified from 2009-2016			
	50-69 years	149.2	163.7	14.5 (9.7%)	**1.1% (0.2, 2.1)**	2009-2011	**9.9% (4.7, 15.3)**	2011-2016	**-2.1% (-3.1, -1.2)**
	70+ years	169.7	174.2	4.5 (2.7%)	0.8% (-2, 3.7)	2009-2011	6.4% (-7.6, 22.5)	2011-2016	-1.3% (-4.2, 1.7)

Table 3.17 (continued) Joinpoint regression analysis results for breast cancer incidence rates for women of all ages and for three age groups (20 to 49 years, 50 to 69 years and 70 years or more) for luminal A and luminal B with and without grade in Scotland from 2009 to 2016 (page 2 of 2)

Molecular subtype	Age groups	Incidence in 2009 per 100,000 women	Incidence in 2016 per 100,000 women	Change in Incidence from 2009 to 2016 N per 100,000 (%)	AAPC (95% CI)	Years before joinpoint	EAPC (95% CI) for period before joinpoint	Years after joinpoint	EAPC (95% CI) for period after joinpoint
Luminal B (all grade)	All ages	18.9	13.8	-5.1 (27%)	**-4.5% (-7.3, -1.6)**	No significant change point identified from 2009-2016			
	20-49 years	10.6	10.4	0.2 (1.9%)	-0.9% (-2.8, 1.0)	No significant change point identified from 2009-2016			
	50-69 years	36.1	27.6	-8.5 (24%)	**-5% (-8.5, -1.3)**	No significant change point identified from 2009-2016			
	70+ years	35.7	20.1	-15.6 (44%)	**-7.3% (-12.6, -1.7)**	No significant change point identified from 2009-2016			
Luminal B (all grade) + Luminal A (high grade)	All ages	46.3	37	-9.3 (20%)	**-3.3% (-4.5, -2.1)**	No significant change point identified from 2009-2016			
	20-49 years	27.3	24.5	-2.8 (10%)	-1.7% (-3.6, 0.2)	No significant change point identified from 2009-2016			
	50-69 years	91.8	74.8	-17 (19%)	**-3.7% (-5.8, -1.5)**	No significant change point identified from 2009-2016			
	70+ years	89.7	61.4	-28.3 (32%)	**-4.2% (-6.6, -1.9)**	No significant change point identified from 2009-2016			

Bold results are significantly different from 0 ($p<0.05$). AAPC average annual percent change, EAPC estimated annual percentage change. Joinpoint regression was performed using the estimated counts corrected for missing ER and HER2 status, and analysis corrects for multiple testing using Bonferroni correction (See methods section).

3.4.6 Incidence trends by method of detection

Table 3.18 below presents the characteristics of women with screen detected tumours and women with non-screen detected tumours that form the dataset for the analysis of incidence rates by method of detection.

Table 3.18 Descriptive characteristics of screen detected and non-screen detected tumours in women aged 50 to 69 years (approximate screening age) diagnosed with BC in Scotland between 1997 and 2016 (page 1 of 2)

Factor	Values	Screen detected 17,134	% [49]	Non-screen detected 17,930	% [51]
NHS Scottish region	North	4,527	[49]	4,753	[51]
	South East	4,912	[51]	4,808	[49]
	West	8,491	[53]	7,572	[47]
Year of diagnosis	1997-2001	3,016	[40]	4,548	[60]
	2002-2006	3,685	[46]	4,268	[54]
	2007-2011	5,135	[54]	4,343	[46]
	2012-2016	5,298	[53]	4,771	[47]
Deprivation quintile	1-Most deprived	2,830	[44]	3,604	[56]
	2	3,389	[48]	3,627	[52]
	3	3,696	[50]	3,689	[50]
	4	3,651	[51]	3,507	[49]
	5-Least deprived	3,567	[50]	3,503	[50]
Grade	I-Well differentiated	3,873	(23)	1,716	(10)
	II- Moderately differentiated	8,433	(49)	6,531	(36)
	III- Poorly differentiated	3,696	(22)	7,406	(41)
	Unknown	1,132	(7)	2,277	(13)
TNM stage	I	10,420	(61)	4,696	(26)
	II	4,537	(26)	7,127	(40)
	III	981	(6)	3,190	(18)
	IV	142	(1)	1,248	(7)
	Unknown	1,054	(6)	1,669	(9)
Nodal Status	No	12,748	(74)	8,772	(49)
	Yes	3,769	(22)	6,955	(39)
	Unknown	617	(4)	2,203	(12)
Tumour size	Less than 10mm	4,273	(25)	1,241	(7)
	10 to 20 mm	8,453	(49)	6,011	(34)
	More than 20mm	3,404	(20)	7,479	(42)
	Unknown	1,004	(6)	3,199	(18)

Table 3.18 (continued) Descriptive characteristics of screen detected and non-screen detected tumours in women aged 50 to 69 years (approximate screening age) diagnosed with BC in Scotland between 1997 and 2016 (page 2 of 2)

Factor	Values	Screen detected 17,134	% [49]	Non-screen detected 17,930	% [51]
ER status	Negative	1,700	(10)	3,897	(22)
	Positive	15,028	(88)	13,134	(73)
	Unknown	406	(2)	899	(5)
PR status*	Negative	1,343	(16)	2,144	(29)
	Positive	4,930	(58)	3,548	(48)
	Unknown	2,273	(27)	1,729	(23)
HER2 status*	Negative	6,799	(80)	5,554	(75)
	Positive	837	(10)	1,247	(17)
	Unknown	910	(11)	620	(8)
Molecular subtype*	Luminal A	5,398	(63)	3,079	(41)
	Luminal B	1,591	(19)	2,300	(31)
	HER2-enriched	212	(2)	442	(6)
	Triple Negative	428	(5)	962	(13)
	Unknown	917	(11)	638	(9)

*Markers available from 2009. Brackets are row percentages and parenthesis are column percentages. ER= oestrogen receptor, HER2= human epidermal growth factor 2, NHS= National Health Service, PR=progesterone receptor, TNM= tumour, nodes, metastases.

The distribution of screen and non-screen detected BC tumours was very similar amongst the three Scottish regions (roughly 50% screen and 50% non-screen detected) but the West region had a lightly higher % of screen detected tumours (53%) than the other two regions. The proportion of screen detected tumours increased over time with 40% of all tumours being screen detected in 1997-2001 and 53% in 2012-2016 period. Deprivation was associated with screening with women in the most deprived areas being less likely to have screen detected tumours (44%) compared to women in the least deprived areas of Scotland (50%).

Furthermore, screen detected tumours had characteristics of a less aggressive disease than tumours that were non-screen detected. They were less likely to be poorly differentiated (22% vs 41%), more likely to be stage I (61% vs 26%) and less likely to be stage II-IV, less likely to have positive nodal status (22% vs 39%), more likely to be smaller than 10mm (25% vs 7%) and more likely to be ER+, PR+ and HER2- than

non-screen detected tumours. The distribution of molecular subtypes was also different between screen and non-screen detected tumours with a higher % of screen detected tumours being luminal A tumours (63% vs 41%), whereas the % of non-screen detected tumours that were luminal B, HER2-enriched and TNBC was highest than that observed for screen detected tumours.

Figure 3.30 shows that the incidence of screen detected tumours sharply increased from 1997 to 2011 but decreased after that time. In contrast, incidence of non-screen detected tumours was constant or slightly declined over the study period.

Figure 3.30 Incidence by method of detection for women aged 50 to 69 years diagnosed in Scotland from 1997 to 2016

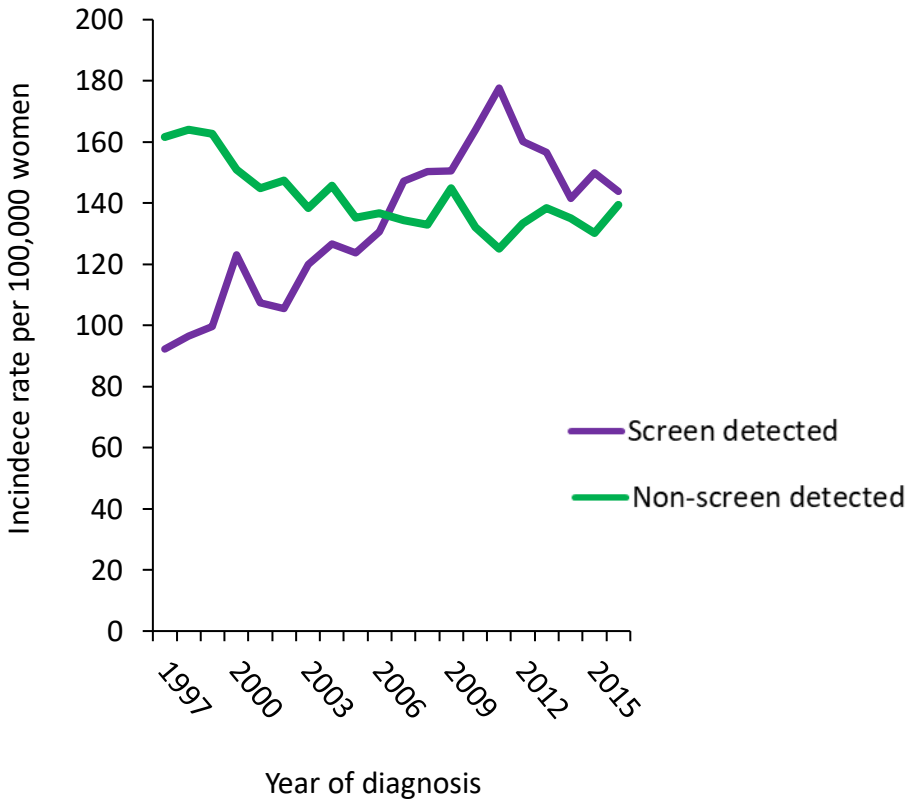


Figure 3.31 of the incidence trends by method of detection and ER status shows that screen detected ER+ tumours have the same pattern to that observed for all screen detected tumours with a rapid increase in incidence whereas ER+ non-screen detected tumours showed a constant trend over time. In contrast, the overall decreasing

incidence observed for ER- tumours is driven by non-screen detected tumours and ER- screen detected tumours show a constant or slightly increasing trend.

Figure 3.31 Incidence by method of detection and ER status in women aged 50 to 69 years diagnosed with BC in Scotland from 1997 to 2016

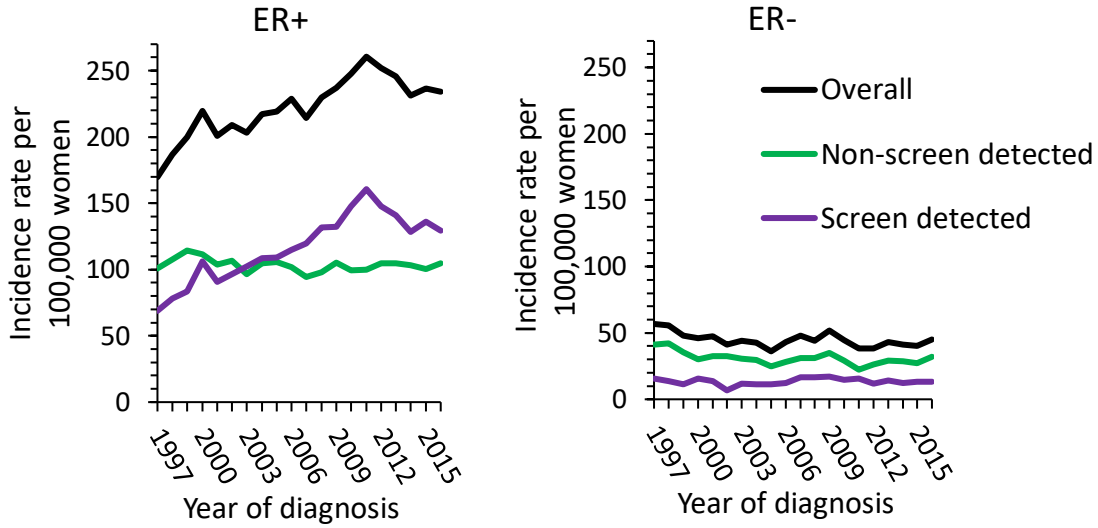
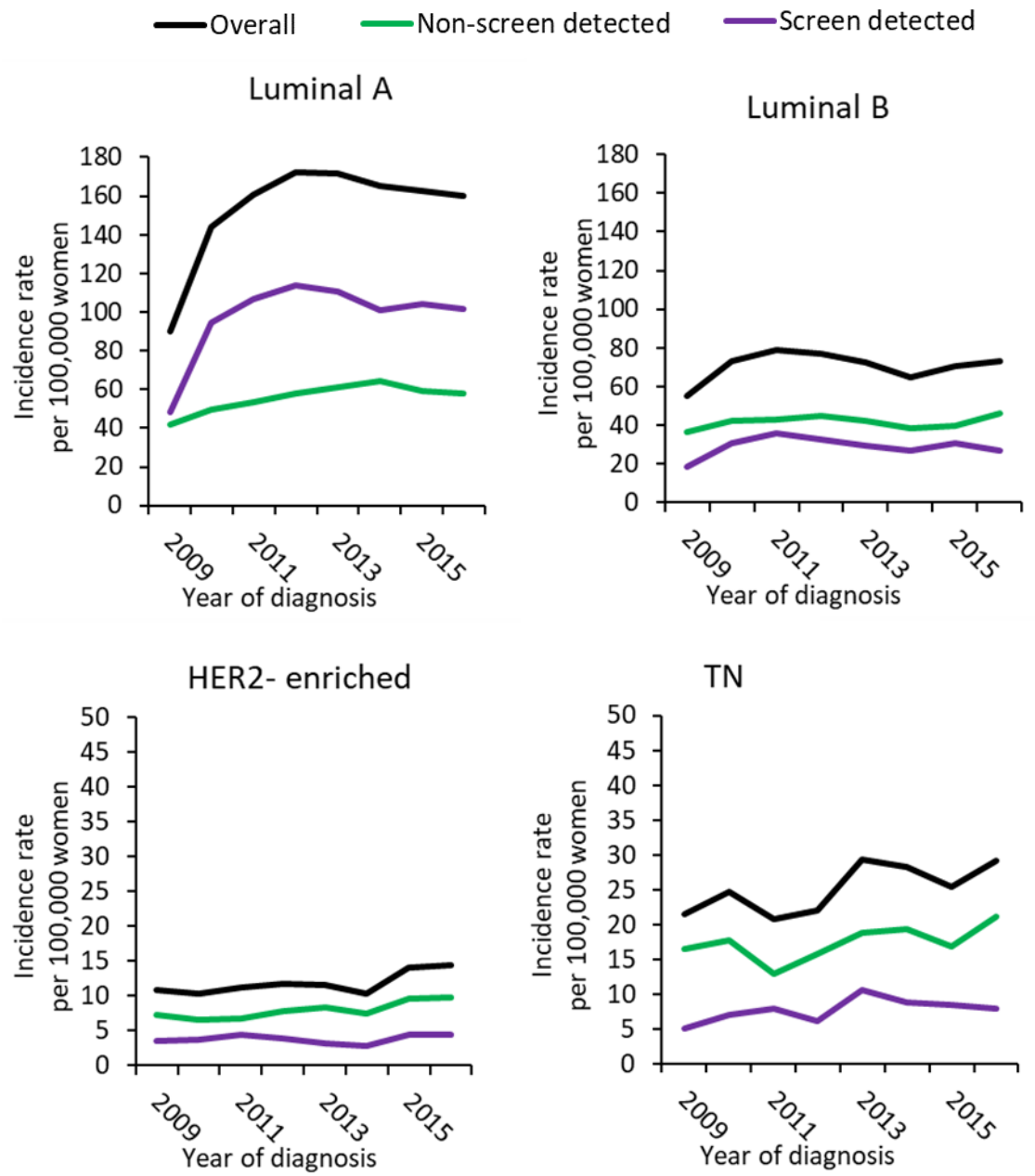


Figure 3.32 of the incidence trends by method of detection and IHC defined subtype showed that the overall trend observed for luminal A tumours was similar to that observed for screen detected tumours. Non-screen detected luminal A tumours also showed an increasing trend over time. Although the incidence of luminal B tumours was higher for non-screen detected tumours, the pattern observed for all luminal B tumours was more similar to that observed for screen detected tumours than for non-screen detected tumours. HER2-enriched and TNBC incidence by method of detection showed that the overall trends were driven by non-screen detected tumours and suggested a slightly increasing trend for these two subtypes.

Figure 3.32 Trends in incidence by method of detection for each IHC defined molecular subtype in women aged 50 to 69 years diagnosed with BC in Scotland from 2009 to 2016



3.5 Discussion

3.5.1 Summary of key findings

In Scotland, trends of BC incidence showed a divergent pattern between ER+ and ER- tumours, with increases of ER+ tumours and decreases for ER- tumours for the study period from 1997 to 2016. However, incidence of ER+ tumours showed a decreasing trend in the last five years (from 2011 to 2016). Increases in incidence of ER+ tumours were observed for women of all ages but particularly for women of screening age (50 to 69 years). In contrast, incidence of ER- tumours declined over time for all age groups and the declines were sharpest in early years, between 1997 and 2002. APC models results indicated that, in addition to the period effects already described above from time trends in ASiR, cohort effects were also observed for ER+ tumours. Women born in the 1920s to 1940s had a lower incidence of ER+ breast tumours than women born around 1950. There was also a suggestion that younger cohorts born between 1960s and 1980s had lower incidence of ER- tumours in comparison to those born around 1950, however this effect was not statistically significant.

The analysis of incidence by HER2 status showed similar incidence patterns to those observed by ER with HER2- tumour incidence patterns similar to ER+ tumours and HER2+ incidence patterns similar to ER- tumours. This was an expected result for HER2- tumours as they are mostly ER+ but was unexpected for HER2+ tumours as most of them are also ER+ tumours. The limited study period for which HER2 data is available in the cancer registry (from 2009 to 2016) influenced the significance of the jointpoint and APC results due to lack of power and supported that monitoring HER2 status by itself does not much more aetiological clues and that its influence on the incidence rates is more clear in combination with other molecular subtypes.

Further analysis of the incidence trends by HER2 in combination with ER and PR status as a surrogate for the intrinsic molecular subtypes of BC suggested differences in secular incidence trends. Luminal A tumours had similar incidence patterns to those observed for ER+ tumours, with increases until 2011 after which incidence declined. In contrast, incidence of luminal B tumour declined over time, particularly in women over 50 years of age. There was no clear time trend in incidence for HER2-enriched tumours but TNBC tumours showed an increasing trend from 2011 that was driven by

increases of 4.6% per year (95% CI: 1.2 to 8.2) in younger women aged 20 to 49 years. APC model results suggested that incidence of luminal A was influenced by age, period and cohort effects but that period effects were the main driver of the changes in incidence, hence suggesting screening as a possible factor contributing to the increasing trends. For luminal B tumours, there was a significant cohort effect with women born in the 1920s and 1930s having higher incidence of luminal B tumours than women born in later years, with the largest declines of luminal B tumours experienced by women aged 60-70 years old. Sensitivity analysis for incidence of the luminal tumours using grade as and additional marker for cell proliferation showed results consistent with those previously observed. However, estimates of the annual percentage change from joinpoint became more precise due to the larger number of luminal B tumours than prior to re-classification of high grade luminal A tumours as luminal B.

3.5.2 Comparison with previous studies and possible explanation for the observed trends

Consistent with previous studies of time trends in BC incidence by ER status in the United States for 1980-2008, Denmark for 1993-2010 and Ireland for 2004 to 2013 [135, 136, 146], incidence trends in Scotland differ by ER status that suggests aetiological heterogeneity with distinct patterns by age at diagnosis, year of diagnosis and birth cohort. Previous studies showed increases for ER+ tumours that ranged from 0.1% in the US [135] to 3% in Denmark [136] and decreases for ER- tumours that ranged from -2% in the US [135] to -3.4% in Ireland [146]. Although consistent in the direction of the trend, differences in the estimates between countries were observed that might be related to the different definition of a BC and the use of tumours as the basis for the incidence trends in the rest of studies instead of women in our study.

The declines due to MHT might also be responsible for the differences observed between countries. In the United States, the almost constant overall trend for ER+ tumours was influenced by periods of increases from 1980 to early 2000s followed by the sharp drop in incidence attributed to the declines in MHT use [54, 55] after MHT use was found to be associated with an increased risk of BC. In Scotland, the decreases in the incidence of ER+ tumours around the year 2000 were previously reported by Sharpe et al.[148]. They observed statistically significant declines of 11.2% per year

in women aged 50 to 64 years (the screening age range up to 2003) that started in the year 2000 and continued up to 2005. Compared to the previous study by Sharpe et al [148], this study showed similar but smaller declines in ER+ tumours around the year 2000. The differences between the two studies in Scotland may have arisen from excluding women with a previous malignancy, imputing missing ER marker and computing incidence rates with number of women instead of number of tumours as the numerator in this analysis. Declines in BC incidence, particularly for ER+ tumours, in the early 2000s were also seen in other European countries, such as France [139], Sweden [145] and Norway [183]. A recent study of the effect of MHT in BC risk suggests a causal association and estimate that approximately one BCs out of 20 million global BCs since 1990 have been caused by MHT [63]. In Scotland, MHT is estimated to account for 2.3% of all BC cases [155].

After the sudden drop in incidence of ER+ tumours in the early 2000s, a further increase in incidence was reported in most countries suggesting other factors might be related to the divergent trends observed. Information on the trends observed in the last decade is very limited but this study shows, for the first time, recent declines in the incidence of ER+ tumours in Scotland that started around the year 2011. In contrast, in the US incidence slightly increased by 0.3% per year from 2012 to 2016, mainly for local stage and HR+ tumours [184] while rates in Ireland remained stable from 2010 to 2013 [146]. These more recent declines in ER+ incidence may at least partly be due to the achievement of stable uptake rates for screening as they are mainly observed in women of screening age from 50 to 69 years [140].

In Scotland, the mammographic screening programme started in 1988 and was one of the first countries in the world to implement a national BC screening programme, earlier than Ireland (2000) and Denmark (2010). After the implementation of a screening programme, incidence rates of the target disease are expected to rapidly increase because prevalent cases are detected and then the effect of the screening programme is expected to decline over time until it reaches a plateau. The saturation of the screening programme has been previously linked to declines in incidence [135, 143]. In Scotland, full implementation of the screening programme was completed by 1991 and uptake has been consistently high with proportions accepting screening over 75% during the study period. In the last three years of the study period (2013-2016) a

slight decrease of 0.6% in uptake has been reported but this is unlikely to have contributed to the recent declines in ER+ tumours [185]. This study supports that mammographic screening is likely to have contributed to the increases in incidence of ER+ tumours from 1997 to 2011. In Scotland, ER+ tumours were more likely to be screen detected than ER- tumours (53% vs. 30%) which is consistent with the fact that ER- tumours are likely to be detected in younger women who are not screened. The APC models showed that the greatest increases were observed among women aged 65 to 72 years which coincides with the extension of the mammographic programme in 2003 to include women aged 65 to 70 years of age. Further, additional analysis in women of screening age (50 to 69 years) showed that increases in the overall incidence of ER+ tumours were driven by increases of screen detected ER+ tumours, whereas not screen detected tumours remained constant for the study period which strongly suggests the important effect of screening in the increasing trends observed. In contrast, ER- tumours were less likely to be screen detected and the overall declining trend was more similar to that observed for not screened detected tumours suggesting that screening is more likely to detect ER+ than ER- tumours. One possible explanation for this could be the rapid cell growth and proliferation of these aggressive subtypes that become symptomatic between screening appointments and are known as interval breast cancers. Interval breast cancers are more likely to be ER/PR negative [186-188], HER2+ [189] and TNBC [187, 190], which could suggest that interval cancers are biologically different to screen detected tumours rather than more difficult to detect. Other studies suggest that ER- or TNBC interval cancers lack the typical radiological features of malignancy seeing in screen detected ER+ tumours [191, 192] and that they are associated with a higher breast density which makes them more difficult to be screen detected [193]. Future efforts should be considered to improve detection of ER- or aggressive tumours subtypes (HER2-enriched, TNBC).

Apart from screening, other factors may have also contributed to the observed divergent trends by ER status. APC models suggest a clear cohort effect for ER+ tumours with older generations having a lower risk of ER+ tumours than younger generations. Cohort effects are those that affect a whole generation of women, such as, changes in reproductive factors and increasing obesity prevalence. The cohort effect

found for ER+ cancers in Scotland was also observed in Denmark and Ireland suggesting similar RF patterns in these countries.

Reproductive factors are a likely contributor to the increases in BC incidence amongst younger generations. For example, delayed childbearing, younger age at menarche, older age at menopause, lack of breastfeeding and nulliparity have been closely related to increased risk of ER+ breast tumours [6, 194]. In Scotland, as in many European and Western countries, reproductive factors have changed considerably over time. Fertility rates have dropped considerably and the number and proportion of nulliparous women has increased [14]. Childbirth has also been delayed with women having children for the first time at the age of 30.5 years in 2017 compared to an average age of 26 years in 1975 [195, 196]. These and other factors, such as earlier age at menarche and delayed menopause, may be associated with the gains in incidence of ER+ tumours.

Another likely contributing factor to changes in BC incidence is obesity. Obesity is clearly associated with increased risk of BC in postmenopausal women, particularly for ER+ tumours [64-66], with limited evidence of an association between obesity and reduced risk of BC in premenopausal women that could be at least partly confounded by reproductive factors including nulliparity, OC use and oligomenorrhoea [197]. In Scotland, overweight and obesity prevalence increased from 52% in 1995 to 63% in 2008 and then remained approximately stable [198]. Recent obesity prevalence trends in Scotland seem to follow similar patterns to those observed for incidence of ER+ tumours and estimates from a recent study suggest that obesity and alcohol consumption may account for 9% of all BC cases diagnosed in Scotland [155].

Evidence of the association of established RFs of BC with more aggressive subtypes of BC (ER-, HER2-enriched and TNBC) is still very limited and hence investigating the RFs that might be driving the observed trends for these subtypes is more difficult than for ER+/luminal tumours. There is some evidence of a possible association between premenopausal obesity and increased risk of TNBC [150, 199, 200]. In this study, increases in TNBC amongst women aged 20 to 49 years were found that could be associated with increased premenopausal obesity. Other factor that has been associated with a reduced risk of the most aggressive subtypes of BC is breastfeeding,

particularly ER- and TNBC [194, 201]. Although, breastfeeding in Scotland remains below the recommended proportions, in the last two decades the percentage of babies that are being breastfed at 6 to 8 weeks increased from 36% in 2001 to 42% in 2018 which may have partially contributed to the declines observed for ER- tumours [202].

3.5.3 Strengths

3.5.3.1 Scottish cancer registry data

First, the Scottish cancer registry data presents an excellent opportunity for the analysis of incidence trends by ER and other molecular markers. Scotland was the first country in the UK to collect data on ER and it has 10 more years of data than other parts of the UK. Further, the cancer registry has high quality longitudinal data with a case ascertainment over 98%. Overall BC incidence is routinely reported by ISD (https://www.isdscotland.org/). However, the heterogeneity observed in BC suggests the need for specific monitoring of time trends in incidence for the different subtypes of BC as they have different aetiology and prognosis. This could lead to the identification of high risk groups of women and to the implementation of new prevention and treatment programmes.

Both statistical modelling techniques used for this part of the PhD (joinpoint analysis and APC models) have been successfully used to monitor and summarise cancer rates in many countries but I have used them for the first time to analyse UK BC data. Both methods are practical and complementary to analyse BC incidence trends. Joinpoint modelling is a useful tool for monitoring overall trends and estimate periods of time with changes in the rates. However it does not provide any aetiological clue about which factors might be driving the observed trends. For that reason, APC models, which decompose the trends into age, period and cohort effects are an important additional tool to describe the trends and estimate whether changes are related to age, period or cohort effects. APC models have been particularly useful to identify further signals in incidence and mortality that may be missed by traditional descriptive methods and that can lead to further hypotheses to be explored. I used the latest joinpoint analysis and APC model tools developed by NCI which are more informative and robust than traditional models.

3.5.4 Limitations

3.5.4.1 Data

The validity of the studies conducted using population-based cancer registry data depends on the accuracy and quality of the data collected by the registry and the quality control procedures in place [203]. There are three main indicators to evaluate the quality of registry data: comparability, completeness, validity or accuracy [204].

- The comparability of cancer registry data is related to whether the results between cancer registries are comparable since the registry follows international guidelines in terms of classification and coding of the neoplasms, the definition of incidence and data of incidence, the distinction between a primary cancer and an extension, recurrence or metastasis of the primary cancer. The Scottish cancer registry is considered comparable to other cancer registries as it follows the standard classification and coding of disease (the International Classification of Disease), and a protocol has been established for the definition of incidence and date of incidence [205] which has been recently updated to use the European network of cancer registries (ENCR) recommended definition of incidence from 2019. One of the main limitations for comparability is the recording of multiple tumours. The Scottish cancer registry collects all tumours identified in a person but there is no distinction as to whether secondary tumours are extensions, metastasis or recurrence from the primary tumour and incidence rates reported by ISD are using all tumours available. However, this method is also used in most other countries and the analysis of incidence in this PhD aimed to correct the overestimation of the breast cancer incidence rates due to multiple tumours in the same woman.
- The completeness is the extent to which the registry is able to ascertain almost all cancer cases within the Scottish population. Completeness ensures that cancer registrations are highly representative of the general population and that estimates of incidence and survival trends are accurate. Case ascertainment or completeness in the Scottish cancer registry has been estimated to be very high, with over 98% of cases ascertained by the registry [157].

- The accuracy or validity of the data refers to the proportion of cases that truly have the characteristics recorded in the registry. Validity of the data can be checked by evaluating indicators such as, agreement with medical records, histological verification, missing information and internal consistency of the records [206]. The accuracy of the Scottish cancer registration has been previously estimated to be high with only an approximate 3% of cases showing discrepancies between the cancer registry and available medical records [207]. In terms of missing information, the registry had low percentages of missing data for the molecular markers (approximately 7% for ER status and 11% for HER2). However, as observed in Appendix Figure A.1, missing molecular marker data can have an important impact in the rates of BC incidence by ER status, underestimating the incidence rates of ER+ and ER- tumours, particularly for earlier years (when missing molecular marker data was higher). Not correcting for missing data could lead to incorrectly estimating a higher increasing incidence of ER+ tumours than the one observed and a lower declining rate for the ER- tumours.

Apart from the intrinsic limitations associated with cancer registry data, there were other issues particular to the BC data and the analysis of the incidence rates for the different molecular subtypes of BC:

- Molecular data availability for HER2 and PR markers precluded estimation of the long term trends in incidence as these markers are only available from 2009 and therefore, time trends are restricted to seven years of data with the additional limitation of relatively high proportions of missing data in the years soon after data became available. The results from joinpoint regression and APC models for the HER2 alone and the molecular subtypes of BC had estimates with wide CIs common for less prevalent subtypes. Future study of the incidence by molecular subtypes in Scotland should continue as better estimation of trends will be possible once more years of data are available.
- Individual level data were not available so investigation of possible RFs associated with the BC incidence in the study cohort was not possible. However, the Scottish cancer registry can be easily linked to other national

records datasets, such as maternity and prescription data and future studies should look at the effect of those RFs in the observed trends. Another limitation of the study is the lack of mRNA expression assays for the classification of the molecular subtypes of BC. In this study, markers measured by IHC are used as surrogates for the molecular subtypes, which are reasonably good proxies but mRNA profiling data would be considered a gold-standard for intrinsic-subtype classification [208].

- As screening mammography was fully implemented during the study period, I cannot compare incidence rates before and after the implementation of mammographic screening and estimate the impact of screening on the incidence rates. However, in order to describe the effect of screening in the trends I look at different age groups: women with <50 years (before screening), women aged 50 to 69 years (during screening) and women aged 70 years or older (after screening). Further, as method of detection is available in the cancer registry, incidence trends by method of detection were investigated overall and by ER status and IHC defined molecular subtypes.

3.5.4.2 Statistical analysis

Imputation of missing molecular markers ER and HER2 is essential to accurately estimate BC incidence trends for each molecular subtype. In terms of missing information, the registry had low percentages of missing data for the molecular markers (approximately 7% for ER status and 11% for HER2). However, as observed in Appendix Figure A.1, missing molecular marker data can have an important impact in the rates of BC incidence by ER status, underestimating the incidence rates of ER+ and ER- tumours, particularly for earlier years (when missing molecular marker data was higher). Not correcting for missing data could lead to incorrectly estimating a higher increasing incidence of ER+ tumours than the one observed and a lower declining rate for the ER- tumours.

The imputation method assumes that ER and/or HER2 data have the same chance of being missing among each cohort of women by year and age at diagnosis. If this assumption was wrong, a confounder associated with the molecular markers would have to influence whether the markers are tested and recorded. In Scotland, health

service guidelines are used to inform investigation and treatment and missing molecular data is more likely to reflect administrative omissions, and geographic uptake. A more complex imputation model incorporating tumour grade, stage and other covariates found that the overall imputed counts were very similar to those obtained using the simpler model with only age and year of diagnosis [209]. Therefore, redistributing the relatively small percentage of missing molecular data in cases within each year and age at diagnosis has been considered an appropriate method to estimate incidence trends. Further, previous studies using US, Denmark, and Irish data [135, 136, 146] have also considered the assumption of the simple imputation model to be reasonable and the US study has been compared to previous studies using the same SEER data and doing multiple imputation using chained equations giving very similar results [61, 210].

Joinpoint regression analysis and APC models are both ecological methods and a causal relationship between the trends observed and the factors that might be driving those trends cannot be established. However, both methods are useful descriptive tools to generate hypotheses and estimate the overall increase/decrease in the trends and the effect of age, period and cohort effects on the trends. Both methods present limitations intrinsic to the use of statistical methods and based on the assumptions for each method. Joinpoint regression analysis is based on generalised linear theory and assumes that the data follows a Poisson distribution so the results are based on whether this assumption can be considered valid or not. Cancer counts are often model following a Poisson distribution and Poisson regression has been widely used as an appropriate method for the analysis of cancer counts and rates, including breast cancer. Another assumption of the joinpoint regression is that the data can be divided into subsets and that each subset has its own linear trend with a particular intercept and slope, so the model assumes linearity and that the error terms are independent and normally distributed [211, 212]. This assumption depends on the actual number of time points with count data available as few time points would deem the analysis not appropriate. In our study, 20 consecutive years of breast cancer counts were available. Another limitation of the joinpoint regression analysis tool is the fact that the final model can be calculated using different methods and parameters and hence, the results might vary depending on the options selected. For the analysis presented throughout

the thesis, the Grid search method was used to calculate the final model with a maximum of 3 joinpoints (given that we had 20 years of consecutive data) and autocorrelated errors and the permutation test for model selection. Sensitivity analysis using a different modelling method to the Grid search method, different model selection methods to the permutation test and different error options were performed with similar results to the original methods, finding the same number of joinpoints and in a similar location. Another limitation of Joinpoint regression is the number of tests performed. The permutation method consists on a total of 4,499 permutations but the p value test is adjusted for multiple testing using Bonferroni correction [172]. Some studies have indicated that Bayesian methodologies can also be applied to joinpoint modelling and as they incorporate prior information on the number of joinpoints based on information of the counts, they eliminate the issue of multiple testing to find the number of joinpoints and its location [213, 214]. However, models such as the ones presented here have been seen to provide similar results to Bayesian methods. Multiplicity continues to be an issue once you do subgroup analysis and this needs to be further corrected doing Bonferroni correction or other multiplicity adjusted techniques.

The main limitation of the APC models is the identifiability constraint which has been widely discussed in most APC research [177, 215]. The identifiability problem is related to the fact that the rates cannot uniquely be attributed to the effects of age, period and cohorts without imposing some additional constraints due to the fact that age, period and cohort are associated (cohort=period-age) and hence, co-linear. Those additional constraints are usually deem unverifiable and must be carefully selected based on information relevant to the study data and its biological hypothesis. However, the APC models computed through the dissertation and developed by Rosenberg et al. [179] provide a number of estimable functions that do not require additional constraints to be imposed since the cohort deviations are weighted to account for the variable number of periods that they can be observed. This technique ensures that the fitted rates can be expressed in terms of the age, period and cohort effects. Another limitation of the APC models is that some of the functions can be hard to interpret, however, in the new model the estimable functions are all identifiable and closely related to common epidemiological functions. Another limitation specific to the APC

model is the limited number of methods used to assess goodness-of-fit. Others have propose to use a smooth Gaussian process to model the residuals for each of the components [216]. Here, as proposed by the authors of the model used, we graphically assessed the residuals to check goodness of fit for each model. Finally, another important limitation is that the models do not incorporate established RFs for BC such as, reproductive factors and screening that are likely associated with the age, period and cohort effects estimated by the models.

3.6 Conclusion

BC incidence trends differ by ER status and for molecular subtypes in Scotland and showed trends consistent with those observed in other countries. To the best of my knowledge, the recent declines in incidence of ER+ tumours from 2011 have not been shown in any other country. Another important finding is the increasing trend observed for TNBC in young women, which is the subtype with the worst prognosis. This study shows that screening is a likely contributor of the increasing trends observed for ER+ and luminal tumours and that screening programmes should prioritise detection of the more aggressive subtypes that are less likely to be screen detected. New personalised screening programmes could be based on polygenic risk scores that stratify women according to their BC subtype risk. Additional data, particularly on obesity and reproductive factors, are needed to further investigate the RFs associated to the observed trends and future monitoring of BC incidence should be done by subtype. Apart from differences in incidence, BC subtypes are likely to show survival heterogeneity and this will be further assessed in Chapter 4

Chapter 4 Survival for the different subtypes of breast cancer and their association with other tumour characteristics

4.1 Background

According to the latest Globocan estimates, BC is the world's leading cause of cancer mortality among females in over 100 countries [217]. Overall BC mortality trends in Scotland and the UK, have seen consistent declines in recent decades [218] and predictions for the UK estimate further declines by 36% between 2014 and 2035 [219]. However, these data are based on all BCs with limited analysis on mortality trends at the population level for subtypes of BC, including trends among different subgroups of women with BC based on age and other prognostic tumour characteristics (e.g. grade, stage).

4.1.1 Tools to assess cancer progression

Along with cancer incidence and survival, mortality rates are important population-based tools to estimate cancer progression [220]. Mortality rates are usually estimated using number of cancer deaths as the numerator and number of women in the general population as the denominator, however, incidence-based mortality (IBM) can also be estimated using number of women with breast cancer as the denominator for the rates. A study in the US estimating IBM trends overall and by ER status and age at diagnosis found clinically and statistically significant BC IBM rates declines between 1990 and 2003 for ER+ tumours (limited to women younger than 70 years) but not for ER- tumours [221]. Mortality data are usually derived from death certificate records and therefore, information on individual and tumour characteristics is generally not available. Linkage of death records to population-based cancer registries with incidence data allows the estimation of IBM rates by other individual and tumour characteristics and previous research has found that IBM rates are comparable to those from death certificate records [222]. Given the prognostic importance of molecular markers, trends in BC IBM rates for each of the molecular subtypes could further inform the burden of disease and identify

women with specific subtypes or tumour characteristics for which mortality rates are not showing improvements and who could be the target of new prevention and treatment programmes. However, population-based estimates of IBM rates by molecular subtypes require a long follow-up of at least 10-15 years [222] since women who die from BC are likely to be diagnosed many years before and hence, available molecular marker data at the time of diagnosis is necessary to give reliable long-term IBM rates by molecular subtypes. Given the relatively recent routine collection of data on molecular markers in the cancer registries (ER from 1997 in Scotland) reliable long-term IBM rates cannot be estimated yet. For that reason, most studies investigating BC prognosis by molecular subtypes focus on differences in shorter term breast cancer specific survival (BCSS).

4.1.2 Review of previous studies of BC prognosis by molecular subtypes and important prognostic factors

4.1.2.1 Search terms and inclusion criteria

A literature search was performed in Medline using the keywords "breast cancer", "survival or prognosis" and "oestrogen receptor or hormone or molecular subtype". Studies were selected if data were female invasive BCs from population-based, cancer registries or large epidemiological studies with a majority of European ancestry populations. Additionally, they had to report HRs or relative excess risk (RER) for the comparison of the molecular subtypes of BC. Table 4.1 summarises the selected studies from the USA and Table 4.2 from the studies in Europe. A formal search was not conducted to review the prognostic value of the rest of factors.

4.1.2.2 Studies of BC prognosis by molecular subtypes in the USA.

Five previous studies in the US have shown that prognosis of BC clearly differs between molecular markers (Table 4.1). A large population-based study in over 150,000 women diagnosed with BC from 1990 to 2001 using SEER11 data, found that women with ER+/PR-, ER-/PR+ and ER-/PR- have significantly lower survival than women with ER+/PR+ tumours [223]. A more recent study extended the analysis to more than 400,000 women in 18 SEER cancer registries and found a 60% (95% CI: 58 to 65%) increase of death from BC in women with ER- tumours compared to women with ER+ tumours but

that difference was only observed during the first 5 years after diagnosis [224]. Prognostic differences have also been estimated between molecular subtypes (or their IHC surrogates), with luminal subtypes having a better prognosis than TNBC or HER2-enriched subtypes. A recent study in the United States using SEER data from 18 registries on over 190k cases of women diagnosed between 2010 and 2013 showed that HR+ (ER+ and/or PR+)/HER2- (luminal A) subtype had the best survival followed by HR+/HER2+ (luminal B), while TNBC (HR-/HER2-) had the worst survival of all subtypes [225]. Evidence of the prognostic differences between subtypes has also been observed in cohort studies, such as the Nurses' Health Study. This study in around 2,000 women diagnosed between 1967 and 1997 and followed up to 2007 found that luminal B, HER2-enriched and basal-like tumours had hazard ratios (HRs) for BC mortality of 1.90 (95% CI: 1.33 to 2.71), 1.36 (95% CI: 0.87 to 2.12) and 1.58 (95% CI: 1.05 to 2.39) respectively when compared to luminal A subtypes over a median follow up of 15 years [226]. Another study, the Carolina Breast Cancer Study, that used immunohistochemical markers to subtype more than 1,000 invasive BCs found that only the basal-like subtype was associated with a statistically significant HR of 1.7 (95% CI: 1.0 to 2.9) of BC mortality compared to luminal A tumours [227].

Table 4.1 Studies from the US reporting BC survival for the molecular subtypes of BC.

Study and country	Years of diagnosis	n	Breast cancer subtypes categories reported	Hazard Ratio (95% CI)	Confounders adjusted for in the model
Dunnwald et al. [223] 2007, USA	1990-2001	155,175	ER+/PR+ ER+/PR- ER-/PR+ ER-/PR-	Reference 1.4 (1.3-1.5) 1.8 (1.6-1.9) 2.3 (2.2-2.4)	Age of diagnosis, year of diagnosis, SEER registry, race/ethnicity, histologic tumour type, tumour size, stage, grade and lymph node status.
Dawood et al. [226] 2011, the Nurses' Health Study, USA	1976–1997	1,945	Luminal A Luminal B HER2-type Basal-like Unclassified	Reference 1.9 (1.3–2.7) 1.4 (0.9–2.1) 1.6 (1.1–2.4) 1.4 (0.9–2.2)	Age at diagnosis, year of diagnosis, BMI at diagnosis, tumour grade, stage of disease, radiation treatment, chemotherapy and hormonal treatment.
O'Brien et al. [227], 2011 Carolina Breast Cancer Study, USA.	1996-2001	631	Luminal A Luminal B Basal-like HER2+/ER- Unclassified	Reference 1.5 (0.8–2.7) 1.7 (1.1–2.9) 1.4 (0.7–2.9) 1.6 (0.9–3.1)	Age at diagnosis, date of diagnosis, and stage at diagnosis.
Ren et al. [224] 2014, USA	1990-2005	439,444	ER+ ER-	Reference 1.6 (1.6-1.7)	Age at diagnosis, race, tumour grade, T and N stages.
Howlader et al. [225] 2018, USA	2010-2013	196,094	HR+/HER2- HR+/HER2+ HR-/HER2+ Triple negative	Reference 0.8 (0.7-0.8) 1.2 (1.2-1.3) 2.5 (2.4-2.7)	Tumour stage, Bloom-Richardson tumour grade, nodal status, surgery, age at diagnosis, SEER registry, race/ethnicity, poverty index, urban index, insurance status and marital status.

BMI=Body Mass Index, ER= oestrogen receptor, HER2= human epidermal growth factor 2, HR= hormone receptor, PR=progesterone receptor, SEER= Surveillance, Epidemiology, and End Results, USA=United States of America.

4.1.2.3 Studies of BC prognosis by molecular subtypes in Europe.

European countries have also reported differences in BCSS between molecular subtypes (Table 4.2). A study using data from EUROCARE, a database oh high-resolution breast cancer studies from registries in Estonia, France, Italy, Spain, the Netherlands and the UK reported a pooled relative excess risk (RER) of death of 0.32 (95% CI: 0.24 to 0.43) for ER+PR+ compared to ER-PR- tumours. Having one of the molecular markers positive (ER+ or PR+) also had a beneficial effect on the risk of death [RER=0.41 (95% CI: 0.28 to 0.62)]. Apart from the previous study, nine studies from the six countries below reported comparisons of BC survival by molecular subtypes in Europe.

Italy

Results from a population-based study in Italy [228] in 1,400 women diagnosed during the period 2004-2005 showed that 3-year survival rates were highest for luminal A tumours and lowest for HER2-enriched tumours, with HRs for luminal B, TNBC and HER2+ tumours of 1.65 (95% CI: 1.11 to 2.46), 1.68 (95% CI: 1.04 to 2.72) and 2.18 (95% CI: 1.28 to 3.70) respectively when adjusted for age and stage. A more recent study with population-based data in over 3000 women from 9 Italian cancer registries that included the Ki67 marker in the definition of the subtypes found a RERs of death significantly greater in the triple-negative and HER2-enriched subtypes when compared to luminal A tumours [229].

Norway

In Norway, a study in over 20,000 women diagnosed between 2005 and 2015 from the national cancer registry, found that TNBC had the highest mortality HR=3.12 (95% CI: 2.64 to 3.68) compared to luminal A tumours. This study also explored the effect of the subtypes on BC specific mortality for three distinct age groups and found increased likelihood of breast cancer death (BCD) for young women with luminal A tumours and for old women with any subtype compared to women with the same subtype aged 50-69 years [230]. Previous studies using retrospective data in women diagnosed with BC in Norway also showed similar results. Engstrom et al. analysed data in over 900 female BC cases with tumour specimens available and used IHC and in situ hybridisation to assess

ER, PR, HER2, CK5 and Ki67 markers. They found that luminal A subtype had the best BC survival while HER2 and the five negative phenotype had the worst and that the differences in BC survival where restricted to the first 5 years after diagnosis with no significant differences between the subtypes after that time [231]. These results were confirmed by Valla et al [232] in a larger population of BC cases (over 1400).

Spain

A study in Spain using data from 10 regional cancer registries in over 3,000 women diagnosed in 2005 with a median follow-up time of 5 years, showed an increased RR of death in HER2-overexpressed and TNBC of 1.72 (95% CI: 1.15 to 2.57) and 3.16 (95% CI: 2.26 to 4.41) respectively compared to ER+ and/or PR+ and HER2- tumours after adjusting for age, stage and grade [233].

Germany

In Germany, data from the Saarland Cancer registry in over 8000 female invasive BC cases diagnosed between 200 and 2009 was assessed for differences in BC prognosis by HR and HER2 status separately. The study found a statistically significant RER for HR- tumours compared to HR+ tumours [HR= 2.9 (95% CI: 2.3 to 3.7)] but not for HER2 status alone [HR=0.9 (95% CI: 0.8 to 1.2) for HER2+ compared to HER2-] [234].

France

In France, a population-based study with over 4000 BC cases diagnosed in the Cote d'Or region found that ER- and PR- tumours had an increased risk of BC mortality [HR=1.3 (95% CI: 1.0 to 1.6) for ER- and HR= 1.4 (95% CI: 1.2 to 1.8) for PR-] after adjusting for age, number of nodes examined, stage, locoregional extension and multifocality [235].

Ireland

In Ireland, a study in over 7000 postmenopausal women aged 50 to 66 years diagnosed from 2006 to 2011 and with data ascertained from the National Cancer registry estimated that the risk of BC death was 2.0 (95% CI: 1.4 to 2.8) and 3.6 (95% CI: 2.7 to 5.0) times higher in women that over-expressed HER2 and with TNBC subtypes compared to the risk in women with luminal A tumours and after adjusting for age, tumour grade and stage, screening, deprivation and comorbidities [236].

Table 4.2 Studies from Europe reporting BC survival for the molecular subtypes of BC ordered by country and publication date (page 1 of 3).

Study and country	Years of diagnosis	n	Breast cancer subtypes categories reported	Hazard Ratio (95% CI)	Confounders adjusted for in the model
Allemani et al. [237] 2004, EUROCARE high-resolution studies (Estonia, France, Italy, Spain, Netherlands and UK)	1990–1992	4478	ER/PR status ER− and PR− ER+ and PR+ Either ER+ or PR+ Unknown	RER Reference 0.3 (0.2–0.4) 0.4 (0.3–0.6) 0.5 (0.4-0.7)	Age at diagnosis, tumour morphology and tumour stage.
Caldarella et al [228] 2011, Italy	2004-2005	1487	Luminal A: ER/PR+ and HER2- Luminal B: ER/PR+ and HER2+ Triple negative: ER/PR- and HER2- HER2 positive: ER/PR- and HER2+	Reference 1.7 (1.1-2.5) 1.7 (1.0-2.7) 2.2 (1.3-3.7)	T and N status.
Minicozzi et al. [229] 2013, Italy	2003-2005	3,381	 Luminal A (ER+ and/or PR+, HER2-, low Ki67) Luminal B (ER+ and/or PR+, HER2-, high Ki67) Luminal-HER2 (ER+ and/or PR+, HER2+, any Ki67) Triple-negative (ER-,PR-, HER2-, any Ki67) HER2-enriched (ER-,PR-, HER2+, any Ki67).	RER Reference 1.8 (1.2–2.5) 1.9 (1.3–2.9) 2.7 (1.8–4.1) 2.3 (1.4–3.6)	Age at diagnosis, stage, and treatments (surgery, radiotherapy, chemotherapy, hormonotherapy)
Engstrom et al [231] 2013, Norway	1961-2008	909	 Luminal A Luminal B (HER2-) Luminal B (HER2+) HER2 subtype five negative phenotype (5NP) Basal-like phenotype (BP)	Reference 1.3 (0.9–1.8) 2.1 (1.3–3.3) 3.7 (2.4–5.7) 3.2 (1.8–5.5) 2.4 (1.5–3.8)	Stage. They also present model adjusted for age but not for both.

Table 4.2 (continued) Studies from Europe reporting BC survival for the molecular subtypes of BC ordered by country and publication date (page 2 of 3).

Study and country	Years of diagnosis	n	Breast cancer subtypes categories reported	Hazard Ratio (95% CI)	Confounders adjusted for in the model
Valla et al. [232] 2016, Norway	1995-2013	1,423	Luminal A Luminal B (HER2-) Luminal B (HER2+) HER2 type Five negative phenotype Basal phenotype	Reference 2.0 (1.2–3.1) 3.6 (2.1–6.3) 5.1 (2.8–9.3) 4.2 (2.0–8.6) 2.7 (1.4–5.2)	Age at diagnosis, stage and histopathologic grade.
Johansson et al [230] 2019, Norway	2005-2015	21,384	Luminal A-like (ER+PR+HER2-) Luminal B-like HER2-negative (ER+PR-HER2-) Luminal B-like HER2-positive (ER+PR+/-HER2+) HER2-positive (ER-PR-HER2+) Triple-negative (ER-PR-HER2-)	Reference 1.7 (1.4, 2.0) 1.0 (0.8, 1.2) 1.3 (1.1, 1.7) 3.1 (2.6, 3.7)	Age at diagnosis, year of diagnosis, tumour grade, tumour stage and surgery.
Puig-Vives et al [233] 2013, Spain	2005	3480	 ER+ and/or PR+ and HER2- ER+ and/or PR+ and HER2+ HER2-overexpressed Triple negative Unclassified	RER Reference 1.0 (0.7-1.5) 1.7 (1.2-2.6) 3.2 (2.3-4.4) 2.6 (2.0-3.3)	Age at diagnosis, stage and histological grade

Table 4.2 (continued) Studies from Europe reporting BC survival for the molecular subtypes of BC ordered by country and publication date (page 3 of 3).

Study and country	Years of diagnosis	n	Breast cancer subtypes categories reported	Hazard Ratio (95% CI)	Confounders adjusted for in the model
Holleczek et al [234] 2013, Germany	2000-2009	8571	Hormone receptor status: HR+ (ER+PR+) HR mixed (ER+ or PR+) HR- (ER- PR-) HER2/neu expression: HER2- HER2+	RER Reference 1.7 (1.3-2.3) 2.9 (2.3-3.7) RER Reference 0.9 (0.8-1.2)	Age at diagnosis, stage, morphology, tumor grade, hormone receptor status and HER2/neu expression.
Dabakuyo et al [235] 2008 France	1982-2005	4223	ER status ER+ ER- PR status PR+ PR-	 Reference 1.3 (1.0, 1.6) Reference 1.4 (1.2-1.8)	Age at diagnosis, year of diagnosis, number of nodes examined, Stage T and N, ER status, PR status, locoregional extension and multifocality.
O'Brien et al. [236] 2018, Ireland	2006–2011	7160	Luminal A Luminal B, HER2− Luminal B, HER2+ Her2 over-expressing Triple negative Unknown	Reference 1.3 (0.9–1.8) 1.5 (1.1–2.0) 2.0 (1.4–2.8) 3.6 (2.7–5.0) 1.6 (0.9–2.6)	Age at diagnosis, stage, grade, screening, marital status, deprivation and co-morbidities.

Table presents hazard ratios unless states otherwise (Relative Excess Risk). CI= confidence interval, ER= oestrogen receptor, HER2= human epidermal growth factor 2, HR= hormone receptor, PR=progesterone receptor, RER= Relative Excess Risk.

4.1.2.4 The prognostic effect of other tumour characteristics in relation to the BC subtypes

The prognostic effect of age and tumour characteristics such as grade and stage in BC has been previously described [238, 239], however information on prognosis of combinations of age and tumour characteristics by BC molecular subtypes is limited. Several studies have shown that young age is an independent prognostic factor in women with luminal subtypes but not for HER2-enriched or TNBC [240]. For example, Lian et al, in a retrospective study in over 2,000 women with stage I to III BC showed associations of young age (<40 years) with luminal A tumours with worse outcomes [disease free survival (DFS), distant metastasis free survival (DMFS) and BCSS] compared to older women with luminal A tumours [241]. Another study using SEER data with 34,000 women from the US evaluating the prognostic effect of age in the molecular subtypes showed that women 40 years or older with the HR+/HER2+ subtype had worse OS than women younger than 40 years of age. However, in the presence of competing risks, young age at diagnosis of BC (<40 years) was only found to be associated with worse BCSS of TNBC compared to women diagnosed at 40 years or older [242]. A study in over 4,000 women using microarray data found that younger age at diagnosis of BC (< 40 years) was associated with poorer recurrence free survival in TNBC but not for HER2-enriched tumours when compared to women aged 40 years or more with the same subtypes [243]. Prognosis in older women has also been described to differ between subtype, with evidence from RCTs on older women with HR+ tumours showing increased hazards of BCD in women aged 65 to 74 (HR=1.25, 95% CI: 1.01 to 1.54) and women aged 75 or older (HR=1.63, 95% CI: 1.23 to 2.16) compared to women younger than 65 years [244]. The importance of stage and grade in prognosis for the different subtypes has been described in a few previous studies. The effect of histological grade and molecular subtypes in BCSS was evaluated in a recent study in Norway, showing that HER2-enriched tumours of grade 2 were associated with a 6 times increased BC mortality compared to luminal A grade 2 tumours but this association was not observed for grade 3 tumours which might be due to a small sample sizes for grade 3 tumours [231]. A study with over 123,000 women diagnosed with invasive BC from the California Cancer Registry and using grade to further differentiate luminal A and luminal B tumours found

a variability in BCSS amongst women with HER2+ tumours between different stages [245]. This study also found better survival for all ER+ subtypes than for the ER- subtypes irrespective of tumour stage [245]. Method of detection in countries where screening mammography is available has been established as an independent prognostic factor for BC [246] but statistically significant associations have only been found for luminal A subtypes [247].

4.1.3 Survival chapter layout

In Scotland, survival analyses based on cancer registry data in women diagnosed with BC have been previously published [248] but survival (overall and BC specific) for the different hormone subtypes or molecular subtypes has not previously been described using population-based cancer registry data. For that reason, this chapter describes trends in survival of BC in Scotland by ER status and the IHC defined molecular subtypes of BC. I will also investigate the main individual and tumour characteristics associated with survival for each ER status and IHC defined molecular subtype. Within the survival analysis results, I will describe trends in 5-year BCSS for the different subtypes within the different subgroups of key prognostic factors to assess whether survival has improved over time in Scotland. This chapter aims to identify high-risk groups of patients with worse outcomes that may benefit from further treatment or prevention interventions.

4.2 Objectives and hypotheses

Objectives:

- To determine the association of the different IHC defined subtypes with BCD in Scotland
- To assess the prognostic effect of age, pathologic grade, node status, tumour size and stage by molecular subtype
- To investigate whether time trends in BC survival differ by molecular subtypes and stratified by important prognostic factors

- To describe characteristics of groups of women diagnosed with BC whose BCSS has not improved over time

Hypotheses:

BC survival will differ according to ER status and other important individual and tumour characteristics, such as, age, stage grade and deprivation. I hypothesise that:

- ER+ tumours have better prognosis than ER- tumours
- Luminal A tumours have better prognosis than luminal B, HER2-enriched and TNBC
- BC survival trends have improved in Scotland over time in recent decades and may be related to screening and targeted treatment improvements, particularly for women with HER2+ tumours
- Women diagnosed with BC living in least deprived areas of Scotland have better prognosis than women living in the most deprived areas and prognosis might depend on tumour subtype.

4.3 Methods

4.3.1 Study population

The same population (n= 72,217) used for the analysis of incidence rates was the starting point for developing the cohort to investigate survival trends in women diagnosed with BC from 1997 to 2016 in Scotland. This cohort included only the first BC diagnosis for each woman (see section on incidence). Further exclusion criteria were applied following ISD guidelines [249] for survival analyses as described below.

Women with missing postcode, living outside Scotland or aged more than 99 years were excluded from the analysis (n=55). Those women who had missing vital status (n=154) or who were diagnosed with BC only from death certificates (n=126) were also excluded from the survival analysis. A further 99 women (all with vital status recorded as dead) had the same date of incidence and death and therefore, I assumed that their diagnosis was also estimated at the time of death and, hence, I excluded them from the analysis. The

total number of excluded cases was 434 (0.6% of the total) and the final population consisted of 71,784 women diagnosed from 1997 to 2016 (Table 4.3).

Table 4.3 Total breast cancer registrations from 1997 to 2016 in Scotland and final selection of women for survival analysis with exclusion criteria

Total registrations from 1997 to 2016 (after exclusion of multiple tumours)	**72,217**
Excluded from survival analysis	
Missing postcode, living outside Scotland or older than 99 years	55
Vital status unknown	154
Death certificate only	126
Same date of incidence as date of death	99
Total excluded (% excluded)	*434 (0.6%)*
Total included (% included)	**71,784 (99.4%)**

4.3.2 Statistical analyses

4.3.2.1 Outcome definition

Breast cancer specific survival (BCSS) was the primary outcome of the survival analysis. Breast cancer deaths were derived using only the underlying (primary) cause of death as recorded in the registry. Date of incidence in the Scottish Registry is normally recorded as the date of first consultation or admission at the hospital for that cancer. This date is a definite point in time that can be verified from the records and is the most consistent and reliable date to use [205]. Duration of follow-up was defined as time from date of diagnosis of BC to the first of: date of death from BC, 31st December 2017 for women still alive at the end of the study period or embarkation date if women moved from Scotland. The 31st of December 2017 was selected, the data was obtained in April of 2018 and completion of the data for the year 2016 should be the 31st December 2017 in accordance with the United Kingdom and Ireland Association of Cancer Registries (UKIACR) guidelines. Primary and secondary causes of death are derived from death records linked to the cancer registry [250], with the potential for up to 5 causes of death

(extended to up to 8 causes in 2013). ISD official statistics use a cause specific death variable derived from underlying or primary cause of death with quite a broad selection of ICD9 (until 31.12.1999) and ICD10 codes (from 1.1.2000) specific to the disease [249]. The approach taken for this analysis is similar to that described by Skyrud et al. [251] in that only ICD9 174 and ICD10 C50 codes from primary cause of death were used to derive BC specific death. Other primary causes of death were regarded as censored observations for the calculation of BCSS. Overall survival (OS) was used as a secondary outcome with death from any cause defined as the outcome and censoring at end of follow-up or at embarkation.

4.3.2.2 Breast cancer specific survival analysis by ER status and IHC defined molecular subtypes

Baseline characteristics for all women and by age group are described. No formal tests were carried out to look for differences between groups given that large sample sizes highly influence the p-value for those type of tests.

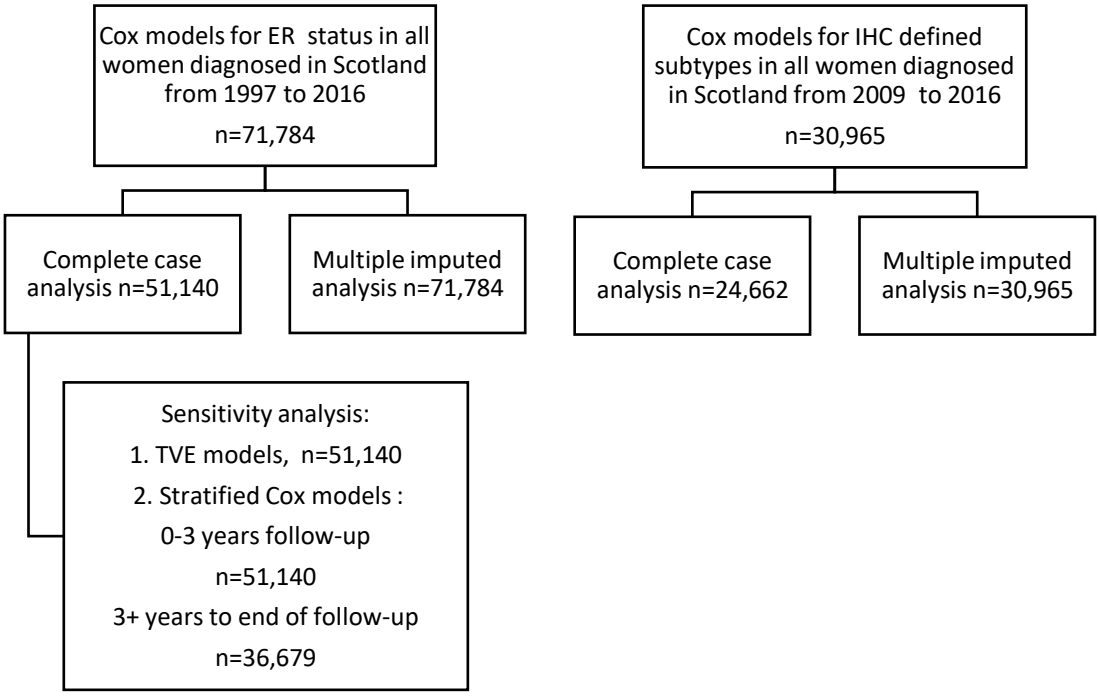
Non- parametric **Kaplan-Meier (KM)** estimates [252] were used to estimate BCSS and OS at 5 and 10 years by ER status. Given the short follow up for the IHC defined molecular subtypes (maximum follow up of 8 years) only estimates at 5 years for BCSS and OS were computed using the KM method. Five-year survival is a typical endpoint use for population cancer statistics and recommended as a quality performance indicator by NHS Scotland [253]. The effect of individual and other tumour characteristics and the effect of treatments on BCSS was also described using KM curves (Appendix C.5) and compared using long rank tests [254]. Five and 10-year BCSS and OS for the combinations of the most important prognostic factors (age, grade and stage) by ER and the IHC defined molecular subtypes were also estimated.

BCD was selected as the primary event of interest in the survival analysis, however other causes of death that were recorded as primary (underlying) cause for cohort member were also described. KM estimates of survival can be biased in the presence of competing risks as this method assumes that all events are independent and regards all other events (non BCD) as censored observations [255]. In addition to calculating OS estimates and BCSS

estimates, I estimated **cumulative incidence functions (CIF)** for other causes of death [including cardiovascular diseases (CVDs)] for the different combinations of age and ER status in order to assess whether competing risks were present.

Traditional Cox proportional hazards models [256] were fitted to investigate the association between ER status and IHC defined molecular subtypes (main exposures) and BCSS among Scottish women with BC after controlling for other covariates. Hazard Ratio (HR) with 95% CI are presented. The proportional hazards (PH) assumption was assessed using log-minus-log plots (Appendix Figure C.1) and formally tested for the fully adjusted model with ER as the main exposure (Appendix Table C.2) as an example. CCA of ER+ and ER- tumours (with ER+ as reference) diagnosed between 1997 and 2016 and CCA of IHC defined molecular subtypes (with luminal A tumours as reference) from 2009 are presented. The flowchart below (Figure 4.1) summarises the type of analysis and number of women for each analysis.

Figure 4.1 Flowchart of the survival analysis carried out using Cox models for ER status and IHC defined molecular subtypes as main exposures



Covariates adjusted for in the models were defined as described in section 3.3.1 of the incidence chapter. All confounding factors were included in the models based on clinical importance and for consistency with previous literature. Further, univariate models were performed to check if they were statistically significant (at the 5% level). As before, IHC defined subtypes were defined using grade to further differentiate luminal A and luminal B subtypes. Covariates in the models were: year of diagnosis (categorised into four 5-calendar year groups: 1997-2001, 2002-2006, 2007-2011 and 2012-2016) for the ER models and 2009-2011 and 2012-2016 for the molecular subtypes models, age at diagnosis separating those of screening age (<50, 50 to 69 and 70+ years), NHS Scottish region, tumour characteristics (grade, TNM stage, and method of detection), treatment regimens (surgery, radiotherapy, chemotherapy and HT) and area-based deprivation (SIMD) and comorbidity measures (Charlson index of comorbidity). Tumour markers included in the molecular subtype definition (ER, PR, HER2 status and grade) were not modelled as covariates in the molecular subtype models. Model 0 included the main exposure (ER status or the molecular subtypes) unadjusted. Model 1 included model 0 plus age at diagnosis, year of diagnosis and NHS Scottish region. Model 2 included model 1 plus tumour characteristics. Model 3 included model 2 plus treatment regimens. Lastly, model 4 included model 3 plus deprivation and comorbidity measures.

Sensitivity analysis was carried out to investigate alternatives to the traditional Cox model when the PH assumption did not hold for ER status and the molecular subtypes. The overall HR in a Cox model is estimated over the complete follow-up period, for that reason represents an average of all HRs, from very early HRs that affect almost all individuals to the HR at the end of the follow up were fewer individuals are still at risk. Given that the same weights are given to HRs at any time point, the overall HR is only a good estimate when the HR does not change over time. The PH assumption of the traditional Cox model implies that the effect of the prognostic factors in the risk (hazard) of BCD is constant over time. However, this assumption is often violated in the presence of markers for BC [257, 258]. Although a violation of the PH assumption can considerably bias [259] the estimates from the Cox model and lead to misleading conclusions, the assumption has been often ignored in the cancer literature [260].

For this study two approaches were taken to investigate alternatives to the Cox model:

1. An extended Cox regression model with time-varying effects (TVE) [258] for those variables for which the PH assumption did not hold was fitted introducing the interaction of each one of those variables with follow-up time. A likelihood ratio test was used to compare the original Cox model with the TVE model.
2. Stratified models: two independent models partitioning the follow up time up to 3 years for model 1 and after 3 years for model 2 were also fitted

A comparison of the results from the traditional Cox model, the extended model with TVE and the stratified models for the fully adjusted (model 4) with ER as the main exposure is presented in Appendix C.3. Number of women included in each analysis can be found in Figure 4.1.

4.3.2.3 Dealing with missing covariate data

MI was performed and findings of CCA models were compared with those of imputed models analysis (MIA). The data were assumed to be MAR after checking for missing data patterns. Missing covariate data were imputed by chained equation models [261] using a model compatible with the analysis model for ER status, PR status, HER2, IHC defined molecular subtypes, tumour grade, tumour stage, screening and for the treatment regimens (surgery, chemotherapy, radiotherapy and HT). Thirty imputed datasets were created using the missing covariate prognostic factors and outcome variables. The outcome used was the Nelson-Aalen estimator for time to BCD and a censoring indicator as described by White and Royston [262]. Missing values for ER status, PR status, HER2 status, screening and treatments (binary variables) were imputed from a logistic model and missing values for tumour grade and tumour stage (ordinal variable) from an ordinal multinomial model. Additionally, the complete variables (age at diagnosis, NHS region, year of incidence, SIMD and Charlson comorbidity Index) were included in the imputation model. Distributions of imputed variables were checked and compare to observed data. Cox regression analysis was performed on each of the 30 imputed datasets and the Rubin's rule was used to combine the coefficients from the models [263].

Comparison of CCA and MIA was restricted to the traditional Cox models for the main outcomes (ER and the IHC defined subtypes). MIA was not performed for TVE models as imputation of interactions of the covariates with time are computationally intensive, particularly within the safe haven environment.

4.3.2.4 Analysis of the association of other important prognostic factors with breast cancer death by molecular subtypes

To assess the prognostic value of other covariates within each subtype, additional Cox regression models were fitted for each ER status and each IHC molecular subtype separately. Fitting separate models for each subtype allows adjusting for appropriate confounders for that specific subtype, for example, women with ER+ tumours are usually treated with HT but women with ER- tumours are not, for that reason, HT was only adjusted for in the ER+ and luminal models. Tumour grade was excluded from the luminal A and luminal B tumours as it was used to define these subtypes. Separate models of each subtype also help with the fundamental assumption from Cox models, the proportionality assumption, as no comparison between subtypes is directly estimated. However, other covariates can still fail to show proportional hazards over time. For that reason, those models for which the PH assumption was violated, were compared to extended Cox models with TVE as explained in the sensitivity analysis below.

4.3.2.5 Trends of breast cancer survival over time for combinations of important prognostic factors

The Cox models showed that the most important prognostic factors of survival for all ER subtypes were age, grade and stage. The latest guidance from AJCC TNM stage classification (8^{th} edition) [163] highlights the need to use not only TNM stage but to incorporate molecular markers (ER, PR, HER2, ki67) and histological grade into the staging system to improve prognostic value [264]. I used combinations of age, ER, grade and TNM stage to investigate trends in 5-year BCSS and identify the combination of characteristics where survival is poorest, that could be targeted in future interventions. ER/grade and ER/stage combinations were analysed by age group except for those combinations for which sample size was deemed too small to give precise estimates (such

as, ER- low grade tumours with n<50). Further, due to small numbers, grade was categorised into 2 groups, low grade (grade I and grade II) and high grade (grade III: poorly differentiated) and TNM stages III and IV were collapsed into the same category.

OS and BCSS probabilities at five years (with 95% CI) for each combination and incidence year were calculated using KM and plotted against year of diagnosis to graphically assess trends over time. Graphs of survival trends were smoothed using a three-year moving average, with incidence year in the graphs representing the middle year for that three-year period (for example, year 2000 in the graph represents the average of years 1999-2001). Survival probabilities were also calculated for the combinations of those four important prognostic factors (age, ER, grade and stage) and method of detection to inspect whether survival improvements were related to the tumour being screen detected or not.

Joinpoint regression analysis was used to estimate changes in BCSS probability for each combination of tumour factors at 5 years and AAPC is presented (in the graphs) with 95% CI for those combinations that showed statistically significant trends over time for the whole study period or EAPC for each of the time periods identified through joinpoint. Bonferroni correction [265] was used to correct for type I errors resulting from multiple Joinpoint regression analyses (one regression for each combination of age, ER status and grade/stage). For example, for the combination of ER status and grade for each age group, 9 different regressions were performed, hence the significance level was corrected to be $\alpha = \frac{0.05}{9} = 0.0056$. Complete joinpoint results are presented in Appendix C.10 and Appendix C.13. Survival trends for the IHC defined subtypes in combination with age, grade and TNM stage are not presented due to small sample sizes for the rare subtypes (HER2-enriched and TNBC) and due to the short follow-up (given that molecular subtypes are only available from 2009, 5-year BCSS can only be computed from 2009-2011).

Given the important effect of treatment regimens in survival and that treatment decisions are guided by molecular markers and age, trends in treatments over time are presented by ER status and age group.

4.4 Results

4.4.1 Description of the population for survival analysis

Table 4.4 provides a description of the individual and tumour characteristics by age group for the cohort of women diagnosed with BC between 1997 and 2016 in Scotland included in the survival analysis.

Table 4.4 Individual and tumour characteristics for the population of women included in the survival analysis by age group (page 1 of 2)

	<50 years (N=14,379) [20%]	**50-69 years (N=35,592) [50%]**	**70 years or older (N=21,813) [30%]**	**Total (N=71,784)**
Region of Scotland				
North	3,639 (25%)	9,288 (26%)	5,665 (26%)	18,592 (26%)
South East	4,020 (28%)	9,769 (27%)	6,044 (28%)	19,833 (28%)
West	6,720 (47%)	16,535 (47%)	10,104 (46%)	33,359 (46%)
Year of diagnosis				
1997-2001	3,324 (23%)	7,741 (22%)	5,239 (24%)	16,304 (23%)
2002-2006	3,543 (25%)	8,319 (23%)	5,427 (25%)	17,289 (24%)
2007-2011	3,739 (26%)	9,473 (27%)	5,467 (25%)	18,679 (26%)
2012-2016	3,773 (26%)	10,059 (28%)	5,680 (26%)	19,512 (27%)
SIMD quintile				
Least deprived	3,218 (23%)	7,570 (21%)	4,101 (19%)	14,889 (21%)
4	3,080 (21%)	7,594 (21%)	4,252 (20%)	14,926 (21%)
3	2,878 (20%)	7,387 (21%)	4,593 (21%)	14,858 (20%)
2	2,726 (19%)	6,803 (19%)	4,729 (21%)	14,258 (20%)
Most deprived	2,477 (17%)	6,238 (18%)	4,138 (19%)	12,853 (18%)
TNM stage 4 categories				
I	3,851 (27%)	15,576 (44%)	4,682 (22%)	24,109 (34%)
II	6,111 (42%)	11,875 (33%)	7,256 (33%)	25,242 (35%)
III	2,573 (18%)	4,221 (12%)	3,540 (16%)	10,334 (14%)
IV	601 (4%)	1,398 (4%)	1,612 (7%)	3,611 (5%)
Unknown	1,243 (9%)	2,522 (7%)	4,723 (22%)	8,488 (12%)
Tumour grade				
Grade I	1,196 (8%)	5,625 (16%)	1,863 (8%)	8,684 (12%)
Grade II	5,012 (35%)	15,063 (42%)	7,902 (36%)	27,977 (39%)
Grade III Poorly differentiated	6,658 (46%)	11,237 (32%)	5,590 (26%)	23,485 (33%)
Unknown	1,513 (11%)	3,667 (10%)	6,458 (30%)	11,638 (16%)

Table 4.4 (continued) Individual and tumour characteristics for the population of women included in the survival analysis by age group (page 2of 2)

	<50 years (N=14,379) [20%]	50-69 years (N=35,592) [50%]	70 years or older (N=21,813) [30%]	Total (N=71784)
Tumour size (in cm)				
Less than 10mm	1,108 (8%)	5,543 (16%)	1,016 (5%)	7,667 (11%)
10 to 20mm	5,034 (35%)	14,571 (41%)	4,698 (22%)	24,303 (34%)
More than 20mm	6,164 (43%)	11,014 (31%)	6,404 (29%)	23,582 (33%)
Unknown	2,073 (14%)	4,464 (12%)	9,695 (44%)	16,232 (22%)
Positive nodal status?				
Yes	6,216 (43%)	10,858 (30%)	4,769 (22%)	21,843 (30%)
No	7,172 (50%)	21,674 (61%)	7,525 (34%)	36,371 (51%)
Unknown	991 (7%)	3,060 (9%)	9,519 (44%)	13,570 (19%)
Diagnosed through screening?				
Yes	269 (2%)	17,101 (48%)	2,143 (10%)	19,513 (27%)
No	13,786 (96%)	17,877 (50%)	19,138 (88%)	50,801 (71%)
Unknown	324 (2%)	614 (2%)	532 (2%)	1,470 (2%)
ER status				
Positive	10,505 (73%)	28,385 (80%)	16,113 (74%)	55,003 (77%)
Negative	3,188 (22%)	5,655 (16%)	2,855 (13%)	11,698 (16%)
Unknown	686 (5%)	1,552 (4%)	2,845 (13%)	5,083 (7%)
PR status*				
Positive	3,096 (51%)	8,471 (53%)	4,499 (50%)	16,066 (51%)
Negative	1,482 (25%)	3,482 (22%)	1,868 (21%)	6,832 (22%)
Unknown	1,458 (24%)	3,997 (25%)	2,612 (29%)	8,067 (26%)
HER2 status*				
Positive	1,142 (19%)	2,082 (13%)	981 (11%)	4,205 (14%)
Negative	4,466 (74%)	12,337 (77%)	6,571 (73%)	23,374 (75%)
Unknown	428 (7%)	1,531 (10%)	1,427 (16%)	3,386 (11%)
Molecular subtype*				
Luminal A	2,337 (39%)	8,464 (53%)	4,623 (51%)	15,424 (50%)
Luminal B	2,147 (36%)	3,888 (24%)	1,893 (21%)	7,928 (26%)
HER2-Enriched	310 (5%)	654 (4%)	321 (4%)	1,285 (4%)
Triple Negative	802 (13%)	1,388 (9%)	705 (8%)	2,895 (9%)
Unknown	440 (7%)	1,556 (10%)	1,437 (16%)	3,433 (11%)

*restricted to years 2009 to 2016 (total n=30,965, by age group: <50 years (N=6,036), 50-69 years (N=15,950) and 70 years or older (N=8,979), parenthesis () are column percentages and brackets [] are row percentages. ER= oestrogen receptor, HER2= human epidermal growth factor 2, NHS= National Health Service, PR=progesterone receptor, SIMD= Scottish Index of Multiple Deprivation, TNM= tumour, nodes, metastases.

The majority, over 75% of tumours in the study population, were ER+ with slightly higher proportions of ER+ tumours in women of screening ages 50 to 69 years than in other age groups. The most common IHC defined molecular subtype was luminal A for all age groups but women aged 50 to 69 years and women aged 70 years or older had a higher % of luminal A tumours (53% and 52% respectively) than younger women (39%). Luminal B and TNBC were more prevalent in women younger than 50 years compared to older women.

Of the 71,784 women diagnosed with BC between 1997 and 2016 in Scotland with a median follow-up of 5.5 years (7.1 years for censored observations) with available information on death, 26,280 (37%) died during the study period with a median follow-up of 3.4 years (Table 4.5). Of those who died, 53% had BC as their primary cause of death recorded. However, the proportion of all deaths that were attributed to BC varied greatly by age, with 85% of women younger than 50 years dying from BC compared to 57% and 43% of BCDs in the 50-69 years and 70 years or older groups respectively.

Table 4.5 Breast cancer deaths by age group amongst women with breast cancer diagnosed in Scotland from 1997 to 2016

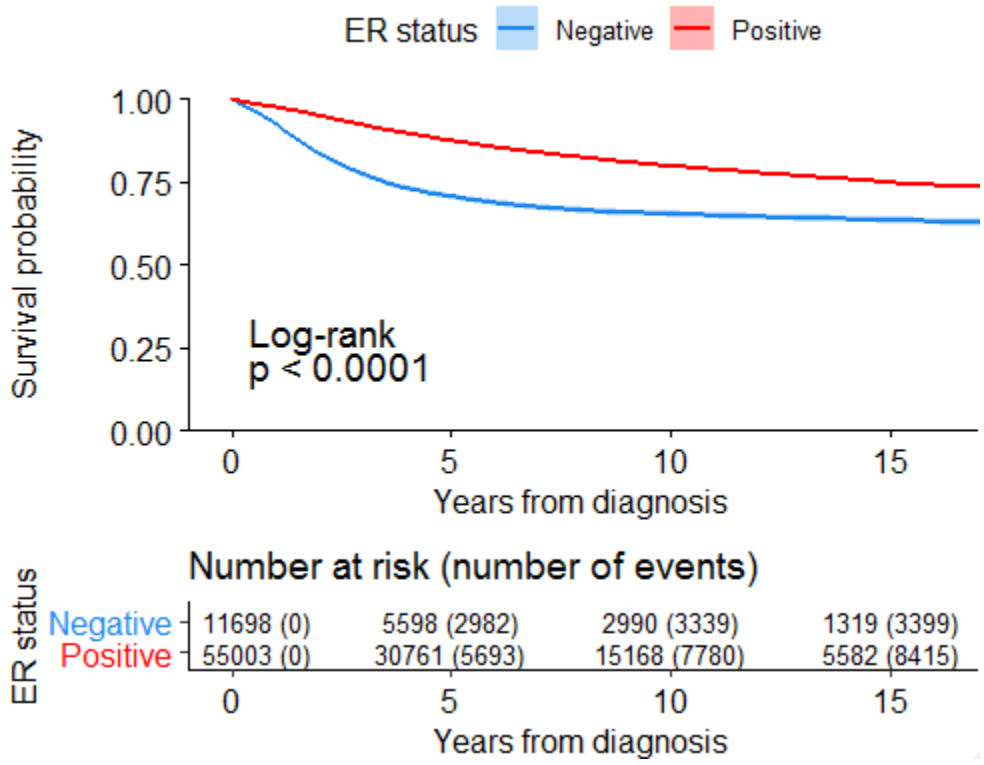
	<50 years (N=3,213)	**50-69 years (N=8,894)**	**70 years or older (N=14,173)**	**Total (N=26,280)**
Breast cancer death				
No	465 (14%)	3,799 (43%)	8,034 (57%)	12,298 (47%)
Yes	2,748 (86%)	5,095 (57%)	6,139 (43%)	13,982 (53%)
Follow-up time (in years)				
Median	3.8	4.5	2.8	3.4
Q1,Q3	2.0, 7.0	2.0, 8.7	1.0, 6.0	1.4, 7.1

Q1=quartile 1, Q3=quartile 3

4.4.2 Does breast cancer survival differ by ER status?

BCSS differed significantly by ER status with women with ER- tumours having lower survival than women with ER+ tumours (Figure 4.2).

Figure 4.2 Kaplan-Meier curves by ER status



The difference in BCSS between women with ER+ and ER- tumours was highest during the first 5 years of follow-up with 87.4% (95%CI: 87.1 to 87.7) of women with ER+ tumours and 70.6% (95%CI: 69.7 to 71.5) of women with ER- tumours surviving 5 years (Table 4.6). Survival differences between ER+ and ER- were still notable at 10 years of follow-up (14.5% absolute difference between women with ER+ and ER- tumours).

BCSS declined with age and the difference in survival between women with ER+ and ER- tumours was highest in women aged 70 years or older (21.7% difference at 5 years). Women aged 50 to 69 years (screening age group) with ER+ tumours had the highest

survival at 5 and 10 years (92.1% and 85.8% respectively), in contrast women aged 70 years or older with ER- tumours had the worst survival of all age and ER combinations.

Table 4.6 Breast cancer specific survival estimates (in %) at 5 and 10 years after diagnosis (with 95% CI) by ER status and age group

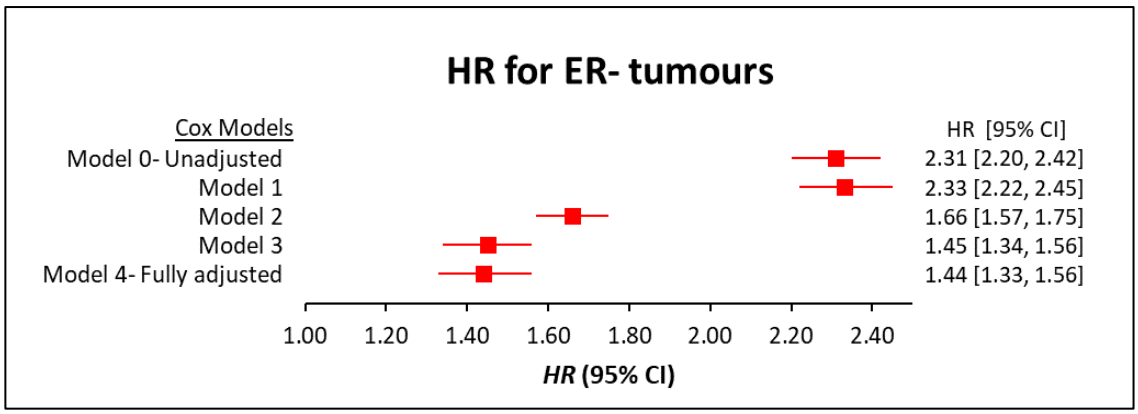
BREAST CANCER SPECIFIC SURVIVAL	<50 YEARS	50-69 YEARS	70 YEARS OR OLDER	TOTAL
ER+				
cases/deaths	6,691/857	17,511/1,548	6,566/1,973	30,768/4,378
5-year BCSS (95% CI)	89.2 (88.6, 89.9)	92.1 (91.7, 92.4)	77.1 (76.4, 77.9)	87.4 (87.1, 87.7)
ER-				
cases/deaths	1,774/587	2,963/944	865/601	5,602/2,132
5-year BCSS (95% CI)	75.1 (73.5, 76.7)	75.1 (73.9, 76.4)	55.4 (53.4, 57.5)	70.6 (69.7, 71.5)
% difference at 5 years (ER+ minus ER-)	14.1 %	17.0%	21.7%	16.8%
ER+				
cases/deaths	3,678/527	9,165/933	2,330/627	15,173/2087
10-year BCSS (95% CI)	80.7 (79.8, 81.6)	85.8 (85.2, 86.3)	66.9 (65.9, 67.9)	79.8 (79.3, 80.2)
ER-				
cases/deaths	1,063/113	1,604/160	327/84	2,994/357
10-year BCSS (95% CI)	69.6 (67.8, 71.4)	70.4 (69.0, 71.7)	48.8 (46.6, 51.1)	65.3 (64.3, 66.3)
% difference at 10 years (ER+ minus ER-)	11.1%	15.4%	18.1%	14.5%

BCSS=Breast cancer specific survival, CI= confidence interval, ER= oestrogen receptor.

Similar patterns in OS at 5 and 10 years between women with ER+ and ER- tumours were seen to those observed for BCSS (Appendix C.1). However, OS for women aged 70 years or older was 24% lower at 10 years than BCSS (31.8% for ER+ and 24.4% for ER-).

Although overall only 18% of all cases were ER- tumours, they accounted for 31% of the total deaths from BC (Table 4.7). Results from Cox models (Figure 4.3) showed that ER- tumours had a higher BC specific mortality than ER+ tumours [HR= 1.44 (95% CI: 1.33 to 1.56)] after adjusting for other tumour characteristics, individual characteristics, treatments and comorbidities.

Figure 4.3 Cox regression model results for the comparison of risk of death from breast cancer for ER- tumours and ER+ tumours (as reference)



Footnote: Model 1 was adjusted for age, incidence year and NHS region. Model 2: model 1 + tumour characteristics (grade, TNM stage and method of detection. Model 3= model 2 + treatments (surgery, radiotherapy, chemotherapy and hormone therapy). Model 4= model 3 + SIMD and Charlson score index. Models carried out in the complete case dataset with n=51,140 women, number of BC deaths=7,592. CI= confidence interval, ER= oestrogen receptor, HR=hazard ratio.

Table 4.7 shows all models (from unadjusted to fully adjusted model) and the effect of the adjustment of other prognostic factors in BCD in women with ER- tumours compared to women with ER+ tumours. Tumour characteristics were the most important prognostic factors of BCD and adjusting for these factors (model 2) reduced the HR for BCD for ER- compared to ER+ tumours from 2.33 (95% CI: 2.22 to 2.45) to 1.66 (95% CI: 1.57 to 1.75). Further adjustment for treatments (model 3) reduced the HR to 1.45 (95% CI: 1.34 to 1.56) and further adjusting for deprivation and comorbidities (model 4) had little effect on the HR for women with ER- tumours (compared to women with ER+).

Table 4.7 Traditional unadjusted and adjusted Cox models for the association of ER status with breast cancer death (page 1 of 2)

	No women (%)	No deaths (%)	Model 0 unadjusted	Model 1	Model 2	Model 3	Model 4
ER Status							
Positive	42,146 (82%)	5,238 (69%)	Ref	Ref	Ref	Ref	Ref
Negative	8,994 (18%)	2,354 (31%)	2.31 (2.20-2.42)	2.33 (2.22-2.45)	1.66 (1.57-1.75)	1.45 (1.34-1.56)	1.44 (1.33-1.56)
Age							
<50 years	10,886 (21%)	1,823 (24%)	1.37 (1.30-1.45)	1.27 (1.20-1.34)	0.86 (0.81-0.91)	0.89 (0.83-0.94)	0.89 (0.84-0.95)
50-69 years	27,869 (55%)	3,248 (43%)	Ref	Ref	Ref	Ref	Ref
70 years or older	12,385 (24%)	2,521 (33%)	2.23 (2.12-2.35)	2.26 (2.14-2.38)	1.59 (1.51-1.68,)	1.36 (1.27-1.45)	1.36 (1.27-1.45)
NHS Scottish region							
West	20,738 (41%)	2,800 (37%)	Ref	Ref	Ref	Ref	Ref
North	14,545 (28%)	2,388 (31%)	1.20 (1.14-1.27)	1.19 (1.13-1.26)	1.17 (1.11-1.24)	1.07 (1.01-1.13, p=0.015)	1.12 (1.06-1.19)
South East	15,857 (31%)	2,404 (32%)	1.08 (1.03-1.14, p=0.004)	1.11 (1.06-1.18)	1.09 (1.03-1.15)	0.97 (0.92-1.03, p=0.331)	1.01 (0.96-1.07, p=0.665)
Year of diagnosis							
1997-2001	10,390 (20%)	2,705 (36%)	Ref	Ref	Ref	Ref	Ref
2002-2006	11,077 (22%)	2,209 (29%)	0.84 (0.79-0.89)	0.86 (0.81-0.91)	0.86 (0.81-0.91)	0.79 (0.75-0.84)	0.79 (0.75-0.84)
2007-2011	13,302 (26%)	1,761 (23%)	0.69 (0.65-0.73)	0.73 (0.68-0.77)	0.72 (0.67-0.76)	0.68 (0.64-0.72)	0.68 (0.64-0.73)
2012-2016	16,371 (32%)	917 (12%)	0.73 (0.67-0.79)	0.77 (0.71-0.83)	0.80 (0.74-0.87)	0.73 (0.67-0.79)	0.73 (0.67-0.79)
Grade							
Grade I-(Well) differentiated	7,465 (14%)	351 (5%)	Ref		Ref	Ref	Ref
Grade II- Moderately (well) differentiated	23,878 (47%)	2,701 (35%)	2.78 (2.49-3.11)		1.86 (1.66-2.08)	1.84 (1.65-2.06)	1.84 (1.64-2.06)
Grade III-Poorly differentiated	19,797 (39%)	4,540 (60%)	6.08 (5.45-6.78)		3.02 (2.70-3.38)	3.04 (2.71-3.41)	3.02 (2.70-3.39)
TNM stage							
I	20,598 (40%)	930 (12%)	Ref		Ref	Ref	Ref
II	20,722 (40%)	2,736 (36%)	2.46 (2.27-2.67)		2.16 (2.00-2.33)	2.12 (1.96-2.29)	2.11 (1.95-2.28)
III	7,935 (16%)	2,801 (37%)	10.13 (9.21-11.16)		6.33 (5.86-6.84)	5.95 (5.48-6.45)	5.90 (5.44-6.40)
IV	1,885 (4%)	1,125 (15%)	30.13 (27.61-32.89)		20.94 (19.13-22.91)	11.05 (9.96-12.25)	11.09 (10.00-12.30)
Screening							
Yes	16,227 (32%)	853 (11%)	Ref		Ref	Ref	Ref
No	34,913 (68%)	6,739 (89%)	3.88 (3.62-4.17)		1.76 (1.63-1.90)	1.63 (1.51-1.76)	1.62 (1.50-1.75)

Table 4.7 (continued) Traditional unadjusted and adjusted models Cox models for the association of ER status with breast cancer death (page 2 of 2)

	No women (%)	No deaths (%)	Model 0 unadjusted	Model 1	Model 2	Model 3	Model 4
Surgery							
Yes	48,343 (94%)	6,204 (82%)	Ref			Ref	Ref
No	2,797 (6%)	1,388 (18%)	10.17 (9.58-10.78)			4.04 (3.74-4.37)	4.01 (3.70-4.33)
Radiotherapy							
Yes	35,250 (69%)	4,863 (64%)	Ref			Ref	
No	15,890 (31%)	2,729 (36%)	1.25 (1.19-1.31)			1.04 (0.99-1.10, p=0.096)	1.03 (0.98-1.09, p=0.201)
Chemotherapy							
Yes	20,329 (40%)	4,216 (56%)	Ref			Ref	Ref
No	30,811 (60%)	3,376 (44%)	0.55 (0.53-0.58)			0.98 (0.92-1.04, p=0.488)	0.96 (0.90-1.02, p=0.218)
Hormone therapy							
Yes	40,136 (79%)	5,098 (67%)	Ref			Ref	Ref
No	11,004 (21%)	2,494 (33%)	1.97 (1.87-2.06)			1.31 (1.21-1.41)	1.31 (1.21-1.41)
SIMD quintile							
Least deprived	11,083 (22%)	1,481 (19%)	Ref				Ref
4	11,121 (22%)	1,556 (20%)	1.07 (0.99-1.15, p=0.075)				1.03 (0.96-1.11, p=0.307)
3	10,858 (21%)	1,643 (22%)	1.18 (1.10-1.27)				1.12 (1.04-1.20, p=0.002)
2	9,862 (19%)	1,576 (21%)	1.28 (1.19-1.37)				1.19 (1.11-1.28)
Most deprived	8,216 (16%)	1,336 (18%)	1.32 (1.23-1.42)				1.24 (1.15-1.34)
Charlson Score							
Mean (SD)	0.0 (0.3)	0.1 (0.3)	1.34 (1.24-1.44)				1.23 (1.13-1.33)

Footnote: Model 1 was adjusted for age, incidence year and NHS region. Model 2: model 1 + tumour characteristics (grade, TNM stage and method of detection. Model 3= model 2 + treatments (surgery, radiotherapy, chemotherapy and hormone therapy). Model 4= model 3 + SIMD and Charlson score index. Models carried out in the complete case dataset with n=51,140 women, number of BC deaths=7,592. All HRs were statistically significant at the 0.1% level unless stated otherwise. ER= oestrogen receptor, NHS= National Health Service, Ref= reference category, SD= standard deviation, SIMD= Scottish Index of Multiple Deprivation, TNM= tumour, nodes, metastases.

Results from the fully adjusted model (model 4) show that all covariates in the model were statistically significant independent prognostic factors for BCD, with the exception of radiotherapy and chemotherapy. BC mortality was particularly high among women older than 70 years (HR= 1.36, 95% CI: 1.27 to 1.45, compared to women aged 50 to 69 years), women with grade III tumours (HR= 3.02, 95% CI: 2.70 to 3.39, compared to women with grade I tumours), women with stage IV tumours (HR= 11.09, 95% CI: 10.00 to 12.30, compared to women with stage I), women with non-screen detected tumours (HR= 1.62, 95% CI: 1.50 to 1.75, compared to women with screen detected tumours), women who did not have surgery (HR= 4.01, 95% CI: 3.70 to 4.33, compared to women who had surgery), women who did not received HT (HR= 1.31, 95% CI: 1.21 to 1.41, compared to women who received HT), women in the most deprived areas of Scotland (HR= 1.24, 95% CI: 1.15 to 1.34, compared to women in the least deprived areas) and women with a higher comorbidity index (HR= 1.23, 95% CI: 1.13 to 1.33 per unit increase).

Log-minus-log plots for all covariates included in the fully adjusted model (model 4) with ER status as the main exposure are presented in Appendix Figure C.1 along with formal testing of the PH assumption (Appendix Table C.2). Log-minus-log plots showed small deviations from proportionality for ER- tumours, grade III, stage IV and the treatment variables that were confirmed by the global and individual covariates PH assumption tests.

Sensitivity analysis

Extended Cox model with TVEs for the fully adjusted model in Appendix C.3 shows that there was a statistically significant interaction of time with ER status [HR for time*ER=0.83 (95% CI: 0.81 to 0.85)] indicating that the HR for the comparison of ER- and ER+ tumours was not constant over time. Estimates of the HR at different times after diagnosis (1, 3, 5 and 10 years) presented in Appendix Table C.4 show that the HR for the comparison of women with ER- tumours vs ER+ tumours decreases over time: the risk of BCD amongst women with ER- tumours is 2.39, 1.63, 1.12 and 0.43 times that for women with ER+ tumours at 1, 3, 5 and 10 years respectively.

The stratified model for 0 to 3 years of follow-up also shows higher BC mortality amongst women with ER- tumours (vs women with ER+ tumours) [HR=2.26 (95% CI: 2.02 to

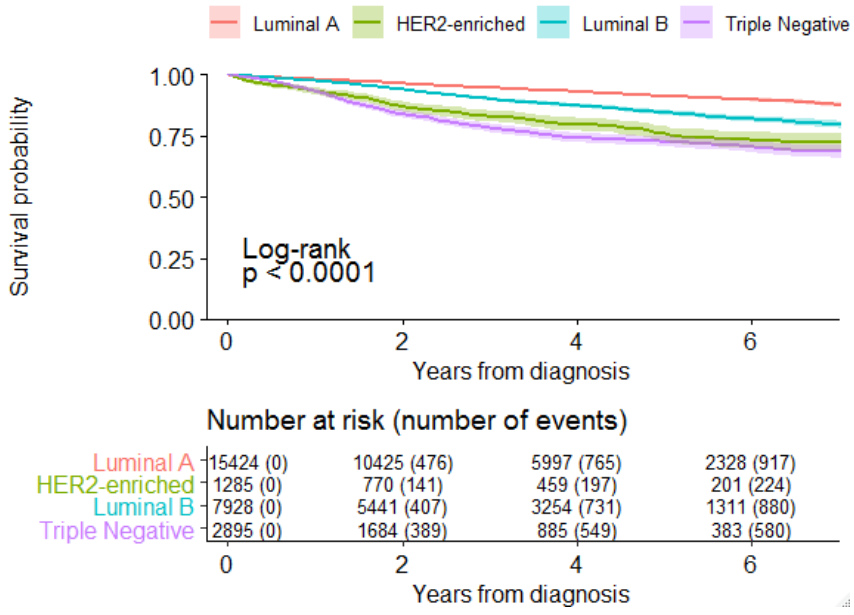
2.52)] compared to the overall HR from the traditional Cox model. Restricting the follow-up time to more than 3 years suggested that BC mortality in women with ER- tumours was actually lower than for women with ER+ tumours [HR=0.85 (95%CI: 0.76 to 0.96)] after adjusting for all other covariates.

Other tumour characteristics (grade, stage, screening) and treatments (surgery, radiotherapy, chemotherapy and HT) also showed non-constant HRs. For example, radiotherapy and chemotherapy showed statistically significant results for both TVE models and stratified models with a beneficial effect of having these treatments for all women that was hidden by the traditional Cox model and that was restricted to early follow-up years (up to 3 years).

4.4.3 Does breast cancer survival differ between IHC defined molecular subtypes?

Women with luminal A tumours had the best survival of all subtypes, followed by women with luminal B tumours and HER2-enriched subtypes (Figure 4.4). Women with a TNBC had the worst survival with 72.6% (95% CI: 70.5 to 74.6) of all women with that subtype surviving at 5 years, considerably lower than 91.3% (95% CI: 90.1 to 91.9) of women with luminal A tumours surviving that time (Table 4.8).

Figure 4.4 Kaplan-Meier curves by IHC defined molecular subtypes



The differences in survival between the IHC defined subtypes were consistent across the three age groups. Women aged less than 50 years and women aged 50 to 69 years had very similar BCSS at 5 years within each subtype. However, women aged 70 years or older had considerably lower survival than women younger than 70 years of age, particularly for the most aggressive HER2-enriched and TNBC subtypes.

Table 4.8 Breast cancer specific survival estimates (in %) at 5 years after diagnosis (with 95% CI) by IHC defined molecular subtypes and age group

IHC defined subtype	<50 YEARS	50- 69 YEARS	70 YEARS OR OLDER	TOTAL
Luminal A				
cases/deaths	749/75	2,413/183	888/331	4,050/589
5-year BCSS (95% CI)	93.6 (92.1, 94.9)	95.5 (94.8, 96.0)	81.6 (80.0, 83.1)	91.3 (90.1, 91.9)
Luminal B				
cases/deaths	679/169	1,176/229	379/242	2,234/640
5-year BCSS (95% CI)	86.2 (84.2, 88.0)	88.8 (87.5, 90.1)	73.3 (70.5, 75.8)	84.5 (83.5, 85.5)
HER2-enriched				
cases/deaths	89/29	173/50	49/54	311/133
5-year BCSS (95% CI)	81.5 (74.9, 86.6)	81.7 (77.4, 85.3)	58.4 (51.1, 65.0)	76.0 (72.7, 78.8)
Triple Negative				
cases/deaths	187/112	327/155	95/117	609/384
5-year BCSS (95% CI)	74.5 (70.5, 78.1)	78.6 (75.7, 81.1)	57.4 (52.4, 62.1)	72.6 (70.5, 74.6)

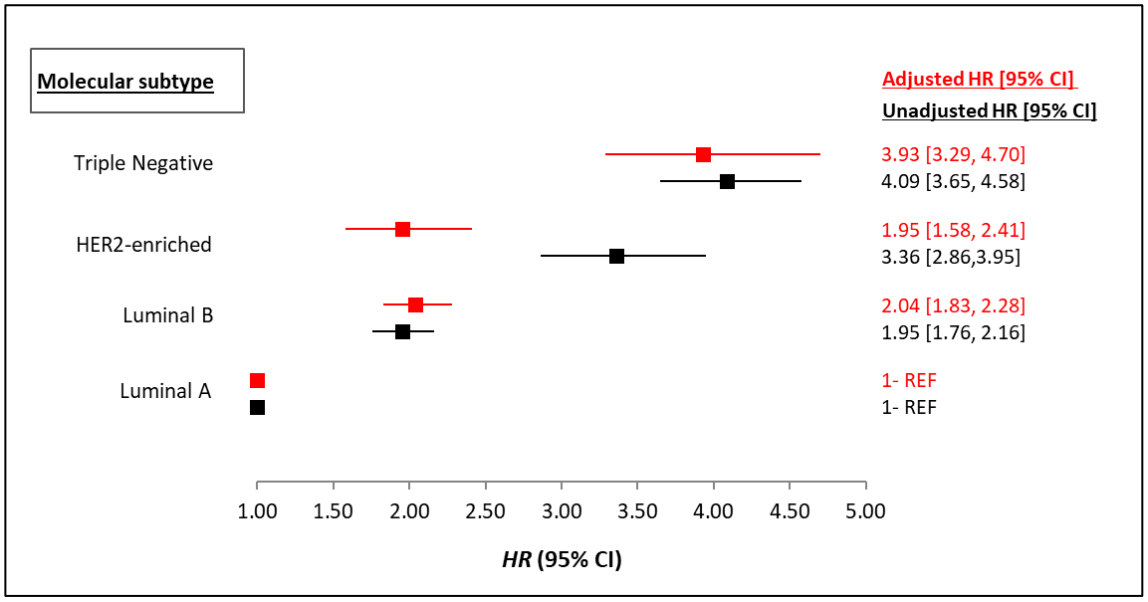
BCSS= breast cancer specific survival, HER2= human epidermal growth factor 2, IHC= immunohistochemistry.

OS estimates (Appendix Table C.5) were similar to BCSS estimates for women younger than 70 years of age and the differences between subtypes were consistent. For women aged 70 years or older OS was considerably lower than BCSS, and the difference was more striking between the luminal subtypes. For example, women aged 70 years or more with luminal A tumours had a BCSS at 5 years of 81.6% and an OS at 5 years of 58.5%.

Results from Cox models (Figure 4.5) showed that BCD was significantly higher for women with TNBC [HR=3.93 (95% CI: 3.29 to 4.70)] compared to women with luminal A tumours. Women with luminal B and HER2-enriched tumours had double BC-specific

mortality than women with luminal A tumours after adjustment for other individual and tumour characteristics. Adjusting for other characteristics had the largest effect, attenuating the HR of BCD for women with HER2-enriched tumours compared to women with luminal A tumours from 3.36 (95% CI: 2.86 to 3.95) in the unadjusted model to 1.95 (95% CI: 1.58 to 2.16) in the fully adjusted model. Full results for all models are presented in Table 4.9. The PH assumption held for IHC defined subtypes and for that reason TVE models were not used for the models with the molecular subtypes as the main exposure. Small deviations from proportionality were observed for other covariates but as I was mainly interested in the effect of the subtypes in BCD the traditional Cox model is presented and deemed adequate.

Figure 4.5 Unadjusted and fully adjusted Cox regression models results for breast cancer death for the IHC defined molecular subtypes with luminal A tumours as the reference



Footnote: Fully adjusted model has age, incidence year, NHS region, tumour characteristics (TNM stage and method of detection, treatments (surgery, radiotherapy, chemotherapy and hormone therapy), SIMD and Charlson score index. Models carried out in the complete case dataset with n=24,266 women diagnosed between 2009 and 2016, number of BC deaths=2,168. CI= confidence interval, HER2= human epidermal growth factor 2, HR=hazard ratio, REF= reference category.

Table 4.9 Traditional unadjusted and adjusted Cox models for the association of IHC defined molecular subtypes with breast cancer death (page 1 of 2)

	No women (%)	No deaths (%)	Model 0- Unadjusted	Model 1	Model 2	Model 3	Model 4
IHC defined subtype							
Luminal A	13,755 (56%)	723 (33%)	Ref	Ref	Ref	Ref	Ref
Luminal B	7,132 (29%)	753 (35%)	1.95 (1.76-2.16)	2.03 (1.83-2.25)	1.71 (1.54-1.89)	2.06 (1.84-2.29)	2.04 (1.83-2.28)
HER2-enriched	1,140 (4%)	188 (9%)	3.36 (2.86-3.95)	3.48 (2.96-4.09)	2.44 (2.08-2.87)	1.97 (1.60-2.44)	1.95 (1.58-2.41,)
Triple Negative	2,635 (11%)	504 (23%)	4.09 (3.65-4.58)	4.31 (3.85-4.84)	4.54 (4.03-5.10)	4.02 (3.36-4.80)	3.93 (3.29-4.70)
Age							
<50 years	5,037 (20%)	441 (20%)	1.47 (1.31-1.66)	1.26 (1.12-1.42)	0.87 (0.78-0.99, p=0.029)	0.94 (0.83-1.06, p=0.287)	0.94 (0.83-1.07, p=0.350)
50-69 years	13,332 (54%)	764 (35%)	Ref	Ref	Ref	Ref	Ref
70 years or older	6,293 (26%)	963 (45%)	3.19 (2.90-3.50)	3.23 (2.94-3.55)	2.08 (1.88-2.29)	1.49 (1.33-1.66)	1.49 (1.33-1.67)
NHS Scottish region							
West	11,072 (45%)	962 (44%)	Ref	Ref	Ref	Ref	Ref
North	6,699 (27%)	630 (29%)	1.04 (0.94-1.15, p=0.418)	1.07 (0.97-1.19, p=0.165)	1.15 (1.04-1.27, p=0.007)	1.19 (1.07-1.32, p=0.001)	1.26 (1.13-1.40)
South East	6,891 (28%)	576 (27%)	0.93 (0.83-1.03, p=0.141)	0.98 (0.88-1.08, p=0.675)	0.96 (0.86-1.06, p=0.415)	1.00 (0.91-1.12, p=0.977)	1.04 (0.94-1.16, p=0.462)
Year of diagnosis							
2009-2011	7,425 (30%)	1,005 (46%)	Ref	Ref	Ref	Ref	Ref
2012-2016	17,237 (70%)	1,163 (54%)	1.16 (1.06-1.27, p=0.003)	1.15 (1.05-1.27)	1.24 (1.13-1.36)	1.19 (1.08-1.31)	1.19 (1.08-1.31)
TNM stage							
I	10,081 (41%)	167 (8%)	Ref		Ref	Ref	Ref
II	9,824 (40%)	622 (29%)	3.92 (3.30-4.65)		2.52 (2.12-3.00)	2.54 (2.12-3.03)	2.51 (2.10-3.00)
III	3,323 (13%)	631 (29%)	12.33 (10.40-14.62)		7.52 (6.31-8.96)	7.30 (6.09-8.76)	7.26 (6.06-8.71)
IV	1,434 (6%)	748 (34%)	57.34 (48.46-67.85)		38.12 (32.00-45.41)	14.54 (11.91-17.76)	14.72 (12.05-17.97)
Screening							
Yes	8,359 (34%)	157 (7%)	Ref		Ref	Ref	Ref
No	16,303 (66%)	2,011 (93%)	7.27 (6.18-8.56)		2.58 (2.17-3.06)	2.16 (1.81-2.58)	2.13 (1.78-2.53)

Table 4.9 (continued) Traditional unadjusted and adjusted Cox models for the association of IHC defined molecular subtypes with breast cancer death (page 2 of 2)

	No women (%)	No deaths (%)	Model 0- Unadjusted	Model 1	Model 2	Model 3	Model 4
Surgery							
Yes	22,178 (90%)	1,212 (56%)	Ref			Ref	Ref
No	2,484 (10%)	956 (44%)	13.92 (12.76-15.19)			5.06 (4.43-5.76)	4.98 (4.37-5.68)
Radiotherapy							
Yes	17,726 (72%)	1,173 (54%)	Ref			Ref	
No	6,936 (28%)	995 (46%)	2.48 (2.27-2.69)			1.06 (0.96-1.17, p=0.215)	1.05 (0.95-1.16, p=0.310)
Chemotherapy							
Yes	9,261 (38%)	1,026 (47%)	Ref			Ref	Ref
No	15,401 (62%)	1,142 (53%)	0.71 (0.65-0.77)			1.28 (1.14-1.44)	1.25 (1.11-1.40)
Hormone therapy							
Yes	19,475 (79%)	1,300 (60%)	Ref			Ref	Ref
No	5,187 (21%)	868 (40%)	2.76 (2.54-3.01)			1.84 (1.59-2.14)	1.85 (1.59-2.14)
SIMD quintile							
Least deprived	5,260 (21%)	399 (18%)	Ref				Ref
4	5,277 (21%)	393 (18%)	0.98 (0.85-1.13, p=0.792)				0.96 (0.83-1.10, p=0.536)
3	5,126 (21%)	453 (21%)	1.19 (1.04-1.37, p=0.010)				1.11 (0.97-1.27, p=0.136)
2	4,784 (20%)	478 (22%)	1.39 (1.22-1.59)				1.16 (1.01-1.32, p=0.035)
Most deprived	4,215 (17%)	445 (21%)	1.49 (1.30-1.71)				1.29 (1.12-1.48)
Charlson Score							
Mean (SD)	0.0 (0.3)	0.1 (0.3)	1.51 (1.34-1.70)				1.23 (1.08-1.40)

Footnote: Model 1 was adjusted for age, incidence year and NHS region. Model 2: model 1 + tumour characteristics (grade, TNM stage and method of detection. Model 3= model 2 + treatments (surgery, radiotherapy, chemotherapy and hormone therapy). Model 4= model 3 + SIMD and Charlson score index. Models carried out in the complete case dataset with n=24,662 women, number of BC deaths=2,168. All HRs were statistically significant at the 0.1% level unless stated otherwise. IHC= immunohistochemistry, NHS= National Health Service, Ref= reference category, SD= standard deviation, SIMD= Scottish Index of Multiple Deprivation, TNM= tumour, nodes, metastases.

4.4.4 Association of other important prognostic factors with breast cancer survival within each subtype

Table 4.10 presents the effect of those covariates in BCD in women with ER+ and ER- tumours separately from multivariate Cox regression analysis.

Table 4.10 Unadjusted and adjusted Cox models results for breast cancer specific mortality stratified by ER status (page 1 of 2)

Characteristic	ER+ no. cases=42,146 no. failures=5,238		ER- no. cases=9,105 no. failures=2,378	
	Unadjusted HR (95% CI)	**Adjusted HR (95% CI)**	**Unadjusted HR (95% CI)**	**Adjusted HR (95% CI)**
Age				
<50 years	1.43 (1.33-1.53)	0.89 (0.82-0.96, p=0.002)	1.07 (0.96-1.18, p=0.212)	0.92 (0.83-1.02, p=0.130)
50-69 years	Ref	Ref	Ref	Ref
70 years or older	2.35 (2.21-2.50)	1.34 (1.24-1.45)	2.09 (1.90-2.29)	1.30 (1.16-1.46)
NHS Scottish region				
West	Ref	Ref	Ref	Ref
North	1.18 (1.11-1.26)	1.11 (1.03-1.19, p=0.005)	1.25 (1.14-1.37)	1.13 (1.02-1.25, p=0.014)
South East	1.13 (1.87-1.20)	1.00 (0.93-1.07, p=0.948)	1.14 (1.03-1.26, p=0.013)	1.04 (0.93-1.15, p=0.493)
Year of diagnosis				
1997-2001	Ref	Ref	Ref	Ref
2002-2006	0.86 (0.80-0.92)	0.80 (0.74-0.85)	0.89 (0.80-0.99, p=0.026)	0.83 (0.75-0.92)
2007-2011	0.71 (0.66-0.77)	0.69 (0.64-0.74)	0.74 (0.67-0.83)	0.71 (0.63-0.79)
2012-2016	0.76 (0.69-0.85)	0.73 (0.66-0.81)	0.82 (0.72-0.93, p=0.003)	0.83 (0.73-0.94, p=0.004)
Grade				
Grade I-(Well) differentiated	Ref	Ref	Ref	Ref
Grade II- Moderately (well) differentiated	2.65 (2.37-2.98)	1.80 (1.61-2.02)	3.86 (2.22-6.70)	3.08 (1.77-5.37)
Poorly differentiated	5.61 (5.01-6.28)	3.14 (2.79-3.53)	4.55 (2.63-7.84)	4.03 (2.33-6.98)
TNM stage				
I	Ref	Ref	Ref	Ref
II	3.26 (2.99-3.56)	2.20 (2.00-2.42)	2.10 (1.84-2.41)	1.88 (1.63-2.16)
III	9.97 (9.12-10.89)	5.51 (4.99-6.08)	7.20 (6.29-8.24)	6.03 (5.23-6.96)
IV	35.09 (31.65-38.90)	10.66 (9.41-12.08)	18.90 (16.03-22.29)	10.58 (8.78-12.75)
Screening				
Yes	Ref	Ref	Ref	Ref
No	3.87 (3.56-4.20)	1.66 (1.51-1.82)	2.66 (2.30-3.07)	1.60 (1.37-1.86)

Table 4.10 (continued) Unadjusted and adjusted Cox models results for breast cancer specific mortality stratified by ER status (page 2 of 2)

Characteristic	ER+ no. cases=42,146 no. failures=5,238		ER- no. cases=9,105 no. failures=2,378	
	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Surgery				
Yes	Ref	Ref	Ref	Ref
No	12.17 (11.36-13.05)	4.55 (4.14-5.00)	13.53 (11.95-15.31)	4.41 (3.81-5.10)
Radiotherapy				
Yes	Ref	Ref	Ref	Ref
No	1.27 (1.20-1.35)	0.97 (0.91-1.03, p=0.320)	1.15 (1.05-1.25, p=0.001)	1.19 (1.09-1.30)
Chemotherapy				
Yes	Ref	Ref	Ref	Ref
No	0.55 (0.52-0.58)	0.87 (0.81-0.94)	1.18 (1.08-1.28)	1.28 (1.14-1.43)
Hormone therapy				
Yes	Ref	Ref		
No	1.44 (1.31-1.57)	1.50 (1.36-1.64)		
SIMD quintile				
Least deprived	Ref	Ref	Ref	Ref
4	1.02 (0.94-1.11, p=0.607)	1.03 (0.95-1.12, p=0.507)	1.12 (0.98-1.28, p=0.0j86)	1.04 (0.91-1.18, p=0.583)
3	1.12 (1.03-1.21, p=0.010)	1.07 (0.99-1.17, p=0.100)	1.29 (1.13-1.47, p<0.001)	1.21 (1.07-1.38, p=0.003)
2	1.24 (1.14-1.35)	1.18 (1.08-1.28)	1.28 (1.13-1.46)	1.23 (1.08-1.41, p=0.002)
Most deprived	1.30 (1.19-1.42)	1.26 (1.15-1.38)	1.24 (1.08-1.41)	1.20 (1.04-1.38)
Charlson Score				
Mean (SD)	1.27 (1.14-1.39)	1.17 (1.06-1.30, p=0.003)	1.45 (1.28-1.64)	1.24 (1.09-1.40, p=0.001)

Footnote: Adjusted model includes age, incidence year, NHS region, grade, TNM stage, method of detection, surgery, radiotherapy, chemotherapy, hormone therapy (only for ER+ model), SIMD and Charlson score index. Models carried out in the complete case dataset separately by ER status. All HRs were statistically significant at the 0.1% level unless stated otherwise. CI= confidence interval, ER= oestrogen receptor, HR= hazard ratio, NHS= National Health Service, Ref= reference category, SD= standard deviation, SIMD= Scottish Index of Multiple Deprivation, TNM= tumour, nodes, metastases.

Results from the Cox models stratified by ER status (Table 4.10) and by the IHC defined molecular subtypes (Table 4.12), with follow-up times for each subtype presented in Table 4.11, showed that age, grade, stage, detection by screening and surgery were the most important prognostic factors irrespective of tumour subtype.

Table 4.11 Median (IQR) follow-up times for all women and for women with each subtype diagnosed in Scotland

	Median (IQR) follow-up time in years	Diagnosis years
ER status		1997-2016
ER+	5.8 (2.7, 10.6)	
ER-	4.6 (1.8, 10.2)	
IHC defined subtype		2009-2016
Luminal A	3.2 (1.5, 5.1)	
Luminal B	3.3 (1.6, 5.3)	
HER2-enriched	2.8 (1.2, 4.9)	
Triple Negative	2.6 (1.2, 4.6)	
All women	5.5 (2.3, 10.5)	1997-2016

ER= oestrogen receptor, IHC= immunohistochemistry, IQR= interquartile range, HER2= human epidermal growth factor 2.

Table 4.12 Unadjusted and adjusted Cox models results for breast cancer specific mortality stratified by molecular subtype in women diagnosed in Scotland from 2009 to 2016 (page 1 of 2)

Characteristic		Luminal A no. cases=13,755 no. failures=723		Luminal B no. cases=7,132 no. failures=753		HER2-enriched no. cases=1,034 no. failures=147		Triple Negative no. cases=2,519 no. failures=453	
		Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Age									
<50 years		1.42 (1.11-1.81, p=0.005)	0.66 (0.51-0.86, p=0.002)	1.21 (1.01-1.46, p=0.038)	1.10 (0.90-1.34, p=0.353)	0.88 (0.55-1.42, p=0.606)	0.64 (0.39-1.06, p=0.085)	1.24 (0.98-1.56, p=0.067)	1.07 (0.84-1.37, p=0.574)
50-69 years		Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
70 years or older		4.60 (3.91-5.42)	1.49 (1.23-1.80)	2.62 (2.22-3.09)	1.39 (1.14-1.69, p=0.001)	2.86 (2.01-4.09)	0.72 (0.45-1.16, p=0.181)	2.32 (1.87-2.88)	1.49 (1.15-1.94, p=0.003)
NHS Scottish region									
West		Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
North		0.86 (0.72-1.02, p=0.087)	1.13 (0.95-1.35, p=0.178)	1.20 (1.01-1.42, p=0.038)	1.37 (1.14-1.63, p=0.001)	2.15 (1.44-3.22, p<0.001)	1.98 (1.29-3.05, p=0.002)	1.26 (1.01-1.58, p=0.038)	0.95 (0.74-1.20, p=0.649)
South East		0.72 (0.60-0.86)	0.89 (0.74-1.07, p=0.230)	1.14 (0.95-1.35, p=0.150)	1.10 (0.92-1.32, p=0.283)	2.03 (1.35-3.05, p=0.001)	1.96 (1.28-3.02, p=0.002)	1.26 (1.01-1.58, p=0.041)	0.96 (0.75-1.22, p=0.730)
Year of diagnosis		1.07 (1.02-1.12, p=0.005)	1.05 (1.01-1.10, p=0.026)	1.08 (1.03-1.13, p=0.001)	1.07 (1.30-2.25, p=0.002)	1.08 (0.99-1.19, p=0.100)	1.03 (0.93-1.14, p=0.310)	1.07 (1.01-1.13, p=0.015)	1.09 (1.04-1.15, p=0.001)
Grade									
Grade I-(Well) differentiated						-	-	0.22 (0.03-1.56, p=0.129)	0.41 (0.06-3.02, p=0.385)
Grade II- Moderately (well) differentiated						Ref	Ref	Ref	Ref
Poorly differentiated						1.76 (1.08-2.84, p=0.022)	2.52 (1.48-4.28, p=0.001)	1.37 (1.03-1.82, p=0.030)	1.47 (1.10-1.97, p=0.009)
TNM stage	I	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
	II	3.86 (2.89-5.17)	2.05 (1.51-2.79)	3.47 (2.53-4.75)	3.30 (2.39-4.57)	2.22 (1.11-4.46, p=0.025)	2.40 (1.17-4.96, p=0.018)	2.36 (1.71-3.25)	1.87 (1.34-2.60)
	III	12.51 (9.31-16.80)	4.77 (3.46-6.59)	8.97 (6.55-12.30)	8.03 (5.76-11.20)	7.01 (3.58-13.70)	8.71 (4.22-17.99)	9.60 (6.96-13.26)	7.61 (5.43-10.67)
	IV	81.31 (62.13-106.43)	10.74 (7.78-14.81)	48.74 (35.41-67.08)	17.94 (12.32-26.12)	28.76 (14.35-57.64)	19.44 (8.24-45.86)	45.50 (31.57-65.55)	15.95 (10.25-24.82)
Screening	Yes	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
	No	11.18 (8.39-14.91)	2.69 (1.95-3.71)	3.80 (2.94-4.91)	1.71 (1.30-2.25)	3.24 (1.83-5.72)	1.80 (0.98-3.32, p=0.058)	4.18 (2.83-6.17)	2.34 (1.55-3.52)

Table 4.12 (continued) Unadjusted and adjusted Cox modes results for breast cancer specific mortality stratified by molecular subtype in women diagnosed in Scotland from 2009 to 2016 (page 2 of 2)

Characteristic	Luminal A no. cases=13,755 no. failures=723		Luminal B no. cases=7,132 no. failures=753		HER2-enriched no. cases=1,034 no. failures=147		Triple Negative no. cases=2,519 no. failures=453	
	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Surgery								
Yes	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
No	32.92 (27.95-38.77)	8.83 (6.97-11.20)	14.17 (12.14-16.55)	3.68 (2.92-4.63)	19.67 (13.56-28.53)	9.31 (5.47-15.85)	30.17 (22.81-39.91)	6.12 (4.24-8.83)
Radiotherapy								
Yes	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
No	4.12 (3.55-4.77)	0.82 (0.68-0.98, p=0.027)	1.88 (1.63-2.18)	0.97 (0.82-1.15, p=0.745)	1.89 (1.37-2.61)	1.49 (1.04-2.14, p=0.029)	1.70 (1.40-2.07)	1.28 (1.04-1.59, p=0.020)
Chemotherapy								
Yes	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
No	0.64 (0.55-0.76)	0.72 (0.59-0.89, p=0.002)	1.39 (1.20-1.60)	1.57 (1.30-1.91)	3.08 (2.22-4.27)	5.66 (3.50-9.15)	1.16 (0.95-1.40, p=0.144)	1.17 (0.91-1.52, p=0.222)
Hormone therapy								
Yes	Ref	Ref	Ref	Ref				
No	1.95 (1.55-2.47)	2.28 (1.78-2.94)	1.90 (1.57-2.30)	1.79 (1.46-2.19)				
SIMD quintile								
Least deprived	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
4	0.90 (0.70-1.14, p=0.374)	0.99 (0.77-1.26, p=0.915)	1.00 (0.79-1.26, p=0.979)	0.91 (0.72-1.15, p=0.444)	1.10 (0.64-1.89, p=0.736)	1.22 (0.69-2.15, p=0.504)	0.87 (0.64-1.18, p=0.368)	1.00 (0.74-1.37, p=0.976)
3	1.09 (0.86-1.38, p=0.473)	0.98 (0.77-1.24, p=0.872)	1.19 (0.95-1.49, p=0.129)	1.13 (0.90-1.42, p=0.274)	1.52 (0.90-2.56, p=0.118)	1.33 (0.77-2.29, p=0.314)	1.16 (0.87-1.55, p=0.319)	1.31 (0.97-1.76, p=0.077)
2	1.47 (1.17-1.84, p=0.001)	1.23 (0.98-1.55, p=0.072)	1.28 (1.02-1.60, p=0.033)	1.13 (0.90-1.41, p=0.305)	1.27 (0.74-2.18, p=0.377)	1.35 (0.78-2.34, p=0.283)	1.19 (0.89-1.60, p=0.239)	1.25 (0.93-1.69, p=0.136)
Most deprived	1.60 (1.27-2.01)	1.39 (1.10-1.76, p=0.006)	1.30 (1.03-1.63, p=0.028)	1.20 (0.95-1.52, p=0.128)	1.65 (0.97-2.81, p=0.066)	2.05 (1.17-3.61, p=0.013)	1.13 (0.84-1.52, p=0.427)	1.10 (0.80-1.49, p=0.560)
Charlson Score								
Mean (SD)	1.41 (1.13-1.76)	1.16 (0.90-1.49)	1.57 (1.30-1.91)	1.28 (1.03-1.58)	2.29 (1.46-3.60)	2.57 (1.47-4.51)	1.33 (1.01-1.75)	1.30 (0.99-1.71)

Footnote: Adjusted model includes age, incidence year, NHS region, grade (only in the HER2=enriched and TNBC models), TNM stage, method of detection, surgery, radiotherapy, chemotherapy, hormone therapy (only for luminal models), SIMD and Charlson score index. Models carried out in the complete case dataset separately by molecular subtype. All HRs were statistically significant at the 0.1% level unless stated otherwise. CI= confidence interval, HR= hazard ratio, NHS= National Health Service, Ref= reference category, SD= standard deviation, SIMD= Scottish Index of Multiple Deprivation, TNM= tumour, nodes, metastases.

Age

Older age was associated with higher BCD for all subtypes. Women older than 70 years had higher BC-specific mortality when compared to women aged 50 to 69 years for all ER status and tumour subtypes except for HER2-enriched tumours (HR=0.72, 95% CI: 0.45 to 1.16). BC specific mortality differed between the subtypes for women younger than 50 years. After adjustment for all other covariates only ER+ and luminal A tumours had lower risk of BC mortality when compared to the same subtype in women aged 50 to 69 years. HER2-enriched subtypes in women younger than 50 years also showed a lower risk of BC mortality but HR did not reach statistical significance. Year of diagnosis was associated with BC death, with women diagnosed in more recent years, between 2012-2016, having a lower BC-specific mortality than those diagnosed between 1997 and 2001 [HR=0.73 for ER+ tumours and 0.83 for ER- tumours] independent of follow-up time.

Other tumour characteristics

Grade III- poorly differentiated tumours were associated with higher BC-specific mortality than lower grade tumours and the association was stronger for those subtypes that did not express ER (HR=4.03, 95% CI: 2.33 to 6.98 for ER- tumours) and those that overexpressed HER2 (HR=2.52, 95% CI: 1.48 to 4.28). Higher stage was associated with poorer BCSS than for lower stage for all subtypes. Women with stage IV tumours had the highest BC-specific mortality that ranged from 10 times (95% CI: 7.8 to 14.8 for luminal A tumours) to 19 times (95% CI: 8.2 to 45.9 for HER2-enriched tumours) the mortality for a stage I tumour of the same subtype.

Screen detection showed a consistent inverse association with death across all subtypes with women with tumours that were not diagnosed through screening being more likely to die from BC (HR=1.66, 95% CI: 1.51 to 1.82 for ER+ tumours and HR=1.60, 95% CI: 1.37 to 1.86 for ER- tumours). Amongst IHC defined molecular subtypes luminal A tumours and TNBC that were not screen detected had the highest increased mortality, HR=2.69 (95% CI: 1.95 to 3.71) and 2.34 (95% CI: 1.55 to 3.52) respectively, compared to screen detected tumours of the same subtype.

Treatments

Women who did not receive surgery had an increased BC-specific mortality compared to those who received surgery that was similar for ER+ and ER- subtypes (HR=4.55, 95% CI: 4.14 to 5.00 for ER+ and HR=4.41, 95% CI: 3.81 to 5.10 for ER-). Surgery had a greater beneficial effect in luminal A and HER2-enriched tumours with women who did not receive surgery having an increase mortality of 8.83 (95% CI: 7.0 to 11.2) and 9.3 (95% CI: 5.5 to 15.9) times the mortality for those who had surgery.

The benefit of radiotherapy in women with ER+ tumours and women with luminal A tumours was only observed in the unadjusted models but was attenuated after adjustment for all other covariates. However, in women with more aggressive subtypes, such as, HER2-enriched and TNBC those that did not receive radiotherapy had an HR of 1.49 (95% CI: 1.04 to 2.14) and 1.28 (95% CI: 1.04 to 1.59) respectively compared to those who had radiotherapy.

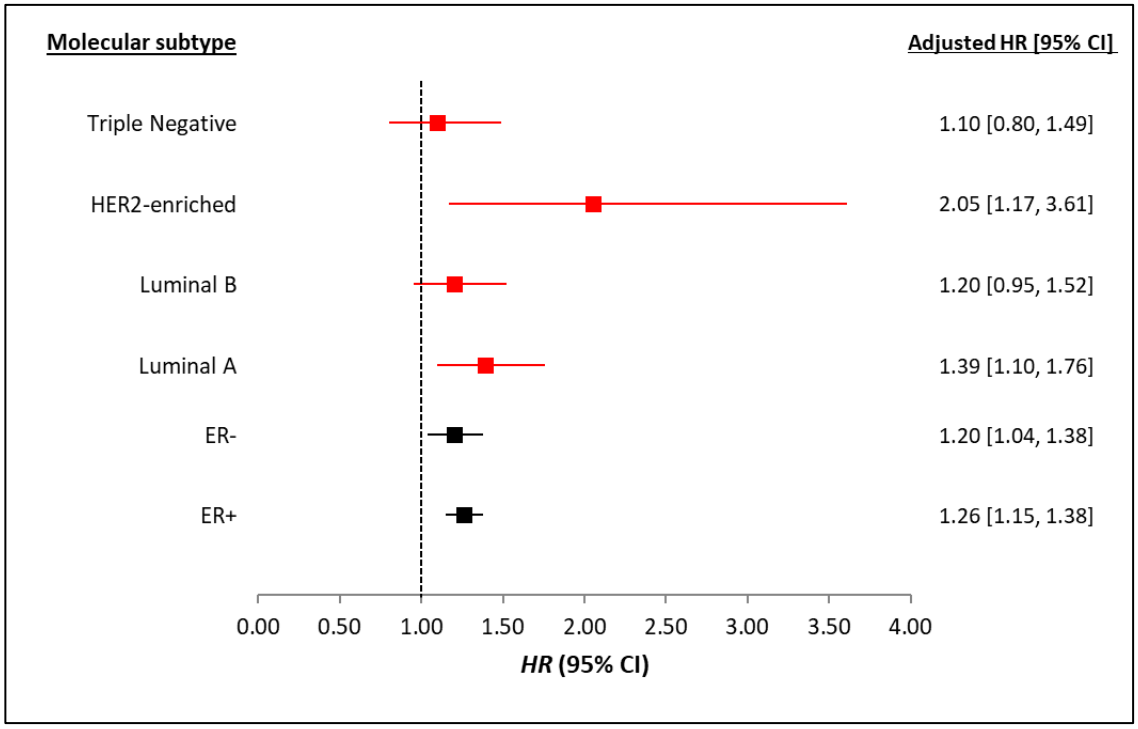
The effect of chemotherapy in BCD also differ between subtypes. Women with ER-, luminal B and HER2-enriched tumours who did not have chemotherapy had increased mortality (HR=1.28, 95% CI: 1.14 to 1.43 for ER-, HR=1.57, 95% CI: 1.30 to 1.91 for luminal B and HR=5.66, 95% CI: 3.50 to 9.15 for HER2-enriched) compared to those who had chemotherapy. In contrast, chemotherapy had the opposite effect on women with ER+ and luminal A tumours and women who did not receive this treatment were less likely to die from BC (HR=0.87, 95% CI: 0.81 to 0.94 for ER+ and HR=0.72, 95% CI: 0.59 to 0.89 for luminal A).

HT was an important factor for those women with hormone sensitive tumours. Women with ER+, luminal A and luminal B tumours who did not receive hormone therapy had higher BC-specific mortality compared to those who received HT. (HR=1.5, 95% CI: 1.36 to 1.64 for ER+, HR=2.28, 95% CI: 1.78 to 2.94 for luminal A and HR=1.79, 95% CI: 1.46 to 2.19 for luminal B).

Deprivation and comorbidities

Deprivation and comorbidities were also associated with BCD amongst all subtypes. Women in the most deprived areas of Scotland were more likely to die from BC compared to those in the least deprived areas of Scotland (Figure 4.6).

Figure 4.6 Hazard ratio (with 95% CI) for the association of women in the most deprived fifth of areas of Scotland with risk of breast cancer death compared to women in the least deprived fifth of areas of Scotland separately by ER status and IHC defined molecular subtypes



Footnote: Fully adjusted model includes age, incidence year, NHS region, tumour characteristics (TNM stage and method of detection, treatments (surgery, radiotherapy, chemotherapy and hormone therapy), SIMD and Charlson comorbidity index. Models carried out separately by subtype with n=42,146 for ER+, n=9,105 for ER-, n=13,755 for luminal A, n=7,132 for luminal B, n=1,034 for HER2-enriched and n=2,519 for Triple Negative. CI= confidence interval, HR= hazard ratio.

The increased mortality associated with being in the most deprived compared to the least deprived areas was highest amongst women with HER2-enriched tumours for whom the risk was doubled. Women in the most deprived areas with ER+, ER- and luminal A tumours also had higher mortality compared to women with the same subtype in the least deprived areas that ranged from a 20% increase for ER- to 40% increase for luminal A tumours. For women with luminal B and TNBC in the most deprived areas there was a suggestion of a negative effect of deprivation in survival but the association was no longer significant after adjustment for other tumour characteristics.

Sensitivity analysis

Results for the comparison of traditional Cox models with TVE models that adjust for non-proportional hazards are presented in Appendix C.6 for ER+ and ER- tumours and in Appendix C.7 for the luminal tumours. The PH assumption for the stratified models for HER2-enriched and TNBC held. The effect of screening, stage, grade, radiotherapy, chemotherapy and HT in the hazards of BCD decreased over time for ER+ tumours. TVE models showed a more plausible results for chemotherapy and radiotherapy that were found to have a beneficial effect on survival that was restricted to early years, compared to the opposite effect suggested by the traditional Cox model.

4.4.5 Missing data patterns and multiple imputation results

Patterns of missing data are reported in Table 3.1. Proportions of missing data were particularly high for tumour characteristics such as, grade and T, N and M pathological and clinical stages. However, the composite TNM stage variable was derived as described in Appendix B.2 using other tumour characteristics such us, tumour size and nodal status that considerably reduced the amount of missing data (from 50 to 12%). The average number of missing variables per woman was low (0.5) but almost 30% of all women included in the survival analyses had at least one missing covariate (Table 4.13).

Table 4.13 Number of missing variables for each woman in the survival dataset

Number of missing variables	Frequency	Percentage
0	51,140	71.2%
1	12,263	17.1%
2	4,601	6.4%
3	2,270	3.2%
4	562	0.8%
5	270	0.4%
6	249	0.4%
7	268	0.4%
8	161	0.2%

The correlation of missingness between covariates is presented in Table 4.14. The highest correlation was observed amongst the treatment variables, for example between chemotherapy and radiotherapy (0.56). Correlation of missingness between tumour characteristics was also high, especially between ER status, grade and stage.

Table 4.14 Correlation of missingness between breast cancer prognostic variables

	ER status	Grade	Stage	Screening	Surgery	Radio	Chemo
Grade	0.41						
TNM stage	0.34	0.35					
Screening	0.23	0.14	0.19				
Surgery	0.18	0.14	0.19	0.28			
Radio	0.18	0.09	0.14	0.40	0.34		
Chemo	0.18	0.10	0.14	0.39	0.43	0.56	
HT	0.17	0.07	0.13	0.37	0.27	0.51	0.43

Chemo= chemotherapy, HT=hormone therapy, Radio=radiotherapy, TNM= tumour, nodes, metastases.

Descriptive characteristics of the women with complete and incomplete data (Table 4.15) were compared to investigate whether data were missing completely at random (MCAR). Differences were observed for most characteristics, except for ER status. The percentage of women with incomplete data (incomplete cases) was higher in women older than 70 years, women living in the West of Scotland, women living in the most deprived areas, women diagnosed in earlier years, women with stage III and IV tumours, not diagnosed through screening and not having surgery, radiation, chemotherapy or HT than in complete cases. Incomplete cases also had a slightly higher mean comorbidity index (0.1 vs 0.0).

Table 4.15 Descriptive characteristics of women with complete and incomplete data

Characteristic		Complete data (N=51,140)	Incomplete data (N=20,644)
Age in years	<50 years	10,886 (21%)	3,493 (17%)
	50-69 years	27,869 (55%)	7,723 (37%)
	70 years or older	12,385 (24%)	9,428 (46%)
NHS Scottish region	North	14,545 (28%)	4,047 (20%)
	South East	15,857 (31%)	3,976 (19%)
	West	20,738 (41%)	12,621 (61%)
Year of diagnosis			
	1997-2001	10,390 (20%)	5,914 (29%)
	2002-2006	11,077 (22%)	6,212 (30%)
	2007-2011	13,302 (26%)	5,377 (26%)
	2012-2016	16,371 (32%)	3,141 (15%)
ER status			
	Negative	8,994 (18%)	2,704 (17%)
	Positive	42,146 (82%)	12,857 (83%)
Tumour grade			
	Grade I- (Well) differentiated	7,465 (15%)	1,219 (14%)
	Grade II- Moderately (well) differentiated	23,878 (47%)	4,099 (46%)
	Poorly differentiated	19,797 (39%)	3,688 (41%)
TNM stage			
	I	20,598 (40%)	3,511 (29%)
	II	20,722 (41%)	4,520 (37%)
	III	7,935 (15%)	2,399 (20%)
	IV	1,885 (4%)	1,726 (14%)
Diagnosed through screening?			
	No	34,913 (68%)	15,888 (83%)
	Yes	16,227 (32%)	3,286 (17%)
Surgery			
	No	2,797 (6%)	8,036 (40%)
	Yes	48,343 (94%)	12,018 (60%)
Radiation			
	No	15,890 (31%)	10,911 (63%)
	Yes	35,250 (69%)	6,494 (37%)
Chemotherapy			
	No	30,811 (60%)	12,717 (67%)
	Yes	20,329 (40%)	6,147 (33%)
Hormone therapy			
	No	11,004 (22%)	4,615 (28%)
	Yes	40,136 (79%)	11,786 (72%)
SIMD quintile			
	Least deprived	11,083 (22%)	3,806 (18%)
	4	11,121 (22%)	3,805 (18%)
	3	10,858 (21%)	4,000 (19%)
	2	9,862 (19%)	4,396 (21%)
	Most deprived	8,216 (16%)	4,637 (23%)
Charlson Comorbidity Index			
	Mean (SD)	0.0 (0.3)	0.1 (0.4)

ER= oestrogen receptor, NHS=National Health Service, SD= standard deviation, SIMD= Scottish Index of Multiple Deprivation, TNM=tumour, node, metastases.

The outcome was also different amongst women with incomplete data with 57% of them having died at the end of the study period compared to 28% of women with complete data (Table 4.16). Incomplete cases were also more likely to die from BC than those with complete data (31% vs 15%). Median follow-up amongst women who died during the study period was 4.7 years for women with incomplete data and 5.8 years for women with complete data. Missingness was clearly related to individual and tumour characteristics and worse outcome was associated with missingness in covariate data, for that reason, data were not MCAR.

Table 4.16 Outcomes and follow-up times for women with complete and incomplete data

	Complete case (N=51,140)	Incomplete case (N=20,644)	Total (N=71,784)
Vital status			
Alive	36,656 (72%)	8,848 (43%)	45,504 (63%)
Dead	14,484 (28%)	11,796 (57%)	26,280 (37%)
Breast cancer death			
No	43,548 (85%)	14,254 (69%)	57,802 (80.5%)
Yes	7,592 (15%)	6,390 (31%)	13,982 (19.5%)
Follow-up time (in years)			
Median	5.8	4.7	5.5
Q1, Q3	2.7, 10.6	1.6, 10.1	2.3, 10.5

Q1=quartile 1, Q3=quartile 3

The MAR assumption states that the missing patterns observed depend on the observed data but not on the unobserved data. The MAR assumption is an empiric concept and therefore, not testable [266]. Further, distinguishing between MAR and MNAR is not possible as it depends on non-measured data. Previous studies using BC registry data have used MI of missing data under the MAR assumption [267] successfully and sensitivity analysis assuming that data was MNAR has shown very similar results [168]. In my study, I assumed that a MAR mechanism was more plausible than MNAR as missingness seemed to be greatly explained by observed covariates. In order to adjust for MAR data which bias the results when using CCA, MI was performed and the comparison of the traditional

fully adjusted Cox model (model 4) with ER status as the main exposure for the CCA and the multiple imputation analysis (MIA) is presented in Table 4.17.

Table 4.17 Comparison of results from complete case analysis model and multiple imputation analysis model (page 1 of 2)

Characteristic		Complete case analysis N=51,140 HR (95%CI)	Multiple imputation N=71,714 HR (95%CI)
ER Status			
	Positive	Ref	Ref
	Negative	1.44 (1.33-1.56)	1.38 (1.29-1.48)
Age			
	<50 years	0.89 (0.84-0.95)	0.87 (0.83-0.92)
	50-69 years	Ref	Ref
	70 years or older	1.36 (1.27-1.45)	1.25 (1.19-1.32)
NHS Scottish region			
	West	Ref	Ref
	North	1.12 (1.06-1.19)	1.11 (1.06-1.16)
	South East	1.01 (0.96-1.07, p=0.665)	0.98 (0.94-1.02, p=0.665)
Year of diagnosis			
	1997-2001	Ref	Ref
	2002-2006	0.79 (0.75-0.84)	0.85 (0.82-0.89)
	2007-2011	0.68 (0.64-0.73)	0.70 (0.67-0.74)
	2012-2016	0.73 (0.67-0.79)	0.74 (0.69-0.78)
Grade			
	Grade I-(Well) differentiated	Ref	Ref
	Grade II- Moderately (well) differentiated	1.84 (1.64-2.06)	1.64. (1.48-1.81)
	Poorly differentiated	3.02 (2.70-3.39)	2.54 (2.30-2.81)
TNM stage			
	I	Ref	Ref
	II	2.11 (1.95-2.28)	2.21 (2.07-2.37)
	III	5.90 (5.44-6.40)	5.61 (5.23-6.03)
	IV	11.09 (10.00-12.30)	10.22 (9.37-11.16)
Screening			
	Yes	Ref	Ref
	No	1.62 (1.50-1.75)	1.71 (1.60-1.83)

Table 4.17 (continued) Comparison of results from complete case analysis model and multiple imputation analysis model (page 2 of 2)

Characteristic		Complete case analysis N=51,140 HR (95%CI)	Multiple imputation N=71,714 HR (95%CI)
Surgery			
	Yes	Ref	Ref
	No	4.01 (3.70-4.33)	3.85 (3.64-4.07)
Radiotherapy			
	Yes		
	No	1.03 (0.98-1.09, p=0.201)	0.96 (0.92-1.01, p=0.086)
Chemotherapy			
	Yes	Ref	Ref
	No	0.96 (0.90-1.02, p=0.218)	1.05 (0.99-1.10, p=0.094)
Hormone therapy			
	Yes	Ref	Ref
	No	1.31 (1.21-1.41)	1.49 (1.40-1.59)
SIMD quintile			
	Least deprived	Ref	Ref
	4	1.03 (0.96-1.11, p=0.371)	1.04 (0.98-1.11, p=0.371)
	3	1.12 (1.04-1.20, p=0.002)	1.12 (1.06-1.19, p=0.002)
	2	1.19 (1.11-1.28)	1.15 (1.08-1.21)
	Most deprived	1.24 (1.15-1.34)	1.20 (1.13-1.27)
Charlson Score			
	Mean (SD)	1.23 (1.13-1.33)	1.10 (1.04-1.16)

Both models are fully adjusted and include age, incidence year, NHS region, tumour characteristics (ER status, TNM stage and method of detection, treatments (surgery, radiotherapy, chemotherapy and hormone therapy), SIMD and Charlson comorbidity index. All HRs were statistically significant at the 0.1% level unless stated otherwise. CI= confidence interval, ER= oestrogen receptor, HR= Hazard Ratio, NHS=National Health Service, Ref= reference category, SD= standard deviation, SIMD= Scottish Index of Multiple Deprivation, TNM=tumour, node, metastases.

Estimates for the association of BCD with individual and tumour characteristics, were similar in the analysis based on imputed data to the CCA. In particular the association of the main exposure comparison (ER- vs ER+ tumours) with BCD was very similar in both the CCA and the MIA but slightly attenuated. For the rest of covariates estimates were slightly lower for age <50 years and age 70 years or older (compared to age 50 to 69 years), grades II and III (compared to grade I), stages III-IV (compared to stage I), not having surgery (compared to having surgery), not having radiotherapy (compared to having radiotherapy), living in the 40% most deprived areas (compared to living in the 20% least deprived areas) and having a higher Charlson comorbidity index, whereas estimates for being diagnosed in 2002 or after (compared to being diagnosed in 1997-

2001, stage II (compared to stage I) , having a screen detected tumour (compare to a non-screen detected tumours), not having chemotherapy (compared to having chemotherapy) and not having HT (compared to having HT) were slightly higher for the analysis with imputed data. This divergent effect in the HRs is likely reflecting that missingness is related to those characteristics for which the effect is attenuated but not so much to those characteristics for which the effect is strengthened. MI estimates were all in the same direction as those for CCA and imputation did not change the statistical significance of any covariate in the model. As expected from the larger sample size, CIs for the estimates based on imputed data were narrower than the CI based on CCA and they overlapped suggesting only minor differences in the estimates from CCA or MIA.

Comparison of results from CCA and MIA for the Cox model with IHC defined molecular subtypes as the main exposure can be found in Appendix C.8. The association of BCD with the molecular subtypes was also slightly attenuated in the analysis with imputed data showing higher BC-specific mortality for luminal B, HER2-enriched and TNBC subtypes compared to luminal A subtype. CI for the estimates were narrower and overlapped with those for the CCA.

4.4.6 Is breast cancer survival improving over time?

4.4.6.1 Descriptive characteristics by year of diagnosis

The percentage of breast tumours amongst women younger than 50 years remained constant over time at around 20% of all breast tumours diagnosed in Scotland (Table 4.18). BCs in women of screening age (50 to 69 years) accounted for a smaller proportion in earlier years, 47% in 1997 to 2001, than in more recent years, 52% in 2012-2016. The percentage of breast tumours that occurred in women of 70 years or older slightly declined over time.

Table 4.18 Description of the most important individual and tumour characteristics amongst women with breast cancer by year of diagnosis

Characteristic	1997-2001	2002-2006	2007-2011	2012-2016	Total
Age					
<50 years	3,324 (21)	3,543 (21)	3,739 (20)	3,773 (19)	14,379 (20)
50-69 years	7,741 (47)	8,319 (48)	9,473 (51)	10,059 (52)	35,592 (50)
70+ years	5,239 (32)	5,427 (31)	5,467 (29)	5,680 (29)	21,813 (30)
ER status					
Positive	10,978 (78)	13,116 (83)	14,912 (83)	15,997 (84)	55,003 (82)
Negative	3,037 (22)	2,665 (17)	2,966 (17)	3,030 (16)	11,698 (18)
Grade					
I- well differentiated	2,143 (19)	2,185 (15)	2,138 (13)	2,218 (13)	8,684 (14)
II- Moderately differentiated	4,790 (41)	6,580 (46)	7,932 (48)	8,675 (49)	27,977 (47)
III- Poorly differentiated	4,701 (40)	5,629 (39)	6,562 (39)	6,593 (38)	23,485 (39)
TNM stage					
I	5,187 (36)	5,178 (35)	6,353 (40)	7,391 (41)	24,109 (38)
II	5,560 (39)	6,275 (42)	6,223 (39)	7,184 (40)	25,242 (40)
III	2,958 (20)	2,669 (18)	2,365 (15)	2,342 (13)	10,334 (16)
IV	710 (5)	666 (5)	1,050 (6)	1,185 (6)	3,611 (6)

Values in the table are frequency (% by column) amongst women with known characteristics. ER= oestrogen receptor, TNM=tumour, node, metastases.

Tumour characteristics also changed with time (Table 4.18). Proportions of women diagnosed with an ER+ tumour increased and proportions of ER- tumours declined over time. The proportion of grade I and grade III tumours declined over time but the proportion of grade II tumours increased from 41% in 1997 to 2001 to 50% in 2012-2016. The proportion of early stage tumours (stage I) increased from 1997 to 2016, in contrast a considerable decline was observed for stage III tumours (from 21% in 1997 to 2001 to 13% in 2012-2016). Proportions of stage II and IV tumours remained approximately constant over the study period.

4.4.6.2 Breast cancer specific survival by combinations of age, ER and grade

BCSS estimates for the combination of ER status and grade for all women and by age groups are presented in Table 4.19.

Table 4.19 Breast cancer specific survival estimates at 5 and 10 years after diagnosis with 95% CI by ER status and grade (low or high) for all women and by age group

ER STATUS	GRADE	<50 YEARS	50-69 YEARS	70 YEARS OR OLDER	TOTAL
5-year BCSS (95% CI)					
ER+	Low	94.8 (94.1, 95.4)	96.1 (95.8, 96.4)	86.3 (85.4, 87.1)	93.4 (93.1, 93.7)
	High	84.4 (83.1, 85.6)	85.4 (84.4, 86.3)	72.4 (70.7, 74.1)	82.1 (81.4, 82.8)
ER-	Low	81.4 (76.8, 85.2)	83.1 (80.4, 85.5)	68.9 (64.4, 73.0)	78.8 (76.7, 80.8)
	High	75.3 (73.4, 77.1)	75.1 (73.6, 76.5)	56.6 (54.0, 59.2)	71.4 (7.30, 72.5)
10-year BCSS (95% CI)					
ER+	Low	88.3 (87.2, 89.3)	91.2 (90.6, 91.7)	77.2 (75.9, 78.4)	87.3 (86.9, 87.8)
	High	73.0 (71.3, 74.7)	75.7 (74.4, 77.0)	61.4 (59.2, 63.6)	71.7 (70.7, 72.6)
ER-	Low	72.7 (67.1, 77.4)	76.6 (73.3, 79.5)	61.7 (56.4, 66.4)	71.6 (69.1, 73.9)
	High	70.1 (68.0, 72.0)	70.8 (69.2, 72.4)	50.5 (47.6, 53.3)	66.5 (65.4, 67.7)

BCSS= breast cancer specific survival, ER=oestrogen receptor.

Women with ER+, low grade tumours had the best survival at 5 and 10 years after diagnosis and women with ER-, high grade tumours the worst survival of all the ER and grade combinations. There was a difference of over 20% in absolute survival between these two groups of women at both 5 and 10 years after diagnosis. Differences in BCSS between ER and grade combinations were consistent across age groups but women older than 70 years had considerably worse survival for all combinations than women in younger age groups. BCSS at 10 years still differed by ER and grade combinations but seem to be very similar for women aged less than 50 years for all combinations except ER+, low grade tumours which had better survival than other subgroups.

4.4.6.3 Breast cancer specific survival by combinations of age, ER and stage

BCSS by ER status and stage showed that ER+ stage I and II tumours had a survival over 90% at 5 years and so did ER- stage I tumours. However, BCSS at 5 years for stage III-IV tumours was considerably worse for both ER+ (65% at 5 years) and ER- (40% at 5 years) tumours. The same pattern was observed at 10 years after diagnosis with ER- stage III-IV tumours having the worst survival, with only 34% survival at 10 years.

Differences in BCSS between ER and stage combinations were consistent across age groups with ER+, stage I tumours having the best survival and ER-, stage III-IV tumours having the worst survival at both 5 and 10 years (Table 4.20). BCSS in women aged <50 years was very similar to that in women of screening age (50 to 69 years). Women of screening age had better survival if they had a low stage tumour irrespective of ER status than women younger than 50. The opposite was observed for more advanced stage tumours (ER+, stage III-IV tumours and ER-, stage II, III and IV) in women younger than 50 years who had better survival than women aged 50 to 69 years with the same ER and stage combination. Women aged 70 years or older, had considerably lower survival than women younger than 70 years of age.

Table 4.20 Breast cancer specific survival estimates at 5 and 10 years after diagnosis with 95% CI by ER status and TNM stage for all women and by age group

ER STATUS	STAGE	<50 YEARS	50-69 YEARS	70 YEARS OR OLDER	TOTAL
5-year BCSS (95% CI)					
ER+	I	97.9 (97.3, 98.4)	98.7 (98.5, 98.9)	94.6 (93.7, 95.4)	97.9 (97.6, 98.1)
	II	93.2 (92.3, 94.0)	94.0 (93.5, 94.6)	86.3 (85.2, 87.3)	91.7 (91.3, 92.2)
	III-IV	72.7 (70.6, 74.6)	69.6 (68.0, 71.1)	54.2 (52.4, 56.1)	64.9 (63.9, 65.9)
ER-	I	88.6 (85.8, 90.9)	92.5 (91.0, 93.8)	88.2 (84.4, 91.1)	90.9 (89.7, 92.0)
	II	83.9 (818, 85.8)	81.8 (79.9, 83.4)	67.6 (64.3, 70.7)	79.4 (78.1, 80.6)
	III-IV	46.6 (42.7, 50.5)	42.8 (39.8, 45.7)	28.7 (25.3, 32.2)	39.6 (37.6, 41.6)
10-year BCSS (95% CI)					
ER+	I	94.7 (93.6, 95.6)	96.4 (96.0, 96.8)	90.1 (88.7, 91.4)	95.1 (94.7, 95.5)
	II	84.6 (83.2, 85.9)	87.1 (86.1, 87.9)	75.7 (74.1, 77.3)	83.6 (82.9, 84.2)
	III-IV	58.1 (55.6, 60.4)	53.2 (51.3, 55.1)	39.0 (36.8, 41.2)	49.6 (48.4, 50.9)
ER-	I	85.4 (82.2, 88.1)	88.6 (86.6, 90.2)	82.0 (77.0, 86.0)	86.8 (85.3, 88.2)
	II	77.3 (74.8, 79.7)	76.7 (74.6, 78.6)	60.9 (57.2, 64.3)	73.5 (72.0, 74.9)
	III-IV	40.7 (36.7, 44.7)	37.4 (34.3, 40.5)	22.0 (18.6, 25.6)	33.9 (31.9, 35.9)

BCSS= breast cancer specific survival, ER=oestrogen receptor.

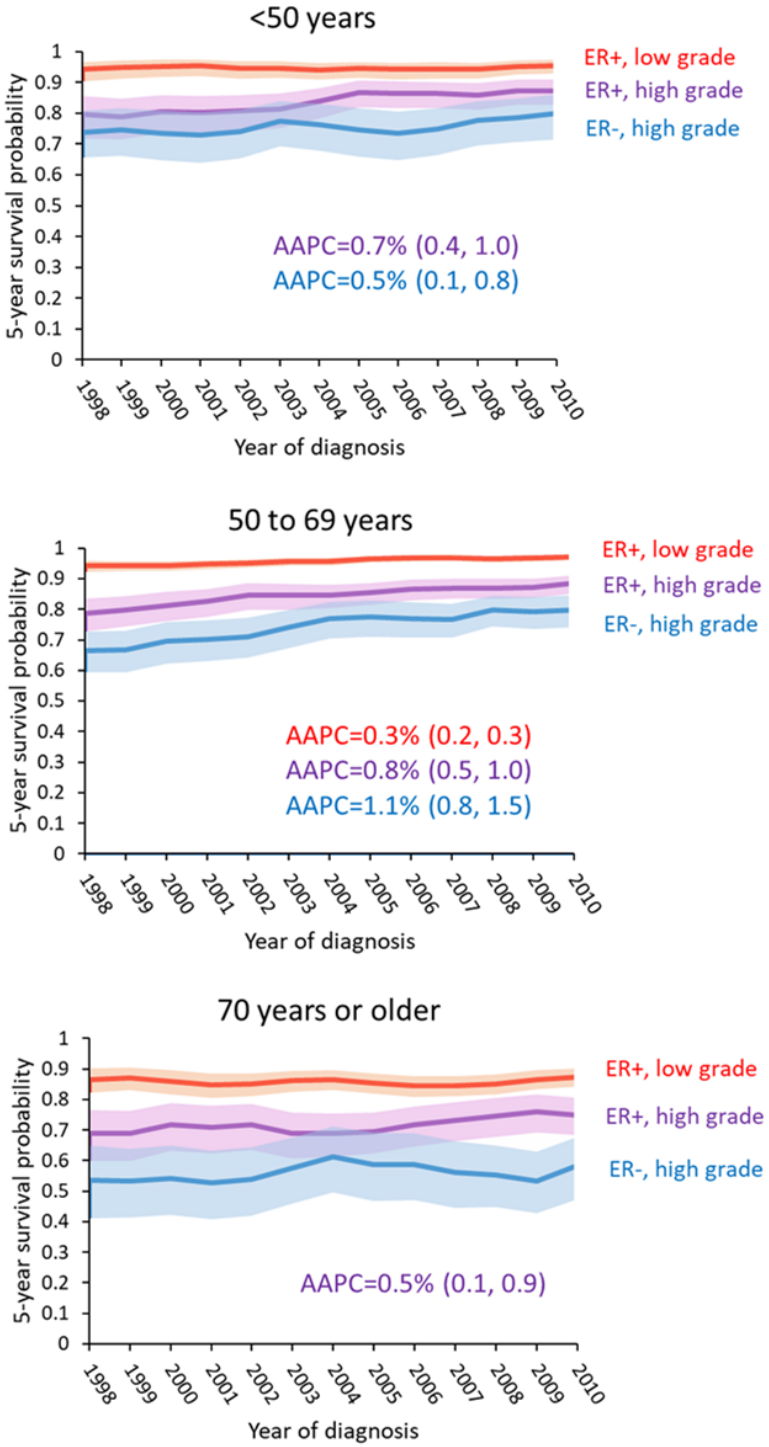
4.4.6.4 Comparison of breast cancer specific survival and overall survival

OS probabilities for ER, grade and ER, stage combinations (Appendix C.9) showed the same patterns as for BCSS but consistently lower estimates. The difference between OS and BCSS for all combinations increased with aged: younger women had very similar OS and BCSS irrespective of ER, grade and stage characteristics, while OS for women with 70 years or older was much lower than BCSS, especially for ER+ tumours with low grade or earlier stage.

4.4.6.5 Time trends in survival by important prognostic factors

Five-year BCSS probabilities increased over time for ER and grade combinations across age groups (Figure 4.7) and estimates from joinpoint regression (Appendix C.10) show that women aged 50 to 69 years had the greatest gains in survival over the study period. Improvements in survival in women aged 50 to 69 years were highest amongst women with high grade tumours and were more striking for ER- tumours (AAPC=1.1%, 95% CI: 0.8 to 1.5) than for ER+ tumours (AAPC=0.8%, 95% CI: 0.5 to 1.0). Women younger than 50 years had improved survival for ER+ high grade tumours (AAPC=0.7%, 95% CI: 0.4 to 1.0) but no consistent trend in improved survival was observed for ER+ low grade tumours. Survival in women younger than 50 years with ER- high grade tumours slightly improved during the study period by 0.5% increase per year (95% CI: 0.1 to 0.8). Five-year BCSS probabilities for women aged 70 years or older slightly increased in women with ER+ high grade tumours (AAPC=0.5%, 95% CI: 0.1 to 0.9) but were constant over time with no noticeable gains observed in women with ER+ low grade and ER- high grade tumours. Estimates of 5-year OS probabilities by ER and grade for the three age groups (Appendix C.11, left column) show the same survival trends patterns than those observed for BCSS but consistently lower survival, especially in women aged 70 years or older.

Figure 4.7 Trends in 5-year survival probabilities by ER and grade combinations for women < 50 years, women aged 50 to 69 years and women aged 70 years or older



Shaded area represents the 95% CI around the BCSS estimate. Year of diagnosis in the graphs represents the middle year for that three-year average. AAPC= average annual percent change, ER= oestrogen receptor.

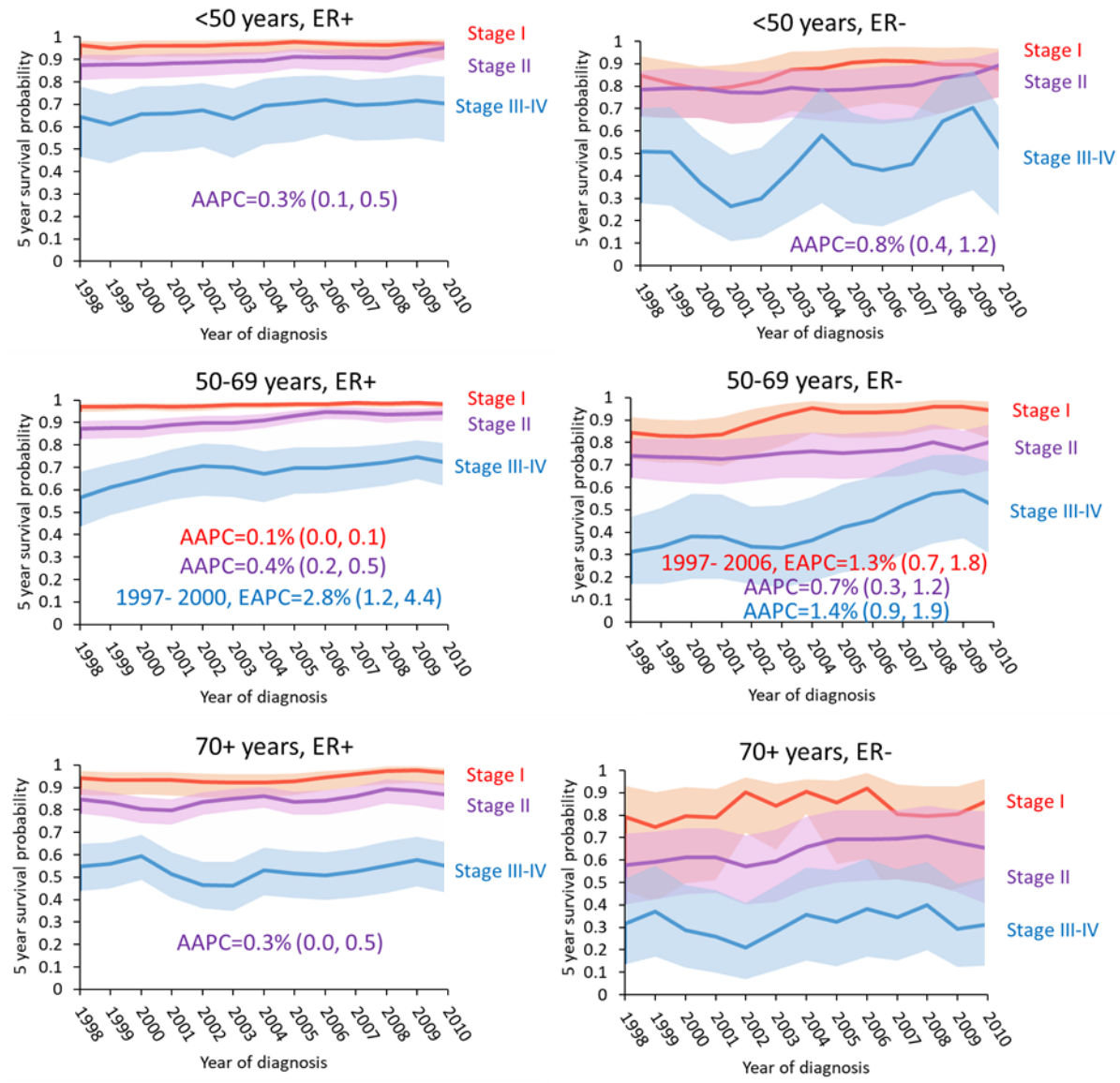
When looking at 5-year BCSS probabilities for the combinations of ER and stage (Figure 4.8), women aged 50 to 69 years old with advanced tumour stages (III-IV) had the highest improvements in survival that were particularly high for ER- tumours for which survival increased by 1.4% per year (95% CI: 0.9 to 1.9) over the study period. Survival probabilities in women aged 50 to 69 years with ER+ advanced stage tumours only improved between years 1997 and 2000, showing no significant trend after that time. Stage I and stage II tumours in women of 50 to 69 years also showed improvements in BCSS over time that were higher in ER- tumours than in ER+ tumours of the same stage, although the increasing trend observed for ER- stage I tumours was only seen from 1997 to 2006.

Survival probabilities amongst women younger than 50 years improved significantly for stage II tumours and were higher in ER- tumours (AAPC=0.8%, 95% CI: 0.4 to 1.2) than in ER+ tumours (AAPC=0.3%, 95% CI: 0.1 to 0.5). There were no statistically significant survival improvements for women aged less than 50 years with stage I or stages III-IV tumours over the study period.

Older women, aged 70 years or more, showed no consistent improvements between ER and stage combinations over the study period, except for stage II ER+ tumours that slightly increased (AAPC=0.3%, 95% CI: 0.0 to 0.5).

Five-year OS probabilities by ER and stage for the three age groups (Appendix C.12) showed the same survival trends patterns than those observed for BCSS but consistently lower survival, especially in women aged 70 years or older.

Figure 4.8 Trends in 5- year survival probabilities by ER status and stage combinations for the three age groups



Shaded area represents the 95% CI around the BCSS estimate. Year of diagnosis in the graphs represents the middle year for that three-year average. AAPC= average annual percent change, EAPC= estimated annual percentage change, ER= oestrogen receptor.

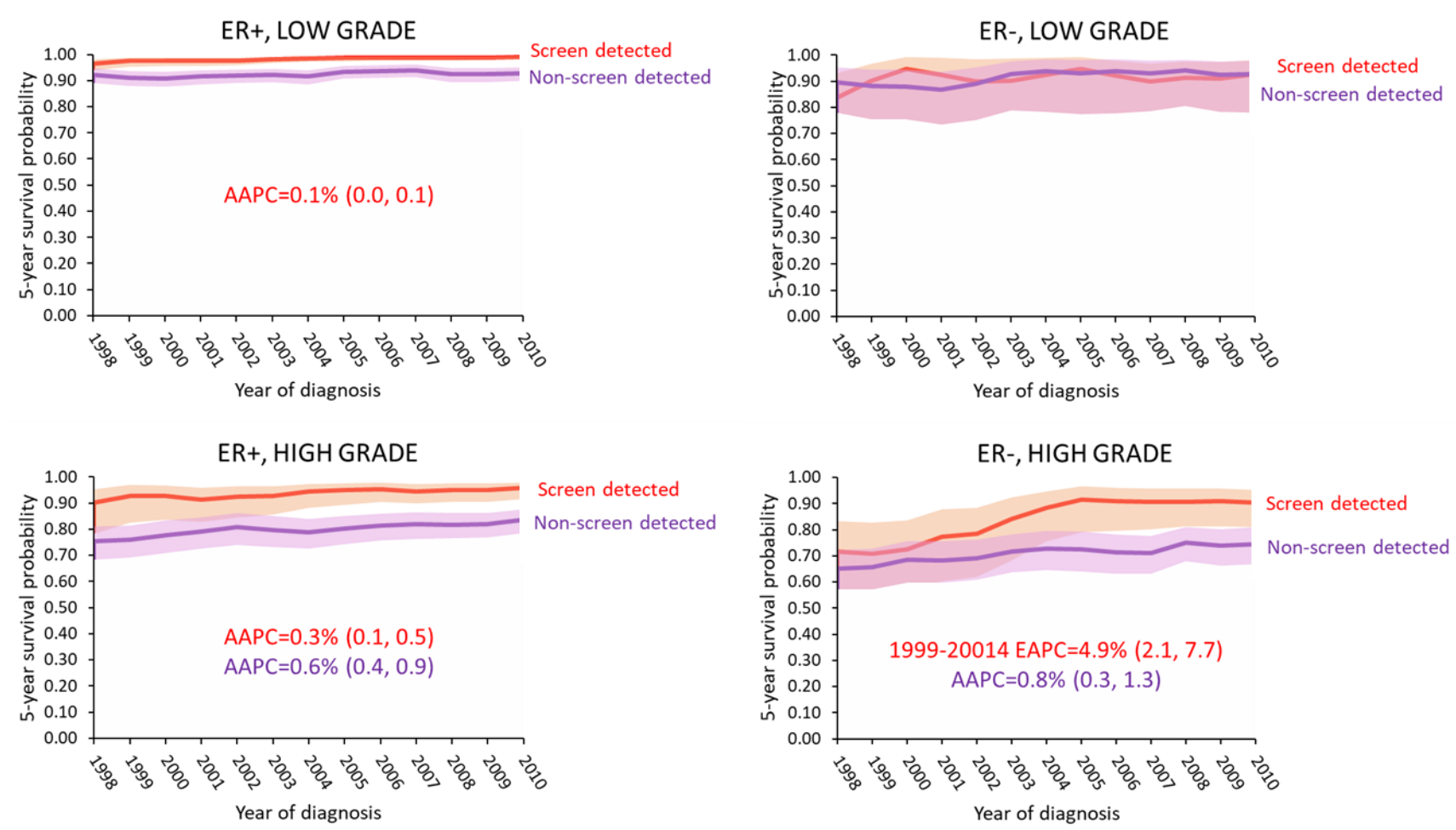
4.4.6.6 Was method of detection associated with improved survival?

BCSS improvements in women of screening age with high grade tumours (both ER+ and ER-) were observed regardless of method of detection (Figure 4.9). The increasing survival pattern for ER+, high grade tumours was slightly higher for tumours that were non-screen detected (AAPC=0.6%, 95% CI: 0.4 to 0.9) than for screen detected tumours (AAPC=0.3%, 95% CI: 0.1 to 0.5). ER- high grade screen detected tumours showed the greatest improvements in survival between 1999 and 2005 with an average 4.9% increase each year (95% CI: 2.1 to 7.7) and no further improvements after that time. BCSS of women with ER- high grade tumours that were not screen detected showed a steady consistent improvement trend over the study period (AAPC=0.8%, 95% CI: 0.3 to 1.3).

BCSS trends among women of screening age by ER and stage (Figure 4.10), showed improvements in women with ER+ earlier stage tumours (stage I and II) regardless of method of detection that were slightly higher for tumours that were not screen detected. Women with ER- tumours showed survival improvements for stage I, screen detected tumours between 1997 and 2004 (EAPC=1.8%, 95% CI: 0.9 to 2.8) and for stage II non-screen detected tumours over the study period (AAPC=0.6%, 95% CI: 0.1 to 1.1) and no statistically significant trend for stage I non-screen detected and stage II screen detected tumours.

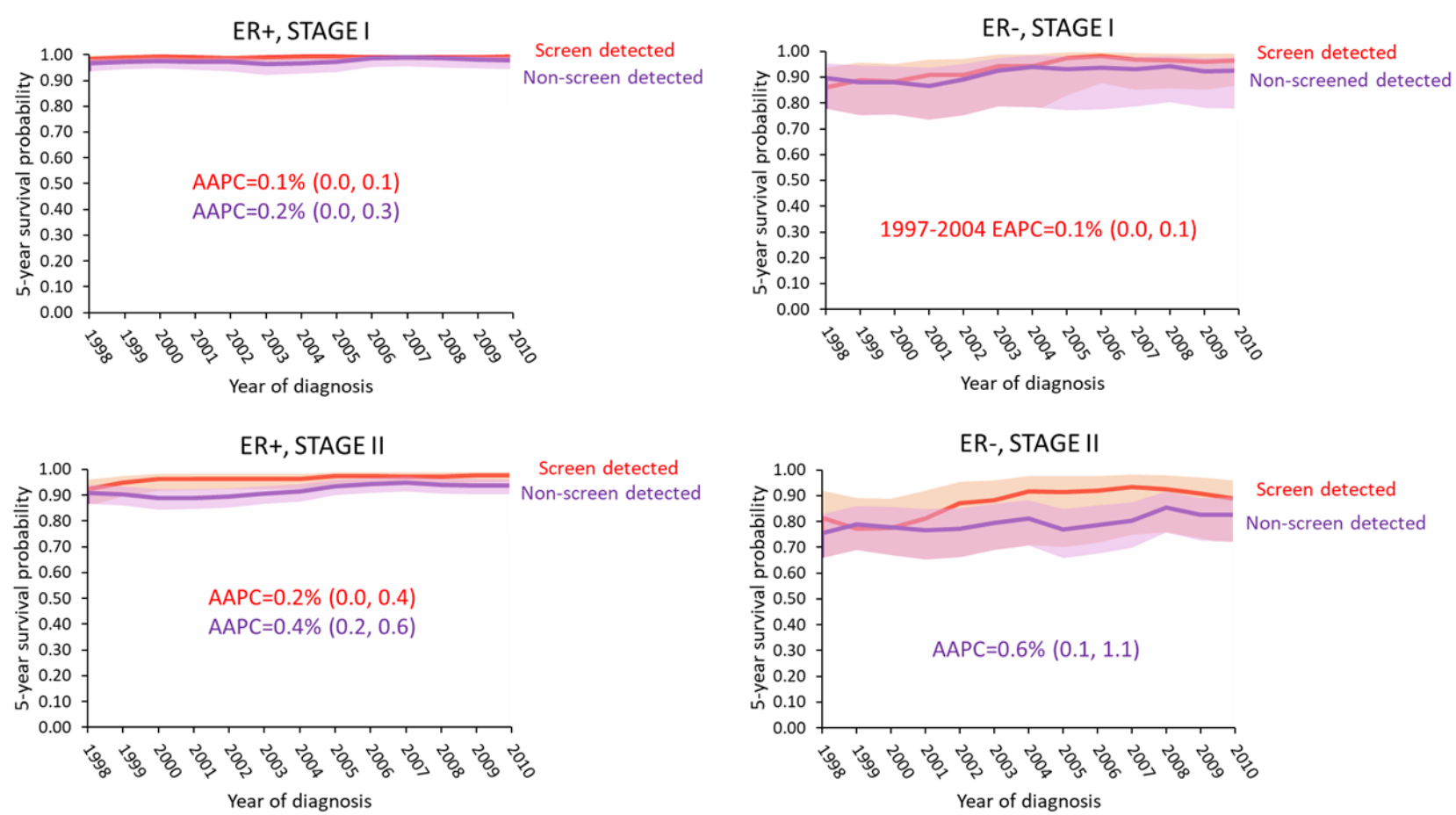
Women of screening age with advanced tumour stages (III-IV) showed improvements in BCSS over time for both screen detected and non-screen detected tumours (Figure 4.11). ER+ stage III-IV tumours that were screened detected showed a constant 5-year BCSS increase over the study period by 1.1%/year (95% CI: 0.4 to 1.8), while the improvements in ER+ stage III-IV non-screen detected tumours were only observed between years 1997 and 2002 (EAPC=3.2%, 95% CI: 1.0 to 5.3), with no significant changes afterwards. Improvements in survival for the ER- advanced tumours were observed regardless of mode of detection but the rise was sharper amongst women with screen detected tumours (AAPC=3.2%, 95% CI: 2.0 to 4.3).

Figure 4.9 Comparison of trends in 5-year survival probabilities by method of detection in women with breast cancer aged 50 to 69 years by ER status and grade combinations



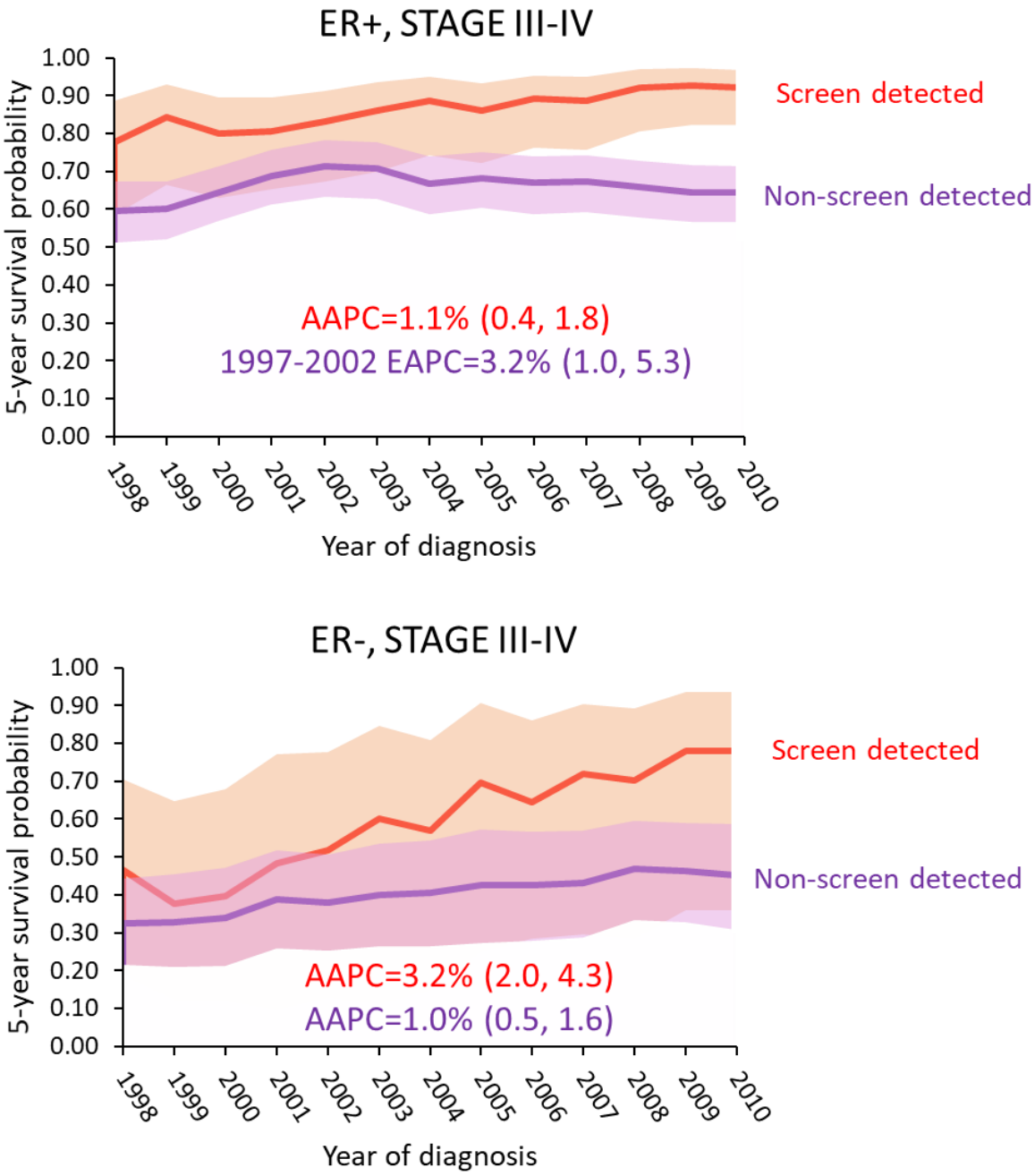
Shaded area represents the 95% CI around the BCSS estimate. Year of diagnosis in the graphs represents the middle year for that three-year average. AAPC= average annual percent change, EAPC= estimated annual percentage change, ER= oestrogen receptor.

Figure 4.10 Comparison of trends in 5-year survival probabilities by method of detection in women with breast cancer aged 50 to 69 years by ER status and stage (I and II) combinations.



Shaded area represents the 95% CI around the BCSS estimate. Year of diagnosis in the graphs represents the middle year for that three-year average. AAPC= average annual percent change, EAPC= estimated annual percentage change, ER= oestrogen receptor.

Figure 4.11 Comparison of trends in 5-year survival probabilities by method of detection in women with breast cancer aged 50 to 69 years by ER status and stage III-IV combinations



Shaded area represents the 95% CI around the BCSS estimate. Year of diagnosis in the graphs represents the middle year for that three-year average. AAPC= average annual percent change, EAPC= estimated annual percentage change, ER= oestrogen receptor.

4.4.7 Competing risk analysis

Primary causes of death amongst women diagnosed with BC in Scotland between 1997 and 2016 are presented in Table 4.21 overall and for each age group.

Table 4.21 Primary cause of death amongst women diagnosed with breast cancer in Scotland from 1997 to 2016 by age group

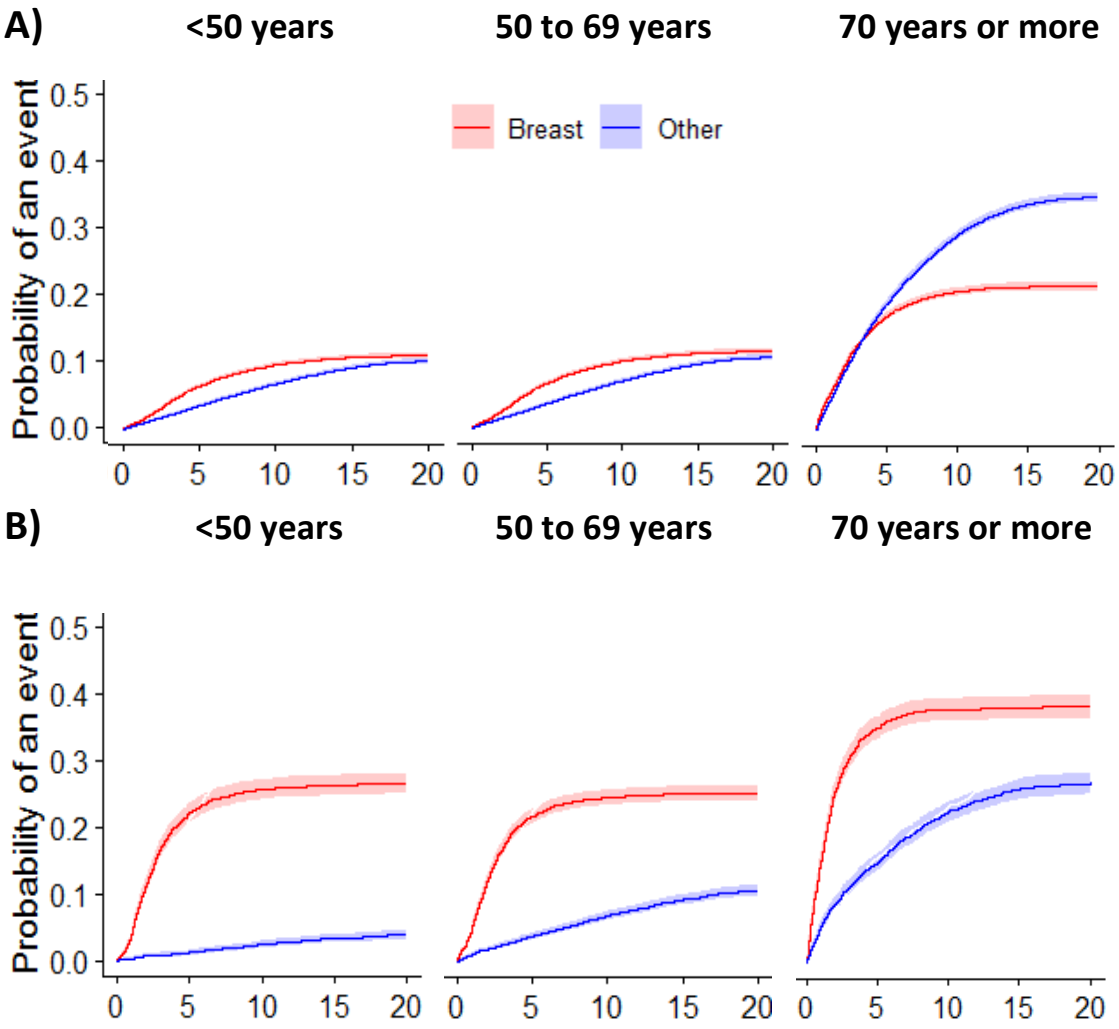
Primary cause of death	<50 years (N=3,213)	50-69 years (N=8,894)	70 years or older (N=14,173)	Total (N=26,280)
Breast cancer	2,748 (86%)	5,095 (57.3%)	6,139 (43%)	13,982 (53%)
Other cancer	193 (6%)	1,449 (16.3%)	1,157 (8%)	2,799 (11%)
CVDs	**59 (2%)**	**916 (10%)**	**3,098 (22%)**	**4,073 (16%)**
Acute Myocardial Infarction	15 (<1%)	242 (3%)	690 (5%)	947 (4%)
Ischaemic Heart Disease	10 (<1%)	167 (2%)	499 (4%)	676 (3%)
Other heart diseases	19 (<1%)	243 (3%)	745 (5%)	1,007 (4%)
Stroke and other	15 (<1%)	264 (3%)	1,164 (8%)	1,443 (5%)
COPD and other respiratory diseases	42 (1%)	440 (5%)	1,141 (8%)	1,623 (6%)
Alzheimer's/Dementia	0 (0%)	134 (2%)	1,036 (7%)	1,170 (5%)
Mental Health (depression, alcohol disorders, schizophrenia and suicide)	17 (<1%)	56 (<1%)	21 (<1%)	94 (<1%)
Diabetes and other endocrine diseases	<10 (<1%)	<70 (<1%)	158 (1%)	230 (<1%)
Miscellaneous	**99 (3%)**	**622 (7%)**	**1,232 (9%)**	**1,953 (8%)**
Accidents	12 (<1%)	54 (<1%)	192 (1%)	258 (1%)
Any other cause of death	13 (<1%)	17 (<1%)	18 (<1%)	48 (<1%)
Benign neoplasm	<10 (<1%)	<20 (<1%)	46 (<1%)	70 (<1%)
Blood diseases	0 (0%)	11 (<1%)	17 (<1%)	28 (<1%)
Digestive system diseases	33 (1%)	270 (3%)	412 (3%)	715 (<1%)
Infectious disease	11 (<1%)	73 (<1%)	140 (1%)	224 (<1%)
Kidney and genitourinary system diseases	<10 (<1%)	<70 (<1%)	243 (2%)	318 (1%)
Medical and surgical complications	<10 (<1%)	<10 (<1%)	10 (<1%)	21 (<1%)
Musculoskeletal system diseases	<10 (<1%)	<30 (<1%)	57 (<1%)	81 (<1%)
Other endocrine diseases	<10 (<1%)	<10 (<1%)	36 (<1%)	50 (<1%)
Other nervous system	13 (<1%)	88 (1%)	116 (<1%)	217 (<1%)
Skin diseases	<10 (<1%)	<20 (<1%)	27 (<1%)	43 (<1%)
Unknown	40 (1%)	101 (1%)	145 (1%)	286 (1%)

Values are frequency (% by column) with some cells showing approximate counts to comply with statistical disclosure.
CVD= Cardiovascular Disease, COPD= Chronic Obstructive Pulmonary Disease.

Apart from BC, CVD (which includes acute myocardial infarction, ischaemic heart disease and stroke), other cancers, COPD and Alzheimer's disease or dementia were the most common primary causes of death in Scottish women diagnosed with BC. There was a clear relationship between age and other primary causes of death, with a higher proportion of women aged 70 years or older dying from these conditions than the proportion observed in women aged less than 70 years. For example, CVDs accounted for 22% of all deaths in women aged 70 years or older compared to 10% of total deaths in women aged 50 to 69 and 2% in women aged less than 50 years.

Figure 4.12 presents the probability of dying from BC depending on ER status and age.

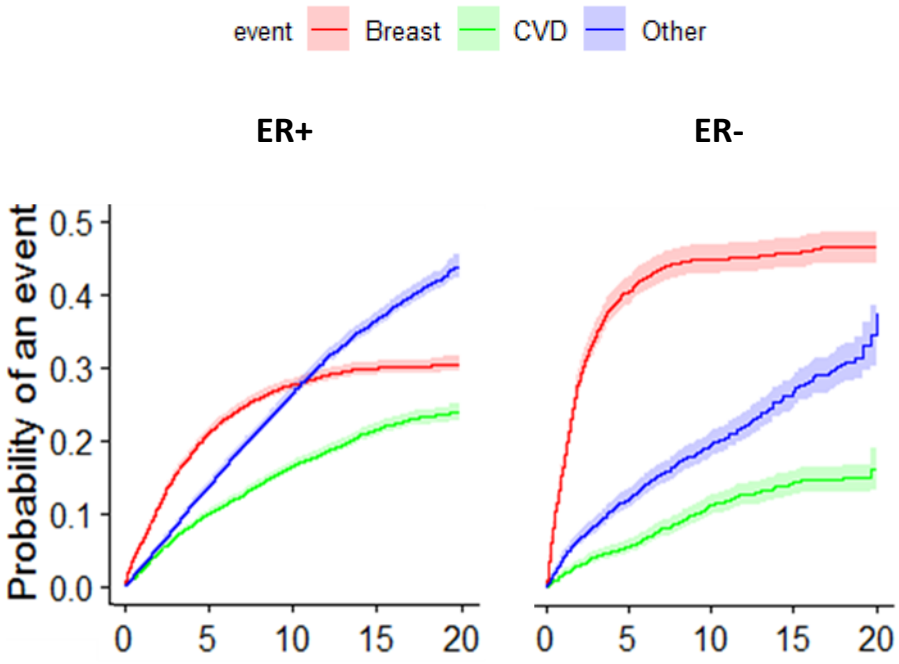
Figure 4.12 Cumulative incidence graph of BC death (breast: red line) and other cause of death (other: blue line) by age group for (A) ER+ tumours and (B) ER- tumours



The probability of dying from BC was highest in women aged 70 years or older with ER- tumours, but probability of BCD was also high amongst younger women, particularly during the first 5 years after BC diagnosis (Figure 4.12, panel b). Women aged less than 50 years with ER+ tumours had considerably higher probability of dying from BC than dying from other causes (Figure 4.12, panel a). In contrast, women with ER+ tumours of screening age (50 to 69 years) had a similar probability of dying from BC as from other causes and women with ER+ tumours aged 70 years or older had the same probability of dying from BC than from other causes during the first 3-4 years after diagnosis and higher probability of dying from other causes after that time.

Figure 4.13 shows that the probability of dying from CVD in women aged 70 years or older was higher amongst women with ER+ tumours and represented almost half of the probability of dying from causes other than BC.

Figure 4.13 Cumulative incidence graph of BC death (breast: red line), CVD death (CVD: green line) and other cause of death (other: blue line) in women aged 70 years or older



4.4.8 Trends in BC treatment by age and ER status in Scotland

The majority of women had surgery to remove the tumour (84% of all women) and were treated with HT (72%). Radiation and chemotherapy were less common treatments with 58% and 37% of all women receiving these treatments (Table 4.22).

Table 4.22 Treatments by age group in women diagnosed with BC in Scotland from 1997 to 2016

Treatment		<50 years (N=14,379)	50-69 years (N=35,592)	70 years or older (N=21,813)	Total (N=71,784)
Surgery					
	Yes	13,753 (96%)	33,509 (94%)	13,099 (60%)	60,361 (84%)
	No	527 (4%)	1917 (5%)	8,389 (38%)	10,833 (15%)
	Unknown	99 (<1%)	166 (<1%)	325 (2%)	590 (<1%)
Radiotherapy					
	Yes	9,906 (69%)	24,164 (68%)	7,674 (35%)	41,744 (58%)
	No	3,738 (26%)	9,842 (28%)	13,221 (61%)	26,801 (37%)
	Unknown	735 (5%)	1,586 (4%)	918 (4%)	3,239 (5%)
Chemotherapy					
	Yes	10,539 (73%)	14,180 (39.8%)	1,757 (8%)	26,476 (37%)
	No	3,574 (25%)	20,622 (57.9%)	19,332 (89%)	43,528 (61%)
	Unknown	266 (2%)	790 (2.2%)	724 (3%)	1,780 (2%)
Hormone therapy					
	Yes	9,298 (65%)	25,953 (72.9%)	16,671 (76%)	51,922 (72%)
	No	4,065 (28%)	7,583 (21.3%)	3,971 (18%)	15,619 (22%)
	Unknown	1,016 (7%)	2,056 (5.8%)	1,171 (5%)	4,243 (6%)
Neoadjuvant therapy					
	No	11,562 (80%)	31,948 (90%)	20,025 (92%)	63,535 (88%)
	Yes	2,817 (20%)	3,644 (10%)	1,788 (8%)	8,249 (12%)
Type of neoadjuvant therapy*					
Chemotherapy alone		2,464 (88%)	2,174 (60%)	204 (11%)	4,842 (59%)
HT alone		163 (6%)	1,207 (33%)	1,482 (83%)	2,852 (35%)
Radiotherapy alone		<10 (<1%)	<20 (<1%)	17 (1%)	33 (<1%)
Chemotherapy and HT		114 (4%)	140 (4%)	34 (2%)	288 (4%)
Chemotherapy and radiotherapy		<50 (<2%)	44 (1%)	<10 (<1%)	95 (1%)
Radiotherapy and HT		<10 (<1%)	<40 (<1%)	35 (2%)	73 (<1%)
All (chemo, radio, HT)		<30 (<1%)	38 (1%)	<10 (<1%)	66 (<1%)

*Frequencies and %s amongst women who had neoadjuvant therapy, n=8,249 (12% of the total). HT=hormone therapy.

Treatment differences were observed between age groups (Table 4.22). Older women aged 70 years or more were less likely to have surgery, radiation and chemotherapy and more likely to have HT and neoadjuvant HT than women younger than 70 years. The

greatest differences between age groups were observed for chemotherapy, with 73% of women aged less than 50 years receiving this treatment, in contrast to 40% and 8% receiving chemotherapy if aged 50 to 69 years and 70 years or older respectively. The use of HT and neoadjuvant therapy increased with age and chemotherapy was the most used neoadjuvant therapy for women younger than 50 years and HT the most used in women aged 70 years or older.

Given the importance of ER status in treatment decisions, Figure 4.14 presents trends of the treatments for BC given to Scottish women diagnosed with BC from 1997 to 2016 by ER status and age group. In Scotland, the percentage of women who had surgery to treat BC was very high (approximately 95%) regardless of ER status and remained constant over time for women aged less than 70 years. In older women aged 70 years or more, the proportion having surgery was lower at approximately 80% in 1998 for both ER+ and ER- tumours. This percentage remained constant in older women with ER- tumours but decreased over time for older women with ER+ tumours (62% in 2016).

HT use was high for all women with ER+ tumours and remained constant over time. Women with ER- tumours still received HT in the late 90s, especially if they were aged 70 years or more but the use of HT among women with an ER- tumour declined sharply over time and only 3-6% of them received HT in 2016.

Chemotherapy treatment was consistently more widely used in women with ER- tumours than in women with ER+ tumours of the same age. The proportion of women receiving chemotherapy remained constant over time for ER+ tumours, whereas it increased in women with ER- tumours, especially in women aged 50 to 69 years and women aged 70 years or older. Radiotherapy use was slightly higher for ER- tumours than for ER+ tumours in the late 1990s, but greater increases in the use of radiotherapy in women with ER+ tumours (especially if they were younger than 70 years) were observed during the study period. These increases have made the proportions receiving radiotherapy slightly higher in women with ER+ tumours than in women with ER- tumours, with the exception of women with ER+ tumours aged 70 years or more that are less treated with radiotherapy than women with ER- tumours of the same age.

Figure 4.14 Trends over time of the percentage of Scottish women diagnosed with BC who were treated with A) surgery and B) hormone therapy C) chemotherapy and D) radiotherapy by ER status (page 1 of 2)

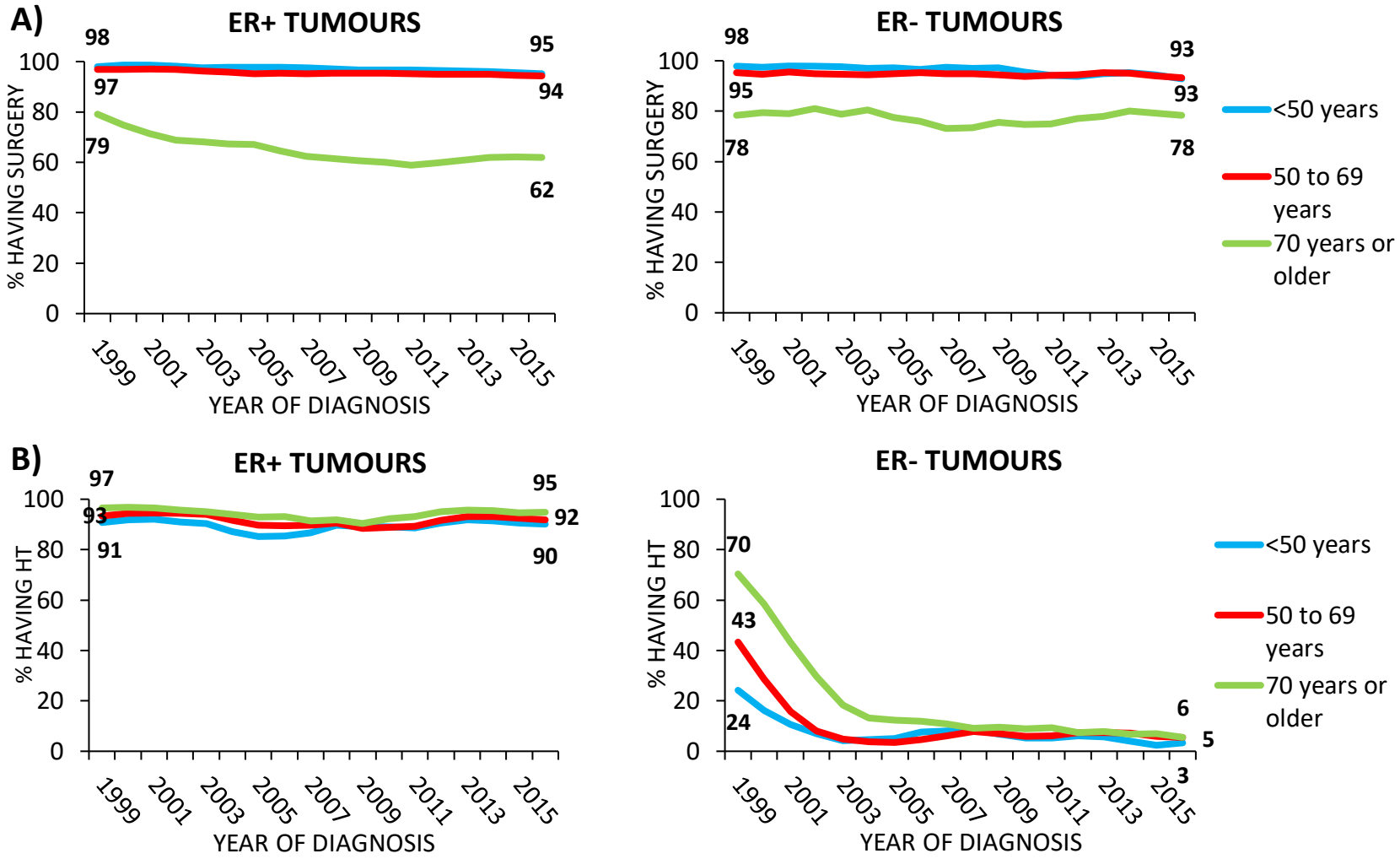
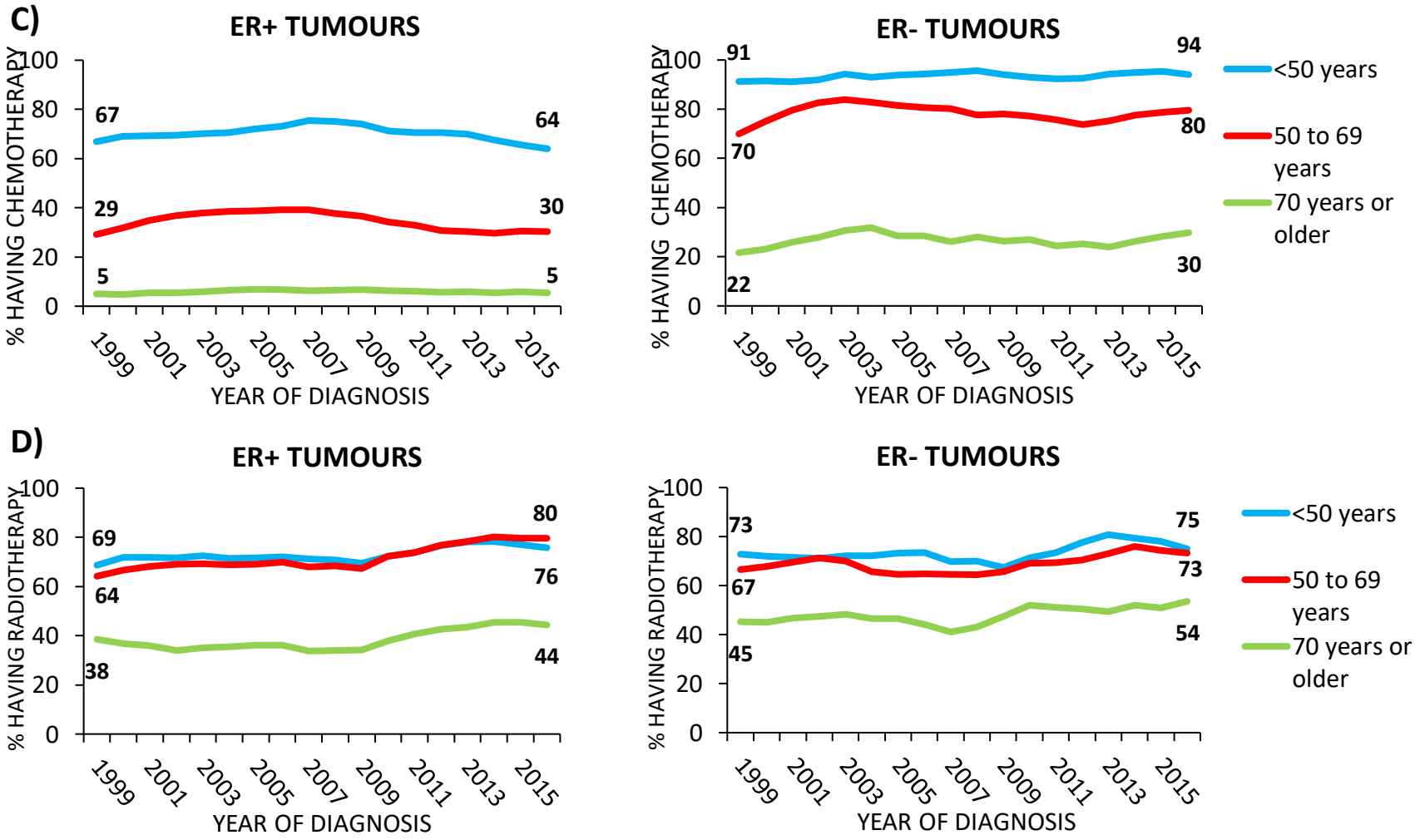


Figure 4. 14 (continued) Trends over time of the percentage of Scottish women diagnosed with BC who were treated with A) surgery and B) hormone therapy C) chemotherapy and D) radiotherapy by ER status (page 2 of 2)



4.5 Discussion

4.5.1 Summary of key findings

4.5.1.1 Survival differences between subtypes

In this large study in over 70,000 women diagnosed with BC in Scotland between 1997 and 2016, prognosis differed between molecular subtypes. Scottish women with ER- tumours had considerably lower survival at 5 years compared to women with ER+ tumours (70.6% vs 87.4%) and an overall 44% higher risk of BCD after adjusting for other individual and tumour characteristics, treatments and comorbidities. The largest previous similar study conducted using SEER data for over 150,000 women found an increased mortality (adjusted HR=2.3, 95% CI: 2.2 to 2.4) in ER-/PR- tumours when compared to ER+/PR+ tumours. In comparison with this study, I found a smaller effect size for the comparison of ER+ and ER- tumours, which might be due to the use of a single hormone marker (only ER instead of ER/PR) and to further adjusting for screening, comorbidities and deprivation. The effect size for the TVE models (adjusted HR at 1 year=2.39, 95% CI: 2.08 to 2.80 and adjusted HR at 3 years=1.63, 95% CI: 1.23 to 2. 30) or for the model with follow-up restricted to 3 years (adjusted HR=2.26, 95% CI: 2.02 to 2.52) in my study shows similar HR for BCD in women with ER- compared to that observed in the US study that is restricted to early follow-up years. This highlights the potential for generating misleading estimates if non-constant hazards are ignored.

The analysis of IHC defined molecular subtypes showed that Scottish women with luminal A subtype had the best survival (91% at 5 years), whereas women with TNBC subtypes had the worst survival (73% at 5 years). Although surrogate IHC definitions have been used in most previous studies there was heterogeneity in the definition of each molecular subtype and also in the outcome assessed between studies. In contrast to other studies, I used grade to further differentiate between luminal A and luminal B tumours and did not differentiate between luminal B HER2- and luminal B HER2+ tumours. Further, I use BCSS as outcome instead of relative survival estimates, DFS or local recurrence rate, as recurrence data are not available in routine cancer data in Scotland. Despite study heterogeneity, our results were similar to those observed from the US [225, 226], Canada

[268], Italy [228], Germany [269], Spain [233], Switzerland [270] and Norway [230]. Scottish women with TNBC subtypes were 4 times more likely to die from BC than women with luminal A tumours which was in the higher end of HRs obtained in other studies that ranged from HR=1.6 in the US [226] to HR=4.2 in Switzerland [270]. The risk of BCD was very similar for Scottish women who expressed HER2 (luminal B and HER2-enriched tumours) regardless of ER status. Our data is consistent with an Italian study (HR=1.7 for luminal B and HER2-enriched subtypes when compared to women with luminal A tumours) [228]. In contrast, a large population-based study in the US found lower BC-specific mortality for luminal B tumours but higher risk for HER2-enriched tumours compared to luminal A tumours [225]. International comparisons are challenging due to the different health care systems and access to targeted therapy drugs. Furthermore, the rare subtypes (HER2-enriched and TNBC) have lack of power and definition of the molecular subtypes differed between countries. However, the highest mortality in women with TNBC subtypes was consistent across studies. This finding might be related to the lack of targeted therapy for TNBC in contrast to hormone positive subtypes or HER2-enriched subtypes for which targeted treatments, Tamoxifen [117] and Trastuzumab [271], have been available since the late 1990s and early 2000s respectively.

Apart from BCSS estimates, secondary analysis of OS at 5 and 10 years showed a similar pattern across the molecular subtypes to that observed for BCSS but with lower estimates of survival, particularly in older women aged 70 years or more with aggressive subtypes (ER-, HER2-enriched or TNBC). Assessment of other underlying causes of death showed that over half of Scottish women diagnosed with BC at 70 years or older died from other causes of death with CVDs contributing to 22% of the total deaths. A recent review estimated that the absolute risk of death from CVDs after BC diagnosis ranges from 1.6% to 10.4% with older age being an important RF [272]. The mechanisms contributing to the increase risk of CVD death amongst BC survivors are not fully understood but shared aetiology such as increased obesity and diabetes prevalence [273] and the toxicity of BC treatments, especially radiotherapy, seem to be likely contributors [274].

4.5.1.2 Association of other prognostic factors with breast cancer death for the different subtypes

A handful of studies have examined the prognostic value of different individual, tumour and treatment covariates stratified by BC subtypes [225, 268]. This analysis is the first in Scotland and the UK using population-based data.

Age

Older age at diagnosis was found to be associated with worse survival for all subtypes. Young age (<40 years) has previously been associated with poorer prognosis of luminal A and TNBC [241-243] when compared to women older than 40 years. However, our results suggest that after adjusting for potential confounders women aged less than 50 years were less likely to die from BC than women aged 50 to 69 years for ER+, ER-, luminal A and HER2-enriched subtypes. In contrast, luminal B and TNBC had a slightly increased mortality but was not statistically significant after adjustment for other covariates. Differences with previous studies might be due to the different age groups used for the analysis and to the inclusion of additional covariates, such as, comorbidities and deprivation in our analysis.

Other tumour characteristics

Histological grade and tumour stage are key prognostic factors for clinical practice and they are important for treatment decisions. In this study, grade and stage were independent prognostic factors for all subtypes and a dose- response effect was observed with higher grade and stage associated with increased BC-specific mortality. Women with poorly differentiated (grade III) tumours had lower survival than women with grade I tumours irrespective of the tumour subtype but the association was stronger for ER- and HER2-enriched tumours. Higher stage was also associated with poorer outcome for all subtypes and had the greatest effect on BCD of all factors. Women with stage IV tumours had an increased BC-specific mortality that ranged from HR=10.7 for luminal A tumours to HR=19.4 for HER2-enriched tumours compared to mortality in women with stage I tumours of the same subtype.

Screening advances the time of tumour detection and tumours detected through screening have been associated with favourable characteristics, such as, low grade, early stage, hormone positive and HER2 negative, hence have better prognosis than symptomatic tumours [246, 275]. In our study, method of detection was associated with BC prognosis irrespective of tumour subtype. Women with tumours that were not screen detected had 60-70% increased BC-specific mortality compared to women with screen detected tumours (for both ER+ and ER- tumours). Slightly different HRs were found between molecular subtypes with luminal A and TNBC non-screen detected tumours having the highest HRs (HR=2.7 and HR=2.3 respectively) and luminal B and HER2-enriched having 1.7 and 1.8 times the HR for a screen detected tumour of the same subtype. The estimate for HER2-enriched subtype did not reach significance after adjusting for all other tumour characteristics and treatments and comorbidities.

Treatments

Surgery was the most important treatment prognostic factor, and women who did not have surgery were more likely to die from BC for all subtypes compared to women with the same subtype receiving surgery. The highest beneficial effect of surgery was seeing in women with luminal A and HER2-enriched tumours. HT also had a beneficial effect on BC mortality in hormone positive tumours.

The effect of radiotherapy and chemotherapy differ between subtypes. Radiotherapy had a beneficial effect on women with HER2-enriched and TNBC subtypes. However, for women with ER+ and luminal A tumours, adjusted models showed no benefit or even a detrimental effect of radiotherapy on luminal A tumours which is unlikely and might have been due to confounding by indication. TVE models seemed to correct, at least partially, this bias.

Similar results were observed for chemotherapy that had a beneficial effect for women with ER-, luminal B and HER2-enriched subtypes but opposite effect (higher death risk) for ER+, luminal A tumours and no effect for TNBC.

Treatment data had limitations as they are usually first treatment and more comprehensive and detailed datasets would be needed to assess the benefits of the different treatments on BCSS and recurrence. Further, treatment effects might have been biased due to confounding by indication. An explanation of this type of bias is presented in section 4.5.3.1.

Deprivation and comorbidities

Previous studies from Scotland and other countries have found a clear association between SES (at both individual and neighbourhood level) and BC mortality, with women with low SES having a higher BC mortality [276-278]. Further, women with low SES are more likely to be diagnosed with more aggressive BC subtypes, particularly ER- and TNBC subtypes [278-280]. However, evidence of the extent to which the distinct prevalence of subtypes within deprived and non-deprived areas contribute to the worse prognosis observed for women with low SES is inconsistent. In the current analysis, deprivation was associated with a higher BCD with those women living in the most deprived areas of Scotland having a higher BC-specific mortality than those in the least deprived areas and this association was observed for all tumours subtypes except for TNBC. After adjusting for other covariates the association was no longer statistically significant for luminal B tumours, probably due to smaller sample sizes (as for TNBC). HER2-enriched tumours had the highest increase in mortality which was almost double for women in the most deprived areas vs women in the least deprived areas but with wider CI. Given that associations were still significant for this subtype after adjustment for other tumour characteristics (including screening) and treatments, future studies should focus on unmeasured lifestyle factors such as obesity or alcohol consumption which might further explain the differences in BCD observed by SES.

Comorbidities, measured using a continuous comorbidity index, were associated with increased BC-specific mortality for all tumour subtypes (except luminal A), with women with HER2-enriched tumours and a higher comorbidity index having the highest BC-specific mortality (2.5 times increased risk for each unit increase in the comorbidity

index). In contrast, in women with luminal A tumours, having comorbidities did not significantly increased mortality after adjusting for all other covariates.

4.5.1.3 Improvements in survival over time

In Scotland, BCSS improved over the study period from 1997 to 2016, however improvements differed by age and tumour characteristics which might be related to differences in treatment and the effect of screening. The greatest improvements in BCSS were observed for women in the screening age group (50 to 69 years) and particularly in those with high grade and/or stage III-IV tumours. These improvements were observed both for ER+ and ER- tumours but were slightly higher for the ER- tumours which are generally less likely to be screen detected, hence, suggesting an important role of treatments in the improvements of this subtype that might be related to the increases observed in radiotherapy use.

Although younger women of less than 50 years are not routinely invited for mammographic screening, trends over time also showed consistent statistically significant improvements in BCSS for ER+ and ER- high grade tumours and stage II tumours which might be due to increased use of neoadjuvant treatments. Data suggest that younger women were more likely to receive chemotherapy, especially if they had ER- tumours. However the proportion of women who received this treatment did not change over time so improvements might be due to other factors, such as increases in radiotherapy use. Younger women with stage III-IV tumours had an increasing survival trend but improvements did not reach statistical significance possibly due to limited power.

BCSS observed in older women (aged 70 years or more) remained approximately constant over time with no statistically significant increases in survival found, except a slight improvement for ER+ high grade or stage II tumours, with undertreatment being a possible factor. Our analysis shows that the percentage of women aged 70 years that were treated with surgery was considerably lower than that observed for women younger than 70 years. This is in concordance with recent RCT in the UK that report undertreatment in women older than 70 years. Also, almost half of women aged 70+ years diagnosed with BC had a different cause of death with CVD being a major contributor. Previous studies have

indicated the potential role of some BC treatments on CVD risk [281, 282], however the relationship is still unclear and further research would be needed to elucidate whether this might be affecting survival trends in Scotland.

Further stratification by method of detection in the age group of women who are invited for mammographic screening in Scotland (aged 50 to 69 years) showed improved BCSS for both screen detected and non-screen detected tumours although generally screen detected tumours had much better survival. Caution must be given to the interpretation of the trends given the presence of competing risks and possible bias.

4.5.2 Strengths

To my knowledge, this is the first population-based study in Scotland and the UK to assess BCSS by molecular subtypes and investigate trends based on important prognostic factors to assess whether BCSS has improved in recent years. This study expands our understanding of the evolution of BCSS in Scotland. This study has several strengths:

4.5.2.1 Data quality and availability

The Scottish cancer registry data quality has been previously described [157] and linkage to mortality records provides the opportunity to assess trends over time by subtypes (with data on ER collected from 1997 and PR and HER2 from 2009) providing one of the longest follow-up periods of cohorts from any European country. Further, either through data linkage to other national datasets or within the minimal dataset for the registry, possible confounders not usually recorded in other European and North American cancer registries, such as, method of detection, comorbidities and deprivation measures were used to assess the effect of these covariates in BCSS trends.

4.5.2.2 Statistical analysis

Another major strength of this study is the use of the extended Cox PH models and the use of MI.

The Cox PH model is the preferred method to estimate survival in cancer patients when adjusting for multiple covariates. However, the PH assumption is key to obtain correct estimates of the effect of the covariates in survival. In the presence of non-proportional

hazards, HR estimates are averaged across time and, for that, reason might mask differences at different time points. This analysis used extended Cox models that introduce interactions between time and those covariates that had non-proportional hazards over time. The TVE results highlighted the importance of correctly estimating HRs, especially for the molecular characteristics and treatment effects.

Most previous studies have failed to account for missing tumour markers, and their results have been based on CCA. However, missing receptor data can biased the results as those without these markers are likely to have worse prognosis and be more frail and less likely to receive certain treatments [267]. In contrast to those studies, I compared findings from MIA to CCA findings for the traditional Cox model with ER and the subtypes as the main exposures. Estimates were found to be similar but slightly attenuated for the MIA and with narrower CIs which was consistent with findings of a previous study with SEER data that used MI to correct for missing molecular marker data

4.5.3 Limitations

4.5.3.1 Bias in observational studies

Observational studies using population- based cancer registry data provide high quality data in which to assess cancer outcomes. However, the validity of their results must be assessed as they can be prone to confounding and biases.

Unmeasured confounders

Unmeasured confounders or measurement errors on the measured confounders might have biased the results. Although our analysis controlled for potential confounders, such as age, deprivation, tumour characteristics, treatments and comorbidities, other factors, such as lifestyle factors (alcohol and tobacco consumption, physical activity), reproductive factors or anthropometric factors (BMI) might have had an effect on the observed trends. For example, the higher risk of BCD observed in women living in the most deprived areas compared to women in the least deprived areas consistent across all subtypes might be further explained by different obesity prevalence and lifestyle factors, such as, alcohol and tobacco consumption. Given that our analysis included more confounders than most

previous studies and that results were consistent across studies, it is unlikely that unmeasured confounders will explain the observed survival differences. However, future research should aim to explain the effect of tumour, treatment and other confounders/mediators in the differences in survival observed between women in most and least deprived areas through causal mediation analysis.

Selection bias

Confounding by indication is a particular form of selection bias in observational studies that estimate the benefit of treatments in cancer mortality [283]. In our study, confounding by indication might have been present, as women with BC receiving treatments such as chemotherapy and radiotherapy are likely to have more aggressive subtypes, whereas those receiving surgery or HT might be more likely to have early stage/low grade cancers and hormone positive subtypes. Similarly it is not surprising that women who are unfit for surgery have poorer outcomes. For that reason, the effect of treatments that are given to women with less severe diseases could show larger improvements in survival while the effect of chemotherapy and radiotherapy could appear to be associated with an increased risk in BCD. Further, selection bias can also affect comparisons of individuals that went through screening and those who did not. In our analysis, using TVE models seemed to correct, at least partially, the selection bias for the treatment effects. Further, the use of multiple covariates within the models might have also corrected part of this bias.

Lead and length bias

Survival rates can be affected by lead time and length biases [284] usually caused by the introduction of a national screening programme during the period of study. Screening inflates survival by advancing the time at which a tumour is diagnosed (lead time bias), by identifying early stage tumours that have a slow progression (length bias) and also by identifying tumours that may not have been identified otherwise as their progression is so slow that they would not affect survival (overdiagnosis). These three biases can explain survival improvements that are related to screening and not to an increase in the number of deaths that are prevented or delayed. Most cancer registries do not record data about mode of detection of a cancer, however the Scottish cancer registry does hence, survival

trends for the different molecular subtypes can be assessed by method of detection providing a clearer image of the effect of screening in BCSS.

In order to (partially) correct for these biases, I adjusted for possible confounders in all analysis and used 5 year survival estimates that might reduce the impact of lead time bias (estimated to be 3 years). Data in Scottish women suggest that screening is important for BC prognosis regardless of molecular subtype. These results are in line with a recent study in Sweden reporting that women who participated in screening had a 41% reduction in the risk of BCD in the 10 years following diagnosis [285]. However the potential for residual confounding remains as women who accept invitations to screening are likely to differ from women that do not attend screening in ways that may influence survival.

4.5.3.2 Data and Statistical Analysis

As previously stated, cancer registry data presents some limitations inherent to the difficulties of cancer registration (section 3.5.4.1 of the incidence chapter). Survival statistics derived from population-based cancer registries are key to estimate progress against BC. However, the validity of BC specific survival analysis depends on the accuracy of cause of death as recorded in the registry which assumes that the underlying cause of death has been accurately determined for each patient. In Scotland, underlying cause of death is based on death certificate records along with additional information provided by other official sources (pathologists, doctor who certified the death, Procurators Fiscal or the Crown office) and certification is completed by a registered medical practitioner following strict guidelines [286] developed by NRS that cross-checks all data sources in order to improve its coding of death. Despite guidelines and data chequing procedures, the death certification review service which aims to improve the quality and accuracy of Medical Certificates of Cause of Death (MCCD) have reported inaccuracies in 7% of all MCCDs with 43% found to have a cause of death considered too vague and 28% to have an incorrect cause of death. Further, the definition of neoplasms was the most common error (8% of all) identified for those that were considered too vague [287]. For that reason, we cannot rule out that our cause- specific analysis might be biased. The used of relative survival was considered as an alternative but it would require

lifetables for the BC molecular subtypes in order to calculate the expected survival which are currently not available. Further, relative survival can also be prone to bias if the lifetables are not representative of the cancer population, for example if the cancer patients are healthier on average than the population as could be the case for BC patients, or if other causes of death shared risk factors, such as obesity (risk factor for both BC and CVDs) [288, 289].

Treatment data were very limited and there was no data available about recurrence. Further, other possible covariates such as reproductive factors or lifestyle factors were not available. However, the Scottish cancer registry can be linked to other national datasets such as maternity records from 1981 and there is scope to further investigate the effect of these factors in molecular subtypes in future research.

Deprivation and comorbidity data used to estimate the effect of these confounders in BC mortality present some limitations [290]. The SIMD is an area-based measure of deprivation rather than an individual-based measure so it can miss some of the people who experience deprivation but do not live in deprived areas [291]. Hence, an association between deprivation and increased BC mortality for some of the subtypes might not be related to individual deprivation. This is particularly true for rural areas where the index domains, particularly the 'access' domain fails to capture important singularities of the rural areas, such as, frequency and cost of public transport [292]. Therefore, deprivation in rural areas is usually underweighted and they are less likely to be ranked as most deprived.

The Charlson comorbidity index depends on the recording of all individual comorbidities included in the index. In Scotland, the score is derived from hospital admission records dating back to 5 years prior to the index admission, for that reason, if the patient was not hospitalised during those 5 years or if the condition was not recorded that comorbidity might be omitted and not included in the index. Further, one could argue that combining all comorbidities into a single index precludes to investigate the effect of individual comorbidities in BC mortality, such as, that of CVDs. Investigating other comorbidities

was beyond the scope of this PhD but future research should aim to investigate the association of CVDs and BC mortality.

Our analysis included women who were diagnosed with more than one tumour but only the tumour with higher grade/nodal status was kept and is included in the analysis which might have had an effect on the survival estimates. However, further adjusting for the presence of multiple tumours had little effect on the HR estimates for the main comparison of ER- vs ER+ tumours. Statistical power was an issue for some of the stratified analysis, especially for the rare subtypes of BC (power calculations reported in Appendix Table C.15). For example, the effect of some of the covariates in the analysis of luminal B and TNBC subtypes did not reach statistically significance probably due to small numbers.Also, the short follow-up (9 years) for the IHC define subtypes precluded estimation of survival trends over long periods of time.

4.6 Conclusion

This analysis using high quality population- based data in Scotland shows for the first time in the UK differences in prognosis between molecular subtypes of BC that are consistent with previous literature. Further, important prognostic factors for each molecular subtype were identified and groups of women for which probability of BCD was highest were also identified, such as women living in the most deprived areas of Scotland and women with comorbidities. Survival trends over time suggested improvements in BCSS and OS in recent years, particularly for women with more aggressive subtypes with high grade or stage III-IV with improvements likely to be related to screening and treatment.

Chapter 5 Discussion

5.1 Introduction

BC is a model disease for personalised medicine with different subtypes for which specific treatments have been developed over the years, such as the tamoxifen for hormone sensitive tumours or trastuzumab for the treatment of HER2+ tumours. Although BC heterogeneity has been widely recognised, cancer surveillance at the population level is still based on the evaluation of incidence, mortality and survival trends for all BC tumours, irrespective of their subtype.

Previous research has shown that molecular subtypes differ aetiologically and in prognosis and other countries have reported distinct incidence and mortality trends by molecular subtypes. Using data from the Scottish cancer registry, this PhD described incidence and survival trends by molecular subtypes in Scotland and identified individual and tumour characteristics of women that are experiencing increasing incidence and lower survival after a BC diagnosis.

My systematic review of incidence trends by ER status in European ancestry populations identified that only a few cancer registries collect molecular marker data. This represents a gap in the literature for the last decade given that new markers have been introduced and distinct trends in incidence by ER status have been identified from the limited number of studies. Further, in the UK the single study that assessed incidence trends by ER status was published in 2010 and was limited to women over 50 years of age. I used linked Scottish cancer registry and mortality data for 1997-2016 to describe incidence and survival trends in a wider range of BC subtypes to extend our existing knowledge. The strengths and limitations of those analyses are discussed in detail in each specific chapter.

This chapter provides a general discussion for the PhD with the following parts: a summary of the results from the PhD focused on the contribution to our understanding of BC subtype incidence and survival trends, and its implications for research and clinical practice. There is also a discussion of the general strengths and limitations and a section with recommendations for future research.

5.2 Contribution of this project to our understanding of BC incidence and survival trends

This project has contributed to our knowledge of secular trends of BC and highlights the feasibility of assessing BC heterogeneity using population-based data. For the first time in the UK, this study shows distinct temporal trends of BC incidence and survival by molecular subtypes for women diagnosed with BC between 1997 and 2016 in Scotland.

5.2.1 Incidence trends by molecular subtypes

Expanding on the only previous study reporting BC incidence trends by ER status in postmenopausal women, after correcting for missing ER status and for multiple tumours per woman, I investigated BC incidence trends by ER for all women diagnosed in Scotland from 1997 to 2016. I found that ER+ tumour incidence continued to increase until 2011 while ER- tumour incidence decreased for the whole study period. Increases in incidence of ER+ tumours were mainly observed for women aged 50 to 69 years old, who are those invited for mammographic screening in Scotland, indicating a probable contribution of screening to the increasing trends in incidence of ER+ tumours. An important contribution of this analysis was the ability to study the potential impact of screening on the incidence rates, which were investigated by looking at incidence trends by method of detection and ER status. Findings suggested that the observed overall ER+ increasing trends were driven by increases of screen detected ER+ tumours, whereas incidence of non-screen detected tumours remained constant over time. Joinpoint and APC models showed consistent results in incidence trends by ER status. The use of APC models also led to the identification not only of age and period effects (probably related to screening) but of a cohort effect in ER+ tumours that could be related to changes in reproductive factors and/or differences in obesity prevalence between birth cohorts of Scottish women. The declines observed for ER+ tumour incidence since 2011 are likely to be multi-factorial. The consistent declines in incidence of ER- tumours over the whole study period since 1997 for women of all ages, which have also been observed in other countries, represent an important finding as these subtypes have considerably worse prognosis than ER+ tumours. However, more research is needed in order to elucidate the factors that might be

driving these declines which could lead to future interventions aimed at reducing the incidence and/or improving the prognosis of these more aggressive subtypes.

The measurement of PR and HER status in BC patients and the collection of these markers in the Scottish cancer registry, allowed me to investigate trends using combinations of these three IHC markers as surrogates for the four intrinsic molecular subtypes defined by Perou et al. Limited follow-up, with data available from 2009 to 2016 contributed to the absence of statistically significant overall trends in incidence of luminal A, HER2-enriched and TNBC tumours. Joinpoint regression analysis showed decreasing incidence of luminal B tumours that was driven by declines in incidence in women aged 50 years or older. APC models confirmed this result and further identified a cohort effect in women aged 60 years or older which highlights the value of using different methods that can pick up signals that would otherwise be missed. Another important finding from this thesis is the increasing incidence trend of TNBC observed in women younger than 50 years. TNBCs have the worst prognosis of all subtypes, as there is no targeted therapy that can be used in their treatment and aggressive chemotherapy is the only treatment option. Future trends in incidence of TNBC in young women need to be monitored and research on the association of modifiable RFs with this subtype would inform future prevention programmes.

5.2.2 Breast cancer prognosis by molecular subtypes

Univariate KM and multivariate Cox regression models were used to investigate survival by molecular subtypes and trends over time. ER+ and luminal A tumours have the best prognosis and ER- and TNBC the worst, consistent with the findings from previous studies [226, 228, 230, 231, 233, 268, 269]. Five-year survival trends of the most important prognostic factors were assessed to look for survival improvements over time. The most important results and its implication are summarised below.

5.2.2.1 The role of age

As expected, age was an independent prognostic factor for survival in all subtypes and increased age was associated with increased likelihood of BCD. Although ER+, luminal A tumours have been associated with better prognosis, several previous studies have found an increased BC-specific mortality in young women (aged <40 years) with these subtypes [230, 240, 241, 293] compared to older women. In our analysis, this association was not observed and women with ER+ and luminal A tumours younger than 50 years had lower risk of BCD than women aged 50 to 69 years with the same subtype after adjusting for tumour characteristics, treatments, deprivation and comorbidities. These discrepancies in findings are likely due to study heterogeneity with different definitions of age groups and luminal subtypes and different covariates used for the adjusted models. Further analysis is needed to investigate whether the increased risk of BCD amongst young compared to older women with luminal A and ER+ tumours observed in other countries is also observed in Scotland using similar age groups and IHC subtypes definitions.

The analysis of 5-year BCSS trends showed improvements over time for women aged less than 50 years of age at BC diagnosis regardless of ER status, especially in women with high grade tumours and stage II tumours. Improvements in BCSS in Scottish women younger than 50 years of age are not likely to be related to screening as only 2% of all tumours are diagnosed through screening in this age group in Scotland. Improvements in BCSS in younger age women might be related to use of more aggressive chemotherapy treatments in recent years. Our data for Scotland showed that the proportion of women receiving chemotherapy remained constant over the study period in women younger than 50 years but information on type of chemotherapy is not available within the registry. Future research should focused on linkage of the registry to detailed treatment data, such as that in cancer audit data, to further investigate treatment pathways for improved survival.

In contrast to younger women, women aged 70 years or older at the time of diagnosis of BC showed consistently worse BCSS regardless of tumour subtype when compared to women of screening age with the same subtype. Cumulative incidence curves for other

causes of death highlighted the importance of competing risks of death from other causes for this age group, and the important contribution of CVD as a cause of death in this population. Further, there were no significant improvements in survival for Scottish women aged 70 years or older that have also been reported in previous studies [294-296] that could be related to poorer health conditions and the omission of treatment [297, 298] and low adherence to standard treatment in older women [299]. In our study, the proportion of women aged 70 years or older with less aggressive ER+ tumours who received BC surgery declined over the study period, proportions receiving chemotherapy remained low but increased for ER- tumours and proportions treated with radiotherapy increased for both ER+ and ER- tumours. A recent study in the UK in women aged 70 years or older showed that BC surgery is safe for this age group with no increased risk of death but that surgery might affect quality of life in this group of women [300]. Future research should aim to identify women with BC that may benefit from additional treatments.

5.2.2.2 The role of method of detection (screening)

These analyses extended the previous work on BC incidence in Scotland, not just by extending the age range of the study population and the study period but also by considering the role of screening in time trends in both BC incidence and survival. The beneficial effect of screening on BC prognosis in other settings has been previously estimated [301, 302] . In our study, the risk of BCD was higher amongst women with non-screen detected tumours compared to women with screen detected tumours for all tumour subtypes and after adjusting for other individual and tumour characteristics, treatments and deprivation and comorbidities. Furthermore, the effect of screening was observed in improvements of 5-year BCSS and OS in women of screening age (50 to 69 years). Although both survival analyses were adjusted for molecular subtype, grade and stage which might be partly responsible for increasing survival due to detection of less aggressive early stage/grade tumours, the effect of length and lead time bias (explained in Section 4.5.3.1) in the survival estimates cannot be completely ruled out.

5.2.2.3 The role of deprivation

A further important additional contribution of this study was the investigation of the association of neighbourhood deprivation with BC prognosis for the different subtypes. Deprivation was associated with higher risk of BCD for all molecular subtypes. Statistical power (Appendix Table C.15) was limited for the rarest subtypes (TNBC and luminal B) for which the association with deprivation was no longer statistically significant after adjusting for age, tumour characteristics, treatments and comorbidities. HER2-enriched tumours had the highest level of inequality, with women in the most deprived areas of Scotland diagnosed with this subtype having double the risk of BCD when compared to women in the least deprived areas with HER2-enriched tumours after adjusting for other tumour characteristics, screening, treatments and comorbidities. Future research should investigate to what extent access to and uptake of screening and treatment, alcohol consumption, smoking, obesity and other lifestyle factors might contribute to these inequalities.

5.3 Strengths and limitations

5.3.1 The use of the Scottish Cancer registry

The use of Scottish cancer registry data for this PhD has provided an excellent opportunity to investigate BC incidence and survival trends by molecular subtypes in Scotland. The high quality of the data and the availability of molecular marker data, especially for ER status (available from 1997) makes it one of the largest cohorts in Europe (over 70,000 women) in which to investigate heterogeneity of incidence and survival trends in BC subtypes. Further, linkage of death records and comorbidity records has provided the opportunity to investigate survival trends by subtype while adjusting for important covariates and identify subgroups of women with higher incidence and worse survival that would benefit from targeted treatments and prevention programmes.

Moreover, the findings from this PhD are representative of the Scottish female population as the cancer registry ascertains 98% [157] of BC cases and might be generalizable to other populations, particularly to the UK population as prevalence of established RFs for

BC are similar between the UK nations. However, regional differences would need to be further investigated once other cancer registries have sufficient molecular marker data. Findings in the trends were also similar to previous studies for other countries [135, 136, 146] adding to the existing literature and providing evidence of the distinct aetiology and prognosis of the BC subtypes.

The use of population-based cancer registry data had also some limitations inherent to cancer registrations, such as changes in the disease classification, reporting delays and the arbitrary definition of a primary incident invasive cancer which is subject to the examination of histological specimens by a pathologist [303]. Furthermore, treatment data within the registry is very limited and there is no information on recurrence. Since TNBC and HER2-enriched subtypes have been associated with increased recurrence risk compared to other BC subtypes [304, 305], linkage of the registry to available audit data with information on recurrence could help investigate the patterns to recurrence within subtypes. Another limitation of the data is the lack of interval cancer data. Although method of detection is available in the registry, information is very limited (tumour recorded as screen detected vs symptomatic) and, without linkage to screening data it is not possible to identify interval cancers. Furthermore, other tumour markers such as ki67 are not available within the Scottish registry. As cancer heterogeneity is an evolving research area, data on new molecular markers that can be used to assess secular trends and identify subgroups of women in need of additional treatments and prevention interventions will either need to be incorporated into the registry of available through data linkage. Finally, the role of RFs, such as reproductive factors and lifestyle factors, in secular trends in BC incidence and survival by molecular subtypes needs further research. This information could be used to estimate the number of BC that could be prevented for each subtype [155] and, inform cancer surveillance and resource allocation for prevention and treatment.

5.3.2 The use of robust statistical methods

Cancer registries record cancer cases and not individual patients' data, therefore, a woman can have multiple cancer records and hence appear multiple times in the registry. Using cancer records can therefor overestimate incidence rates which should be computed using population estimates at the individual (person) level. In order to correct for multiple tumours per woman being included in the rates, I used a single incident case per woman instead of multiple tumours.

The use of joinpoint regression and APC models to assess incidence trends provided further evidence of the distinct trends as results were consistent regardless of the method. APC models not only estimated the overall trend by subtype but provided additional information and found cohort effects on ER+ tumour incidence, hence generating the hypothesis that such effects women born in particular periods might be related to changes in reproductive factors or obesity patterns.

The methods used for the survival analysis expanded the traditional Cox PH model for which the PH assumption has largely been ignored in the cancer literature [260]. Using extended Cox models with TVE, my analysis highlights the importance of checking the PH assumption and of providing alternative methods to estimate the effect of certain tumour characteristics and treatments on BCSS at different time points after diagnosis. Further, the extended Cox model seemed to partially correct confounding by indication for the effects of radiotherapy and chemotherapy on BCSS.

As previously described, missing data for molecular markers can bias the incidence and survival trends if ignored [135]. During this PhD, incidence and survival trends were corrected for missing molecular markers using simple and MI techniques previously validated for cancer registry data.

5.4 Recommendations for future research

Molecular markers are likely to continue to inform approaches to prevention and treatment of breast and other cancers. This thesis shows the importance of studying BC heterogeneity and of the use of molecular markers for cancer surveillance. As heterogeneity is also displayed for primary tumours in other locations, the study of molecular subtypes can be extended to other tumour types which could lead to the discovery of new molecular markers to target for treatment and hence, improved survival in cancer patients. Linkage of national population-based datasets with high quality pathology and RF could further inform prediction, especially for rare subtypes for which information on established RFs is limited. Risk prediction models are used to identify women at high risk, for treatment decisions and to inform patients about their prognosis in clinical practice [306] but these models might need further stratification by subtype, given the heterogeneity observed for BC subtypes in both aetiology and prognosis. There is also the need to establish risk-stratified screening programmes to help identify women at highest risk of aggressive subtypes that would benefit from earlier or more frequent screening and potentially reduce screening frequency for low-risk women.

The novel coronavirus (COVID-19) pandemic and lockdown are a global public health concern and time trends in cancer incidence and survival will be affected by the current pandemic. Cancer patients are at high risk to develop severe complications from COVID-19, including invasive ventilation and death [307, 308]. In Scotland and in most countries in the world, the pandemic has resulted in cessation of routine cancer screening which will result in delayed diagnosis and treatment for many people with cancer. Cancer referrals from primary to secondary care are also being affected and many patients diagnosed with cancer prior to lockdown are currently waiting to receive treatment which could have an effect on their chances of survival. The work presented in this thesis provides a foundation for pre-pandemic BC incidence and survival patterns and assessing future cancer trends will help estimate the effect of the pandemic in patients with BC in Scotland and beyond, further illustrating the value of descriptive epidemiology.

Chapter 6 Conclusion

Although heterogeneity of BC has been established for years, cancer progression is still assessed in many countries overall and not by subtype. Further, few cancer registries in the world collect molecular marker data that could be used to assess incidence and survival trends by the molecular subtypes to further our understanding of BC epidemiology.

Using the excellent data resources within Scotland, this PhD has improved our understanding of the current incidence and survival trends observed in Scotland. The results from this study suggest that molecular subtypes are different diseases with different aetiology and prognosis and that divergent trends currently exist. While decreasing incidence of ER- tumours are cause for celebration, the increases in incidence of ER+ tumours suggest an important effect of screening and possibly other RFs as obesity and changes in reproductive factors. Additionally, this analysis found two important trends that should be carefully monitored in the future: the recent declines observed for ER+ tumours and the increases in TNBC in young women.

This PhD has also identified clear prognostic differences between BC subtypes and highlights the importance of not only molecular markers, but also age, grade, stage and deprivation. Although, survival trends improved in the last two decades, improvements were higher for women of screening age. In contrast, survival in older women did not seem to improve and undertreatment might have been one of the reasons. The adoption of new treatments might have also played a role but more detailed data would be required to assess this contribution.

Considering the importance of BC for public health, this PhD recommends looking at incidence and survival trends by molecular subtypes and future research should focused on the development of targeted screening for women with more aggressive tumours and in improving outcomes in older women and in women living in the most deprived areas of Scotland, through prevention, enhanced screening and treatments and access to care.

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Appendices

Appendix A Systematic Review

Appendix A.1 List of countries included in the systematic review

Europe: Albania, Andorra, Austria, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Macedonia, Malta, Moldova, Monaco, Montenegro, The Netherlands, Norway, Poland, Portugal, Romania, Russian Federation, San Marino, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, UK, Ukraine.

North America: the USA and Canada.

Oceania: Australia and New Zealand.

Appendix A.2 Systematic review search terms

List for Pubmed

#1 *Breast Neoplasms/

#2 breast neoplasm$ OR breast cancer$ OR breast tumor$ OR breast tumour$ OR breast carcinoma$

#3 incidence.mp OR Incidence/

#4 trend$.mp

#5 Receptors, Estrogen/

#6 hormone replacement therapy/ or estrogen replacement therapy/

#7 1 OR 2

#8 3 OR 4

#9 5 OR 6

#10 7 AND 8 AND 9

#11 limit 10 to (English language and humans)

List for Embase

#1 breast cancer/ OR breast tumor/

#2 breast neoplasm$ OR breast cancer$ OR breast tumor$ OR breast tumour$ OR breast carcinoma$

#3 incidence/

#4 trend study/ OR trend$.mp

#5 estrogen receptor/

#6 hormone substitution/ OR estrogen therapy/

#7 1 OR 2

#8 3 OR 4

#9 5 OR 6

#10 7 AND 8 AND 9

#11 limit 10 to (human and English language)

List for Web of Science

#1 breast

#2 cancer OR tumor OR tumour OR neoplasm

#3 incidence OR trend$

#4 estrogen receptor OR hormone replacement therapy OR estrogen replacement therapy

#5 1 AND 2

#6 5 AND 3 AND 4

#7 su=oncology

#8 ti=breast cancer

#9 6 AND 7 AND 8

Appendix A.3 Inclusion and exclusion criteria for systematic review

INCLUSION CRITERIA:

Types of studies
- English language.
- Studies from European ancestry majority countries, i.e. Europe, the US, Canada, Australia and New Zealand.

Studies using data from cancer registries or population-based studies.

Types of participants
- Female adults (>18 years)

Diagnosed with invasive breast cancer

Types of outcome measures
Incidence of invasive breast cancer stratified by ER status.

EXCLUSION CRITERIA:

Types of studies
- Non English language.
- Studies from Non-European ancestry majority countries, i.e. Africa, Asia, South America.
- Quantitative studies without population-based data.
- Reviews (systematic, narrative or qualitative).
- Editorial comments with no research or additional data reported.
- Conference or meeting abstracts.
- Qualitative studies.

Duplicate studies (with the same population data).

Types of participants
- Children (<18 years) and males.

No breast cancer diagnosis or rare types of breast cancer.

Types of outcome measures
Any other than incidence rates in any of its forms OR without stratification by ER status and year of diagnosis.

Appendix A.4 National screening programmes in the countries included in the systematic review

Appendix Table A.1 Characteristics of screening programmes in the countries included in the systematic review

Country	Has a national or regional screening program been implemented?	Year program started	Method of detection	Age groups covered	Recommended time interval for screening
Denmark	YES	1991	Mammography	50 to 69	2 years
Ireland	YES	2000	Mammography	50 to 64	2 years
France	YES	1989	Mammography and clinical breast exam	50 to 74	2 years
Germany	YES	2002	Mammography	50 to 69	2 years
Norway	YES	1996	Mammography	50 to 69	2 years
Scotland	YES	1988	Mammography	50 to 70	3 years
Sweden	YES	1986	Mammography	40 to 74	18 months (age 40 to 49) and 2 years (age 50+)
United States	NO	1995	Mammography and clinical breast exam	40 to 75+	1-2 years

Appendix B Incidence Chapter

Appendix B.1 Classification of breast cancer molecular subtypes based on IHC markers ER, PR and HER2 in Scotland from 2009 to 2016 that would be used during the dissertation

Appendix Table B.1 Intrinsic molecular subtypes as defined by IHC markers throughout the thesis (without the use of tumour grade)

Intrinsic molecular subtype	IHC subtype	ER status	PR status	HR status	HER2 status	Frequency
Luminal A	HR+/HER2- n=20,484 (66%)	Positive	Positive	Positive	Negative	13,344
		Positive	Negative	Positive	Negative	2,173
		Positive	Unknown	Positive	Negative	4,812
		Negative	Positive	Positive	Negative	<200
		Unknown	Positive	Positive	Negative	<10
Luminal B	HR+/HER2+ n=2,915 (9%)	Positive	Positive	Positive	Positive	1,599
		Positive	Negative	Positive	Positive	678
		Positive	Unknown	Positive	Positive	567
		Negative	Positive	Positive	Positive	<100
		Unknown	Positive	Positive	Positive	<10
HER2-enriched	HR-/HER2+ n=1,288 (4%)	Negative	Negative	Negative	Positive	1,077
		Negative	Unknown	Negative	Positive	211
		Unknown	Negative	Negative	Positive	0
Basal-like	HR-/HER2- Triple Negative n=2,899 (9%)	Negative	Negative	Negative	Negative	2,557
		Negative	Unknown	Negative	Negative	342
		Unknown	Negative	Negative	Negative	0
Unknown	Unknown n=3,513 11%)	Positive	Positive	Positive	Unknown	926
		Positive	Negative	Positive	Unknown	185
		Positive	Unknown	Positive	Unknown	1,110
		Negative	Positive	Positive	Unknown	<10
		Negative	Negative	Negative	Unknown	169
		Negative	Unknown	Negative	Unknown	211
		Unknown	Positive	Positive	Unknown	<10
		Unknown	Negative	Negative	Unknown	<10
		Unknown	Unknown	Unknown	Positive	0
		Unknown	Unknown	Unknown	Negative	0
		Unknown	Unknown	Unknown	Unknown	901

ER=oestrogen receptor, PR=progesterone receptor, HR=hormone receptor, HER2= human epidermal growth factor 2, IHC=immunohistochemistry.

Appendix B.2 Algorithm to derive final TNM stage

If pT = X or blank but pathological tumour size is recorded, derive pT as follows:
If pathological tumour size ≤ 20mm, pT = 1
If pathological tumour size >20mm ≤ 50mm, pT = 2
If pathological tumour size > 50mm, pT = 3

If pN = X or blank but number (of nodes) positive is recorded, derive pN as follows:
If numbers positive = 0, pN = 0
If numbers positive = 1–3, pN = 1
If numbers positive = 4–9, pN = 2
If numbers positive ≥ 10, pN = 3

Construct **Final TNM** based on cTNM and pTNM as follows:
Assume MX = M0
T4 takes precedence whether from cT or pT
M1 takes precedence whether from cM or pM
If patient had radiotherapy, chemotherapy, biological therapy, or hormone therapy starting at least 4 weeks before surgery:

- use cT unless pT is greater than or equal to cT. If cTX assume TX, unless pT4
- use cN unless pN is greater than or equal to cN. If cNX assume NX, unless pN3
- use cM unless pM is greater than or equal to cM. If cMX or cM0 assume M0, unless pM1

Otherwise, use pTNM values in preference to cTNM values, unless pTNM values are blank or recorded as X.

Once final TNM has been derived, convert to Stage Grouping I–IV as follows:

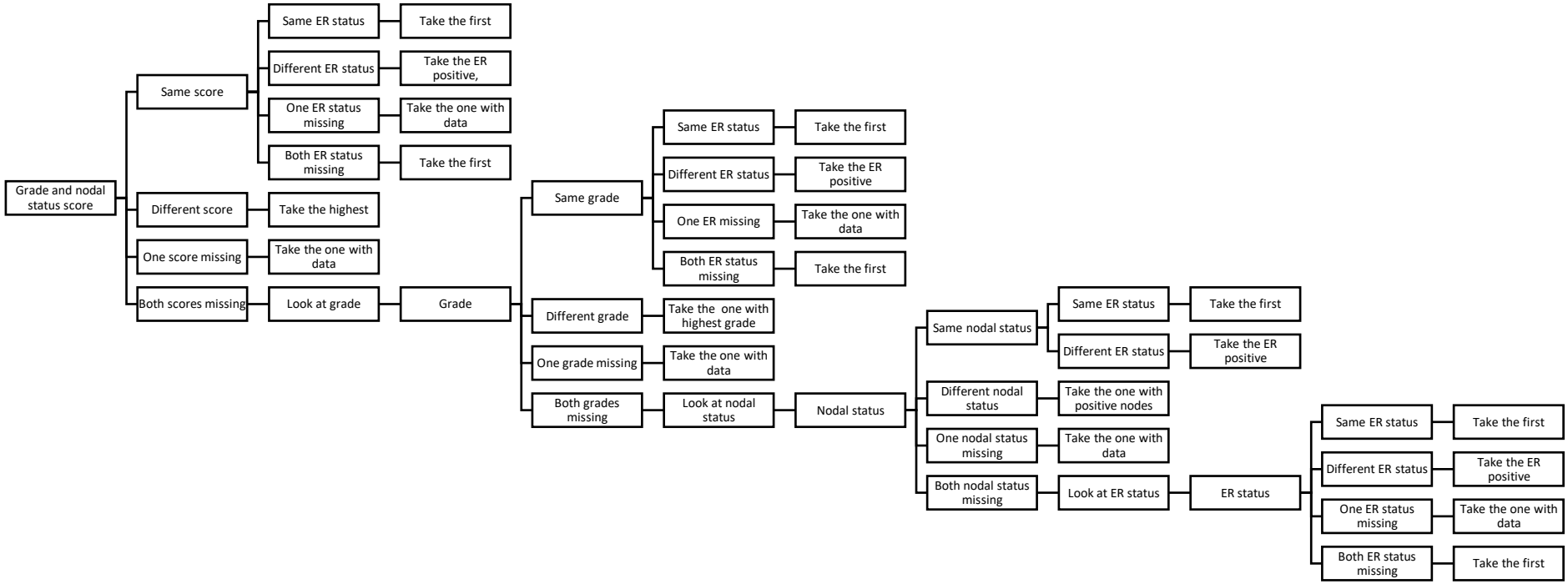
Stage	T	N	M
IA	T1*	N0	M0
IB	T0, T1*	N1mi	M0
IIA	T0, T1*	N1	M0
IIA	T2	N0	M0
IIB	T2	N1	M0
IIB	T3	N0	M0
IIIA	T0, T1*, T2	N2	M0
IIIA	T3	N1, N2	M0
IIIB	T4	N0, N1, N2	M0
IIIC	Any T	N3	M0
IV	Any T	Any N	M1

*T1 includes T1mi

Note: pT = pathological T stage, pN= pathological N stage, pM=pathological M stage, cT = clinical T stage, cN= clinical N stage, cM= clinical M stage.

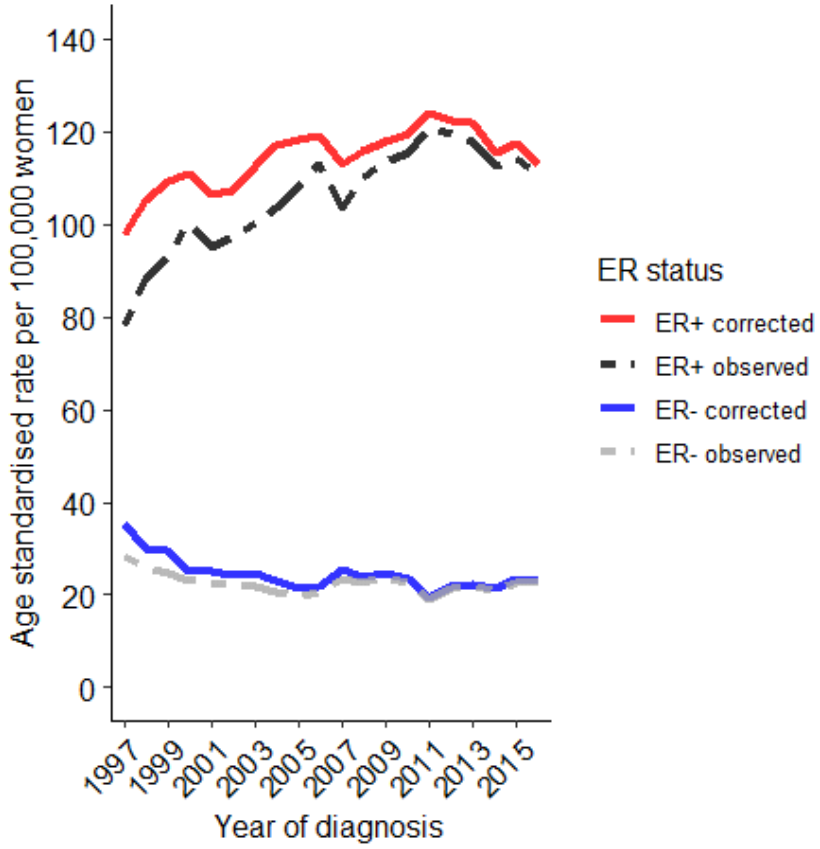
Appendix B.3 Selection of a single invasive tumour in women with two invasive tumours diagnosed within 6 months

Appendix Figure B.1 Flowchart of the selection of one invasive tumour per woman based on grade and nodal status



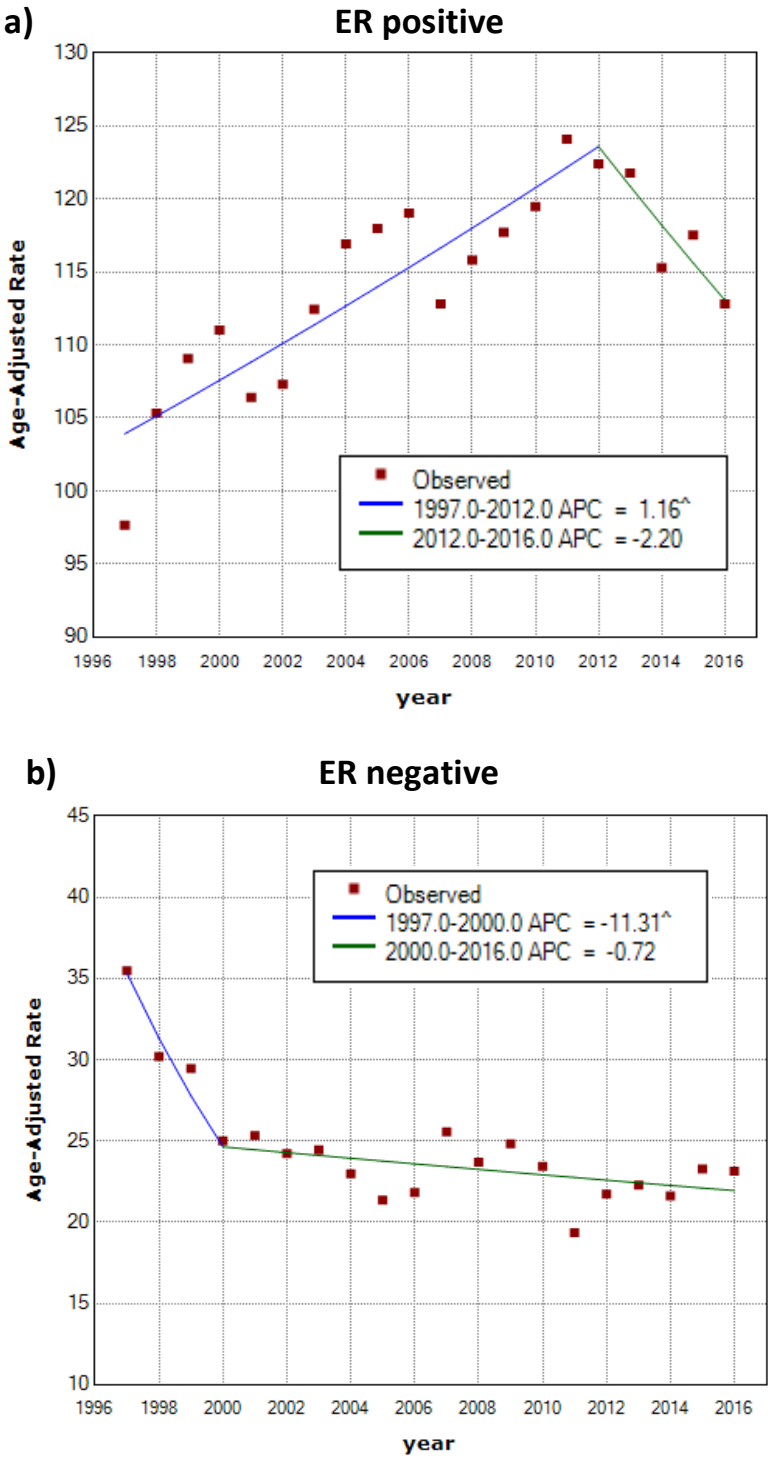
Appendix B.4 Comparison of incidence trends by ER status with and without imputation of missing ER status

Appendix Figure B.2 ASiR for ER+ and ER- tumours with imputation of missing ER status (corrected) and without imputation (observed) from 1997 to 2016 in Scotland



Appendix B.5 Additional results and graphs obtained from Joinpoint regression analysis of the incidence trends by ER status in Scotland

Appendix Figure B.3 Graph of breast cancer incidence trends for ER+ (a) and ER- (b) tumours from joinpoint regression with EAPC estimates



^indicates statistically significant EAPC (p<0.05)

Appendix Table B.2 Modelled age adjusted incidence rates from joinpoint analysis for ER+ and ER- tumours and joinpoint location in the selected final model

ER status	Year	Observed Age-Adjusted Rate	Modelled Age-Adjusted Rate	Standard Error	Joinpoint Location
Positive	1997	97.7	103.9	2.0	
	1998	105.3	105.1	2.1	
	1999	109.1	106.4	2.1	
	2000	111.0	107.6	2.1	
	2001	106.4	108.8	2.1	
	2002	107.3	110.1	2.1	
	2003	112.4	111.4	2.1	
	2004	116.9	112.7	2.2	
	2005	118.0	114.0	2.2	
	2006	119.0	115.3	2.2	
	2007	112.8	116.7	2.1	
	2008	115.8	118.0	2.1	
	2009	117.7	119.4	2.1	
	2010	119.5	120.8	2.1	
	2011	124.1	122.2	2.2	
	2012	**122.4**	**123.6**	**2.1**	**Joinpoint 1**
	2013	121.8	120.9	2.1	
	2014	115.3	118.2	2.0	
	2015	117.5	115.6	2.0	
	2016	112.8	113.1	2.0	
Negative	1997	35.5	35.3	1.2	
	1998	30.2	31.3	1.1	
	1999	29.5	27.8	1.1	
	2000	**25.0**	**24.6**	**1.0**	**Joinpoint 1**
	2001	25.3	24.5	1.0	
	2002	24.2	24.3	1.0	
	2003	24.5	24.1	1.0	
	2004	23.0	23.9	0.9	
	2005	21.4	23.8	0.9	
	2006	21.8	23.6	0.9	
	2007	25.6	23.4	1.0	
	2008	23.7	23.3	1.0	
	2009	24.8	23.1	1.0	
	2010	23.4	22.9	0.9	
	2011	19.4	22.8	0.9	
	2012	21.7	22.6	0.9	
	2013	22.3	22.4	0.9	
	2014	21.6	22.3	0.9	
	2015	23.3	22.1	0.9	
	2016	23.1	21.9	0.9	

Rows in bold indicate Joinpoint location. ER= oestrogen receptor.

Appendix Table B.3 Estimated regression coefficients with general parameterization for the final model fitted with joinpoint regression for trends in incidence of ER+ and ER- tumours in Scotland between 1997 and 2016

ER status	Parameter	Parameter Estimate	Standard Error	Test Statistic (t)	P value
Positive	Intercept 1	-18.43	3.51	-5.26	**<0.0001**
	Intercept 2	49.57	24.50	2.02	0.06
	Slope 1	0.01	<0.01	6.61	**<0.0001**
	Slope 2	-0.02	0.01	-1.83	0.09
Negative	Intercept 1	243.14	83.76	2.90	**0.01**
	Intercept 2	17.67	7.26	2.43	**0.03**
	Slope 1	-0.12	0.04	-2.86	**0.01**
	Slope 2	-0.01	<0.01	-2.00	0.06

Bold results indicate the test was significant at the 5% level. ER= oestrogen receptor.

Appendix Table B.4 Model selection, permutation hypothesis and tests results for ER+ and ER- tumours in joinpoint regression

ER status	Test Number	Null Hypothesis	Alternate Hypothesis	P value	Significance Level~
Positive	#1	0 Joinpoint(s)	3 Joinpoint(s) *	<0.01	0.02
	#2	1 Joinpoint(s) *	3 Joinpoint(s)	0.18	0.03
	#3	1 Joinpoint(s) *	2 Joinpoint(s)	0.09	0.03
Negative	#1	0 Joinpoint(s)	3 Joinpoint(s) *	<0.01	0.02
	#2	1 Joinpoint(s) *	3 Joinpoint(s)	0.28	0.03
	#3	1 Joinpoint(s) *	2 Joinpoint(s)	0.48	0.03

*Selected model after each test. ~ Significance level for individual test (α=0.05). ER= oestrogen receptor.

Appendix B.6 Sensitivity Analysis for joinpoint regression

I used ER+ tumours as an example for the sensitivity analysis. The default methods and parameters are:

- Modeling method: the grid search method with two constraints on the location of the joinpoints (a minimum of 2 observations from a joinpoint to either end of the data, and a minimum of 2 observations between two joinpoints).
- Model selection method: Permutation Test with 4,499 permutations performed and overall significance level of 5%.
- Number of joinpoints: the default number of joinpoints depend on the number of data points. Our data contains 20 consecutive years, therefore, the default maximum number of joinpoints is 3.
    - Errors options: Uncorrelated errors model
    - Estimation of AAPC and EAPC confidence intervals: Parametric method

Appendix Table B.5 Sensitivity analysis of joinpoint regression with uncorrelated and autocorrelated results for ER+ tumours

Autocorrelated errors options	Number of joinpoints	Joinpoint location	EAPC (95% CI) for period before joinpoint	EAPC (95% CI) for period after joinpoint
Uncorrelated model	1 Joinpoint	2012	**1.2% (0.8, 1.5)**	-2.2 (-4.7, 0.4)
Autocorrelated model based on the data	1 Joinpoint	2011	**1.3% (0.8, 1.7)**	-1.6 (-3.4, 0.3)
Autocorrelated model with parameter=0.1	1 Joinpoint	2012	**1.2% (0.8, 1.6)**	-2.2 (-4.7, 0.4)
Autocorrelated model with parameter=0.2	1 Joinpoint	2011	**1.3% (0.8, 1.8)**	-1.6 (-3.4, 0.3)
Autocorrelated model with parameter=0.3	1 Joinpoint	2011	**1.3% (0.8, 1.8)**	-1.6 (-3.5, 0.3)
Autocorrelated model with parameter=0.4	1 Joinpoint	2011	**1.3% (0.8, 1.9)**	-1.7 (-3.7, 0.3)

Bold results indicate the estimate was statistically significant. EAPC= Estimated Annual Percentage Change

Appendix Table B.6 Sensitivity analysis of joinpoint regression for model selection method

Model selection method	Model	Number of joinpoints	Number of observations	Number of parameters	df	SSE	BIC value
BIC	#1	0 Joinpoint(s)	20	2	18	87.40	1.77
	#2	1 Joinpoint(s)	20	4	16	36.71	1.21
	#3	2 Joinpoint(s) *	20	6	14	26.34	**1.17**
	#4	3 Joinpoint(s)	20	8	12	23.83	1.37
BIC3	#1	0 Joinpoint(s)	20	2	18	87.40	1.77
	#2	1 Joinpoint(s) *	20	4	16	36.71	**1.36**
	#3	2 Joinpoint(s)	20	6	14	26.34	1.47
	#4	3 Joinpoint(s)	20	8	12	23.83	1.82
Modified BIC	#1	0 Joinpoint(s) *	20	2	18	87.40	**1.12**
	#2	1 Joinpoint(s)	20	4	16	36.71	1.14
	#3	2 Joinpoint(s)	20	6	14	26.34	1.56
	#4	3 Joinpoint(s)	20	8	12	23.83	2.29

BIC in bold corresponds to smallest BIC value and *selected model. BIC= Bayesian Information Criterion, df= degrees of freedom, SSE= Sum of standard errors.

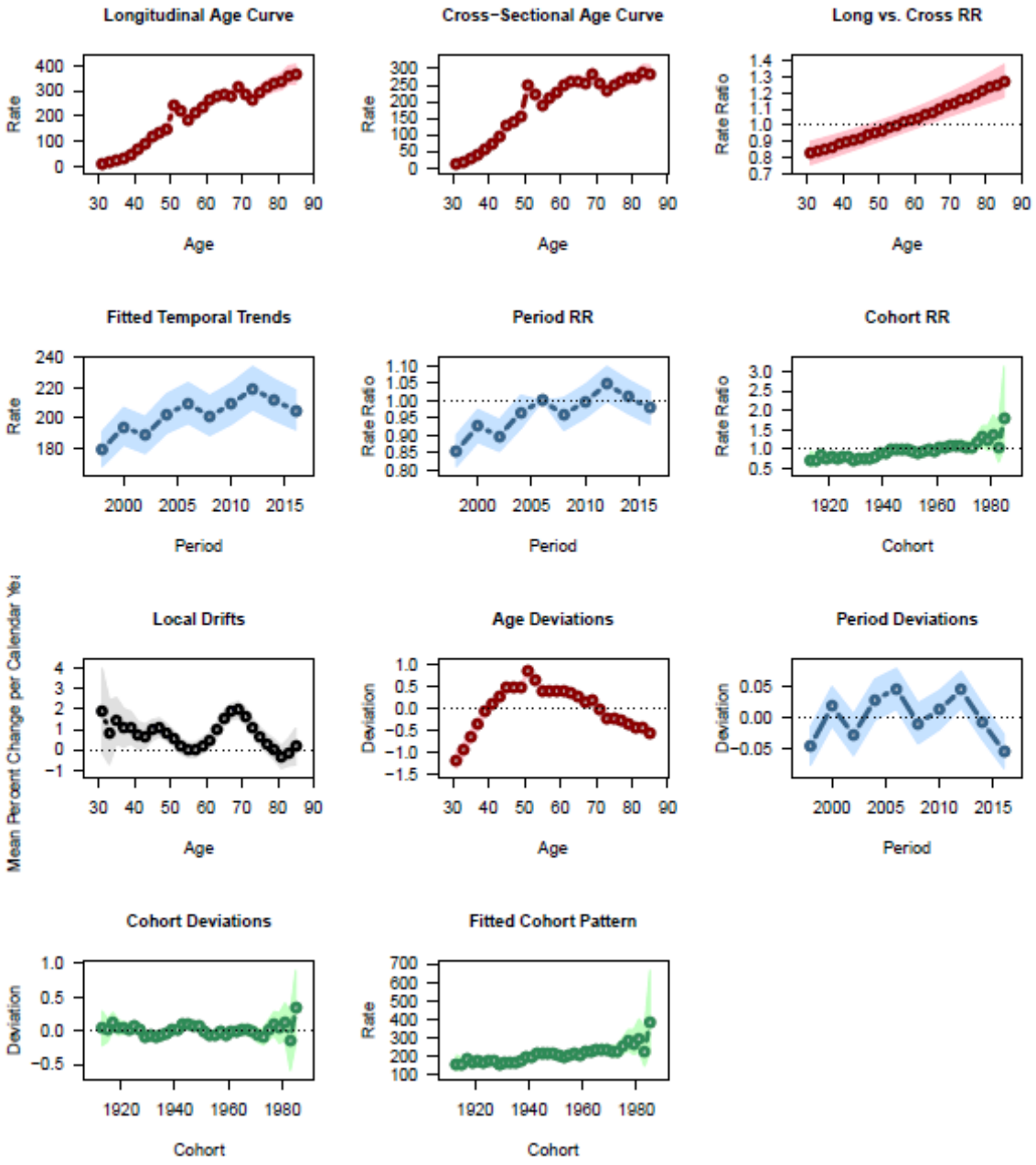
Appendix Table B.7 Sensitivity analysis of joinpoint regression for model selection method and resulting parameters estimates

Model selection method	Number of joinpoints	Time period 1	EAPC (95% CI)	Time period 2	EAPC (95% CI)	Time period 3	EAPC (95% CI)
Permutation test	1 Joinpoint	1997-2012	**1.2% (0.8, 1.5)**	2012-2016	-2.2 (-4.7, 0.4)		
BIC	2 Joinpoints	1997-1999	4.7% (-4.3, 14.6)	1999-2012	**1% (0.5, 1.5)**	2012-2016	-2 (-4.3, 0.5)
BIC3	1 Joinpoint	1997-2012	**1.2% (0.8, 1.5)**	2012-2016	-2.2 (-4.7, 0.4)		
Modified BIC	0 Joinpoint	1997-2016	**0.7% (0.4, 1)**				

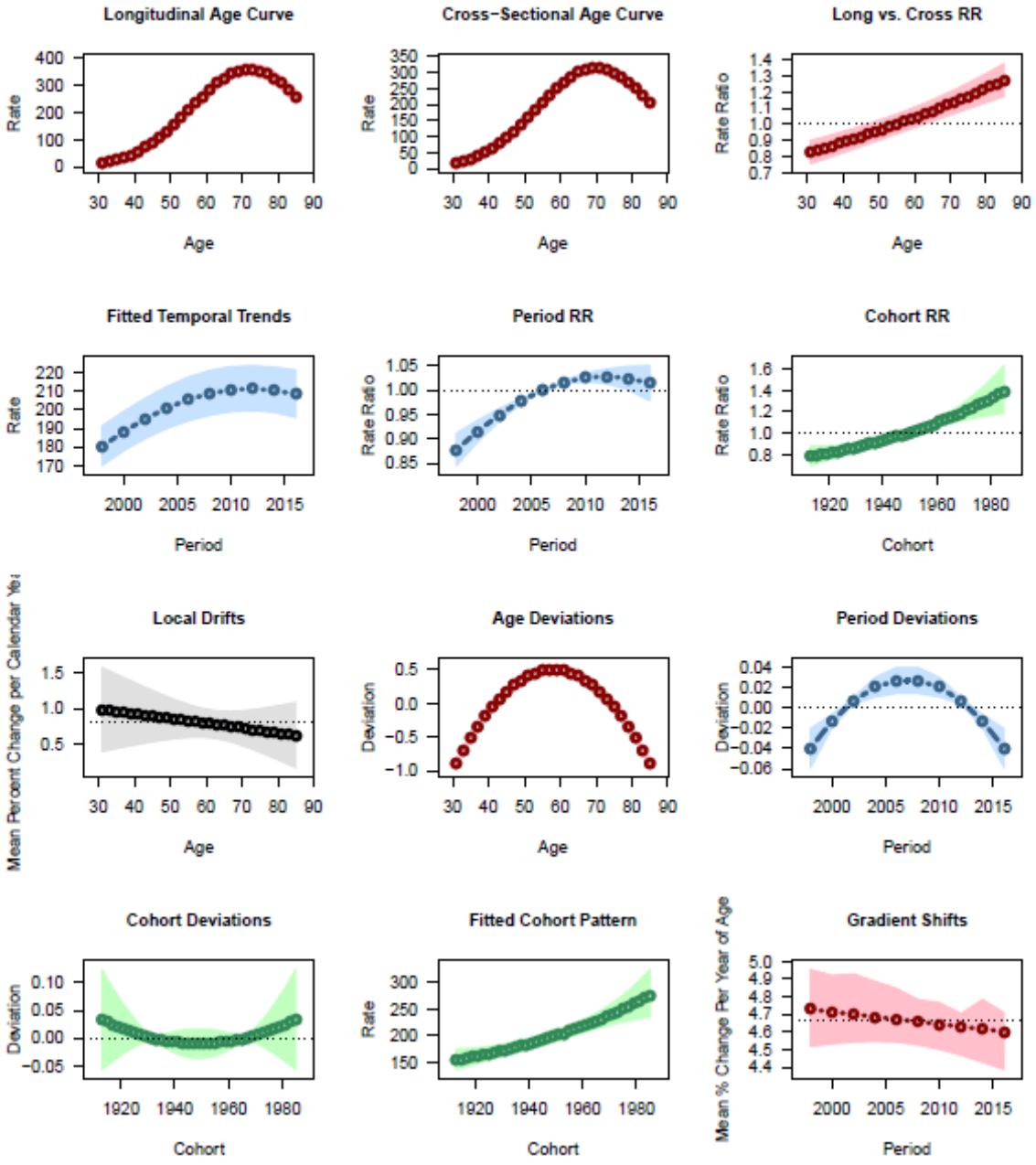
Bold results indicate the estimate was statistically significant. BIC=Bayesian Information Criterion, CI= confidence interval, EAPC= Estimated Annual Percentage Change.

Appendix B.7 Additional graphs and results from APC models for ER+ tumours

Appendix Figure B.4 Graphs for trends in breast cancer incidence for ER+ tumours by age, period and cohort effects



Appendix Figure B.5 Graphs for trends in breast cancer incidence for ER+ tumours by age, period and cohort effects for the curvature parameters



APC models for ER+ tumours have a woman aged 57 years, diagnosed in 2006 and hence born in 1949 as the reference.

Appendix Table B.8 Estimates with 95% CI for all the parameters of the final APC model for ER+ tumours

Parameter	Estimate	SD	Lower CI	Upper CI
Intercept	**-6.56727**	0.00764	-6.58224	-6.55229
LAT	**0.05360**	0.00122	0.05121	0.05598
Net Drift	**0.00800**	0.00106	0.00592	0.01008
CAT	**0.04560**	0.00063	0.04436	0.04683
Age curvature	**-0.00191**	0.00006	-0.00203	-0.00179
Period curvature	**-0.00083**	0.00020	-0.00122	-0.00044
Cohort curvature	0.00003	0.00005	-0.00006	0.00012

Bold results indicate significant estimates. CI=Confidence interval, CAT=Cross-sectional age trend, LAT=Longitudinal age trend, SD=Standard deviation.

Appendix Table B.9 Wald tests results for all key hypotheses on the APC model of ER+ tumours

Hypothesis test	Chi-square	df	P value
Net Drift = 0	56.75	1	**<0.00000000001**
Age curvature = 0	954.37	1	**<0.00000000001**
All Higher-Order Age Deviations = 0	689.03	25	**<0.00000000001**
All Age Deviations = 0	1539.17	26	**<0.00000000001**
Period curvature = 0	17.02	1	**0.00003694340**
All Higher-Order Period Deviations = 0	18.59	7	**0.00956066800**
All Period Deviations = 0	36.33	8	**0.00001526035**
Cohort curvature = 0	0.53	1	0.46596760000
All Higher-Order Cohort Deviations = 0	98.76	34	**0.00000003130**
All Cohort Deviations = 0	99.24	35	**0.00000004633**
All Period RR = 1	85.82	9	**<0.00000000001**
All Cohort RR = 1	151.71	36	**<0.00000000001**
All Local Drifts = Net Drift	93.59	28	**0.00000000540**
All Gradient Shifts = CAT	12.56	10	0.24924410000

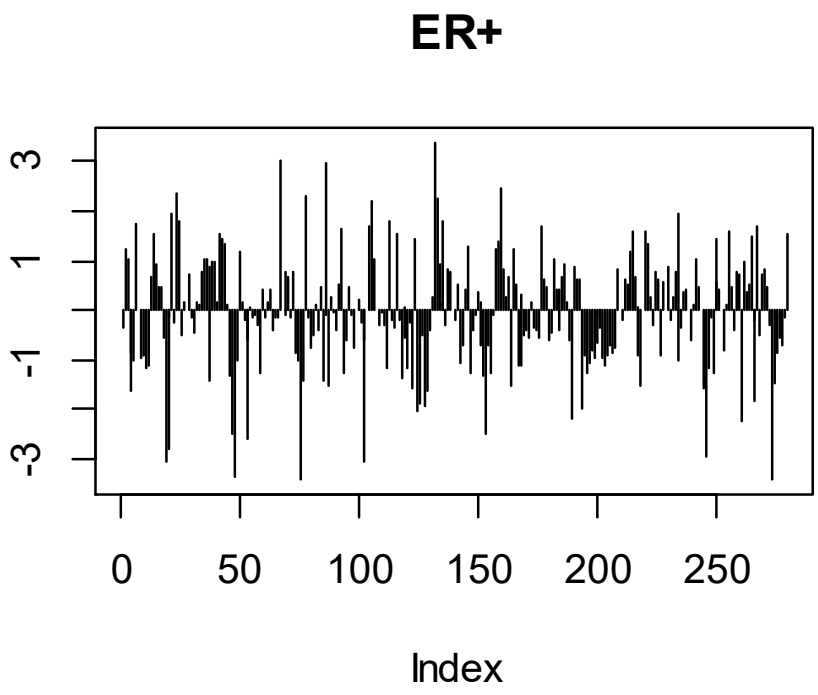
Bold results indicate the test was significant at the 5% level. CAT= Cross-sectional age trend, df= degrees of freedom, RR=rate ratio

Appendix Table B.10 Combination tests for the APC model of ER+ tumours

Combination tests	P value
All Period Deviations = 0	**0.00007388679**
All PRR = 1 <=> FTT = constant	**<0.00000000001**
All Cohort Deviations = 0	**0.00000006260**
All CRR = 1 <=> FCP = constant	**<0.00000000001**

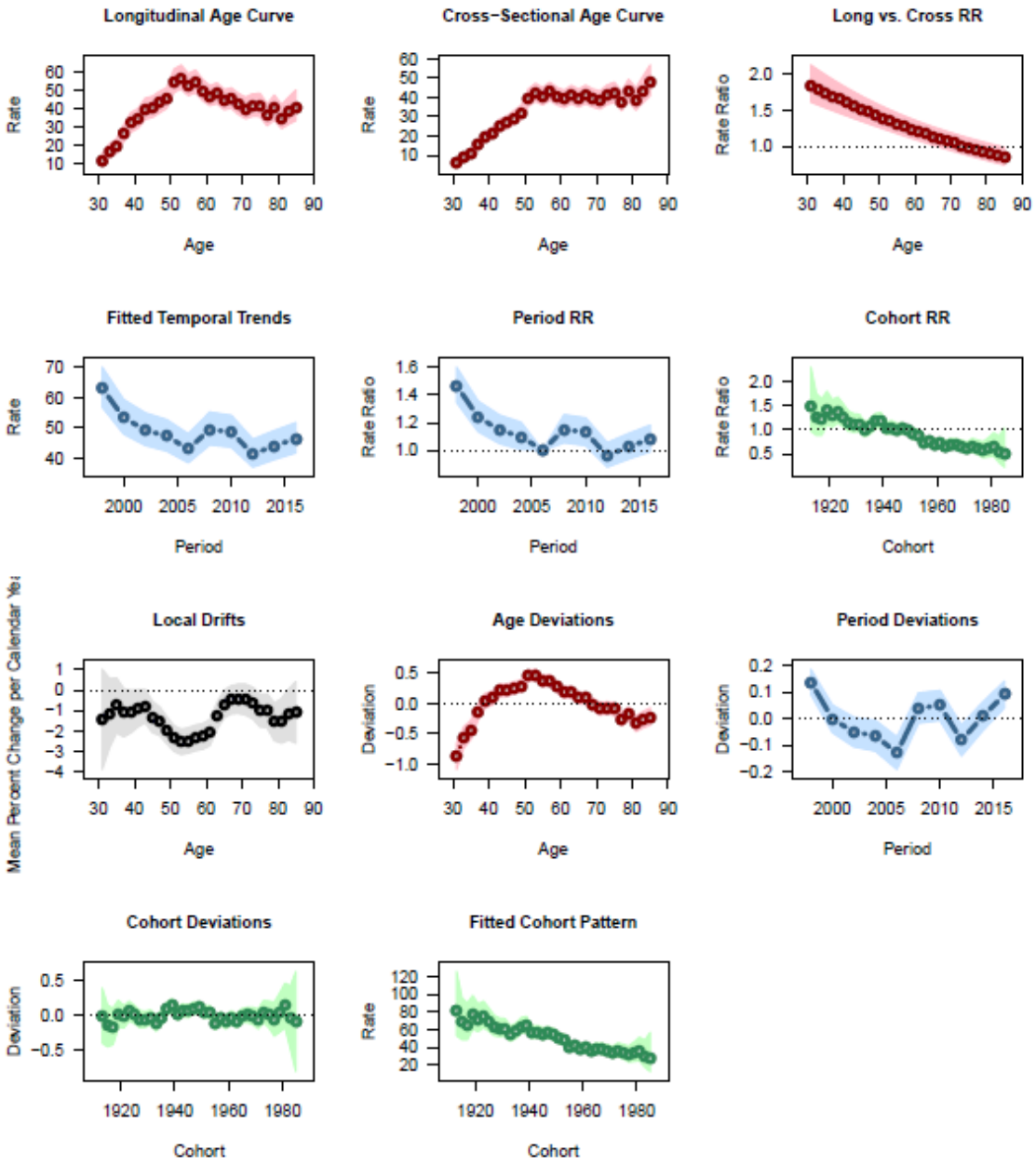
Bold results indicate the test was significant at the 5% level. CRR=Cohort rate ratio, FCP=Fitted cohort pattern, FTT=Fitted temporal trends, PRR=Period rate ratio.

Appendix Figure B.6 Graphs of the deviance residuals for APC models of ER+ tumours

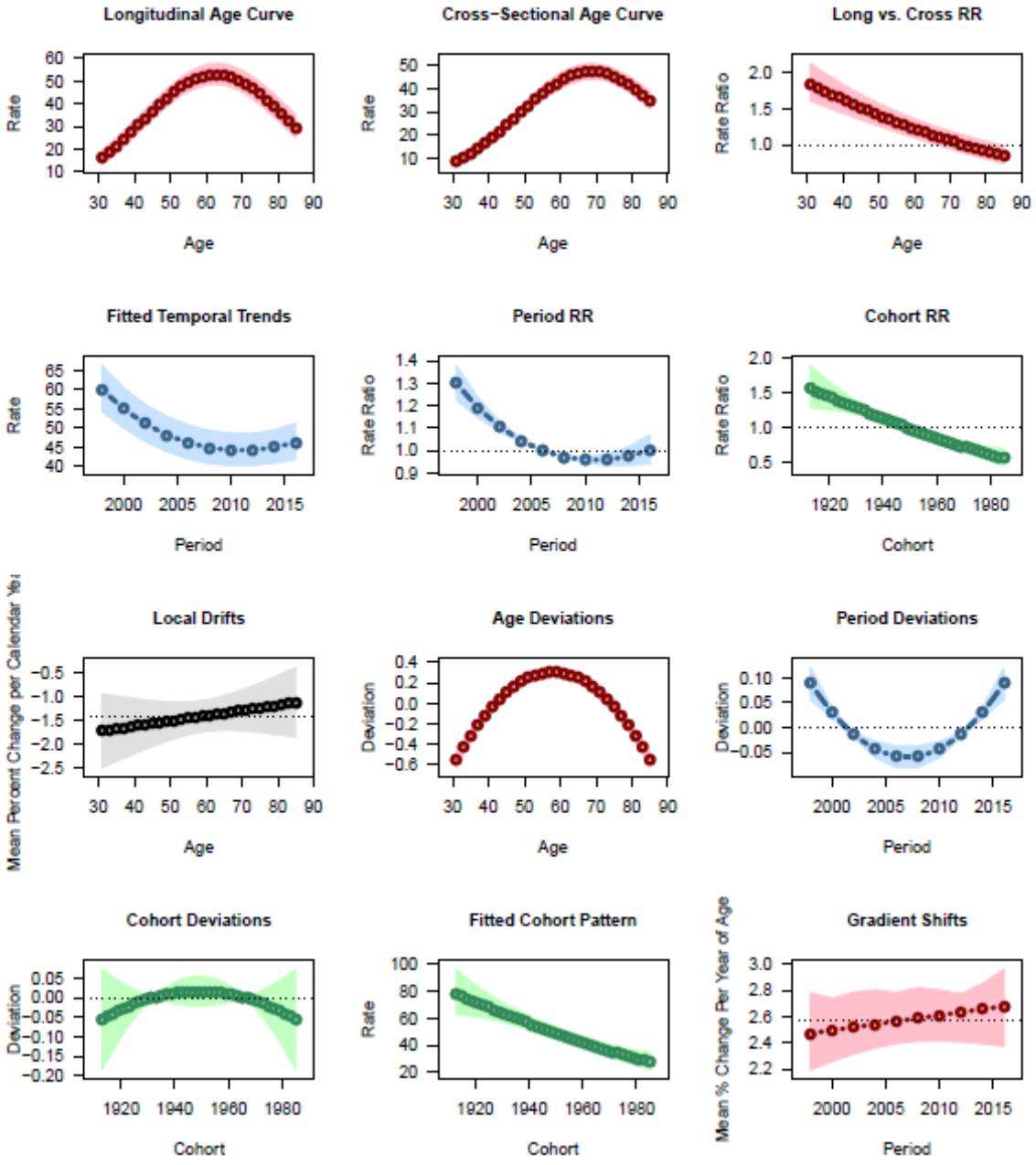


Appendix B.8 Additional graphs and results from APC models for ER- tumours

Appendix Figure B.7 Graphs for trends in breast cancer incidence for ER- tumours by age, period and cohort effects



Appendix Figure B.8 Graphs for trends in breast cancer incidence for ER- tumours by age, period and cohort effects for the curvature parameters



Appendix Table B.11 Estimates with 95% CI for all the parameters of the final APC model for ER- tumours

Parameter	Estimate	SD	Lower CI	Upper CI
Intercept	**-7.98823**	0.01112	-8.01003	-7.96642
LAT	**0.01105**	0.00186	0.00740	0.01470
NetDrift	**-0.01438**	0.00170	-0.01771	-0.01106
CAT	**0.02543**	0.00084	0.02378	0.02708
Age curvature	**-0.00117**	0.00008	-0.00134	-0.00101
Period curvature	**0.00184**	0.00034	0.00117	0.00250
Cohort curvature	-0.00006	0.00007	-0.00018	0.00007

Bold results indicate significant estimates. CAT=Cross-sectional age trend, CI=Confidence interval, LAT=Longitudinal age trend, SD=Standard deviation.

Appendix Table B.12 Wald tests results for all key hypotheses on the APC model of ER- tumours

Hypothesis test	Chi-square	df	P-Value
Net Drift = 0	71.84	1	**<0.00000000001**
Age curvature = 0	191.60	1	**<0.00000000001**
All Higher-Order Age Deviations = 0	117.63	25	**<0.00000000001**
All Age Deviations = 0	293.49	26	**<0.00000000001**
Period curvature = 0	29.22	1	**0.00000006449**
All Higher-Order Period Deviations = 0	34.59	7	**0.00001334335**
All Period Deviations = 0	63.85	8	**0.00000000008**
Cohort curvature = 0	0.74	1	0.38923730000
All Higher-Order Cohort Deviations = 0	46.87	34	0.06987975000
All Cohort Deviations = 0	48.05	35	0.06978012000
All Period RR = 1	140.96	9	**<0.00000000001**
All Cohort RR = 1	125.33	36	**0.00000000001**
All Local Drifts = Net Drift	40.28	28	0.06243669000
All Gradient Shifts = CAT	4.56	10	0.91834280000

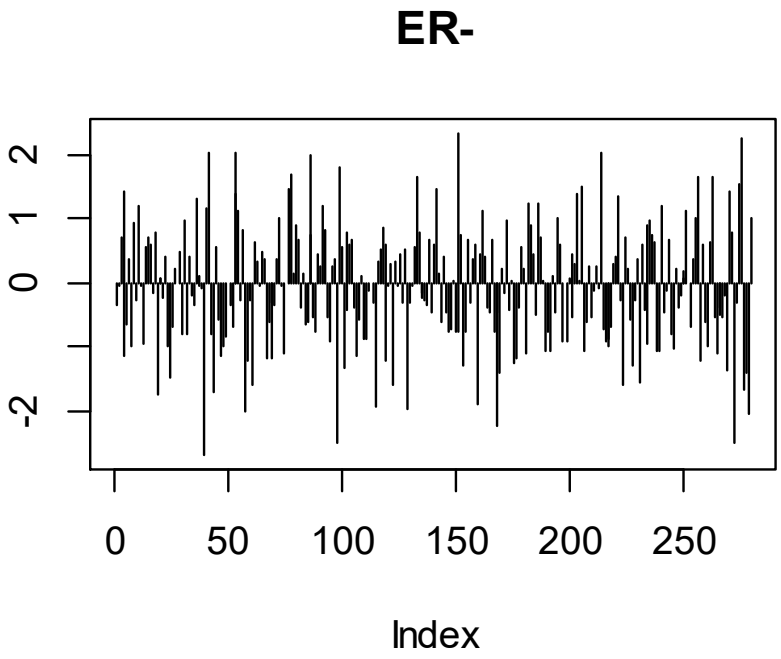
Bold results indicate the test was significant at the 5% level. CAT= Cross-sectional age trend, df= degrees of freedom. RR=rate ratio.

Appendix Table B.13 Combination tests for the APC model of ER- tumours

Combination tests	P value
All Period Deviations = 0	**0.00000012898**
All PRR = 1 <=> FTT = constant	**<0.00000000001**
All Cohort Deviations = 0	0.13975950000
All CRR = 1 <=> FCP = constant	**<0.00000000001**

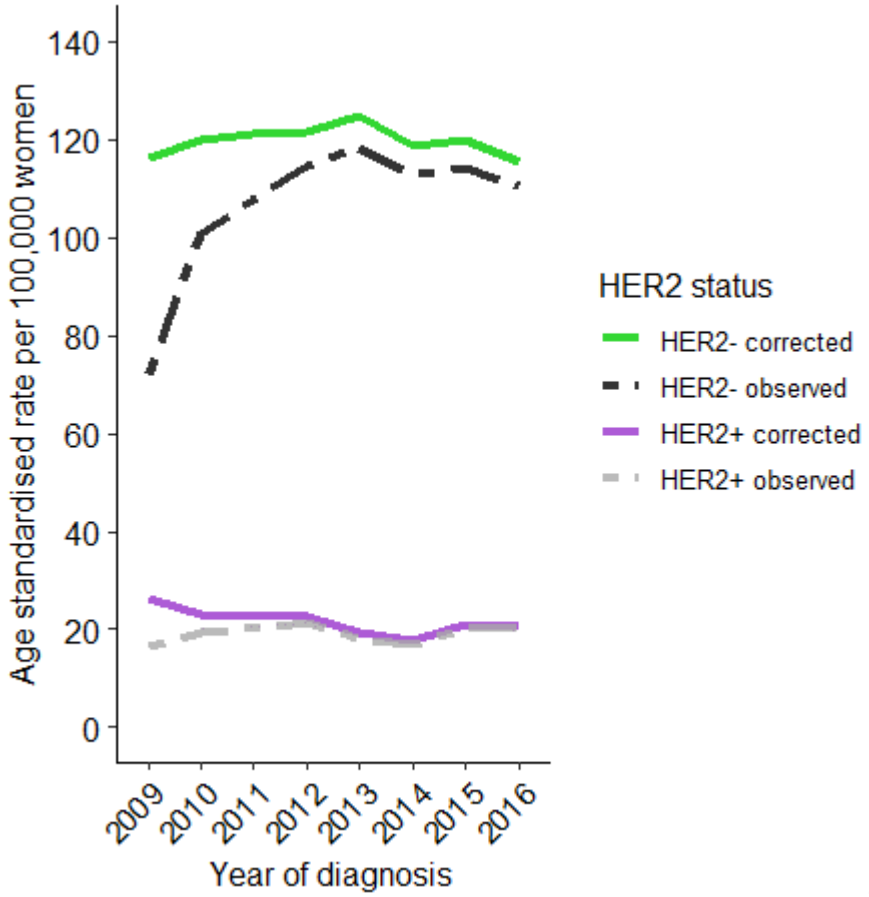
Bold results indicate the test was significant at the 5% level. CRR=Cohort rate ratio, FCP=Fitted cohort pattern, FTT=Fitted temporal trends, PRR=Period rate ratio

Appendix Figure B.9 Graph of deviance residuals for APC model for ER- tumours



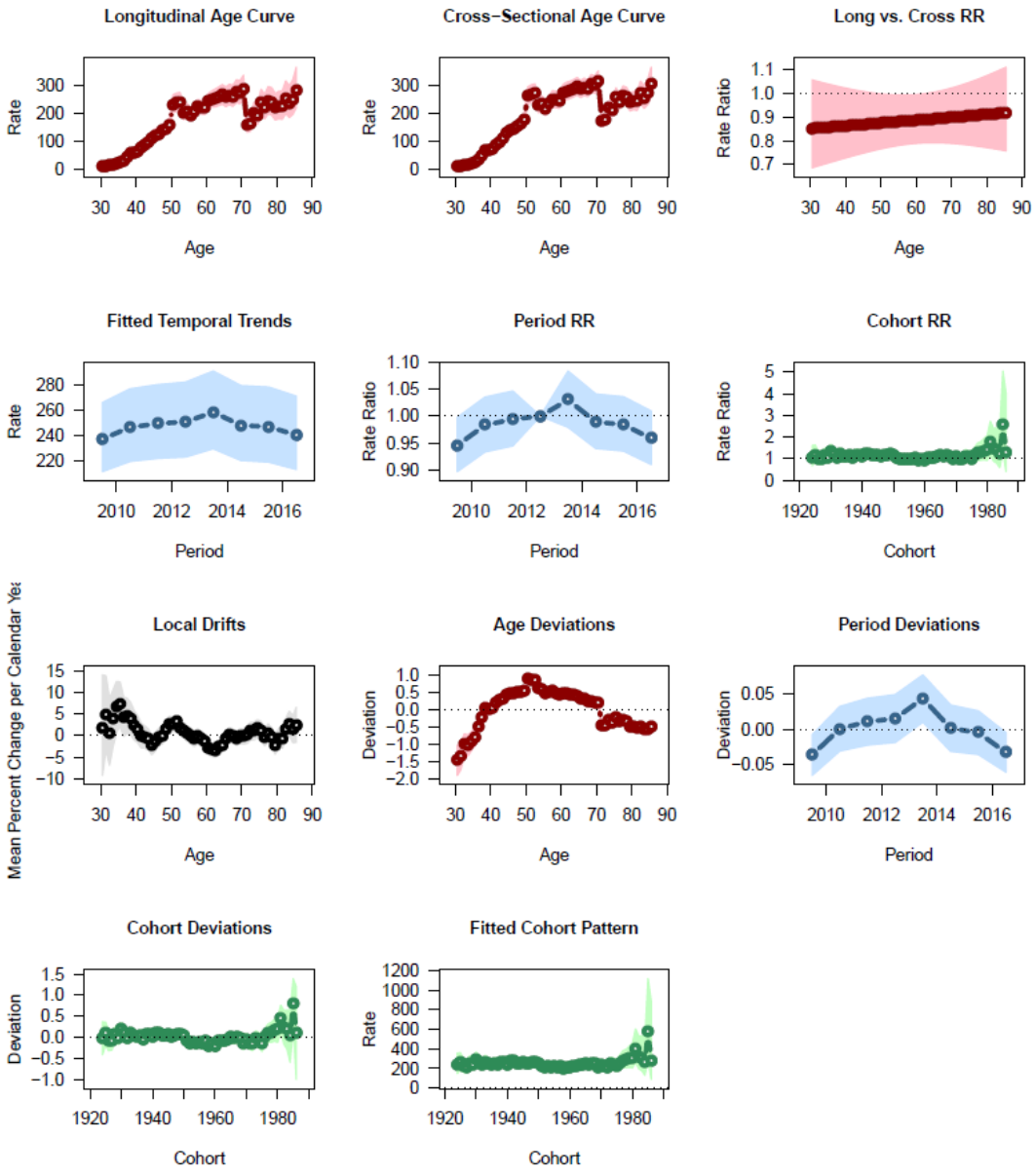
Appendix B.9 Comparison of incidence trends by HER2 status with and without imputation of missing HER2 status

Appendix Figure B.10 ASiR for HER2- and HER2+ tumours with imputation of missing HER2 status (corrected) and without imputation (observed) from 2009 to 2016 in Scotland

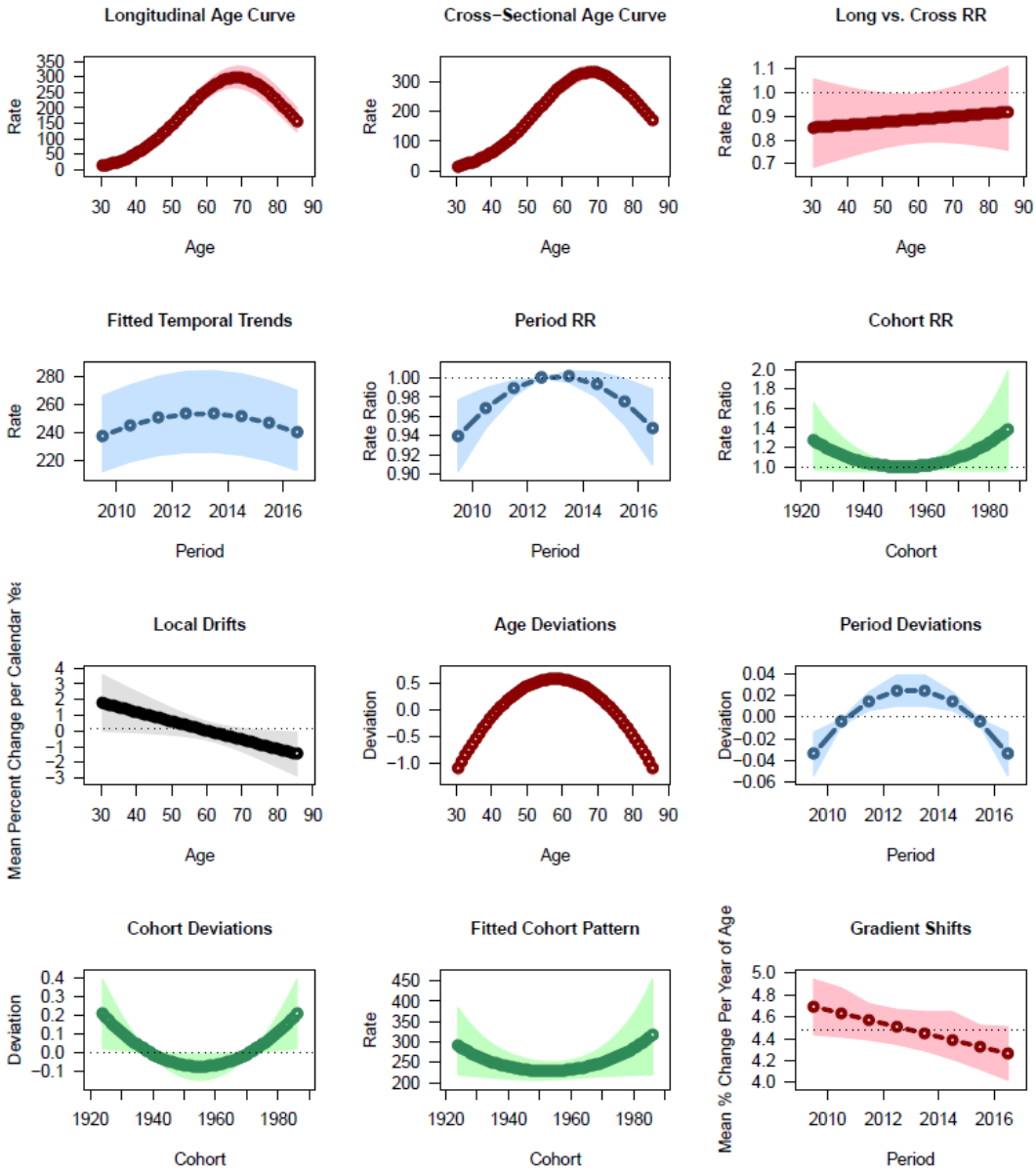


Appendix B.10 Additional graphs and results from APC models for HER2- tumours

Appendix Figure B.11 Graphs for trends in breast cancer incidence for HER2- tumours by age, period and cohort effects



Appendix Figure B.12 Graphs for trends in breast cancer incidence for HER2- tumours by age, period and cohort effects for the curvature parameters



Appendix Table B.14 Estimates with 95% CI for all the parameters of the final APC model for HER2- tumours

Parameter	Estimate	SD	Lower CI	Upper CI
Intercept	**-6.51271**	0.00933	-6.53100	-6.49443
LAT	**0.04517**	0.00322	0.03885	0.05149
Net Drift	0.00136	0.00311	-0.00474	0.00745
CAT	**0.04381**	0.00077	0.04231	0.04532
Age curvature	**-0.00218**	0.00015	-0.00247	-0.00188
Period curvature	**-0.00487**	0.00145	-0.00772	-0.00202
Cohort curvature	**0.00029**	0.00014	0.00003	0.00056

Bold results indicate significant estimates. CAT=Cross-sectional age trend, CI=Confidence interval, LAT=Longitudinal age trend, SD=Standard deviation.

Appendix Table B.15 Wald tests results for all key hypotheses on the APC model of HER2- tumours

Hypothesis test	Chi-square	df	P value
Net Drift = 0	0.19	1	0.06629474
Age curvature = 0	213.79	1	**<0.00000000001**
All Higher-Order Age Deviations = 0	388.92	53	**<0.00000000001**
All Age Deviations = 0	549.00	54	**<0.00000000001**
Period curvature = 0	11.24	1	**0.0008001488**
All Higher-Order Period Deviations = 0	2.02	5	0.8468523
All Period Deviations = 0	13.32	6	**0.03826178**
Cohort curvature = 0	4.65	1	**0.03096644**
All Higher-Order Cohort Deviations = 0	92.96	60	**0.004087221**
All Cohort Deviations = 0	94.92	61	**0.003530879**
All Period RR = 1	13.40	7	0.06285477
All Cohort RR = 1	94.92	62	**0.004528519**
All Local Drifts = Net Drift	93.41	56	**0.001267230**
All Gradient Shifts = CAT	14.57	8	0.06798652

Bold results indicate the test was significant at the 5% level. CAT= Cross-sectional age trend, df= degrees of freedom, RR=rate ratio.

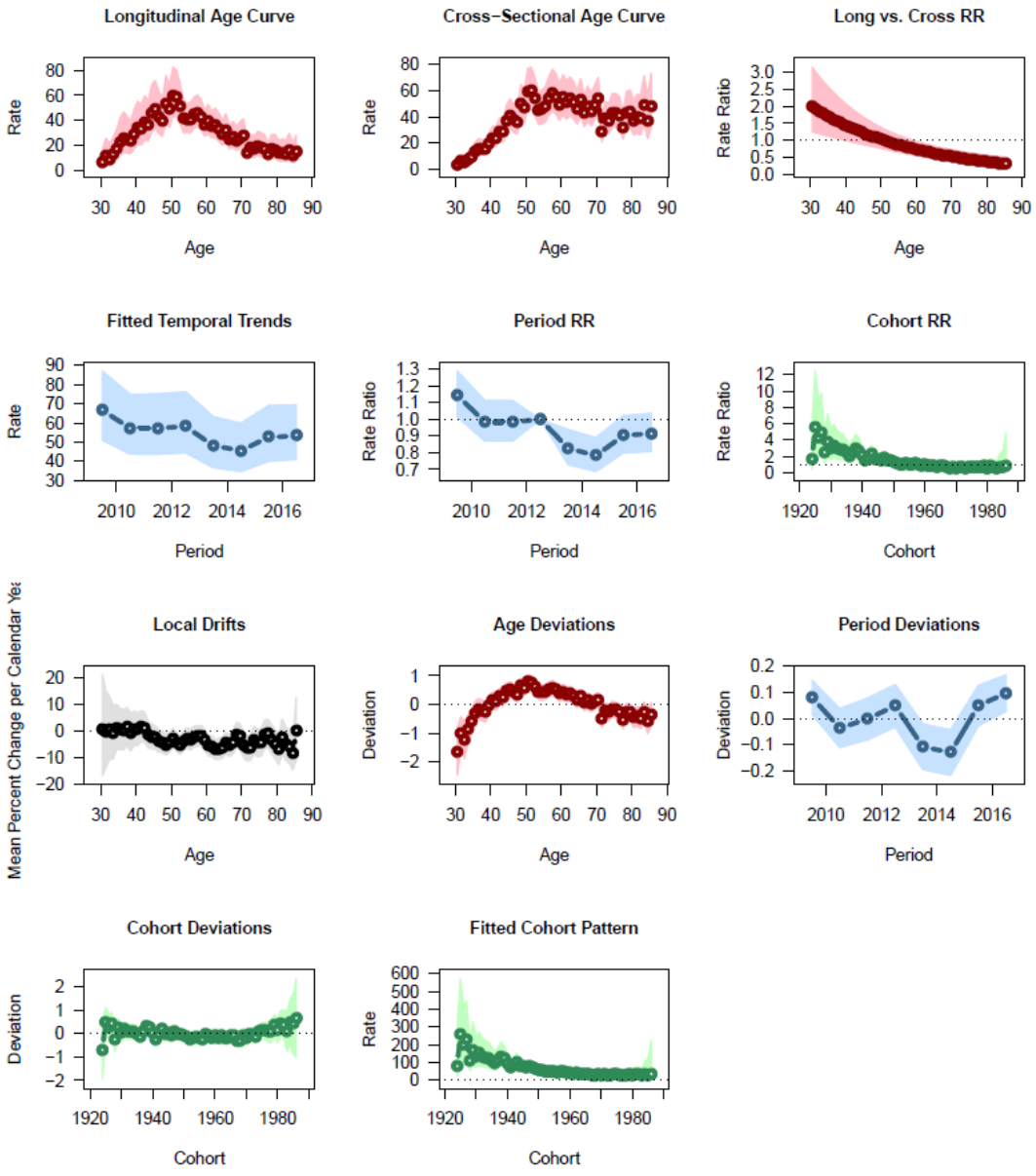
Appendix Table B.16 Combination tests for the APC model of HER2- tumours

Combination tests	P value
All Period Deviations = 0	**0.001600298**
All PRR = 1 <=> FTT = constant	**0.002400447**
All Cohort Deviations = 0	**0.008174443**
All CRR = 1 <=> FCP = constant	0.012261664

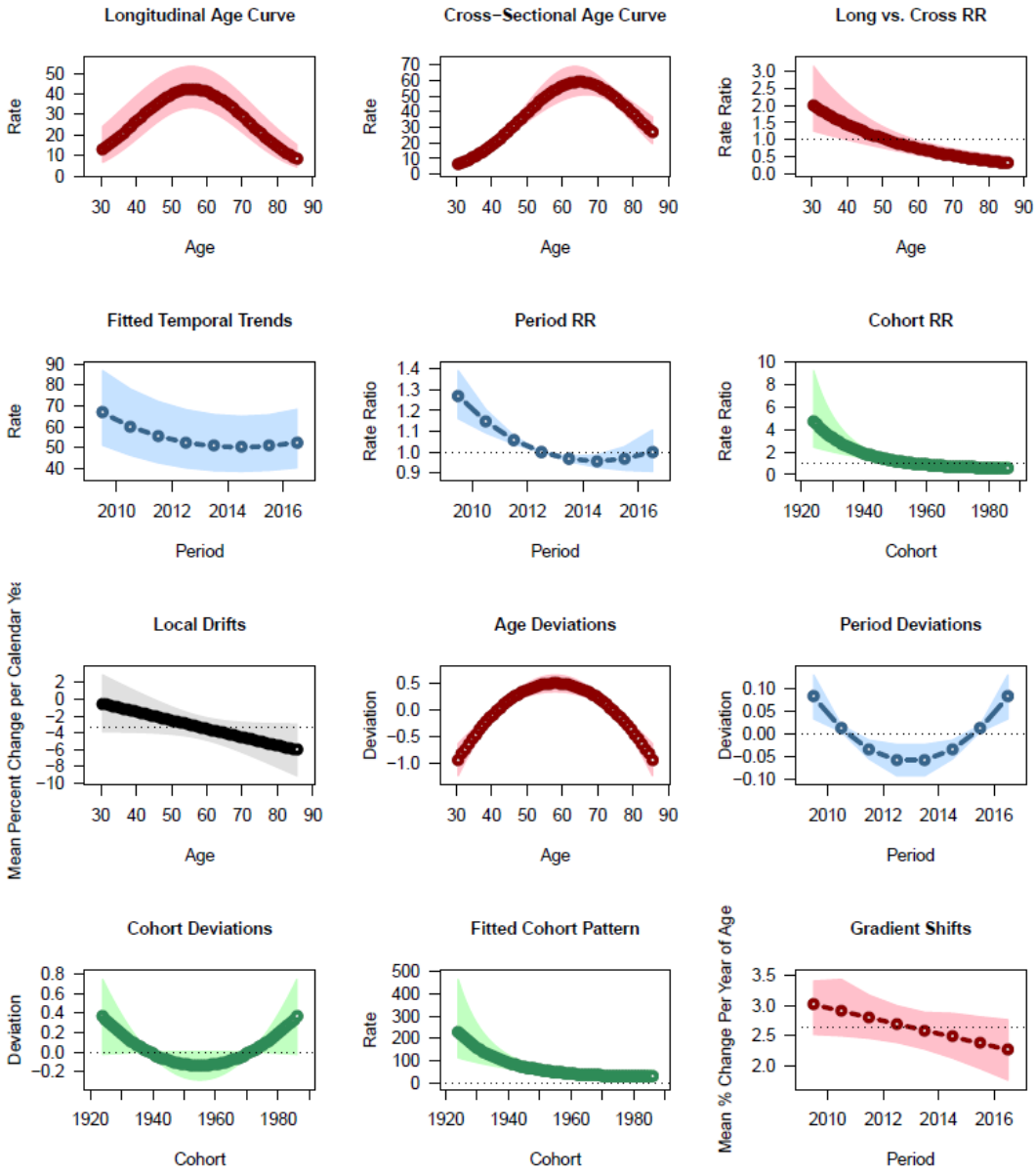
Bold results indicate the test was significant at the 5% level. CRR=Cohort rate ratio, FCP=Fitted cohort pattern, FTT=Fitted temporal trends, PRR=Period rate ratio.

Appendix B.11 Additional graphs and results from APC models for HER2+ tumours

Appendix Figure B.13 Graphs for trends in breast cancer incidence for HER2+ tumours by age, period and cohort effects



Appendix Figure B.14 Graphs for trends in breast cancer incidence for HER2+ tumours by age, period and cohort for the curvature parameters



Appendix Table B.17 Estimates with 95% CI for all the parameters of the final APC model for HER2+ tumours

Parameter	Estimate	SD	Lower CI	Upper CI
Intercept	**-8.08055**	0.01922	-8.11822	-8.04288
LAT	-0.00807	0.00718	-0.02214	0.00600
Net Drift	**-0.03412**	0.00699	-0.04783	-0.02041
CAT	**0.02605**	0.00150	0.02312	0.02898
Age curvature	**-0.00187**	0.00030	-0.00247	-0.00128
Period curvature	**0.01150**	0.00348	0.00468	0.01831
Cohort curvature	0.00052	0.00028	-0.00003	0.00107

Bold results indicate significant estimates. CI=Confidence interval, LAT=Longitudinal age trend, CAT=Cross-sectional age trend, SD=Standard deviation.

Appendix Table B.18 Wald tests results for all key hypotheses on the APC model of HER2+ tumours

Hypothesis test	Chi-square	df	P-Value
Net Drift = 0	23.80	1	**0.00000106989**
Age curvature = 0	37.72	1	**0.00000000082**
All Higher-Order Age Deviations = 0	68.66	53	0.07267159
All Age Deviations = 0	97.61	54	**0.0002577371**
Period curvature = 0	10.94	1	**0.0009418230**
All Higher-Order Period Deviations = 0	12.80	5	**0.02528583**
All Period Deviations = 0	22.74	6	**0.0008894106**
Cohort curvature = 0	3.40	1	0.06529817
All Higher-Order Cohort Deviations = 0	46.43	60	0.9006389
All Cohort Deviations = 0	50.45	61	0.8301537
All Period RR = 1	46.81	7	**0.00000006076**
All Cohort RR = 1	79.82	62	0.06335441
All Local Drifts = Net Drift	47.97	56	0.7685697
All Gradient Shifts = CAT	8.40	8	0.3957113

Bold results indicate the test was significant at the 5% level. CAT= Cross-sectional age trend, df= degrees of freedom, RR=rate ratio.

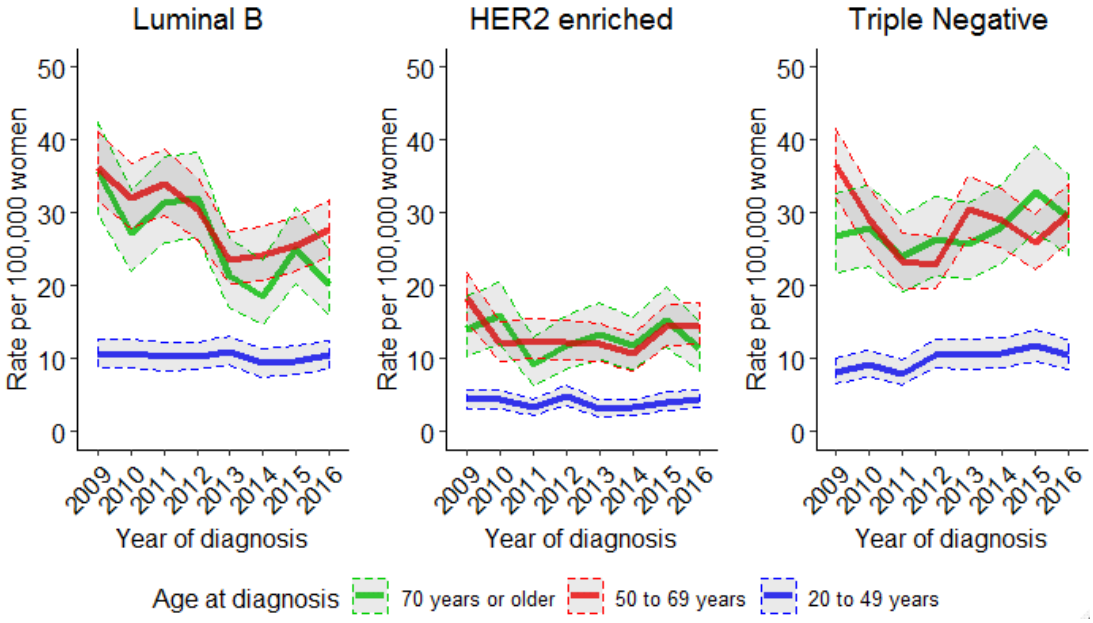
Appendix Table B.19 Combination tests for the APC model of HER2+tumours

Combination tests	P value
All Period Deviations = 0	**0.001883646**
All PRR = 1 <=> FTT = constant	**0.000003209675**
All Cohort Deviations = 0	0.1305963
All CRR = 1 <=> FCP = constant	**0.000003209675**

Bold results indicate the test was significant at the 5% level. CRR=Cohort rate ratio, FCP=Fitted cohort pattern, FTT=Fitted temporal trends, PRR=Period rate ratio.

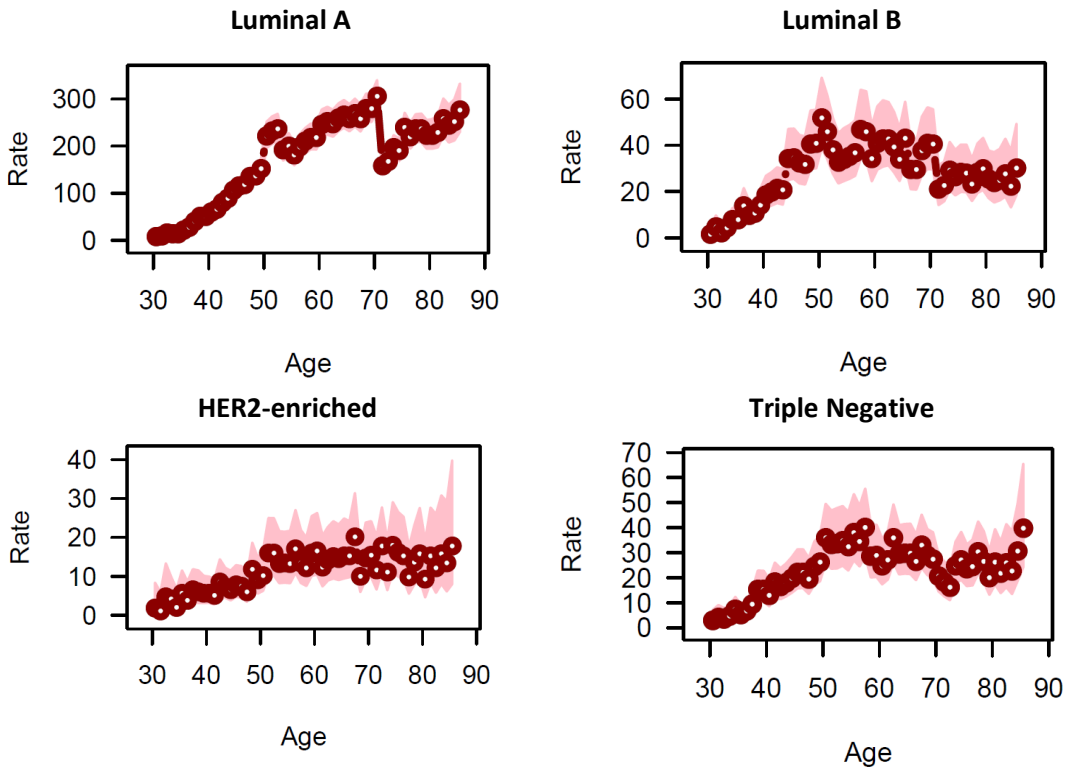
Appendix B.12 Incidence rates of luminal B, HER2-enriched and Triple Negative tumours by age group

Appendix Figure B.15 ASiR for luminal B, HER2-enriched and TNBC tumours by age group from 2009 to 2016 in Scotland (reduced scale for a better description of the trends)

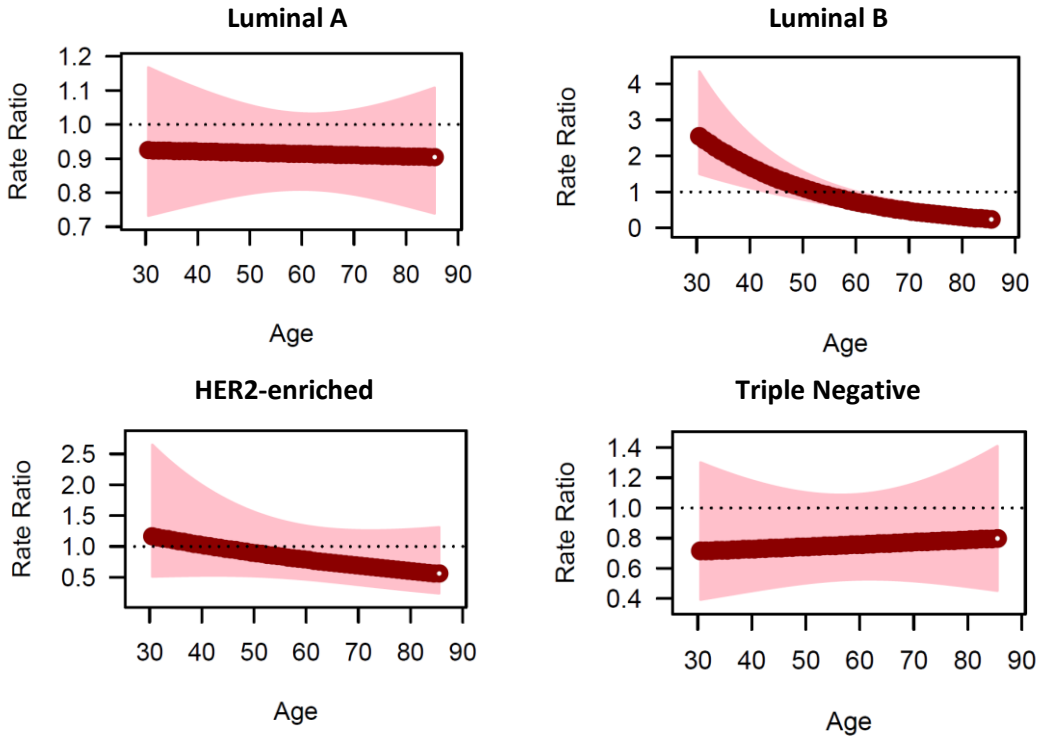


Appendix B.13 Additional graphs and results from APC models for the IHC defined molecular subtypes of breast cancer

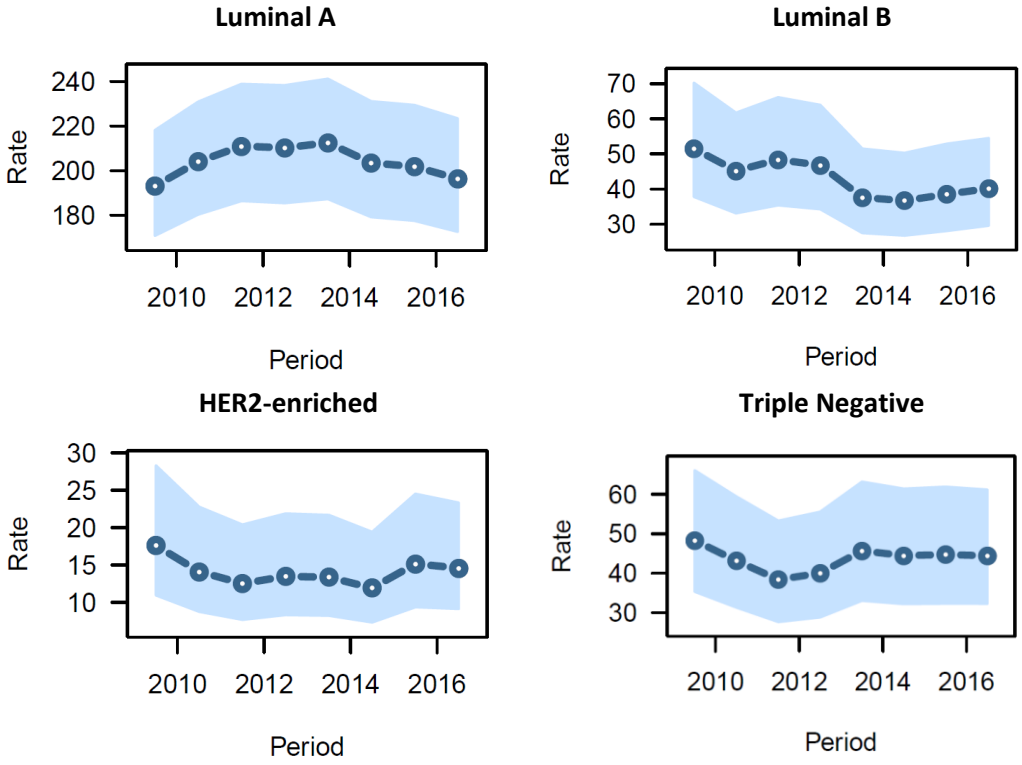
Appendix Figure B.16 Cross-sectional age curve for each molecular subtype



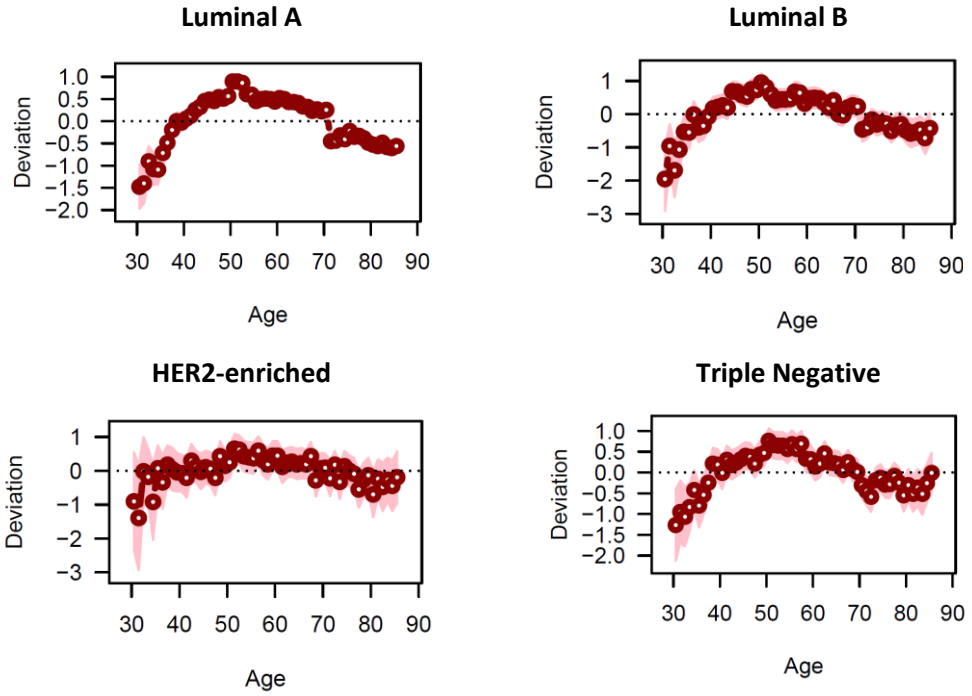
Appendix Figure B.17 Longitudinal vs. cross-sectional rate ratio for each molecular subtype



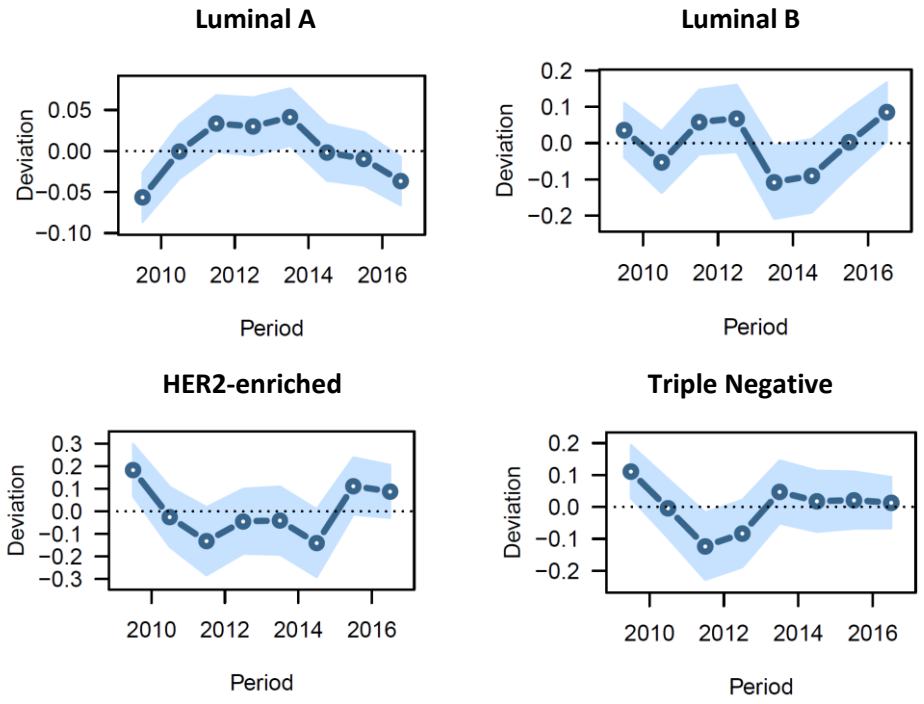
Appendix Figure B.18 Fitted temporal trends for each molecular subtype



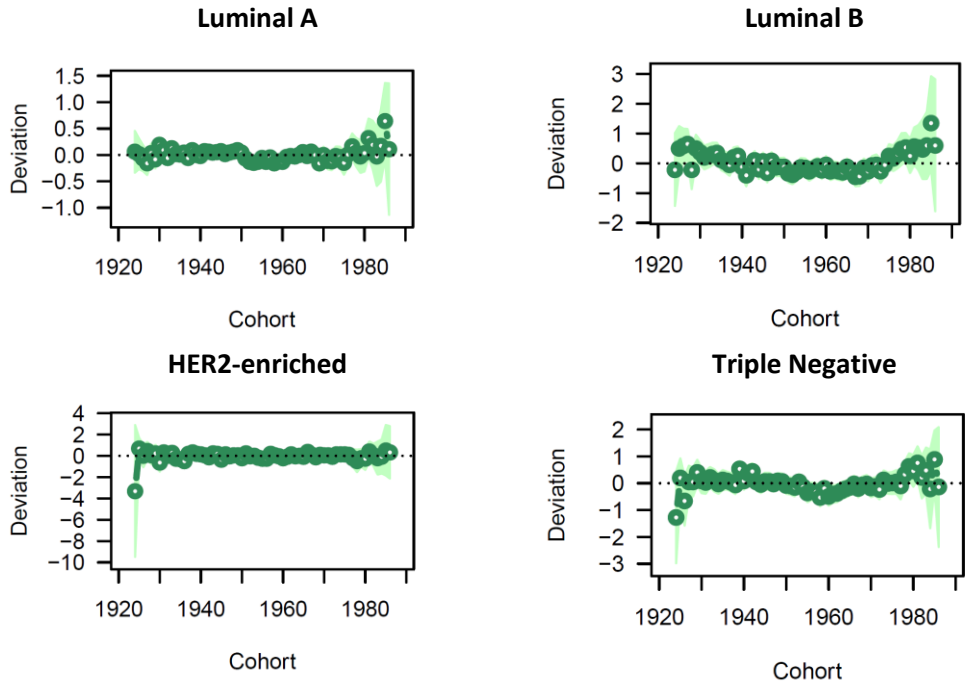
Appendix Figure B.19 Age deviations for each molecular subtype



Appendix Figure B.20 Period deviations for each molecular subtype



Appendix Figure B.21 Cohort deviations for each molecular subtype



Appendix Table B.20 Estimates with 95% CI for all the parameters of the final APC model for each molecular subtype

Molecular subtype	Parameter	Estimate	SD	Lower CI	Upper CI
Luminal A	Intercept	**-6.67943**	0.01048	-6.69997	-6.65889
	LAT	**0.04742**	0.00347	0.04061	0.05422
	Net Drift	-0.00040	0.00335	-0.00695	0.00616
	CAT	**0.04781**	0.00088	0.04609	0.04953
	Age curvature	**-0.00224**	0.00016	-0.00256	-0.00192
	Period curvature	**-0.00662**	0.00153	-0.00963	-0.00362
	Cohort curvature	0.00017	0.00015	-0.00012	0.00047
Luminal B	Intercept	**-8.42779**	0.02233	-8.47157	-8.38402
	LAT	**-0.01872**	0.00846	-0.03530	-0.00215
	Net Drift	**-0.04275**	0.00822	-0.05886	-0.02665
	CAT	**0.02403**	0.00173	0.02063	0.02743
	Age curvature	**-0.00220**	0.00036	-0.00291	-0.00150
	Period curvature	0.00651	0.00404	-0.00142	0.01443
	Cohort curvature	**0.00097**	0.00033	0.00032	0.00162
HER2-enriched	Intercept	**-9.18175**	0.03668	-9.25364	-9.10985
	LAT	0.01497	0.01235	-0.00923	0.03917
	Net Drift	-0.01327	0.01207	-0.03693	0.01039
	CAT	**0.02824**	0.00298	0.02239	0.03409
	Age curvature	**-0.00128**	0.00053	-0.00233	-0.00023
	Period curvature	**0.01920**	0.00608	0.00729	0.03112
	Cohort curvature	-0.00003	0.00049	-0.00100	0.00093
Triple Negative	Intercept	**-8.41950**	0.02277	-8.46413	-8.37486
	LAT	**0.02694**	0.00871	0.00987	0.04401
	Net Drift	0.00200	0.00847	-0.01460	0.01860
	CAT	**0.02494**	0.00175	0.02151	0.02837
	Age curvature	**-0.00166**	0.00037	-0.00238	-0.00094
	Period curvature	0.00822	0.00037	-0.00016	0.01660
	Cohort curvature	0.00056	0.00034	-0.00011	0.00122

Bold results indicate significant estimates. CAT=Cross-sectional age trend, CI=Confidence interval, LAT=Longitudinal age trend, SD=Standard deviation.

Appendix Table B.21 Wald tests results for all key hypotheses on the APC model for each molecular subtype (page 1 of 2)

Molecular subtype	Hypothesis test	Chi-square	df	P value
Luminal A	Net Drift = 0	0.01	1	0.9057431
	Age curvature = 0	185.70	1	**<0.0000000001**
	All Higher-Order Age Deviations = 0	381.80	53	**<0.0000000001**
	All Age Deviations = 0	524.92	54	**<0.0000000001**
	Period curvature = 0	18.72	1	**0.00001513379**
	All Higher-Order Period Deviations = 0	2.54	5	0.7698591
	All Period Deviations = 0	21.11	6	**0.001756117**
	Cohort curvature = 0	1.31	1	0.2515856
	All Higher-Order Cohort Deviations = 0	86.96	60	**0.01301720**
	All Cohort Deviations = 0	87.36	61	**0.01506458**
	All Period RR = 1	21.22	7	**0.003455566**
	All Cohort RR = 1	87.47	62	**0.01828156**
	All Local Drifts = Net Drift	83.83	56	**0.009400891**
	All Gradient Shifts = CAT	7.39	8	0.4947971
Luminal B	Net Drift = 0	27.08	1	**0.0000001953671**
	Age curvature = 0	37.64	1	**0.000000000852**
	All Higher-Order Age Deviations = 0	87.01	53	**0.002232215**
	All Age Deviations = 0	114.95	54	**0.0000026983**
	Period curvature = 0	2.59	1	0.1075124
	All Higher-Order Period Deviations = 0	11.45	5	**0.04314690**
	All Period Deviations = 0	13.34	6	**0.03790295**
	Cohort curvature = 0	8.63	1	**0.003298665**
	All Higher-Order Cohort Deviations = 0	53.44	60	0.7126586
	All Cohort Deviations = 0	61.76	61	0.4486984
	All Period RR = 1	40.63	7	**0.0000009524**
	All Cohort RR = 1	96.57	62	**0.003252102**
	All Local Drifts = Net Drift	56.86	56	0.4427515
	All Gradient Shifts = CAT	10.92	8	0.2060048

Appendix Table B.21 (continued) Wald tests results for all key hypotheses on the APC model for each molecular subtype (page 2 of 2)

Molecular subtype	Hypothesis test	Chi-square	df	P value
HER2-enriched	Net Drift = 0	1.21	1	0.271668649
	Age curvature = 0	5.76	1	**0.016413910**
	All Higher-Order Age Deviations = 0	44.64	53	0.786206109
	All Age Deviations = 0	48.53	54	0.684664247
	Period curvature = 0	9.97	1	**0.001587857**
	All Higher-Order Period Deviations = 0	5.18	5	0.394113156
	All Period Deviations = 0	15.15	6	**0.019157671**
	Cohort curvature = 0	<0.01	1	0.943676832
	All Higher-Order Cohort Deviations = 0	44.40	60	0.934195258
	All Cohort Deviations = 0	44.79	61	0.940723990
	All Period RR = 1	16.36	7	**0.021984728**
	All Cohort RR = 1	47.52	62	0.91256242
	All Local Drifts = Net Drift	42.63	56	0.906051798
	All Gradient Shifts = CAT	4.01	8	0.856553276
Triple Negative	Net Drift = 0	0.06	1	0.8130395
	Age curvature = 0	20.50	1	**0.000005950883**
	All Higher-Order Age Deviations = 0	70.72	53	**0.005225893**
	All Age Deviations = 0	83.80	54	**0.005778583**
	Period curvature = 0	3.70	1	0.05452570
	All Higher-Order Period Deviations = 0	8.21	5	0.1448488
	All Period Deviations = 0	11.37	6	0.07761607
	Cohort curvature = 0	2.71	1	0.09985863
	All Higher-Order Cohort Deviations = 0	65.58	60	0.2893884
	All Cohort Deviations = 0	68.34	61	0.2421994
	All Period RR = 1	11.39	7	0.1223193
	All Cohort RR = 1	68.37	62	0.2700082
	All Local Drifts = Net Drift	62.52	56	0.2557501
	All Gradient Shifts = CAT	13.43	8	0.09795575

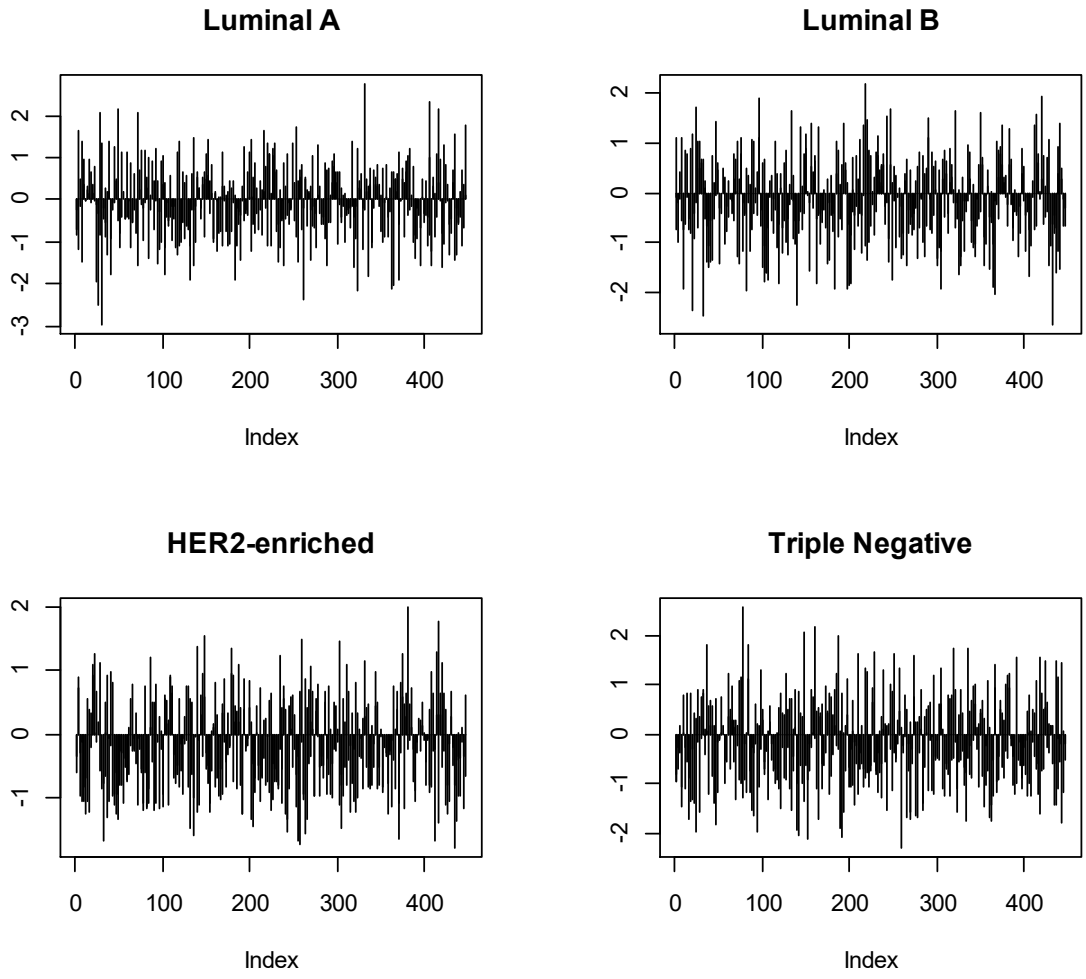
Bold results indicate the test was statistically significant at the 5% level. CAT= Cross-sectional age trend, df= degrees of freedom, RR=rate ratio.

Appendix Table B.22 Combination tests for the APC model for each molecular subtype

Molecular subtype	Combination tests	P value
Luminal A	All Period Deviations = 0	**0.00003026758**
	All PRR = 1 <=> FTT = constant	**0.00004540137**
	All Cohort Deviations = 0	**0.02603439**
	All CRR = 1 <=> FCP = constant	**0.03905159**
Luminal B	All Period Deviations = 0	0.08629380
	All PRR = 1 <=> FTT = constant	**0.000000586101**
	All Cohort Deviations = 0	**0.006597330**
	All CRR = 1 <=> FCP = constant	**0.000000586101**
HER2-enriched	All Period Deviations = 0	**0.003175715**
	All PRR = 1 <=> FTT = constant	**0.004763572**
	All Cohort Deviations = 0	1.0000000
	All CRR = 1 <=> FCP = constant	0.815005948
Triple Negative	All Period Deviations = 0	0.1090514
	All PRR = 1 <=> FTT = constant	0.1635771
	All Cohort Deviations = 0	0.1997173
	All CRR = 1 <=> FCP = constant	0.2995759

Bold results indicate the test was statistically significant at the 5% level. CRR=Cohort rate ratio, FCP=Fitted cohort pattern, FTT=Fitted temporal trends, PRR=Period rate ratio.

Appendix Figure B.22 Graph of deviance residuals for APC models of the IHC defined molecular subtypes



Appendix C Survival Chapter

Appendix C.1 Overall survival at 5 and 10 years by ER status and age group

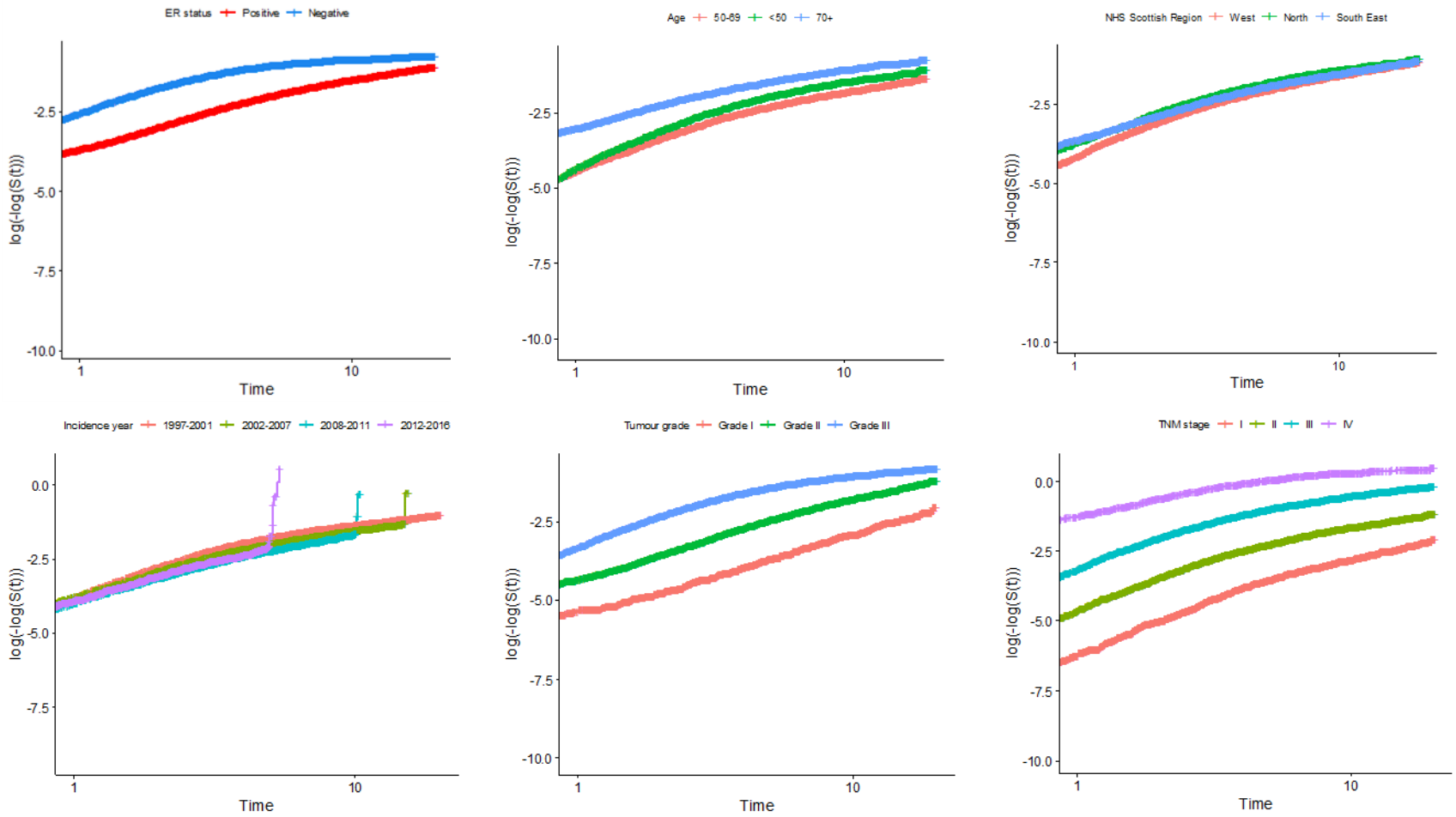
Appendix Table C.1 Overall survival derived from subtraction of proportions of deaths from all causes following a diagnosis of breast cancer among women in Scotland at 5 and 10 years by ER status and age group

OVERALL SURVIVAL	<50 YEARS	50-69 YEARS	70 YEARS OR OLDER	TOTAL
ER+				
cases/deaths	6,691/947	17,511/2,348	6,566/4,412	30,768/7,707
5-year OS (95% CI)	88.1 (87.4, 88.8)	88.0 (87.6, 88.5)	56.9 (56.0, 57.7)	78.9 (78.5, 79.3)
ER-				
cases/deaths	1,774/616	2,963/1,098	865/894	5,602/2,608
5-year OS (95% CI)	74.0 (72.3, 75.5)	71.4 (70.1, 72.6)	41.6 (39.7, 43.6)	64.9 (63.9, 65.8)
% difference at 5 years (ER+ minus ER-)	14.1%	16.6%	15.3%	14%
ER+				
cases/deaths	3,678/624	9,165/1,890	2,330/2,380	15,173/4,894
10-year OS (95% CI)	78.1 (77.1, 79.1)	76.1 (75.4, 76.7)	31.8 (30.9, 32.7)	63.3 (62.8, 63.8)
ER-				
cases/deaths	1,063/152	1,604/335	327/308	2,994/795
10-year OS (95% CI)	66.6 (64.7, 68.4)	61.7 (60.2, 63.1)	24.4 (22.6, 26.3)	54.0 (52.9, 55.0)
% difference at 10 years (ER+ minus ER-)	12.5%	14.4%	7.4%	9.3%

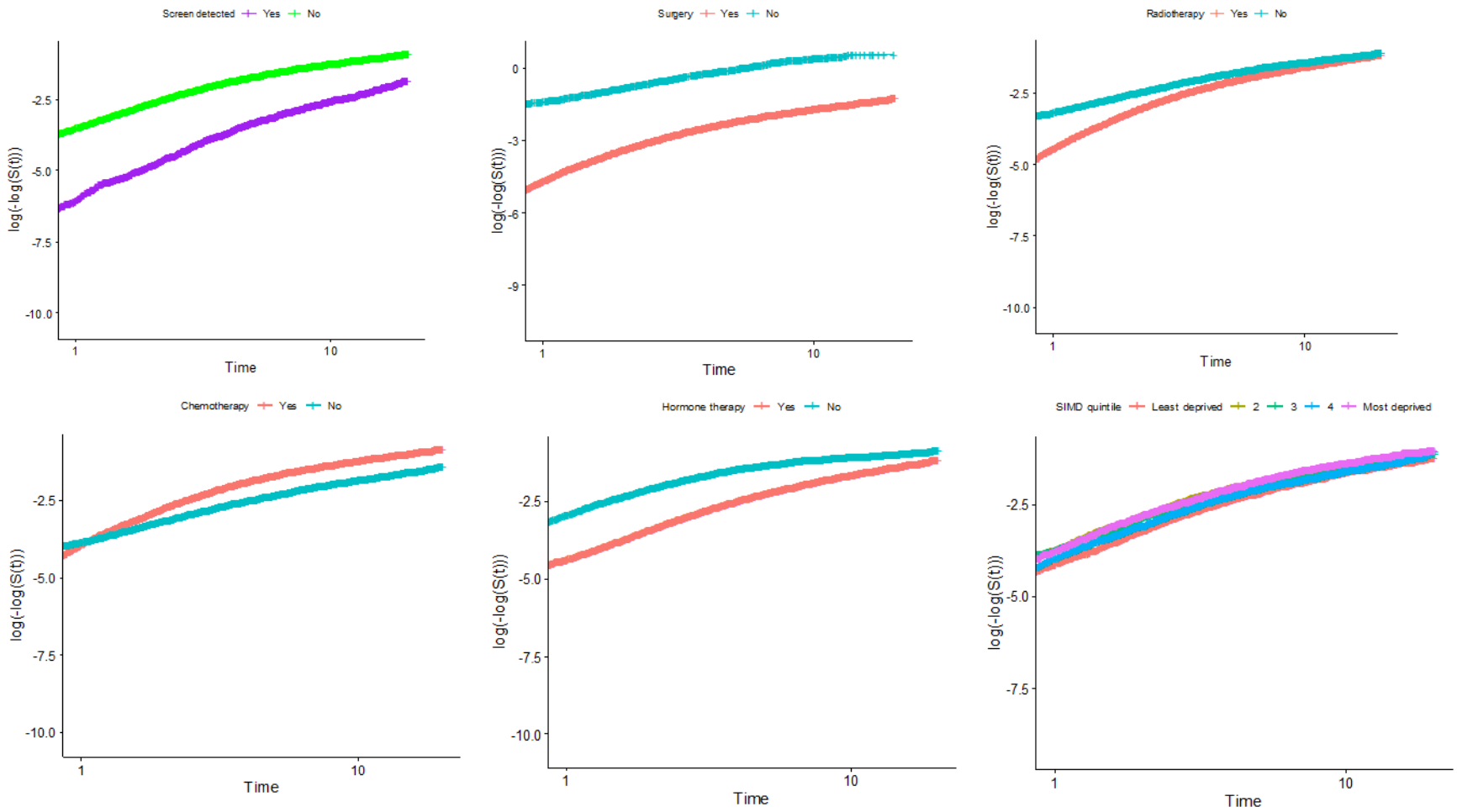
CI= confidence interval, ER= oestrogen receptor, OS= overall survival.

Appendix C.2 Assessing the PH assumption for the fully adjusted model 4 with ER status as the main exposure

Appendix Figure C.1 Log minus log plots to visually inspect the PH assumption for all individual and tumour characteristics and treatment regimes (page 1 of 2)



Appendix Figure C.1 (continued) Log minus log plots to visually inspect the PH assumption for all individual and tumour characteristics and treatment regimes (page 2 of 2)



Appendix Table C.2 Proportional hazards assumption test for fully adjusted Cox model with ER status as an exposure

Variable	Rho	Chi-square	P value
ER status Negative (VS positive)	-0.1381	170	<0.001
Age <50 years (vs 50 -69 years)	0.0026	5.23	0.819
Age 70+ years (vs 50 -69 years)	-0.0174	2.41	0.121
North region (vs West)	-0.0055	0.230	0.632
South East region (vs West)	-0.0076	0.432	0.511
Incidence year 2002-2006 (vs 1997-2001)	0.0214	3.50	0.061
Incidence year 2007-2011 (vs 1997-2001)	0.0286	6.32	0.019
Incidence year 2012-2016 (vs 1997-2001)	0.0076	0.434	0.510
Tumour grade II (vs I)	-0.0076	0.446	0.504
Tumour grade III (vs I)	-0.06282	30.7	<0.001
TNM stage II (vs I)	-0.0384	11.6	<0.001
TNM stage III (vs I)	-0.0870	59.3	<0.001
TNM stage IV (vs I)	-0.1230	115.0	<0.001
Not screen detected (vs screen detected)	-0.0425	14.0	<0.001
No surgery (vs Yes)	-0.0262	5.17	0.023
No radiotherapy (vs Yes)	-0.0639	31.5	<0.001
No chemotherapy (vs Yes)	-0.0643	34.6	<0.001
No hormone therapy (vs Yes)	-0.0635	35.5	<0.001
SIMD quintile 2 (vs least deprived)	-0.0179	2.43	0.119
SIMD quintile 3 (vs least deprived)	-0.0241	4.39	0.036
SIMD quintile 4 (vs least deprived)	-0.0002	0.0003	0.985
Most deprived SIMD quintile (vs least deprived)	-0.0119	1.07	0.301
Charlson score of comorbidity	-0.0175	2.16	0.141
GLOBAL TEST		**1340**	**<0.001**

ER= oestrogen receptor, SIMD= Scottish Index of Multiple Deprivation, TNM=tumour, nodes, metastases.

Appendix C.3 Sensitivity analysis to investigate the PH assumption using extended Cox models with time-varying effects and two independent stratified models by time period

Appendix Table C.3 Comparison of fully adjusted Cox model with model with time-varying effects and independent Cox models stratified by time period (0 to 3 years of follow-up and more than 3 years to the end of the follow-up) (page 1 of 2)

	Fully adjusted Cox model N=51,140, deaths=7,592	Cox model with time by covariate interactions N=51,140, deaths=7,592		Independent Cox models for two time periods	
	HR (95%CI, P value)	HR (95%CI, P value) Main effect	HR (95%CI, P value) Time-varying effect	0-3 years follow-up N=51,140, deaths=3,535 HR (95%CI, P value)	+3 years to end of follow-up N=36,679, deaths=4,041 HR (95%CI, P value)
ER Status			**ER Status*time**		
Positive	Ref	Ref	Ref	Ref	Ref
Negative	1.44 (1.33-1.56)	2.89 (2.55-3.29)	0.83 (0.81-0.85)	2.26 (2.02-2.52)	0.85 (0.76-0.96, p=0.009)
Age					
<50 years	0.89 (0.84-0.95)	0.89 (0.84-0.95)		0.88 (0.80-0.97)	0.89 (0.82-0.97, p=0.005)
50-69 years	Ref	Ref		Ref	Ref
70 years or older	1.36 (1.27-1.45)	1.35 (1.27-1.44)		1.44 (1.32-1.58)	1.25 (1.14-1.38)
NHS region					
West	Ref	Ref		Ref	Ref
North	1.12 (1.06-1.19)	1.11 (1.05-1.17)		1.09 (1.00-1.19, p=0.048)	1.13 (1.05-1.23, p=0.002)
South East	1.01 (0.96-1.07, p=0.665)	0.99 (0.94-1.05, p=0.847)		1.01 (0.92-1.09, p=0.897)	1.00 (0.92-1.08, p=0.901)
Year of diagnosis					
1997-2001	Ref	Ref		Ref	Ref
2002-2006	0.79 (0.75-0.84)	0.79 (0.74-0.84)		0.75 (0.68-0.82)	0.81 (0.75-0.87)
2007-2011	0.68 (0.64-0.73)	0.68 (0.64-0.73)		0.61 (0.56-0.67)	0.73 (0.67-0.80)
2012-2016	0.73 (0.67-0.79)	0.74 (0.68-0.79)		0.71 (0.64-0.78)	0.74 (0.61-0.89)
Grade			**Grade*time**		
Grade I-(Well) differentiated	Ref	Ref	Ref	Ref	Ref
Grade II- Moderately (well) differentiated	1.84 (1.64-2.06)	2.10 (1.72-2.57)	0.99 (0.96-1.01, p=0.369)	1.88 (1.51-2.34)	1.93 (1.69-2.20)
Poorly differentiated	3.02 (2.70-3.39)	4.96 (4.05-6.08)	0.91 (0.89-0.94)	4.06 (3.26-5.05)	2.59 (2.26-2.96)

Appendix Table C.3 (continued) Comparison of fully adjusted Cox model with model with time-varying effects and independent Cox models stratified by time period (0 to 3 years of follow-up and more than 3 years to the end of the follow-up) (page 2 of 2)

		Fully adjusted Cox model N=51,140, deaths=7,592	Cox model with time by covariate interactions N=51,140, deaths=7,592		Independent Cox models for two time periods	
		HR (95%CI, P value)	HR (95%CI, P value) Main effect	HR (95%CI, P value) Time-varying effect	0-3 years follow-up N=51,140, deaths=3,535 HR (95%CI, P value)	+3 years to end of follow-up N=36,679, deaths=4,041 HR (95%CI, P value)
TNM stage				**TNM stage*time**		
	I	Ref	Ref	Ref	Ref	Ref
	II	2.11 (1.95-2.28)	2.61 (2.28-2.99)	0.96 (0.94-0.98)	2.54 (2.20-2.93)	1.94 (1.76-2.13)
	III	5.90 (5.44-6.40)	9.13 (7.94-10.50)	0.91 (0.89-0.93)	8.33 (7.21-9.62)	4.64 (4.18-5.14)
	IV	11.09 (10.00-12.30)	23.54 (19.74-28.08)	0.80 (0.77-0.83)	18.67 (15.89-21.95)	5.99 (5.08-7.06)
Screening	Yes	Ref	Ref	**Screening*time**	Ref	Ref
	No	1.62 (1.50-1.75)	2.07 (1.82-2.36)	0.96 (0.94-0.98)	2.02 (1.76-2.34)	1.52 (1.38-1.67)
Surgery				**Surgery*time**		
	Yes	Ref	Ref	Ref	Ref	Ref
	No	4.01 (3.70-4.33)	5.10 (4.49-5.78)	0.90 (0.87-0.94)	4.11 (3.72-4.54)	3.69 (3.22-4.23)
Radiotherapy				**Radiotherapy*time**		
	Yes			Ref		
	No	1.03 (0.98-1.09, p=0.201)	1.24 (1.15-1.34)	0.96 (0.95-0.97)	1.18 (1.09-1.26)	0.94 (0.88-1.01, p=117)
Chemotherapy				**Chemotherapy*time**		
	Yes	Ref	Ref	Ref	Ref	Ref
	No	0.96 (0.90-1.02, p=0.218)	1.25 (1.14-1.36)	0.94 (0.93-0.96)	1.16 (1.06-1.28)	0.82 (0.75-0.89)
Hormone therapy				**Hormone therapy*time**		
	Yes	Ref	Ref	Ref	Ref	Ref
	No	1.31 (1.21-1.41)	1.69 (1.50-1.92)	0.93 (0.91-0.96)	1.64 (1.47-1.83)	1.07 (0.95-1.19)
SIMD quintile						
	Most deprived	1.24 (1.15-1.34)	1.23 (1.14-1.33)		1.25 (1.12-1.40)	1.23 (1.11-1.36)
	2	1.19 (1.11-1.28)	1.18 (1.10-1.27)		1.27 (1.15-1.42)	1.12 (1.02-1.24)
	3	1.12 (1.04-1.20, p=0.002)	1.12 (1.04-1.20, p=0.002)		1.21 (1.09-1.35, p=0.002)	1.05 (0.95-1.15, p=0.349)
	4	1.03 (0.96-1.11, p=0.371)	1.03 (0.95-1.10, p=0.371)		1.05 (0.94-1.17, p=0.371)	1.02 (0.93-1.12, p=0.702)
	Least deprived	Ref	Ref		Ref	Ref
Charlson Score						
	Mean (SD)	1.23 (1.13-1.33)	1.20 (1.11-1.30)		1.23 (1.11-1.36)	1.13 (0.98-1.30, p=0.084)

Models include age, incidence year, NHS region, grade, TNM stage, method of detection, surgery, radiotherapy, chemotherapy, hormone therapy, SIMD and Charlson score index. All HRs were statistically significant at the 0.1% level unless stated otherwise. CI= confidence interval, HR= hazard ratio, NHS= National Health Service, SD= standard deviation, SIMD= Scottish Index of Multiple Deprivation, TNM= tumour, nodes, metastases.

Appendix Table C.4 Estimates of hazard ratio at 1, 3, 5 and 10 years predicted from Cox model with time-varying covariates

	HR at 1 year	HR at 3 years	HR at 5 years	HR at 10 years
ER Status				
Positive	Ref	Ref	Ref	Ref
Negative	2.39 (2.08, 2.80)	1.63 (1.23, 2.03)	1.12 (0.90, 1.48)	0.43 (0.31, 0.66)
Grade				
Grade I-(Well) differentiated	Ref	Ref	Ref	Ref
Grade II- Moderately (well) differentiated	2.08 (1.65, 2.59)	2.03 (1.52, 2.48)	1.99 (1.40, 2.69)	1.90 (1.15, 2.32)
Poorly differentiated	4.53 (3.60, 5.75)	3.78 (2.83, 5.10)	3.16 (2.23, 4.53)	2.01 (1.22, 3.35)
TNM stage				
I	Ref	Ref	Ref	Ref
II	2.51 (2.27, 2.94)	2.32 (1.90, 2.83)	2.14 (1.68, 2.72)	1.75 (1.25, 2.46)
III	8.33 (7.03, 9.78)	6.96 (5.53, 8.50)	5.81 (4.35, 7.39)	3.71 (2.39, 5.21)
IV	18.92 (15.18, 23.34)	12.18 (9.03, 15.96)	7.85 (5.37, 10.91)	2.61 (1.46, 4.22)
Screening				
Yes	Ref	Ref	Ref	Ref
No	1.99 (1.72, 2.32)	1.84 (1.52, 2.23)	1.70 (1.35, 2.14)	1.39 (1.01, 1.93)
Surgery				
Yes	Ref	Ref	Ref	Ref
No	4.57 (3.90, 5.42)	3.67 (2.94, 4.81)	2.94 (2.26, 4.26)	1.70 (1.11, 3.16)
Radiotherapy				
Yes	Ref	Ref	Ref	Ref
No	1.20 (1.09, 1.30)	1.11 (0.99, 1.22)	1.02 (0.90, 1.15)	0.84 (0.70, 0.99)
Chemotherapy				
Yes	Ref	Ref	Ref	Ref
No	1.17 (1.06, 1.31)	1.04 (0.92, 1.21)	0.92 (0.80, 1.12)	0.68 (0.57,0.91)
Hormone therapy				
Yes	Ref	Ref	Ref	Ref
No	1.57 (1.38, 1.84)	1.36 (1.15, 1.70)	1.19 (0.96, 1.57)	0.84 (0.61, 1.28)

ER= oestrogen receptor, HR= hazard ratio, Ref= reference category, TNM= tumour, nodes, metastases.

The estimated hazard ratio of each covariate as a function of time t is given by: $HR(t)=\exp(\alpha+\beta t)$ where α is the coefficient for the main effect for each specific covariate, β is the coefficient of the interaction of that covariate with time and t represents time in years.

Appendix C.4 Overall (all cause) survival at 5 years by IHC defined molecular subtypes and age groups in women diagnosed with invasive breast cancer from 2009 to 2016 in Scotland, N=30,965

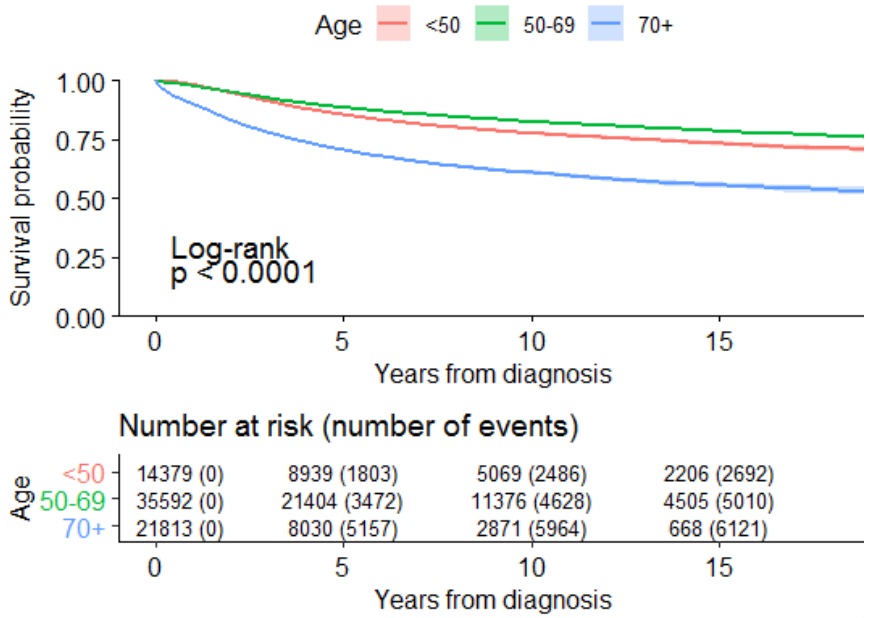
Appendix Table C.5 Overall survival estimates at 5 years (with 95% CI) by IHC defined molecular subtypes and age for women diagnosed from 2009 to 2016

MOLECULAR SUBTYPE	<50 YEARS	50- 69 YEARS	70 YEARS OR OLDER	TOTAL
Luminal A				
cases/deaths	749/90	2,413/334	888/889	4,050/1,313
5-year OS (95% CI)	92.4 (90.8, 93.7)	91.7 (90.9, 92.5)	58.5 (56.6, 60.4)	81.8 (81.0, 82.6)
Luminal B				
cases/deaths	679/185	1,176/297	379/421	2,234/903
5-year OS (95% CI)	84.9 (82.8, 86.7)	85.4 (83.9, 86.8)	57.6 (54.7, 60.4)	78.6 (77.4, 79.7)
HER2-enriched				
cases/deaths	89/29	173/57	49/77	311/163
5-year OS (95% CI)	81.2 (74.6, 86.3)	79.6 (75.2, 83.3)	45.1 (38.0, 51.9)	71.3 (67.9, 74.3)
Triple Negative				
cases/deaths	187/117	327/193	95/176	609/486
5-year OS (95% CI)	72.9 (68.7, 76.6)	73.1 (70.0, 76.0)	41.8 (37.1, 46.5)	65.5 (63.2, 67.6)

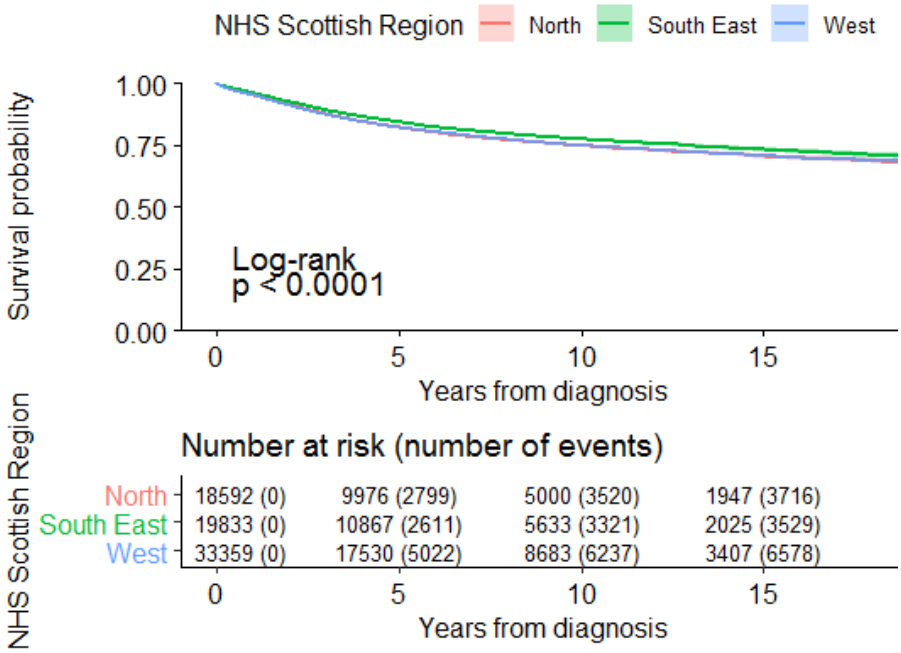
CI= confidence interval, HER2= human epidermal growth factor 2, OS= overall survival.

Appendix C.5 KM curves by individual, tumour characteristics and treatment regimens for women diagnosed in Scotland from 1997 to 2016

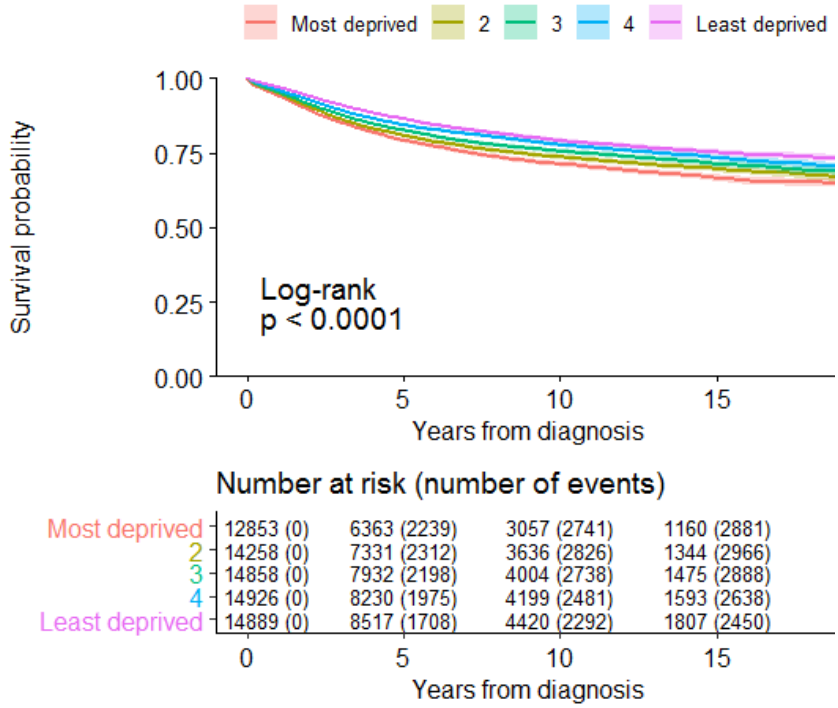
Appendix Figure C.2 KM curves by age



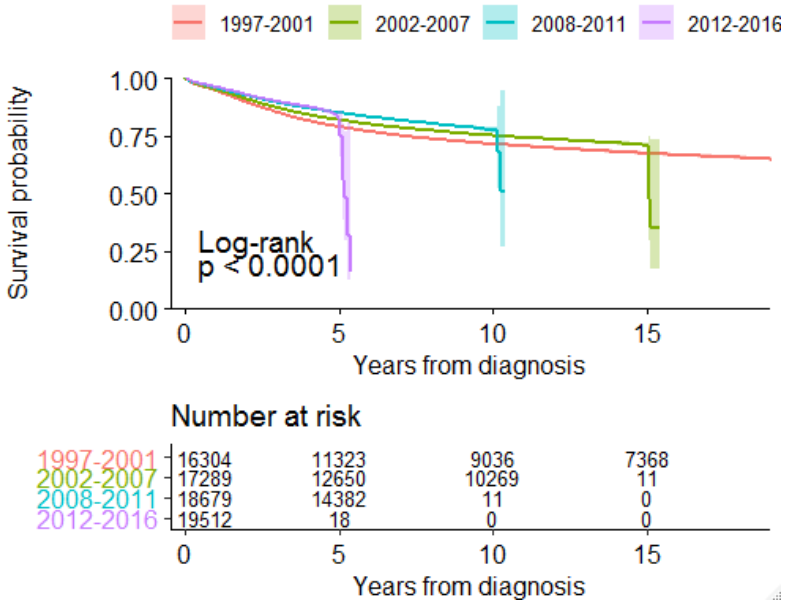
Appendix Figure C.3 KM curves by NHS Scottish region



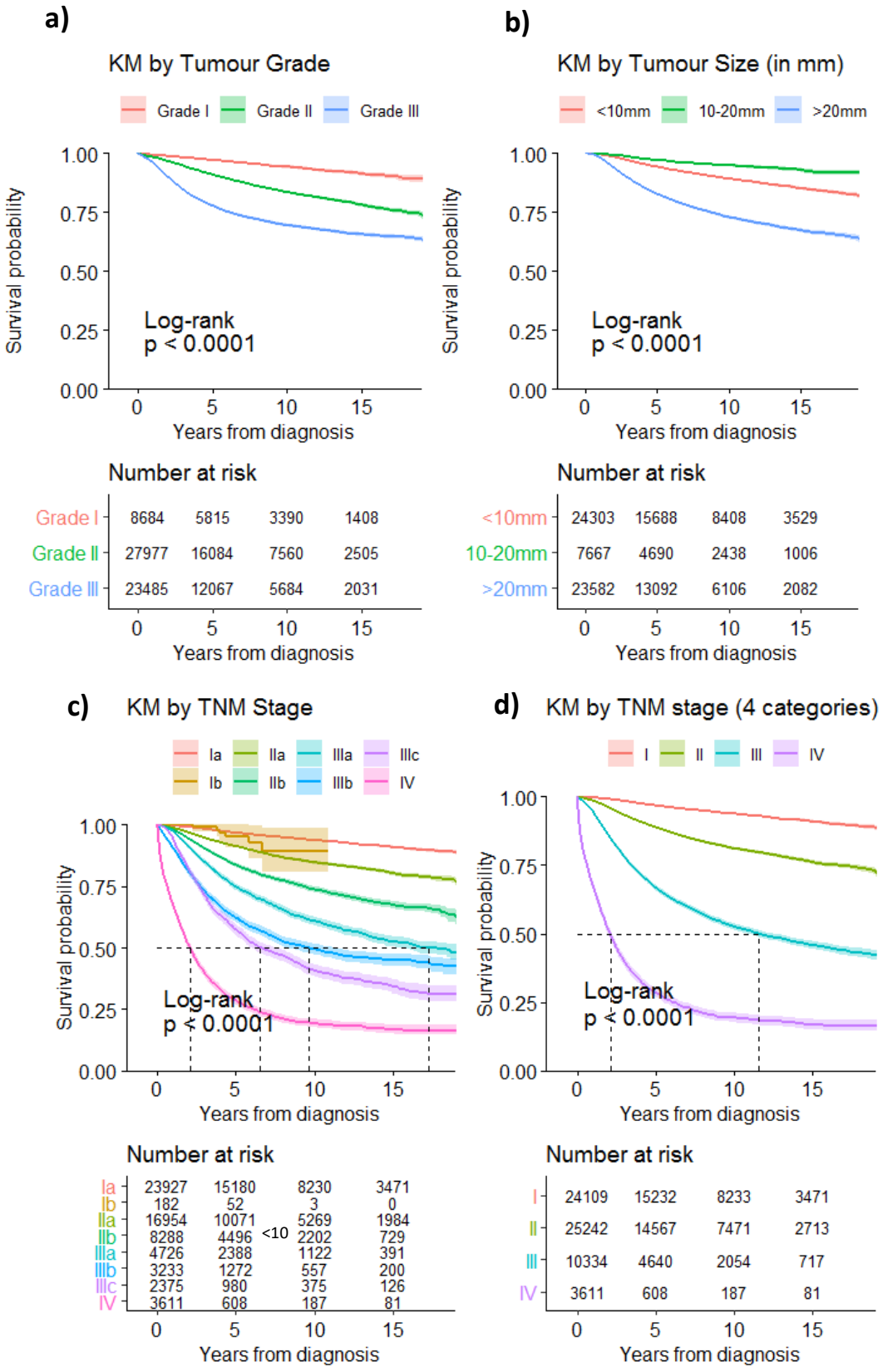
Appendix Figure C.4 KM curves by Scottish index of multiple deprivation



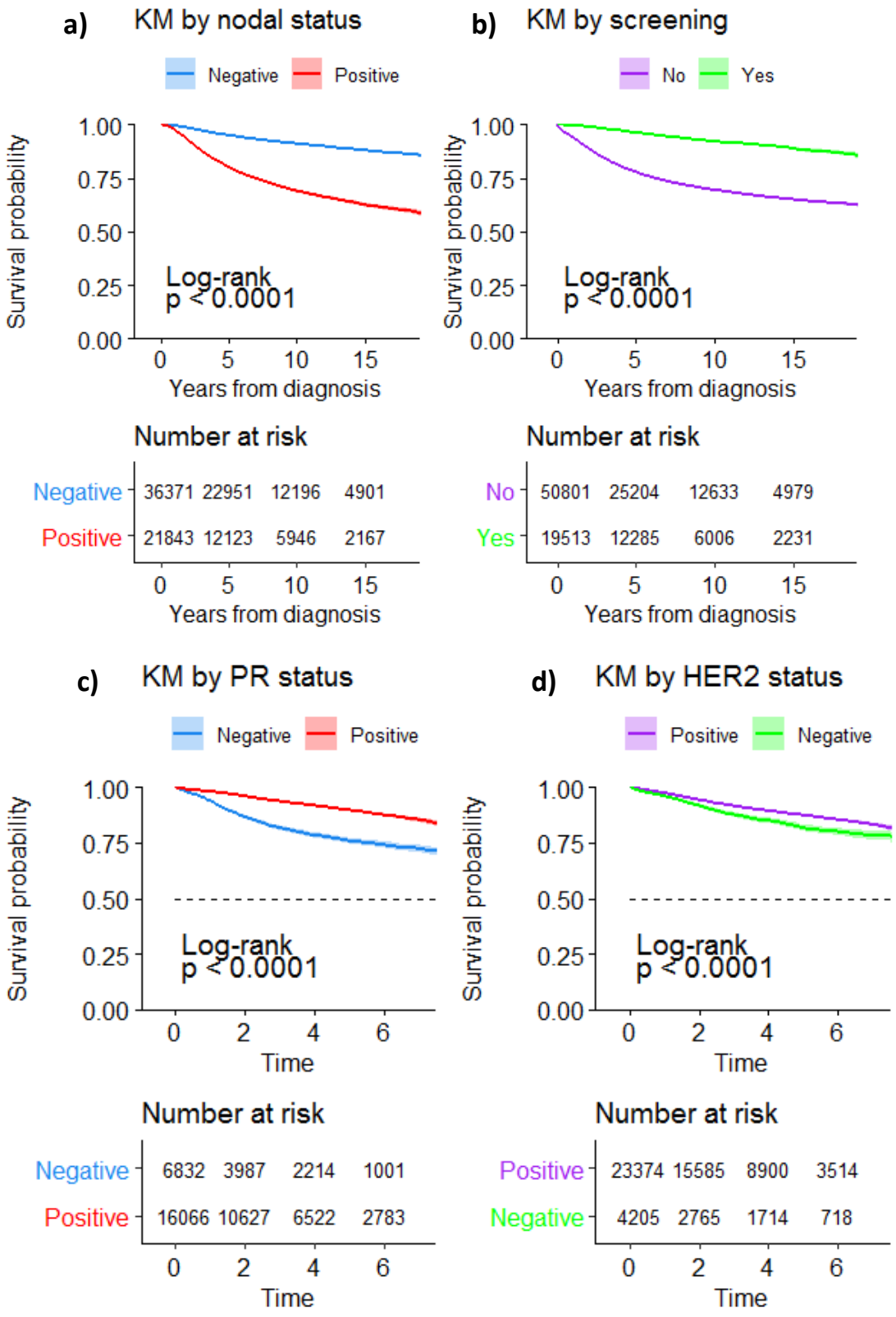
Appendix Figure C.5 KM curves by year of diagnosis (in 5-years groups)



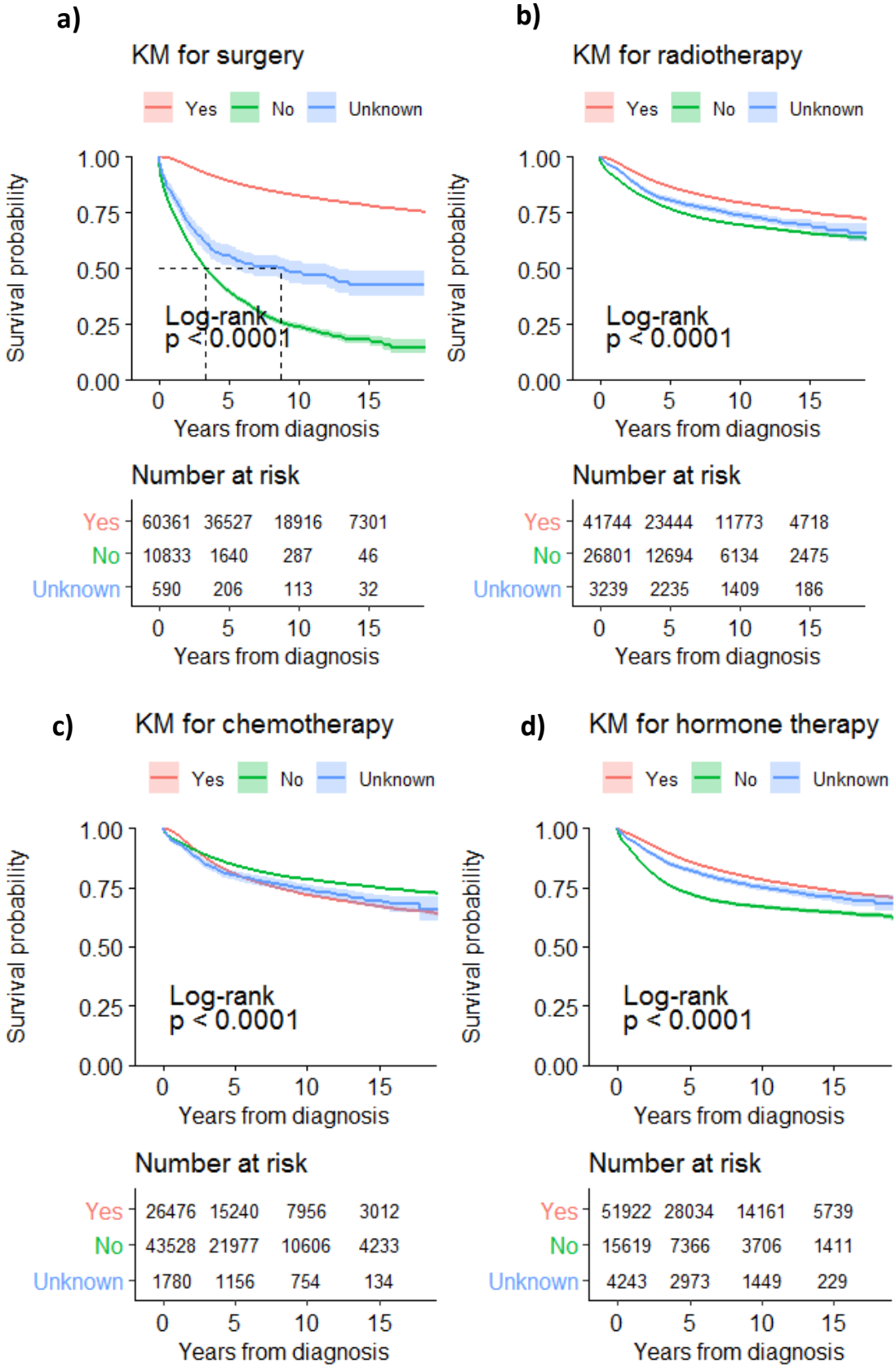
Appendix Figure C.6 KM curves by tumour characteristics: a) tumour grade, b) tumour size (in mm) c) tumour TNM stage (8 categories) d) tumour TNM stage (4 categories)



Appendix Figure C.7 KM curves by tumour characteristics: a) nodal status, b) method of detection (screen detected or not) c) PR status and d) HER2 status



Appendix Figure C.8 KM curves by treatments: a) surgery, b) radiotherapy, c) chemotherapy and d) hormone therapy



Appendix C.6 Sensitivity analysis: traditional and extended Cox models with TVE stratified by ER (1997-2016)

Appendix Table C.6 Comparison of traditional and extended Cox models with TVE for ER+ and ER- tumours (separately) diagnosed from 1997 to 2016 (page 1 of 3)

	ER+ no. cases=42,146 no. failures=5,238		ER- no. cases=9,105 no. failures=2,378	
	Traditional Cox model HR (95%CI)	**Extended Cox model with TVE HR (95%CI)**	**Traditional Cox model HR (95%CI)**	**Extended Cox model with TVE HR (95%CI)**
Age				
<50 years	0.89 (0.82-0.96)	0.89 (0.82-0.95)	0.92 (0.83-1.02)	0.92 (0.83-1.02)
50-69 years	Ref	Ref	Ref	Ref
70 years or older	1.34 (1.24-1.45)	1.36 (1.25-1.46)	1.30 (1.16-1.46)	1.26 (1.12-1.42)
NHS region				
West	Ref	Ref	Ref	Ref
North	1.11 (1.03-1.19)	1.11 (1.03-1.19)	1.13 (1.02-1.25)	1.11 (1.01-1.23)
South East	1.00 (0.93-1.07)	0.99 (0.92-1.06)	1.04 (0.93-1.15)	1.02 (0.91-1.13)
Year of diagnosis				
1997-2001	Ref	Ref	Ref	Ref
2002-2006	0.80 (0.74-0.85,)	0.79 (0.74-0.85)	0.83 (0.75-0.92)	0.83 (0.75-0.93)
2007-2011	0.69 (0.64-0.74)	0.69 (0.64-0.74)	0.71 (0.63-0.79)	0.71 (0.63-0.79)
2012-2016	0.73 (0.66-0.81)	0.73 (0.66-0.81)	0.83 (0.73-0.94)	0.83 (0.73-0.95)
Grade				
Grade I-(Well) differentiated	Ref	Ref	Ref	Ref
Grade II- Moderately (well) differentiated	1.80 (1.61-2.02)	1.95 (1.58-2.41)	3.08 (1.77-5.37)	2.91 (1.67-5.08)
Poorly differentiated	3.14 (2.79-3.53)	4.94 (3.99-6.11)	4.03 (2.33-6.98)	3.76 (2.17-6.52)
TNM stage				
I	Ref	Ref	Ref	Ref
II	2.20 (2.00-2.42)	2.70 (2.27-3.22)	1.88 (1.63-2.16)	2.86 (2.29-3.57)
III	5.51 (4.99-6.08)	8.44 (7.05-10.10)	6.03 (5.23-6.96)	12.07 (9.59-15.18)
IV	10.66 (9.41-12.08)	28.66 (23.132-35.51)	10.58 (8.78-12.75)	27.83 (20.65-37.49)

Appendix Table C.6 (continued) Comparison of traditional and extended Cox models with TVE for ER+ and ER- tumours (separately) diagnosed from 1997 to 2016 (page 2 of 3)

		ER+ no. cases=42,146 no. failures=5,238		ER- no. cases=9,105 no. failures=2,378	
		Traditional Cox model HR (95%CI)	**Extended Cox model with TVE HR (95%CI)**	**Traditional Cox model HR (95%CI)**	**Extended Cox model with TVE HR (95%CI)**
Screening					
	Yes	Ref	Ref	Ref	Ref
	No	1.66 (1.51-1.82)	2.33 (1.98-2.74)	1.60 (1.37-1.86)	1.60 (1.37-1.86)
Surgery					
	Yes	Ref	Ref	Ref	Ref
	No	4.55 (4.14-5.00)	4.09 (3.71-4.51)	4.41 (3.81-5.10)	3.98 (3.42-4.62)
Radiotherapy					
	Yes	Ref	Ref	Ref	Ref
	No	0.97 (0.91-1.03)	1.15 (1.04, 1.27)	1.19 (1.09-1.30)	1.51 (1.32-1.73)
Chemotherapy					
	Yes	Ref	Ref	Ref	Ref
	No	0.87 (0.81-0.94)	1.08 (0.96-1.21)	1.28 (1.14-1.43)	1.58 (1.35-1.85)
Hormone therapy					
	Yes	Ref	Ref		
	No	1.50 (1.36-1.64)	2.20 (1.90-2.56)		
SIMD quintile					
	Least deprived	Ref	Ref	Ref	Ref
	4	1.03 (0.95-1.12)	1.03 (0.94-1.12)	1.04 (0.91-1.18)	1.04 (0.91-1.19)
	3	1.07 (0.99-1.17)	1.08 (0.99-1.17)	1.21 (1.07-1.38)	1.21 (1.07-1.38)
	2	1.18 (1.08-1.28)	1.17 (1.08-1.28)	1.23 (1.08-1.41)	1.23 (1.08-1.40)
	Most deprived	1.26 (1.15-1.38)	1.26 (1.15-1.38)	1.20 (1.04-1.38)	1.19 (1.04-1.37)
Charlson Score					
	Mean (SD)	1.17 (1.06-1.30)	1.17 (1.05-1.29)	1.24 (1.09-1.40)	1.21 (1.07-1.38)

Appendix Table C.6 (continued) Comparison of traditional and extended Cox models with TVE for ER+ and ER- tumours (separately) diagnosed from 1997 to 2016 (page 3 of 3)

	ER+ no. cases=42,146 no. failures=5,238		ER- no. cases=9,105 no. failures=2,378	
	Traditional Cox model HR (95%CI)	**Extended Cox model with TVE HR (95%CI)**	**Traditional Cox model HR (95%CI)**	**Extended Cox model with TVE HR (95%CI)**
TIME-VARYING EFFECTS				
Screening*time				
No		0.95 (0.93-0.97)		
TNM stage*time				
II		0.97 (0.95-0.99)		0.90 (0.86-0.94)
III		0.93 (0.90-0.95)		0.82 (0.77-0.86)
IV		0.76 (0.73-0.80)		0.69 (0.62-0.77)
Grade*time				
Grade II- Moderately (well) differentiated		0.99 (0.96-1.02)		
Poorly differentiated		0.91 (0.89-0.94)		
Radiotherapy*time				
No		0.97 (0.95, 0.98)		0.91 (0.88-0.95)
Chemotherapy*time				
No		0.96 (0.94-0.97)		0.93 (0.89-0.97)
Hormone therapy*time				
No		0.92 (0.89-0.95)		

Footnote: Models are adjusted for age, incidence year, NHS region, grade, TNM stage, method of detection, surgery, radiotherapy, chemotherapy, hormone therapy (only for ER+ model), SIMD and Charlson score index. Models carried out in the complete case dataset separately by ER status. All HRs were statistically significant at the 0.1% level unless stated otherwise. CI= confidence interval, ER= oestrogen receptor, HR= hazard ratio, NHS= National Health Service, Ref= reference category, SD= standard deviation, SIMD= Scottish Index of Multiple Deprivation, TNM= tumour, nodes, metastases, TVE= time varying effects.

Appendix C.7 Sensitivity analysis: traditional and extended Cox models with TVE for luminal A and luminal B subtypes (2009-2016)

Appendix Table C.7 Comparison of traditional Cox model and extended Cox models with time-varying covariates effects for luminal A and luminal B tumours (page 1 of 3)

		Luminal A no. cases=13,755 no. failures=723		Luminal B no. cases=9,105 no. failures=2,378	
		Traditional Cox model HR (95%CI)	**Extended Cox model with TVE HR (95%CI)**	**Traditional Cox model HR (95%CI)**	**Extended Cox model with TVE HR (95%CI)**
Age					
	<50 years	0.65 (0.53-1.01)	0.75 (0.55-1.03)	1.06 (0.87-1.30)	1.03 (0.84-1.26)
	50-69 years	Ref	Ref	Ref	Ref
	70 years or older	1.48 (1.28-2.16)	1.67 (1.29-2.16)	1.41 (1.15-1.74)	1.39 (1.13-1.71)
NHS region					
	West	Ref	Ref	Ref	Ref
	North	1.13 (0.94-1.65)	1.26 (0.95-1.67)	1.33 (1.11-1.61)	1.29 (1.07-1.56)
	South East	0.90 (0.73-1.28)	0.96 (0.73-1.27)	1.06 (0.88-1.28)	1.06 (0.88-1.28)
Year of diagnosis					
	2009-2011	Ref	Ref	Ref	Ref
	2012-2016	1.05 (0.99-1.12)	1.06 (0.99-1.13)	1.05 (1.00-1.11)	1.06 (1.01-1.11)
TNM stage					
	I	Ref	Ref	Ref	Ref
	II	2.02 (1.41-2.88)	2.03 (1.42-2.91)	3.10 (2.23-4.30)	5.96 (2.87-12.34)
	III	4.78 (3.25-7.02,)	4.83 (3.29-7.11)	7.53 (5.38-10.54)	22.16 (10.63-46.19)
	IV	11.33 (7.62-16.85)	11.45 (7.69-17.04)	17.50 (11.88-25.77)	66.70 (30.68-145.01)
Screening					
	Yes	Ref	Ref	Ref	Ref
	No	2.43 (1.70-3.45)	2.42 (1.70-3.45)	1.69 (1.28-2.23)	1.69 (1.28-2.24)

Appendix Table C.7 (continued) Comparison of traditional Cox model and extended Cox models with time-varying covariates effects for luminal A and luminal B tumours (page 2 of 3)

	Luminal A no. cases=13,755 no. failures=723		Luminal B no. cases=9,105 no. failures=2,378	
	Traditional Cox model HR (95%CI)	**Extended Cox model with TVE HR (95%CI)**	**Traditional Cox model HR (95%CI)**	**Extended Cox model with TVE HR (95%CI)**
Surgery				
Yes	Ref	Ref	Ref	Ref
No	7.60 (5.72-10.11)	7.78 (5.85-10.34)	3.94 (3.09-5.01)	3.73 (2.91-4.78)
Radiotherapy				
Yes			Ref	Ref
No	-	-	1.42 (1.16-1.73)	2.61 (1.88-3.63)
Chemotherapy				
Yes	Ref	Ref	Ref	Ref
No	0.67 (0.51-0.88)	0.68 (0.52-0.89)	1.90 (1.54-2.33)	4.29 (2.95-6.24)
Hormone therapy				
Yes	Ref	Ref		
No	1.63 (1.14-2.32)	3.72 (2.13-6.47)		
SIMD quintile				
Least deprived	Ref	Ref	Ref	Ref
4	0.97 (0.71-1.32)	0.97 (0.71-1.33)	0.97 (0.76-1.23)	0.95 (0.75-1.22)
3	0.92 (0.68-1.25)	0.94 (0.70-1.27)	1.16 (0.92-1.48)	1.14 (1.07-1.45)
2	1.27 (0.96-1.70)	1.30 (0.98-1.73)	1.17 (0.92-1.48)	1.15 (0.08-1.46)
Most deprived	1.41 (1.03-1.92)	1.43 (1.05-1.95)	1.25 (0.98-1.61)	1.26 (0.98-1.62)
Charlson Score				
Mean (SD)	1.17 (0.81-1.69)	1.17 (0.81-1.69)	1.30 (1.05-1.60)	1.29 (1.04-1.60)

Appendix Table C.7 (continued) Comparison of traditional Cox model and extended Cox models with time-varying covariates effects for luminal A and luminal B tumours (page 3 of 3)

	Luminal A no. cases=13,755 no. failures=723		Luminal B no. cases=9,105 no. failures=2,378	
	Traditional Cox model HR (95%CI)	**Extended Cox model with TVE HR (95%CI)**	**Traditional Cox model HR (95%CI)**	**Extended Cox model with TVE HR (95%CI)**
TIME-VARYING EFFECTS				
Screening*time				
No				
TNM stage*time				
II				0.81 (0.68-0.98)
III				0.70 (0.58-0.85)
IV				0.60 (0.48-0.75)
Grade*time				
Grade II- Moderately (well) differentiated				
Poorly differentiated				
Radiotherapy*time				
No				
Chemotherapy*time				
No				0.80 (0.72-0.88)
Hormone therapy*time				
No		0.73 (0.60-0.89)		0.73 (0.63-0.83)

Footnote: Models are adjusted for age, incidence year, NHS region, grade, TNM stage, method of detection, surgery, radiotherapy (only for luminal B model), chemotherapy, hormone therapy (only for luminal A model), SIMD and Charlson score index. Models carried out in the complete case dataset separately by molecular subtype. CI= confidence interval, ER= oestrogen receptor, HR= hazard ratio, NHS= National Health Service, Ref= reference category, SD= standard deviation, SIMD= Scottish Index of Multiple Deprivation, TNM= tumour, nodes, metastases, TVE= time varying effects.

Appendix C.8 Comparison of complete case analysis and multiple imputation results for IHC defined subtypes

Appendix Table C.8 Traditional Cox model results from CCA and MIA with IHC defined subtypes as main exposure. Models in women diagnosed from 2009 to 2016

		Complete case analysis N=24,662 HR (95%CI)	Multiple imputation N=30,965 HR (95%CI)
IHC defined subtype	Luminal A	Ref	Ref
	Luminal B	2.04 (1.83-2.28)	1.98 (1.79-2.19)
	HER2-enriched	1.95 (1.58-2.41)	1.73 (1.44-2.08)
	Triple Negative	3.93 (3.29-4.70)	2.86 (2.46-3.33)
Age	<50 years	0.94 (0.83-1.07, p=0.350)	0.95 (0.85-1.05, p=0.304)
	50-69 years	Ref	Ref
	70 years or older	1.49 (1.33-1.67)	1.37 (1.24-1.51)
NHS region			
	West	Ref	Ref
	North	1.26 (1.13-1.40)	1.30 (1.19-1.42)
	South East	1.04 (0.94-1.16, p=0.462)	1.06 (0.97-1.16, p=0.170)
Year of diagnosis			
	2009-2011	Ref	Ref
	2012-2016	1.19 (1.08-1.31)	1.05 (1.03-1.07)
TNM stage	I	Ref	Ref
	II	2.51 (2.10-3.00)	2.63 (2.25-3.09)
	III	7.26 (6.06-8.71)	6.97 (5.90-8.23)
	IV	14.72 (12.05-17.97)	14.29 (11.99-17.02)
Screening			
	Yes	Ref	Ref
	No	2.13 (1.78-2.53)	2.14 (1.84-2.49)
Surgery			
	Yes	Ref	Ref
	No	4.98 (4.37-5.68)	4.08 (3.67-4.53)
Radiotherapy			
	Yes		
	No	1.05 (0.95-1.16, p=0.310)	1.07 (0.96-1.16, p=0.106)
Chemotherapy			
	Yes	Ref	Ref
	No	1.25 (1.11-1.40)	1.28 (1.15-1.42)
Hormone therapy			
	Yes	Ref	Ref
	No	1.85 (1.59-2.14)	2.28 (2.04-2.54)
SIMD quintile			
	Least deprived	Ref	Ref
	4	0.96 (0.83-1.10, p=0.536)	0.98 (0.87-1.10, p=0.718)
	3	1.11 (0.97-1.27, p=0.136)	1.13 (1.01-1.26, p=0.036)
	2	1.16 (1.01-1.32, p=0.035)	1.12 (0.99-1.25, p=0.051)
	Most deprived	1.29 (1.12-1.48)	1.34 (1.20-1.51)
Charlson Score			
	Mean (SD)	1.23 (1.08-1.40, p=0.001)	1.12 (1.01-1.25, p=0.031)

Models are adjusted for age, incidence year, NHS region, grade, TNM stage, method of detection, surgery, radiotherapy, chemotherapy, hormone therapy, SIMD and Charlson score index. All HRs were statistically significant at the 0.1% level unless stated otherwise CI= confidence interval, ER= oestrogen receptor, HR= hazard ratio, NHS= National Health Service, Ref= reference category, SD= standard deviation, SIMD= Scottish Index of Multiple Deprivation, TNM= tumour, nodes, metastases.

Appendix C.9 Overall survival estimates by ER-grade and ER-stage combinations for all women diagnosed in Scotland from 1997 to 2009 and by age groups

Appendix Table C.9 Five and 10-year overall survival by ER status and grade for all women diagnosed in Scotland from 1997 to 2016 and by age groups

	ER STATUS	GRADE	<50 YEARS	50-69 YEARS	70 YEARS OR OLDER	TOTAL
5-year OS (95% CI)	ER+	Low	94.0 (93.2, 94.6)	92.2 (91.8, 92.6)	67.7 (66.7, 68.8)	86.0 (85.6, 86.4)
		High	83.2 (81.9, 84.4)	81.7 (80.7, 82.7)	56.2 (54.2, 57.8)	75.8 (75.0, 76.5)
	ER-	Low	81.2 (76.6, 85.0)	79.2 (76.3, 81.8)	54.4 (49.8, 58.8)	72.6 (70.4, 74.7)
		High	74.1 (72.2, 75.9)	71.6 (70.1, 73.1)	43.8 (41.3, 46.3)	66.4 (65.3, 67.5)
10-year OS (95% CI)	ER+	Low	85.9 (84.7, 87.0)	81.5 (80.7, 82.2)	40.1 (38.7, 41.4)	71.1 (70.5, 71.7)
		High	70.6 (68.8, 72.3)	66.9 (65.5, 68.3)	31.2 (29.3, 33.1)	59.1 (58.1, 60.1)
	ER-	Low	71.3 (65.8, 76.2)	68.0 (64.4, 71.3)	33.6 (28.9, 38.4)	58.9 (56.2, 61.4)
		High	66.9 (64.7, 68.9)	62.2 (60.4, 63.9)	26.1 (23.6, 28.6)	55.9 (54.6, 57.1)

CI= confidence interval, ER= oestrogen receptor, OS= overall survival.

Appendix Table C.10 Five and 10-year overall survival by ER status and stage for all women diagnosed in Scotland from 1997 to 2016 and by age groups

	ER STATUS	STAGE	<50 YEARS	50-69 YEARS	70 YEARS OR OLDER	TOTAL
5-year OS (95% CI)	ER+	I	97.2 (96.5, 97.8)	95.0 (94.6, 95.4)	78.5 (77.0, 79.9)	92.2 (91.8, 92.6)
		II	92.2 (91.3, 93.1)	90.2 (89.5, 90.9)	66.1 (64.7, 67.5)	83.7 (83.1, 84.2)
		III-IV	70.9 (68.9, 72.9)	65.5 (63.9, 67.1)	39.7 (38.0, 41.4)	57.2 (56.2, 58.2)
	ER-	I	87.9 (85.1, 90.3)	88.9 (87.2, 90.4)	74.6 (70.0, 78.6)	86.4 (85.0, 87.7)
		II	82.6 (80.4, 84.6)	77.8 (75.9, 79.6)	52.6 (49.4, 55.7)	73.5 (72.1, 74.8)
		III-IV	45.6 (41.7, 49.4)	40.1 (37.2, 43.0)	20.4 (17.6, 23.3)	35.5 (33.6, 37.3)
10-year OS (95% CI)	ER+	I	92.4 (91.2, 93.5)	86.7 (85.9, 87.4)	52.8 (50.7, 54.9)	81.2 (80.5, 81.9)
		II	82.0 (80.6, 83.4)	77.5 (76.4, 78.5)	37.5 (35.9, 39.1)	66.8 (65.9, 67.6)
		III-IV	55.3 (52.8, 57.6)	45.4 (43.6, 47.3)	17.7 (16.2, 19.2)	37.3 (36.2, 38.5)
	ER-	I	81.7 (78.2, 84.7)	78.8 (76.3, 81.0)	48.9 (43.1, 54.6)	75.0 (73.0, 76.8)
		II	74.6 (72.0, 77.1)	67.8 (65.5, 70.0)	31.6 (28.3, 34.8)	61.5 (59.9, 63.1)
		III-IV	38.5 (34.6, 42.4)	31.0 (28.1, 34.0)	10.1 (7.8, 12.6)	26.5 (24.7, 28.3)

CI= confidence interval, ER= oestrogen receptor, OS= overall survival.

Appendix C.10 Results from Joinpoint regression for ER-grade and ER-stage combinations for the three age groups

Appendix Table C.11 Estimates of 5-year breast cancer specific survival trends from joinpoint regression results by age, ER and grade combinations for all women diagnosed in Scotland from 1997 to 2011

Age	ER status	Grade	5-year BCSS in 1997	5-year BCSS in 2011	Difference 5-year BCSS 1997 to 2011	Period	AAPC (95%CI)	P value
<50 years	ER+	Low	93%	95%	-2%	Full period	0.1 (0.0, 0.3)	0.1318
		High	80%	88%	8%	Full period	0.7 (0.4, 1)	**0.0003***
	ER-	High	72%	80%	8%	Full period	0.4 (0.1, 0.8)	**0.0186**
50-69 years	ER+	Low	94%	98%	4%	Full period	0.3 (0.2, 0.3)	**<0.0001***
		High	75%	90%	5%	Full period	0.7 (0.5, 0.9)	**<0.0001***
	ER-	High	69%	81%	12%	Full period	1.1 (0.7, 1.5)	**0.0001***
70+ years	ER+	Low	84%	89%	5%	Full period	0.1 (-0.1, 0.3)	0.3500
		High	67%	71%	4%	Full period	0.5 (0.1, 0.9)	**0.0278**
	ER-	High	58%	66%	8%	Full period	0.4 (-0.3, 1.1)	0.2445

Bold results indicate the test was statistically significant at the 5% level. * Indicates that the p value is significant after correcting for multiple testing using Bonferroni correction, $\alpha = \frac{0.05}{9} = 0.0056$. AAPC=average annual percentage change, BCSS=Breast cancer specific survival, CI=confidence interval, ER=oestrogen receptor.

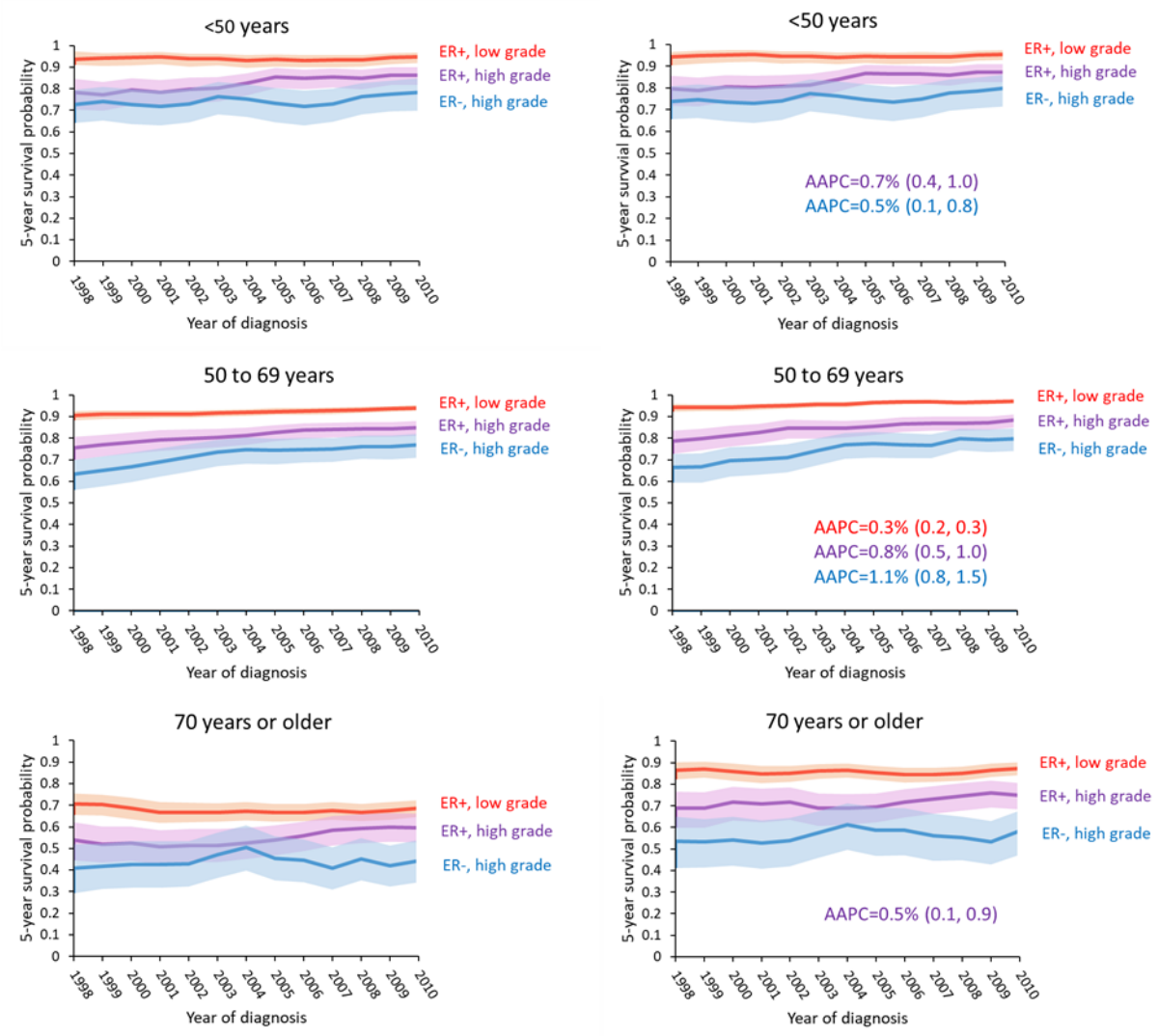
Appendix Table C.12 Estimates of 5-year breast cancer specific survival trends from joinpoint regression results by age, ER and stage combinations for all women diagnosed in Scotland from 1997 to 2011

Age	ER status	Stage	5-year BCSS in 1997	5-year BCSS in 2011	Difference 5-year BCSS 1997 to 2011	Period	AAPC (95%CI)	P value
<50 years	ER+	I	99%	98%	-1%	Full period	0.0 (-0.1, 0.1)	0.9186
		II	90%	93%	3%	Full period	0.3 (0.1, 0.5)	**0.0048**
		III-IV	70%	81%	11%	Full period	0.4 (-0.1, 0.9)	0.1243
	ER-	I	88%	80%	-8%	Full period	-0.1(-0.7, 0.6)	0.8341
		II	80%	90%	10%	Full period	0.8 (0.4, 1.2)	**0.0033**
		III-IV	53%	42%	-9%	Full period	0.3 (-1.1, 1.7)	0.7109
50-69 years	ER+	I	97%	99%	2%	Full period	0.1 (0.0, 0.1)	**0.0053**
		II	90%	95%	5%	Full period	0.4 (0.2, 0.5)	**0.0001***
		III-IV	61%	70%	9%	1997-2000	2.8 (1.2, 4.4)	**0.0063**
						2000-2011	-0.2 (-0.8, 0.4)	0.4672
	ER-	I	87%	95%	8%	1997-2006	1.3 (0.7, 1.8)	**0.0009***
						2000-2011	-0.6 (-1.5, 0.3)	0.2335
		II	75%	85%	10%	Full period	0.7 (0.3, 1.2)	**0.0089**
		III-IV	38%	50%	12%	Full period	1.4 (0.9, 1.9)	**0.0002***
70+ years	ER+	I	97%	97%	0%	Full period	0.2 (-0.1, 0.5)	0.1705
		II	82%	86%	4%	Full period	0.3 (0.0, 0.5)	**0.0434**
		III-IV	54%	52%	2%	Full period	-0.2 (-0.8, 0.5)	0.6222
	ER-	I	85%	86%	1%	Full period	0.6 (-0.1, 1.3)	0.1291
		II	77%	77%	0%	Full period	0.3 (-0.3, 0.9)	0.4010
		III-IV	24%	37%	7%	Full period	0.1 (-1.1, 1.2)	0.9323

Bold results indicate the test was statistically significant at the 5% level. * Indicates that the p value is significant after correcting for multiple testing using Bonferroni correction, $\alpha = \frac{0.05}{18} = 0.0028$. AAPC=average annual percentage change, BCSS=Breast cancer specific survival, CI=confidence interval, ER=oestrogen receptor.

Appendix C.11 Five-year OS and BCSS probabilities (with 95% CI) by ER and grade combinations and by age group

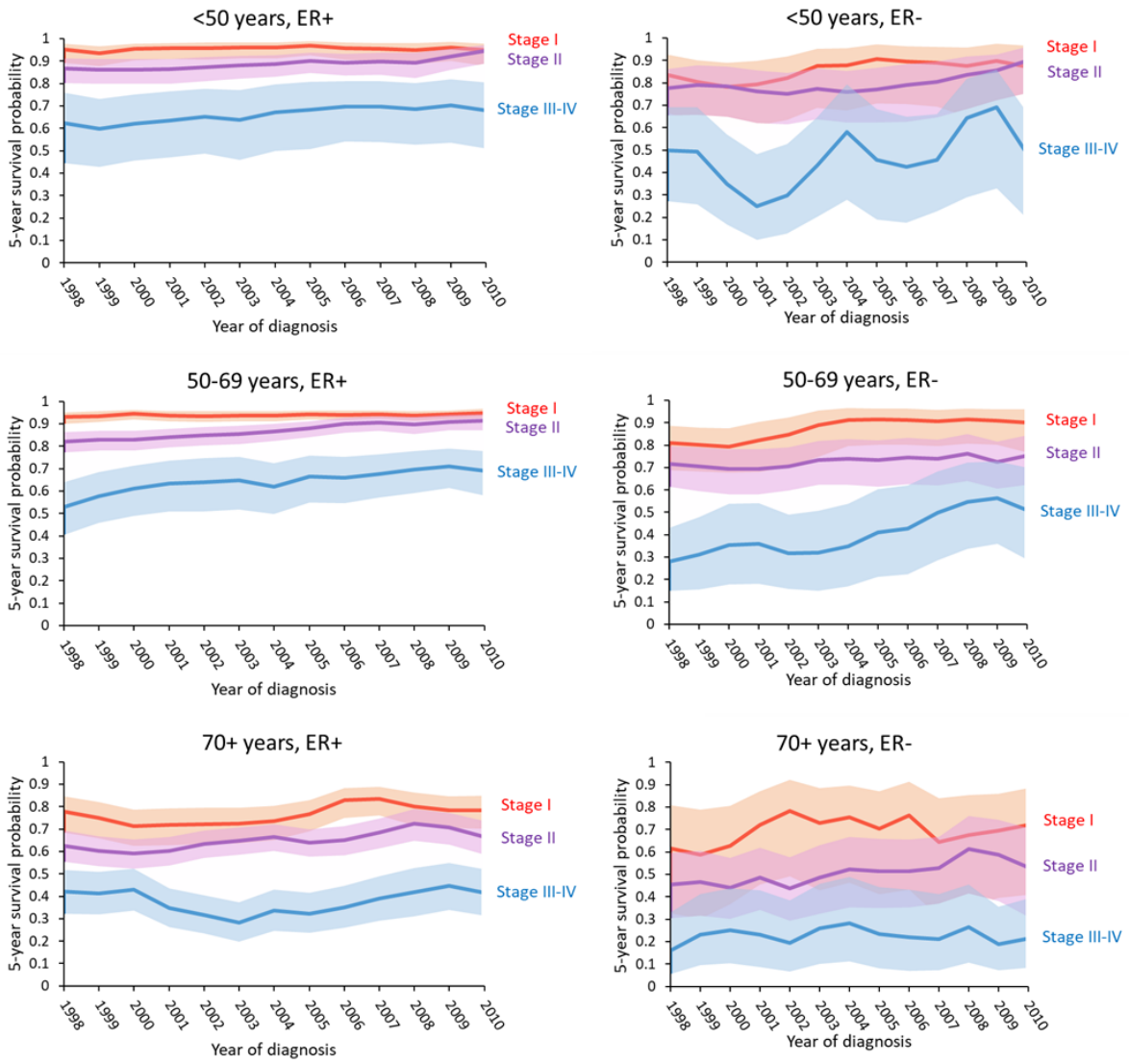
Appendix Figure C.9 Comparison of OS (left column) and BCSS (right column) by age, ER and grade combinations in women diagnosed from 1997 to 2011 in Scotland



Shaded area represents the 95% CI around the estimate. Year of diagnosis in the graphs represents the middle year for that three-year average. AAPC=average annual percentage change, ER=oestrogen receptor.

Appendix C.12 Five-year OS probabilities (with 95% CI) by ER and stage combinations and by age group

Appendix Figure C.10 Five-year OS by age, ER and stage combinations in women diagnosed from 1997 to 2011 in Scotland



Shaded area represents the 95% CI around the OS estimate. Year of diagnosis in the graphs represents the middle year for that three-year average. ER=oestrogen receptor.

Appendix C.13 Results from Joinpoint regression for ER-grade and ER-stage combinations for the three age groups

Appendix Table C.13 Estimates of 5-year breast cancer specific survival trends from joinpoint regression results by screening, ER and grade combinations for women aged 50 to 69 years (screening age) diagnosed in Scotland from 1997 to 2011

Screening	ER status	Grade	5-year BCSS in 1997	5-year BCSS in 2011	Difference 5-year BCSS 1997 to 2011	Period	AAPC (95%CI)	P value
Yes	ER+	Low	96%	99%	3%	Full period	0.1 (0.1, 0.2)	**0.0009***
		High	82%	96%	14%	Full period	0.3 (0.1, 0.5)	**0.0207**
	ER-	Low	81%	97%	16%	1997-2000	4.0 (-0.9, 8.9)	0.1424
						2000-2011	0.0 (-0.5, 0.5)	0.9711
		High	82%	87%	5%	1997-1999	-7.2 (-17.9, 3.4)	0.2257
						1999-2004	4.9 (2.1, 7.7)	**0.0118**
						2004-2011	-0.2 (-1.0, 0.6)	0.6316
No	ER+	Low	93%	95%	2%	Full period	0.2 (0.0, 0.4)	0.1439
		High	73%	84%	11%	Full period	0.6 (0.4, 0.9)	**0.0001***
	ER-	Low	73%	89%	16%	Full period	0.2 (-0.7, 1.2)	0.6488
		High	65%	77%	12%	Full period	0.8 (0.3, 1.3)	**0.0051***

Bold results indicate the test was statistically significant at the 5% level. * Indicates that the p value is significant after correcting for multiple testing using Bonferroni correction, $\alpha = \frac{0.05}{8} = 0.0063$. AAPC=average annual percentage change, BCSS=Breast cancer specific survival, CI=confidence interval, ER=oestrogen receptor.

Appendix Table C.14 Estimates of 5-year breast cancer specific survival trends from joinpoint regression results by screening, ER and stage combinations for women aged 50 to 69 years (screening age) diagnosed in Scotland from 1997 to 2011

Screening	ER status	Stage	5-year BCSS in 1997	5-year BCSS in 2011	Difference 5-year BCSS 1997 to 2011	Period	AAPC (95%CI)	P value
Yes	ER+	I	98%	100%	2%	Full period	0.1 (0.0, 0.1)	**0.0315**
		II	88%	96%	8%	Full period	0.2 (0.0, 0.4)	**0.0410**
		III-IV	67%	89%	22%	Full period	1.1 (0.4, 1.8)	**0.0104**
	ER-	I	83%	97%	14%	1997-2004	1.8 (0.9, 2.8)	**0.0042***
						2004-2011	-0.0 (-0.5, 0.4)	0.8936
		II	91%	84%	-6%	Full period	0.4 (-0.4, 1.3)	0.3487
		III-IV	56%	80%	24%	Full period	3.2 (2.0, 4.3)	**0.0001**
No	ER+	I	96%	98%	2%	Full period	0.2 (0.0, 0.3)	**0.0443**
		II	90%	95%	5%	Full period	0.4 (0.2, 0.6)	**0.0034***
		III-IV	59%	64%	5%	1997-2002	3.2 (1.0, 5.3)	**0.0182**
						2002-2011	-0.9 (-1.8, 0.0)	0.0671
	ER-	I	91%	91%	0%	Full period	0.4 (0.0, 0.8)	0.0918
		II	71%	85%	14%	Full period	0.6 (0.1, 1.1)	0.0259
		III-IV	35%	46%	11%	Full period	1.0 (0.5, 1.6)	**0.0019***

Bold results indicate the test was statistically significant at the 5% level. * Indicates that the p value is significant after correcting for multiple testing using Bonferroni correction, $\alpha = \frac{0.05}{12} = 0.0042$. AAPC=average annual percentage change, BCSS=Breast cancer specific survival, CI=confidence interval, ER=oestrogen receptor.

Appendix C.14 Power and sample size calculations for Cox proportional hazards models.

Appendix Table C.15 Power calculations for the proportional hazards models with BC survival for the rare subtypes as the outcome and deprivation or screening as the main exposure.

BC subtype	Main Exposure	Number of cases	Number of deaths	Probability of being exposed	Probability of dying from BC	Postulated Hazard ratio	Type I error rate, α	Power, β
Luminal B	SIMD quintile (most vs least deprived)	**3103**	**521**	0.45	0.17	1.2	0.05	0.54
		5000	840	0.45	0.17	1.2	0.05	0.75
		7500	1259	0.45	0.17	1.2	0.05	0.90
TNBC	SIMD quintile (most vs least deprived)	**1120**	**292**	0.52	0.26	1.1	0.05	0.13
		5000	1303	0.52	0.26	1.1	0.05	0.40
		15000	3911	0.52	0.26	1.1	0.05	0.85
HER2-enriched	Mode of detection (non-screen vs screen detected)	**1285**	**283**	0.81	0.22	1.8	0.05	0.97

Numbers in bold represent the original sample sizes in the analysis. Numbers in red represent the estimates number of deaths that would be required to obtain the estimated power (also in red). Power calculations have been performed using 'powerSurvEpi' package [309] in R studio [310] which are based on the sample size formulas for the proportional hazards models developed by Schoenfeld [311] and Latouche et al [312].

Appendix D Published article

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ARTICLE

Epidemiology

Distinct temporal trends in breast cancer incidence from 1997 to 2016 by molecular subtypes: a population-based study of Scottish cancer registry data

Ines Mesa-Eguiagaray[1], Sarah H. Wild[1], Philip S. Rosenberg[2], Sheila M. Bird[1,3], David H. Brewster[1], Peter S. Hall[1,4], David A. Cameron[4], David Morrison[5] and Jonine D. Figueroa[1,4]

BACKGROUND: We describe temporal trends in breast cancer incidence by molecular subtypes in Scotland because public health prevention programmes, diagnostic and therapeutic services are shaped by differences in tumour biology.
METHODS: Population-based cancer registry data on 72,217 women diagnosed with incident primary breast cancer from 1997 to 2016 were analysed. Age-standardised rates (ASR) and age-specific incidence were estimated by tumour subtype after imputing the 8% of missing oestrogen receptor (ER) status. Joinpoint regression and age–period–cohort models were used to assess whether significant differences were observed in incidence trends by ER status.
RESULTS: Overall, ER-positive tumour incidence increased by 0.4%/year (95% confidence interval (CI): −0.1, 1.0). Among routinely screened women aged 50–69 years, we observed an increase in ASR from 1997 to 2011 (1.6%/year, 95% CI: 1.2–2.1). ER-negative tumour incidence decreased among all ages by 2.5%/year (95% CI: −3.9 to −1.1%) over the study period. Compared with the 1941–1959 birth cohort, women born in 1912–1940 had lower incidence rate ratios (IRR) for ER+ tumours and women born in 1960–1986 had lower IRR for ER− tumours.
CONCLUSIONS: Future incidence and survival reporting should be monitored by molecular subtypes to inform clinical planning and cancer control programmes.

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BACKGROUND

Breast cancer incidence is rising and it is the most common cancer among women worldwide.[1] Breast cancer is not a single disease, but comprises multiple subtypes, with oestrogen receptor (ER) expression, a key marker of prognostic and aetiologic significance.[2] ER+ tumours, which are amenable to targeted anti-oestrogenic therapies, such as tamoxifen and aromatase inhibitors, are the most common type of breast cancers accounting for 65–75% of breast cancer cases in high-income populations.[3] Progesterone receptor (PR) is also a commonly tested marker of hormone responsiveness that is highly correlated with ER. Tumour overexpression of the human epidermal growth factor receptor 2 (HER2) was identified over two decades ago. The discovery of HER2 laid the foundation for biological therapies, which were shown to be clinically effective in treating tumours expressing this marker. HER2-targeted therapies have been widely available in the United Kingdom since 2006.[4] ER− tumours are rarer, have an earlier age of onset and worse prognosis than ER+ tumours, in part because fewer targeted treatments are available than for ER+ tumours. In addition to prognostic differences, epidemiologic studies have shown aetiologic differences by tumour subtypes.[5,6]

There are relatively few population cancer registries that collect ER, PR and HER2 data, the key distinguishing markers for molecular subtypes of breast cancer. Recent analyses support divergent incidence trends by ER status in the United States, Denmark and Ireland, with ER+ breast cancer incidence increasing and ER− breast cancer incidence decreasing.[7–9] Data on a combination of subtypes using ER, PR and HER2 are even more limited, with few reports from the United Kingdom.[10–12] ER, PR and HER2 molecular markers are used often as surrogates for the intrinsic subtypes of breast cancer defined by mRNA expression profiling[13] because, unlike genetic profiling subtypes, the molecular markers have been measured routinely in recent years. In the age of precision medicine, quantifying and monitoring cancer incidence by molecular subtypes are important in optimising public health prevention programmes, the allocation of resources and availability of screening, diagnostic and therapeutic services and for improving outcomes.[14] An important issue in assessing trends by ER status is the need to account for missing data, as completeness of marker data has improved over time, but imputation methods can be applied to address this limitation.[7–9,15]

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Within Scotland's renowned, high-quality routine electronic health records, the Scottish cancer registry is an excellent resource to investigate temporal trends in cancer incidence. Data collection began for ER in 1997 and PR and HER2 in 2009, and so provides data almost a decade earlier than other UK national registries. While monitoring of breast cancer incidence in the United Kingdom is standard,[16,17] these data have not been presented by molecular subtypes, despite substantial evidence that heterogeneity exists by ER status.[6,18–20]

Here we report on breast cancer incidence trends in Scotland by ER and ER/HER2 combinations using several statistical methods: (1) age-standardised and age-specific incidence rates, which are typically used to report cancer statistics,[21] (2) joinpoint regression models to determine whether significant changes occurred during 1997–2016, and the speed at which they have occurred[22] and (3) age–period–cohort (APC) models[23–25] based on generalised linear model theory to enable description of age, period and birth cohort effects to provide possible clues to potential underlying factors contributing to incidence trends, and thereby inform public health and NHS programmes.

METHODS

Data and cohort definition

All primary invasive breast cancers (defined on the basis of the International Classification of Diseases, 10th revision code of C50) diagnosed in women aged 20+ years, between 1997 and 2016, were ascertained from the Scottish cancer registry held by Information Services Division (ISD) of NHS National Services Scotland. The Scottish cancer registry achieves 98% breast cancer case ascertainment and is over 99% complete.[26] Breast cancer incidence after a previous cancer is considered a different aetiology (i.e. possible different risk factors such as radiation exposure amongst others) and, while an interesting topic, was not the major interest of this analysis. Supplementary Fig. 1 describes how the final study population was derived: notably, by exclusion of men and women who had a prior non-breast primary tumour. ER status was based on Allred scoring.[27] Women with primary breast cancer are the basis for analysis, each characterised by her worst-prognosis tumour. In our study population, 3653 women (5% of the study population) had multiple invasive breast cancers denoted in the cancer registry. The first primary invasive breast cancer was chosen if the time between diagnoses was >6 months, whereas for those with more than 1 diagnosis <6 months apart ($n = 2094$), the more advanced invasive cancer was selected. Of those 2094, 1837 (88%) had the same ER status, 154 (0.07%) had different ER status and the rest had one or more of the records with missing ER status. We therefore prioritised the record with less missing data. Given that only 0.07% cases lacked agreement in ER status, this prioritisation had negligible impact on the results; using tumours (not individuals) as the numerator, which is typically done in regular cancer reporting, overestimates the incidence rates of breast cancer, and we used one tumour per person to minimise bias in time trends. Permission for use of the data was obtained from the Public Benefit and Privacy Panel (PBPP) of NHS Scotland (reference number 1718-0057), and analyses were conducted in the Scottish National Safe Haven.[28]

Additional demographic and tumour data obtained were age at diagnosis, NHS Scotland regions (North, South East and West), tumour grade (grade I—well differentiated to III—poorly differentiated), tumour size (less than 10 mm, 10–20 mm and more than 20 mm), nodal involvement (yes or no), screen-detected tumour (yes or no) and the status of molecular markers ER, PR and HER2 (positive, negative or unknown). ER and PR status are measured using immunohistochemistry (IHC), and HER2 status was assessed using a combination of IHC with fluorescent in situ hybridisation for equivocal (2+) cases. Previous studies have noted that assessment of ER status reliability is high with an error rate below 5%.[29] ER/HER2 combinations were used as surrogates for the four intrinsic subtypes of breast cancer, the gold standard for which uses mRNA expression profiling. ER+/HER2− was used as a surrogate for Luminal A tumours, ER+/HER2+ for Luminal B, ER−/HER2+ for HER2-enriched tumours and ER−HER2− for triple-negative tumours. The high quality of these data has been previously described.[30]

Statistical methods

Missing ER and ER/HER2 status were imputed conditioned on age and year of diagnosis, with the assumption that data were missing at random, using a validated method.[7–9] Age-standardised incidence rates (ASR) per 100,000 women were calculated using the direct method, the European standard population (2013) and mid-year estimates of the Scottish population for each age and year.[31] Age-specific incidence rates were calculated for 5-year age groups (20–24 to 90+) and individual calendar years using two approaches: with the number of tumours as the numerator for consistency with routine reporting, and with one tumour per woman as the numerator for all other analyses. ASRs were calculated for all age groups combined and for three separate age groups, with the middle group defined on the basis of eligibility for routine breast screening in Scotland (20–49 years, 50–69 years and 70 years or older), and for each ER status and ER/HER2 combinations.

Joinpoint regression models were used to describe breast cancer incidence rates overall, by ER status and ER/HER2 combinations for all women in the cohort and for three age groups (20–49, 50–69 and 70+ years). Joinpoint models describe if changes in incidence trends occur and identify the time points at which a change is observed (referred to as joinpoints). The permutation test method, as described by Kim et al.,[22] was used iteratively: it starts by testing the null hypothesis of a simple model with zero joinpoints against the alternative hypothesis of a more complex model with the maximum number of joinpoints previously specified (3 joinpoints for this study). The procedure continues until all possible numbers of joinpoints have been tested. A total of 4,499 permutations are performed, and the p-value test is adjusted for multiple testing using the Bonferroni correction.[32] In the final model, the estimated annual percentage change (EAPC) for each of the periods identified is calculated. The average annual percent change (AAPC) is also reported as a measure of the overall trend from 1997 to 2016. Joinpoint regression software is a free open- access software that can be downloaded at https://surveillance.cancer.gov/joinpoint/.[33]

APC models were fitted for age-standardised incidence of ER+ and ER− tumours. The APC model provides a unique set of best-fitting $\log_{10}$ incidence rates obtained by maximum likelihood estimators for period, age and cohort, which have been shown to provide similar rates to ASR, but allow investigation of differences by birth cohorts—with the middle cohort as referent—which are not investigated in ASR or joinpoint regression analysis. As a consequence of small numbers in some strata, we restricted these models to women aged 30–85 years and used 28 2-year age groups (from 30–31 to 84–85) and 10 2-year periods (from 1997–1998 to 2015–2016) of calendar year of diagnosis, which covered birth cohorts from 1912 to 1986. The net drift, similar to the EAPC and AAPC estimates, is reported with 95% confidence intervals (CI). Local drifts were also estimated and describe the annual percentage change for each age-specific rate over time.[34] In addition, period and cohort rate ratios are also presented to compare the age-specific rates in each period or cohort with the reference points in the middle of the study period and birth cohort (2006 for period and 1949 for cohort). Together with cohort rate ratios (CRR), a combination test of significance for the complete cohort deviations is reported. This new combination test aims to determine if there is an association of the observed rates

Table 1. Descriptive characteristics by ER status for all women with an invasive breast cancer diagnosed between 1997 and 2016 in Scotland.

Characteristics	ER−		ER+		ER unknown	
	n	%	*n*	%	*n*	%
	11,726	[16]	55,144	[76]	5347	[8]
Age at diagnosis						
<50 years	3196	(27)	10,550	(19)	695	(13)
50–69 years	5668	(48)	28,441	(52)	1580	(30)
70 years or older	2862	(24)	16,153	(29)	3072	(57)
Grade						
I—well differentiated	195	(2)	8288	(15)	232	(4)
II—moderately differentiated	1714	(15)	25,734	(47)	602	(11)
III—-poorly differentiated	8308	(71)	14,586	(26)	642	(12)
Unknown	1509	(13)	6536	(12)	3871	(72)
Nodal status						
Uninvolved/negative	6194	(53)	29,400	(53)	869	(16)
Involved/positive	4110	(35)	17,369	(31)	415	(8)
Unknown	1422	(12)	8375	(15)	4063	(76)
Tumour size						
Less than 10 mm	1017	(9)	6470	(12)	202	(4)
10–20 mm	3428	(29)	20,449	(37)	478	(9)
More than 20 mm	4960	(42)	18,168	(33)	512	(10)
Unknown	2321	(20)	10,057	(18)	4155	(78)
PR status[a]						
Negative	3803	(79)	3036	(12)	<10	(<1)
Positive	226	(5)	15,869	(62)	<10	(<1)
Unknown	764	(16)	6489	(26)	901	(99)
HER2 status[a]						
Negative	2761	(66)	18,709	(84)	36	(5)
Positive	1210	(29)	2553	(11)	10	(1)
Unknown	184	(4)	1129	(5)	725	(94)

Brackets [] indicate row percentages and parentheses () indicate column percentages for that category.
[a]Denotes markers that were recorded from 2009 to 2016, and the number of cases for those years = 31,099. Differences by known ER status for all characteristics were significantly different with χ^2 $p < 0.001$.

with the birth cohorts above the linear influences represented by the net drift. The test provides a more robust method than the traditional Wald test while correcting for multiple testing. With the exception of joinpoint regression, all analyses were carried out using R.[35]

RESULTS

Characteristics of the cohort by ER status

Between 1997 and 2016, 72,217 women of 20 years of age or older were diagnosed with at least one invasive breast cancer in Scotland (Table 1). Seventy-six percent of these tumours were ER+, 16% were ER− and 8% had unknown ER status. However, the percentage of missing ER status decreased over time from 20% in 1997 to 2% in 2016. Proportions with unknown ER status differed by region and age: higher in the West compared with the North and Southeast of Scotland and in women aged 70 years or older compared with women younger than 70 years (14% missing vs. 5%). Almost half of breast cancers were diagnosed among women of 50–69 years of age, similar to the range for eligibility for routine breast cancer screening (50–70 years) since 2003.

Tumour characteristics differed by ER status, with ER– tumours having characteristics associated with more advanced/aggressive disease. ER− tumours had higher grade, were larger and more likely to have positive lymph node status. The patterns of other molecular markers also differed by ER status, with ER– tumours more likely to be PR− and HER2+ than ER+ tumours. In contrast, ER+ tumours were more likely to be PR+ and HER2− than ER− tumours.

The combinations of ER/HER2 status after imputing for missing ER and HER2 status are shown in Fig. 1a. Most tumours were ER+/HER2−, with ER−/HER2+ tumours being the least common combination. ER−/HER2− tumours, the most aggressive subtype, were the second most common at 11%. Cross-sectional age-specific curves for the ER/HER2 combinations (Fig. 1b) show incidence of all subtypes increasing rapidly with age, until the approximate age of menopause, age 50 years; thereafter, the increase continued more gradually up to 70 years for ER+/HER2− tumours, but there was no further increase for ER− tumours or ER+/HER2+ tumours.

Age-standardised incidence rates with EAPCs from joinpoint regression. Age-standardised incidence of ER+ tumours increased from 98 per 100,000 women in 1997 to 113 per 100,000 women in 2016 (Table 2, Supplementary Fig. 2), with an average annual percentage change (AAPC) of 0.4% (95% CI: −0.1 to 1%). Incidence was higher for ER+/HER2− tumours than for the rest of the subtypes, similar to that of ER+ tumours, with increases observed up to 2011. Estimates from the join-point analysis (Table 2) show that the increase in incidence of ER+ tumours was

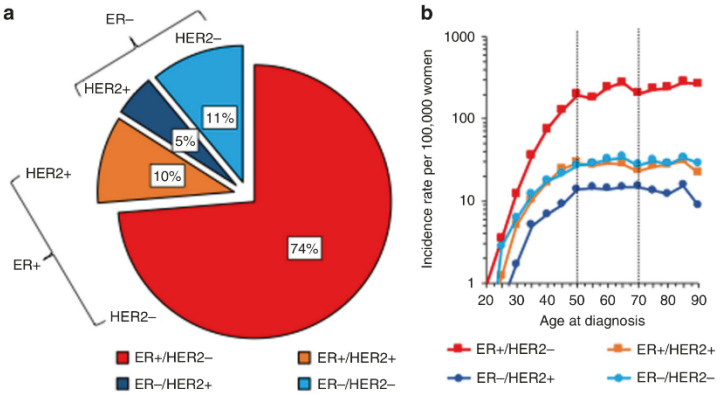

Fig. 1 Distribution of the breast cancer subtypes by ER/HER2 status and their age-specific incidence in Scotland for 2009–2016 ($N = 31,099$). a Shows a pie chart and **b** shows age-specific incidence on the log scale by subtype. **b** Data are for 31,099 breast cancer cases with ER/HER2 missing status imputed for analysis. Dotted lines in the graph denote ages 50–70 years, the age group invited for screening in Scotland every 3 years.

reasonably constant (1.2% increase annually, 95% CI: 0.8–1.5%) from 1997 till around 2012, after which incidence decreased by ~2.2% annually (95% CI: −4.7 to 0.4%). By contrast, ER− tumour incidence decreased over the study period by approximately 2.5% per year (95% CI: −3.9 to −1.1%), but showed a slow rate of decline of 0.7%/year (95% CI: −1.5, 0.0) from 2000 to 2016. ER−/HER2− tumour incidence increased by 3.2% (95% CI: 0.3–6.1%) from 2011 to 2016 (Supplemental Table 1 Supplementary Fig. 3), although the latter finding was based on relatively small numbers.

Women 50–69 years of age had the highest increases in ER+ incidence at a similar period as noted overall (Table 2, Fig. 2a), followed by women aged 20–49 years where ER+ tumour incidence increased by 1.1% annually. For women of 70 years or older rates were stable. The decreases observed in ER− tumours were consistent across the three age groups (Fig. 2b). Differences in time trends in incidence rates were also observed between ER + and ER− tumours, depending on whether the tumour was screen-detected or not. Among women aged 50–69 years with available ER and screening data, 53% of all ER+ tumours were screen-detected compared with 30% of ER− tumours. Further, among women aged 50–69 years with ER+ tumours, the incidence of non-screen-detected tumours was higher in earlier period years of diagnosis (1997–2003) than for screen-detected tumours. ER+ screen-detected tumours mimicked the incidence pattern observed for all ER+ tumours, with consistent increases in incidence until 2011, whereas non-screen-detected ER+ tumours remained constant (Fig. 2c). In women aged 50–69 years, the incidence of ER− tumours that were not screen-detected declined over time, whereas screen-detected ER− tumour incidence remained constant (Fig. 2d).

Age–period–cohort models. The results from APC models were consistent with those observed from joinpoint regression, with net drifts suggesting increases in the overall incidence of ER+ tumours by 0.8% per year (95% CI: 0.6–1.0%/year) from 1997 to 2016, and ER− tumour incidence decreasing by −1.4% (95% CI: −1.8 to −1.1%/year). After adjusting for period and cohort effects, local drifts showed that the highest increase in incidence of ER+ tumours was observed in women around 70 years of age (2% per year, 95% CI: 1.6–2.4%) (Supplementary Fig. 4a). The greatest drop in incidence of ER− tumours was observed in women of screening age 50–69 years (Supplementary Fig. 4b).

Compared with the women born in 1949, ER+ tumour incidence was higher among more recent birth cohorts. In contrast, ER− incidence was lower for more recent birth cohorts compared with the cohort born in 1949. CRRs compared with women born in 1949 ranged from 0.7 for women born in 1913 to 1.8 for women born in 1985 for ER+ tumours, and from 1.5 for women born in 1913 to 0.5 for women born in 1985 for ER− tumours (Fig. 3). The combination test for ER+ tumours revealed cohort effects beyond the log-linear trend shown by the net drift (p value < 0.0001), but the test for ER− tumours failed to reach significance (p value = 0.14).

DISCUSSION

This study demonstrates that, in Scotland, temporal trends of breast cancer incidence were distinct by molecular subtypes, with increases for ER+ and decreases for ER− tumours between 1997 and 2016. With respect to ER+ tumours, their incidence increased for all ages for the study period, but particularly among women of screening ages 50–69 years, with the largest increases occurring from around 1997 to 2011 followed by modest declines. In contrast, the incidence of ER− cancers decreased among all ages till the early 2000s. Finally, we noted cohort effects such that, in comparison with women born around 1950, women of older generations (those born in the 1910s–1940s) had a lower risk of ER+ tumours, whereas there was no significant evidence for cohort effects for ER− tumours. Further analysis of the incidence trends by subtype (as defined by ER/HER2 combinations) generally showed similar results to those observed by ER status only. ER+/HER2− (surrogate for luminal A) tumours followed the same pattern as all ER+ tumours. However, our findings suggest a significant increase in the rarer and more aggressive ER−/HER2− breast cancers among women 20–49 years of age, similar to recent increases noted in the United States that need careful future monitoring.[36] Our data affirm that future incidence and survival reporting should be monitored by molecular subtypes to inform clinical planning and cancer control programmes.

Consistent with reports from the United States, Denmark and Ireland,[7–9] our data show for the first time in a UK national cancer registry, contrasting temporal trends of breast cancer incidence by ER status, and suggest the presence of aetiologic heterogeneity with distinct patterns by period, age at diagnosis and birth cohort.

Table 2. Joinpoint regression analysis stratified by age groups and ER status from 1997 to 2016.

ER status	Age groups	Rate in 1997 per 100,000 women	Rate in 2016 per 100,000 women	Change in rate from 1997 to 2016 per 100,000 women (%)	Average annual percentage change (95% CI)	*N* for complete case analysis	*N* for estimated counts corrected for missing ER status	Years before joinpoint	EAPC (95% CI) for the period before joinpoint	Years after joinpoint	EAPC (95% CI) for the period after joinpoint
Positive	20–49	41.9	52.1	10.2 (20%)	1.1% (0.7, 1.5)	10,550	11,083	*No significant change point identified from 1997 to 2016*			
	50–69	192.3	237.4	45.1 (19%)	0.7% (0.2, 1.3)	28,441	29,758	1997–2011	1.6% (1.2, 2.1)	2011–2016	−1.8 (−3.7, 0.1)
	70+	235.9	234.5	−1.4 (−0.6%)	0.1% (−0.3, 0.5)	16,153	18,763	*No significant change point identified from 1997 to 2016*			
	All ages	97.7	112.8	15.1 (13%)	0.4% (−0.1, 1.0)	55,144	59,604	1997–2012	1.2% (0.8, 1.5)	2012–2016	−2.2 (−4.7, 0.4)
Negative	20–49	23.8	15.2	−8.6 (−36%)	−2.2% (−3.9, −0.6)	3196	3358	1997–2001	−10.0% (−17.0, −3.0)	2001–2016	0% (−1.1, 1.2)
	50–69	64.1	45.5	−18.6 (−29%)	−1.6% (−2.5, −0.8)	5668	5931	*No significant change point identified from 1997 to 2016*			
	70+	71.8	41.2	−30.6 (−43%)	−2.4% (−4.2, −0.7)	2862	3324	1997–2003	−7% (−11.0, −2.0)	2003–2016	−0.3% (−1.9, 1.5)
	All ages	35.5	23.1	−12.4 (−35%)	−2.5% (−3.9, −1.1)	11,726	12,613	1997–2000	−11% (−19.0, −3.0)	2000–2016	−0.7% (−1.5, 0)

EAPC estimated annual percentage change, *AAPC* estimated average annual percentage change.
Joinpoint regression was performed using the estimated counts corrected for missing ER status, and analysis corrects for multiple testing using Bonferroni correction (see 'Methods' section).

Previous studies have shown estimated annual increases in the age-standardised rate of breast cancer from early 1990s to 2010 for ER+ ranging from 0.1 to 3% and declines for ER− ranging from −1.9 to −3.4%.[7–9] The Scottish Cancer Registry's detailed tumour hormone receptor data have been used to describe trends in incidence patterns of breast cancer. Specifically, it was previously reported that there were declines in ER+ tumours among women 50–64 years of age that were statistically significant by 2005.[12] These findings were attributed to reduction in menopausal hormone (MH) use (also known as hormone-replacement therapy), which had been shown to be associated with increased risk of breast cancer. Unlike the previous analysis, we excluded women with a previous malignancy, imputed missing ER status and used individuals rather than tumours as the numerator for incidence rates, but the findings were similar for comparable years, confirming that MH resulted in more women diagnosed with breast cancer. The declines in breast cancer incidence coincident with decreased MH use observed in Scottish data have also been shown in the United States,[37] Sweden, Norway[38] and France.[39] We observed consistent increases over time for ER+ tumour incidence beyond 2002, after which MH use declined. Based on recent reports on the association of MH use and breast cancer risk, MH has been estimated to contribute to 1 in 20 breast cancers diagnosed worldwide since 1990.[40] In more recent years, when MH use has declined, MH has been estimated to have an approximate 5-year lag time to breast cancer incidence, and contribute to 2.3% of breast cancers in Scotland in recent years. Despite reductions in MH use from 2005 to 2011, the incidence of breast cancer continued to increase. In addition to the long-term effects of previous MH use, other factors, such as screening efficiency and obesity, are also likely to contribute to time trends in breast cancer incidence.

Mammographic screening is likely to be an important contributing factor to the increased incidence of ER+ tumours we observed from 1997 to 2011. In Scotland, the breast screening programme was established in 1988 with full national coverage attained in 1991.[41] Scotland's breast screening programme was introduced earlier than in other countries that have evaluated breast cancer incidence trends by ER status (i.e. 2000 in Ireland and 2010 in Denmark; in the United States, although there are no national screening programmes, in the Kaiser Permanente Health Management Organization, uptake of screening to 75% of eligible women was seen starting in 1993)[37]. From 1994 to 2003, women 50–64 years of age in Scotland were invited for screening, with extension in 2003 to include women aged 65–70 years. Over the course of the entire study period in Scotland, the mammographic screening programme had around 75% uptake. Our data showing that ER+ tumours are more likely to be screen-detected than ER− tumours (53% vs. 30%), and our APC model results showing incidence of ER+ tumours greatest for those of screening ages between 65 and 72 years, suggest that some of the increases observed in ER+ tumours are likely to be due to detection of prevalent disease in these older women. A similar pattern was also observed in the previous report.[12] Our analysis among women of screening age showed that the trend for screen-detected ER+ breast cancers is similar to that of the overall ER+ breast cancer incidence seen in this age group, strongly suggesting that mammographic screening is better at detection of ER+ than ER− breast cancers. Detecting ER− breast cancers has remained a challenge—they tend to present at younger ages, as larger tumours, and have fewer targeted treatments unlike ER+ breast cancers.[42] The natural history of breast cancer suggests a complicated aetiology when evaluating screen-detected tumours.[43] Our data suggest that ER+ screen-detected tumours have been significantly increasing over the time period of our study although, in more recent years, the incidence has stabilised or perhaps declined slightly, which we intend to continue monitoring.

Yen and colleagues aimed to determine risk factors and molecular tumour markers that might be associated with screen-

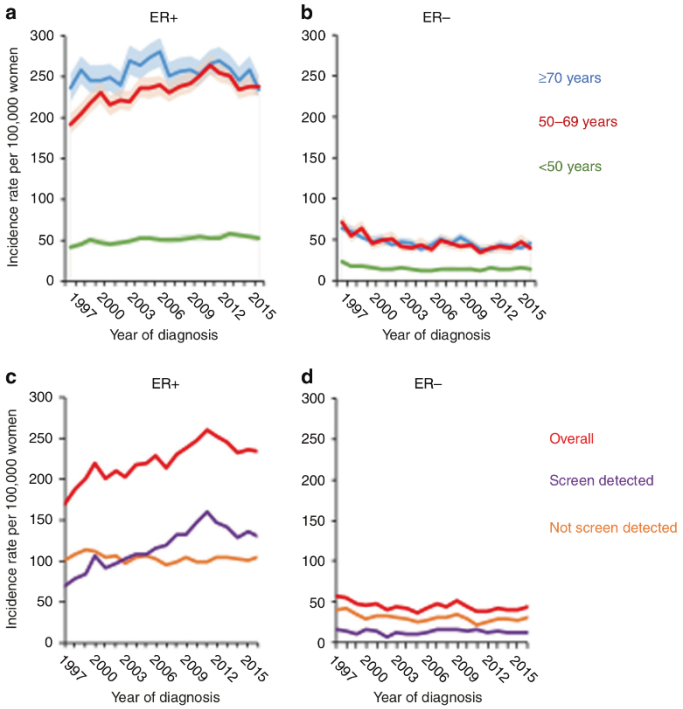

Fig. 2 Age-specific trends in breast cancer incidence stratified by age groups, screen detection and ER status in Scotland for 1997–2016. ER-positive (**a**) and ER-negative (**b**) age-specific trends for age groups 20–49 (green), 50–69 (red) and 70 years old (blue). Shaded areas surrounding lines indicate 95% CI of rates. Panels **c**, **d** are restricted to women aged 50–69 years, and incidence rates overall (red), for screen-detected (purple) and not screen-detected (orange) are shown for ER+ (**c**) and ER− tumours (**d**).

detected tumours using data on 1924 screen-detected and 1001 interval-detected cancer cases diagnosed in Sweden.[44] They found that higher BMI, older age at first birth, higher breast density (the radiologic appearance of the breast) and family history of breast cancer were significant positive contributors to tumours that are diagnosed through mammography screening. These data are consistent with increasing obesity and advancing ages at first birth in the population contributing towards likely increasing risk and therefore incidence of ER+ tumours. Furthermore, specific molecular subtypes, such as ER− breast cancers and the subset of ER− basal-like tumours, were more likely to be interval cancers. Predictive modelling of breast cancer has been proposed as a potential tool for personalised medicine and risk-stratified screening,[45–47] and future efforts might be used within screening programmes to improve the detection of more aggressive ER− breast cancers, particularly amongst those at higher risk of developing such cancers. With increased technologic advances in imaging modalities, it will be important to assess how these impact screen-detected tumours, and whether they can also improve detection for more aggressive ER-negative tumours that are more likely to be diagnosed outside of most screening programmes' age ranges. With increasing emphasis on efficiency in maximising limited resources, modelling studies on stratified screening using UK data suggest that such approaches could improve the cost-effectiveness of the screening programme, reduce overdiagnosis and maintain the benefits of screening.[48]

The strengths of our study are the high quality of the longitudinal data collected within the Scottish cancer registry, the first one in the United Kingdom that routinely started recording molecular marker data (ER status from 1997 and PR and HER2 status from 2009). Marker data can be used to monitor and describe incidence trends in the future and for other types of cancer that display heterogeneity. Further, monitoring breast cancer incidence by molecular subtypes can help the NHS allocate resources for treatment and prevention, and lead to the identification of high-risk groups of women for which to implement future prevention programmes and treatments.

A potential limitation of our study is imputation of ER status for 8% of the population and the assumptions used, which were that ER/HER2 data have the same chance of being missing among each cohort of patients by year and age at diagnosis. For this assumption to be wrong, there would have to be a confounder associated with ER status that would influence whether ER status was tested and recorded. This scenario seems unlikely in Scotland's health service where guidelines are used to inform investigation and treatment. Missingness is more likely to reflect administrative omissions, and geographic uptake in reporting ER status. This assumption has been used in US, Denmark and Irish data.[7–9] Performing multiple imputation using additional individual-level covariates would be more important when describing survival. An extended imputation model for individuals that incorporated the American Joint Committee on Cancer TNM stage[49] and tumour grade in addition to age and year of diagnosis

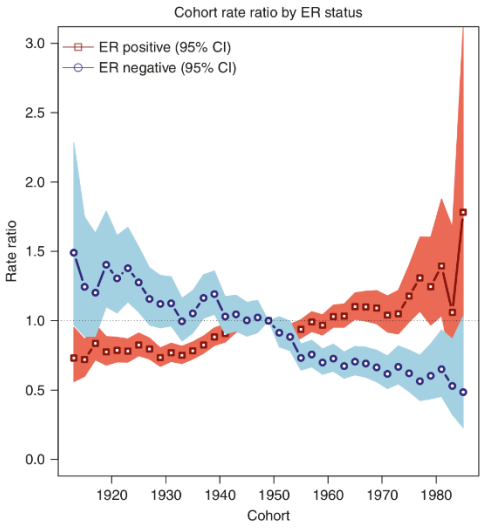

Fig. 3 Birth cohort rate ratios (CRR) for breast cancer incidence rates in Scotland by ER status. CRR describes the incidence rates for each birth cohort relative to the 1949 birth cohort.

found that the overall imputed counts were very similar to those obtained using the simpler model that contained just age and year of diagnosis.[15] Therefore, redistributing the relatively small percentage of missing receptor status in cases within each single year of age at diagnosis and calendar year of diagnosis according to the distribution observed for that specific cohort of patients is appropriate for estimating incidence trends.

Another limitation of our study is the absence of individual-level risk factor data, including participation in breast screening programmes in prior years to define interval breast cancers and stage data. However, in future studies, it should be possible to identify some key factors using linked data including detailed cohort data. The United Kingdom is renowned for its high-quality, longitudinal data and the ability to perform linkage studies using a unique identifier. Hence, we envision future analysis using the cancer registry linked to other datasets, including community prescription drug records, mammography imaging, maternity and hospital records to provide more detailed information on the role and patterns of key risk factors in breast cancer incidence trends. Another limitation of the study is the lack of mRNA expression assays for the classification of the molecular subtypes of breast cancer. In our study, markers measured by IHC are used as surrogates for the molecular subtypes, which are reasonably good proxies, but mRNA profiling data would be considered a gold standard for intrinsic-subtype classification.[13]

In conclusion, incidence trends of breast cancer in Scotland differ by ER status, and are consistent with trends observed in other countries. It will be important to monitor whether ER+ tumour incidence stabilises or reduces over time. Additional data are needed to establish whether incidence of HER2+ tumours, which are ER−, remains low since their treatment involves monoclonal antibodies, such as trastuzumab and pertuzumab,[13,50] which are amongst the more expensive breast cancer treatments used by the NHS. Further research should be focused on monitoring incidence trends by subtype because of the marked risk, detection and treatment differences for breast cancer subtypes.

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AUTHOR CONTRIBUTIONS

Conception and design of the study: J.D.F., S.W., I.M.E. and S.B. Interpretation of data: all authors. Drafting of the paper: I.M.E., J.D.F. and S.W. Revised work and provided important intellectual content: all authors. Final approval of the paper: all authors.

ADDITIONAL INFORMATION

Ethics approval and consent to participate Approval from the Public Benefit and Privacy Panel for Health and Social Care is a requirement for data access. Our project was approved by PBPP reference number 1718-0057.

Consent to publish Not applicable.

Data availability The data used in this study can be accessed through application to electronic Data Research and Innovation Service (eDRIS), a part of the Information Services Division of NHS Scotland.

Competing interests S.M.B. holds shares in GlaxoSmithKline. Other authors declare no competing interests.

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